



# Endocrine Abstracts

8th European Congress of  
Endocrinology incorporating  
the British Endocrine Societies

1–5 April 2006, Glasgow, UK





# **Endocrine Abstracts**

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## **8th European Congress of Endocrinology incorporating the British Endocrine Societies**

1–5 April 2006, Glasgow, UK

**Advance Abstracts of Papers**

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# Plenary Lecturers' Biographical Notes

## **European Journal of Endocrinology Prize Lecture**

S Costagliola, Erasmus Hospital, Brussels, Belgium

Sabine Costagliola completed her PhD in immunology at Aix Marseille University, France in 1991, followed by a PhD in biomedical sciences at the Free University of Brussels, Belgium in 2000. Dr Costagliola is currently a research scientist at the National Scientific Research Foundation, Belgium and Group Leader at the Institute of Interdisciplinary Research, the Free University of Brussels, Belgium. She is also an executive committee member of the European Thyroid Association.

Dr Costagliola has been awarded the Laureat of Haarrington De Vischer Prize from the European Thyroid Association (2001), the Alvarenga Prize from the Royal Academy of Medicine, Belgium (2004) and the Gaetano Salvatore Prize from the Academia dei Lincei, Roma, Italy (2005). She has over 70 peer-reviewed articles published in international journals and has spoken at more than 50 international conferences or symposia.

## **Geoffrey Harris Prize Lecture**

F F Casanueva, Santiago de Compostela University, Complejo Hospitalario,  
Universitario de Santiago, Spain

Dr Casanueva is Professor of Medicine and Head of the Clinical Endocrinology section at the University Hospital at Santiago de Compostela University, Spain. After completing his medical training in Madrid, he moved to Milan, Italy, where he spent three years working with Prof. Eugenio Muller. After this, he moved to Winnipeg, Canada, where he spent two years working with Prof. Henry Friesen. He is also a member of the European Society of Endocrinology and has served as an Executive Committee member, Vice-President and President.

He is a member of numerous other academic societies and serves on the editorial boards of several journals, with more than 250 peer-reviewed papers to his name. Currently, he is the Chairman of the Executive Committee of the International Society of Endocrinology.

His main research areas are regulation of growth hormone secretion, clinical neuroendocrinology and regulation and action of ghrelin.



## **Transatlantic Medal Lecture**

D J Mangelsdorf, University of Texas Southwestern Medical Centre, Dallas,  
Texas, United States

Dr David J Mangelsdorf was born in Davenport, Iowa in 1958. He received his BS in Biology and Chemistry from Northern Arizona University in Flagstaff (1981) and his PhD in Biochemistry from the University of Arizona in Tucson (1987). He did his postdoctoral studies at The Salk Institute for Biological Studies (1987–1993). Since 1993 he has been at the University of Texas Southwestern Medical Center at Dallas, where he is currently a Professor in the Departments of Pharmacology and Biochemistry, and an Investigator of the Howard Hughes Medical Institute. He also holds the Doris and Bryan Wildenthal Distinguished Chair in Medical Science. His awards include the John J Abel Award and Gerald Aurbach Award from the Endocrine Society, the Adolf Windaus Prize from the Falk Foundation, and the Heinrich-Wieland Prize.

Dr Mangelsdorf's research interests are focused on the mechanism of action of orphan nuclear receptors. His discoveries include the RXRs (retinoid X receptors), LXRs (oxysterol receptors) and FXR (bile acid receptor). This work has revealed the existence of a metabolic cascade of responses that govern lipid metabolism, and is helping to lead to the development of a new generation of drugs targeted to these receptors.

## **Clinical Endocrinology Trust Visiting Professor Lecture**

W F Crowley Jr, Harvard Medical School, Boston, United States

Dr William F Crowley Jr is a Professor of Medicine at Harvard Medical School, Director of the Harvard Medical School's Center Reproductive Endocrine Sciences Center and Director of Clinical Research at the Massachusetts General Hospital.

Dr Crowley and his colleagues pioneered the use of GnRH analogues in the treatment of children with central precocious puberty, a therapy now used worldwide for children with this disorder. This was also the key proof of principle for GnRHa-induced therapeutic desensitisation now used widely in prostate cancer, endometriosis, and PCOD. He and his colleagues developed the use of pulsatile GnRH to induce ovulation in infertile women and also sexual maturation and fertility in men with hypogonadotropic hypogonadism and have used this model to define neuroendocrine physiology in the human. Most recently, his group has identified several new genes that underlie the neuroendocrine control of reproduction in the human using genetic and molecular approaches.

Dr Crowley has received the Annual General Clinical Research Centers Award for Excellence in Clinical Research, the Endocrine Society's Award for Clinical Investigation, and the Fred Conrad Koch Award in 2005, the Endocrine Society's highest award. He is an Honorary Fellow of the Royal Society of Physicians in Ireland and was awarded the Mentor of the Year Award from Women in Endocrinology in 2000. He served as the President of the Endocrine Society from 2001–2002.

## **British Thyroid Association Pitt-Rivers Lecture**

B Vennström, Karolinska Institute, Stockholm, Sweden

Björn Vennström studied the retroviral oncogenes v-erbA and v-erbB and their cellular protooncogenes during his post-doctoral training at University of California, San Francisco. With Dr Vennström's own research group, first in Uppsala but later at European Molecular Biology Laboratory in Heidelberg, and in collaboration with others, defined the functions of the two oncogenes in tumorigenesis. While elucidating the mechanism of v-erbA action, he showed that c-erbA encodes a thyroid hormone receptor (TR). Along with several other investigators he subsequently identified the roles of the TRs alpha and beta in mediating the diverse functions of thyroid hormone. The use of mice deficient for TR genes allowed the identification of major roles of TR alpha in heart, bone and neuronal tissues, and found that TR beta is dominant for regulating the hypothalamic-pituitary-thyroid axis, development of sensory organs and homeostasis of cholesterol metabolism. This aids the understanding of thyroid hormone action and augments the development of compounds that selectively target specific tissues. This research has also highlighted the role of unliganded TRs: the aporeceptors exert profoundly deleterious effects both during development and in the adult, thus providing an understanding for how lack of hormone causes hypothyroidism. Lately, his research aims at elucidating the role of TRs in development and adult function of the nervous system.

## **Society for Endocrinology Dale Medal Lecture**

J Findlay, Prince Henry's Institute of Medical Research, Clayton, Victoria, Australia

Professor Jock Findlay is Deputy Director and Head of the Female Reproductive Endocrinology Group of Prince Henry's Institute of Medical Research and a Senior Principal Research Fellow of the National Health and Medical Research Council of Australia (NHMRC). He is Chairperson of the Infertility Treatment Authority of Victoria and the NHMRC Embryo Research Licensing Committee. Professor Findlay was Chair of the Scientific and Technical Advisory Group, Department of Reproductive Health and Research, World Health Organisation, Geneva, Switzerland, from 1998–2003.

His awards include the 1999 Society for Endocrinology (UK) Asia and Oceania Medal and the 2006 Distinguished Scientist Award of the Society for Reproduction and Fertility (UK). He is a Member of the Order of Australia (AM) for services to medical research, particularly reproductive biology, and as a medical administrator.

Professor Findlay's research led to a paradigm shift in understanding the importance of local as well as peripheral regulation of endocrine tissues, particularly by inhibin. The concept of local regulation has been examined in the pituitary, ovary, adrenal and endometrium. His laboratory has been funded by grants from the National Institutes of Health (USA), the Rockefeller Foundation (USA), the Wellcome Trust (UK) and the NHMRC.

## **Clinical Endocrinology Trust Lecture**

A P Weetman, University of Sheffield Medical School, Sheffield, United Kingdom

Tony Weetman is the Sir Arthur Hall Professor of Medicine and Dean of the Medical School at the University of Sheffield, and Consultant Endocrinologist at the Sheffield Teaching Hospitals Foundation Trust. After graduating from the University of Newcastle-upon-Tyne in 1977, he trained with Professor Reg Hall at the Welsh National School of Medicine, Dr Tony Fauci at the Laboratory of Immunoregulation, National Institutes of Health in Bethesda, USA and Professor Sir Keith Peters at the Royal Postgraduate Medical School, London and the University of Cambridge. His main research interests are the immunoregulation and genetics of autoimmune endocrine disorders, especially those involving the thyroid. He is a former Editor of *Clinical Endocrinology*, *The British Medical Bulletin* and *Clinical and Experimental Immunology*, as well as serving as an Associate Editor of *Endocrine Reviews*. He received the Merck Prize of the European Thyroid Association in 2002, and is currently President of the British Thyroid Association and a member of the Executive Committee of the European Thyroid Association.

## **Vitamin D action**

R Bouillon, Katholieke Universiteit Leuven, Leuven, Belgium

Roger Bouillon graduated from the Katholieke Universiteit Leuven, Belgium in 1968 and trained in internal medicine, endocrinology and nuclear medicine at the Academic Hospital Sint Rafael, Leuven until 1975, and completed his PhD in 1977 on 'Advances in Calcium Metabolism'. He is currently a full Professor of the Faculty of Medicine, Katholieke Universiteit Leuven (1990-present) and Chairman of the Unit of Endocrinology, University Hospital Gasthuisberg, Leuven, Belgium (1986-present). Prof Bouillon sits on numerous Boards and is a member of several journals' Editorial Boards. His research activities include bone and calcium metabolism, vitamin D research, including non-calcemic activity of 1,25-(OH)<sub>2</sub>D<sub>3</sub> analogues, and type 1 diabetes. He has authored over 400 peer-reviewed articles.

## **Psychological determinants of metabolic risk**

M Law, University of London, London, United Kingdom

Malcolm Law is Professor of Epidemiology and Preventive Medicine in the Wolfson Institute of Preventive Medicine, Barts and The London School of Medicine at the University of London. He qualified in medicine in 1972 from the University of Adelaide, South Australia. He worked in general medicine and chest medicine for some years, particularly at St Mary's Hospital and the Brompton Hospital in London. He trained in epidemiology and preventive medicine with Geoffrey Rose at the London School of Hygiene in 1983-1984, and has worked at the Barts site with Professor Wald since 1985. Much of his work has been in the prevention of heart disease and stroke, with seminal publications on salt and blood pressure, serum cholesterol and heart disease, passive smoking and heart disease, and the reasons for the low heart disease mortality in France (the French paradox). Quantification of the effects of changing risk factors on the incidence of myocardial infarction and stroke has been a prominent feature of his work. Publications with Professor Wald and other colleagues on the effect of statins (according to specific statin and dose) on LDL cholesterol and on risk of heart disease and stroke, of the five main categories of blood pressure lowering drug according to dose on blood pressure and on risk of heart disease and stroke, and of folic acid on serum homocysteine and risk of heart disease and stroke led to the calculations of the effect of the Polypill in preventing ischaemic heart disease events by 88% and stroke by 80%.

## **Embryonic stem cells**

M Trucco, Children's Hospital of Pittsburgh, Pittsburgh, PA, United States

Massimo Trucco graduated in Medicine and Surgery from the University of Turin, Italy in 1974. From the Institute of Medical Genetics, where he learned the basis of human histocompatibility, he went to Cambridge, UK to work with Cesar Milstein (1977). He then moved to the Basel Institute of Immunology, then directed by Niels Jerne, where he published the characterisation of the first monoclonal antibodies specifically directed against histocompatibility (HLA) molecules. In 1981, Dr Trucco moved to the University of Pennsylvania, where he clarified the molecular basis of HLA-DQ polymorphism. Once at the University of Pittsburgh (1986), in collaboration with Hugh McDevitt, Stanford, he studied the influence of HLA-DQ alleles as potential genetic markers for susceptibility/resistance to autoimmune diseases, like type 1 diabetes, and the transduction molecules of NK cells. In 1991, he became the Hillman Professor of Pediatric Immunology and was honored with the Cristobal Diaz Prize at the International Diabetes Federation Congress. The William Stadie Award of the American Diabetes Association and the University of Michigan Sandoz Prize were received in 1993, while in 1996 the University of Pittsburgh Chancellor's Distinguished Award was given for his work on the etiology of type 1 diabetes.



# Plenary Lectures

## European Journal of Endocrinology Prize Lecture S1

### Evolutionary mechanisms of glycoprotein hormone receptors (TSHr, LH/CGr and FSHr) to cope with hCG in humans

S Costagliola

Erasmus Hospital, Brussels, Belgium.

The glycoprotein hormones are proteins comprising a common alpha subunit and specific beta subunits having substantial sequence similarity. The glycoprotein hormone receptors (GPHr) (FSH, LH/CG and TSH receptors) present a bipartite structure, with a large extracellular domain (ECD) responsible for high affinity hormone binding and a carboxyl-terminal serpentine region, shared by G-protein-coupled-receptors (GPCRs), implicated in transmission of the activation signal. Mutagenesis experiments and the phenotypes of natural GPHr mutants lead to a model for activation, in which the actual agonist of the serpentine domain would be an "active conformation" of the ECD, triggered by binding of the hormone without need for a direct interaction of the ligand with the serpentine region. In agreement with this view, full activation of the TSHr can be achieved by a single amino acid substitution within the ECD, as well as by binding of antibodies with stimulating activity. Despite these similarities, co-evolution of the hormone-receptor couples resulted in the establishment of tight specificity barriers, preventing promiscuous activation under normal physiological conditions. The emergence of chorionic gonadotropin (CG) in primates, constituted an evolutionary challenge to the specificity barrier in GPHr couples. In humans, during the first trimester of pregnancy, hCG reaches concentrations at which it displays some thyrotropic activity, bringing most pregnant women on the fringe of hyperthyroidism. In agreement with these notions, one would expect mutations affecting the hormone binding surface of the TSHr or FSHr to be responsible for rare cases of gestational hyperthyroidism or spontaneous ovarian hyperstimulation syndrome (sOHSS). Whereas this prediction has been verified in one case of gestational hyperthyroidism caused by a mutation in the ECD of the TSHr, up to now, all cases of sOHSS have been associated with mutations in the serpentine domain of the FSHr. From these observations, we hypothesized that establishment of the specificity barrier against CG followed different evolutionary paths in the TSH and FSH receptor lineages.

## Geoffrey Harris Prize Lecture S2

### Ghrelin: a talc of modern endocrinology

Felipe Casanueva

Growth hormone (GH) is regulated by two antagonistic hypothalamic hormones, GHRH and somatostatin, plus the liver-derived hormone IGF-I and metabolic signals. Then GH actions are implicated on somatic growth and in the regulation of general metabolism.

Developed in the seventies of past century, GH secretagogues (GHS) are small artificial molecules, either peptidyl or non-peptidyl, who actively discharge GH in all animal species so far studied and through any route of administration. No similar compounds exist in nature as they were formulated through an iterative process of trial and modification based on its capability to release GH in vitro. Curiously, these invented compounds were instrumental for the cloning of the GHS receptor (GHS-R).

Using this peculiar "orphan receptor", ghrelin was isolated from the gastrointestinal area. Ghrelin activation of its cognate receptor leads to three main type of actions: 1) GH secretion, 2) appetite and energy metabolism regulation, and 3) other CNS actions like regulation of sleep and anxiety. The development of ghrelin antagonists allowed a better understanding of the physiological role of this new hormone, suggesting that the receptor mediating the release of GH is different or that there are different subtypes from those regulating appetite and metabolism. On the other hand, the GHS-R manifests a peculiar pattern of internalization and a cross-talk with other intracellular signalling systems, such as adenosine and GHRH receptors.

Ghrelin anticipates the initiation of meals and releases GH. In catabolic situations, raised ghrelin may induce enhanced food intake, increased gastric emptying and food assimilation, coupled with GH levels which would promote a prompt nutrient incorporation to muscles and to fat reserves.

It is customary that after discovering a new hormone, the following step is the cloning of the cognate receptor and finally the development of analogues for further clinical use. In the history of ghrelin, a "reverse pharmacology" process was used as the GHS can be considered bona fide analogues of ghrelin, generated decades before the natural compound. With the coming in

scene of obestatin, a ghrelin-gene derived peptide, the fascinating interplay of several hormones regulating GH secretion and energy homeostasis may start to be unravelled.

## Transatlantic Medal Lecture S3

### Nuclear receptors and transcriptional control of lipid metabolism

D Mangelsdorf

UT Southwestern Medical Centre at Dallas, Texas, United States.

Nuclear receptors are ligand-dependent transcription factors that govern complex physiologic pathways, including reproduction, development, and metabolism. The importance of this receptor superfamily is emphasized by its conservation throughout evolution, from simple multicellular organisms to humans. Because of their therapeutic potential in governing physiology and disease, targeting nuclear receptors for novel drug discovery has become a major interest in both academia and the pharmaceutical industry. One area of intense focus has been the lipid-sensing receptors that govern metabolism of cholesterol (LXRalpha and LXRbeta), and bile acids (FXR). Previous studies have shown that many of the functions of LXRs and FXR are diametric to one another. For example, in the liver LXR agonists stimulate the pathways leading to bile acid and fatty acid synthesis, while FXR agonists repress both pathways. On the other hand, activation of both receptor systems has a beneficial effect in maintaining cholesterol homeostasis throughout the body. Our current work has focused on evaluating the yin and yang relationship of LXRs and FXR in other tissues, as well as in models for various lipid-related disorders, including obesity, cholesterol gallstone disease, inflammatory bowel diseases, innate immunity, and cancer. The specific regulatory cascades that are governed by LXRs and FXR in each of these models will be discussed. In addition, the therapeutic potential of agonists for these receptors will be explored.

## Clinical Endocrinology Trust Visiting Professor Lecture S4

### Understanding the genetic control of puberty in the human

W Crowley

Harvard Medical School, Boston, United States.

One of the fundamental mysteries of biology has been the genetic control of puberty in the human. High degrees of species specificity in the control of GnRH secretion, the central actor in sexual maturation, and the rarity of a discrete period of childhood quiescence independent of suckling in most animals have compounded this problem.

The clinical condition of Idiopathic Hypogonadotropic Hypogonadism, either with normosmia (nIHH) or with anosmia (Kallmann's Syndrome [KS]), has thus emerged as an invaluable human model in this line of inquiry. IHH is an isolated GnRH deficiency combined with a demonstrable responsiveness to exogenous GnRH administration in the absence of an anatomic cause. While rare, IHH has a rich clinical and genetic heterogeneity that has enabled the elucidation of several single genes which, when mutated, create a monogenic form of delayed puberty. Via this model, the crucial roles of *KAL-1* in GnRH neuronal migration, *DAX-1* in hypothalamic-pituitary development, and *GnRHR* in gonadotrope responsiveness have been identified.

Recently, mutations in a G-coupled protein receptor (GPR54), previously unsuspected as having a role in GnRH secretion, was demonstrated to cause IHH and to be essential for normal puberty in the human as is its peptide ligand, metastin. Initially identified in IHH families, its crucial role in sexual maturation has now been confirmed in rodents and primates.

In addition, a second gene causing an autosomal dominant form of Kallmann's Syndrome has been discovered via deletion mapping studies. The gene encoding the Fibroblast Growth Factor Receptor 1 (*FGFR-1*) often has an accompanying skeletal phenotyping in addition to IHH signaling its presence clinically.

The clinical and genetic heterogeneity of these latter two genes as well as their implication for sexual maturation in general will be the topic of this lecture.

## British Thyroid Association Pitt-Rivers Lecture

S5

### Neurological abnormalities in mice with a dominant negative thyroid hormone alpha 1

B Vennström

CMB, Karolinska Institute, Stockholm, Sweden.

The syndrome of Resistance to Thyroid Hormone (RTH), found in about 300 patient families, is characterized by multiple somatic abnormalities, varying levels of mental retardation, and tachycardia. It is caused by mutations in thyroid hormone receptor beta 1 (TRb) which confer dominant negative effects. No patient with a germline mutant TRa gene has yet been found. We therefore introduced a patient's mutation into the mouse TRa1 gene. The mutation allows T3 binding with a 10x lower affinity thus causing the receptor to act as a transcriptional repressor unless challenged with high levels of T3. The heterozygous knock-in mice have a profound phenotype: a severe retardation of postnatal maturation that however is largely normalized in adult mice.

We have subsequently identified several neurological deficiencies that persist into adulthood. Neuromuscular dysfunctions include abnormal gait, poor hind limb coordination, inability to climb properly, and poor performance on the Rotarod. Some but not all of these deficiencies could be ameliorated by T3 treatment of the animals during postnatal days P10–P35, indicating that the mutant TR causes developmental defects affecting the motor system.

The adult mice also exhibit an extreme anxiety in the open field and elevated plus maze tests, and are impaired in tests for exploration and recognition of novel objects. All tests were fully normalized by adult but not juvenile T3 treatment, suggesting that these deficiencies are caused by an adult, neurophysiological function of the mutant TR.

Our data thus suggest that the mice exhibit two distinct features similar to those of thyroid hormone depletion: a neuromuscular one caused by hormone deficiency during development and that is irreversible in the adult, and a psychiatric one that can be ameliorated by treating the adult animal, suggesting an effect of the mutant receptor on central neurophysiological functions.

## Society for Endocrinology Dale Medal Lecture

S6

### The actions of inhibin in endocrine tissues

JK Findlay

Prince Henry's Institute of Medical Research, Clayton, Victoria 3168, Australia.

Inhibins are members of the Transforming Growth Factor- $\beta$  (TGF- $\beta$ ) superfamily of pleiotrophic growth and differentiation factors that includes activins and bone morphogenetic proteins (BMPs). Inhibins were characterized originally as providing negative endocrine feedback from the gonads to the pituitary gland to regulate Follicle Stimulating Hormone (FSH) secretion. More recently, inhibin has been shown to antagonise activin and BMP action in engineered cell systems through binding to betaglycan, thereby providing a hypothesis for a local autocrine/paracrine role of inhibin in the tissues that produce them. Our focus has been to test this hypothesis of inhibin action in several endocrine cell systems.

Inhibin antagonizes a number of actions of activin: on FSH production by mouse pituitary L $\beta$ T2 cells, GnRH receptor promoter activity in mouse Leydig-like TM3 cells and L $\beta$ T2 cells, 17 $\alpha$ -hydroxylase activity in mouse adrenal cells (AC), and production of latent matrix metalloproteinases in human endometrial cells. However, unlike activin, reversal of the BMP inhibitory action on 17 $\alpha$ -hydroxylase in AC cells by inhibin was reduced by higher concentrations of BMP agonists. This can be attributed in part, to the suppression of betaglycan expression by BMPs, with consequent reduction in inhibin binding to betaglycan. This data confirms the central role of betaglycan in inhibin action in endocrine cells, and shows that betaglycan is dynamically regulated in part by BMPs. We conclude that inhibins integrate the multiple inputs from members of the TGF- $\beta$  superfamily through a hierarchy of interactions involving the regulation and action of betaglycan.

Supported by the NH&MRC of Australia (RegKeys #241000 & #198705).

## Clinical Endocrinology Trust Lecture

S7

### Thyroid disease paradigms and problems

AP Weetman

University of Sheffield Medical School, Sheffield, United Kingdom.

It is the 50th anniversary of the discovery of thyroid autoimmunity by Rose and Witebsky in suitably immunised rabbits and then by Doniach and Roitt who identified thyroglobulin antibodies in the serum of patients with Hashimoto's thyroiditis. This is really the exemplar or paradigm of an autoimmune disease and subsequent discoveries have provided new paradigms. Graves' disease is a prime example of disease apparently caused exclusively by an autoantibody while unravelling the genetics of thyroid autoimmunity has established the role of immunoregulatory genes such as CTLA-4. An international collaboration has recently shown that there are no major genes conferring susceptibility as revealed by linkage studies in a large cohort of families but by applying the candidate gene approach to this collection we were able clearly to show that TSH-receptor polymorphisms confer susceptibility to Graves' disease and not autoimmune hypothyroidism, possibly explaining how these clinically distinct conditions occur together in families. Another paradigm has been the demonstration of the important role that T cell suppressor cells play in preventing thyroid autoimmunity, based on the elegant studies of Penhale and others in T cell deficient animals. It is now known that these renamed T regulatory cells are critical in controlling autoreactive T cells that escape thymic tolerance in all individuals. Reconstitution Graves' disease is a recent example which seems likely to result from an imbalance in T regulatory cells in patients treated with T cell depleting monoclonal antibodies or in those with HIV treated with highly active antiretroviral therapy. The critical role of the target cell in the autoimmune thyroiditis is a final paradigm. The problems that remain to be solved include improved immunological treatments and unravelling the complex interrelationship between thyroid autoimmune responses and those which cause ophthalmopathy, but progress is highly likely in these areas shortly.

## Vitamin D action

S8

### Vitamin D action

R Bouillon

Katholieke Universiteit Leuven, Leuven, Belgium.

Ligand activated VDR regulates about 3% the mouse genome. Its classical function is to regulate calcium and bone homeostasis. 1,25-(OH) $_2$ D is essential for transepithelial calcium transport in the intestine (by an epithelial calcium channel, TRPV6) and in the kidney (TRPV5). Normal osteoclastogenesis and bone resorption as well as bone mineralisation is possible in the absence of VDR when VDR KO men or animals receive a high calcium diet. VDR.1 $\alpha$ ,25-(OH) $_2$ D $_3$  also have major effects on many other non-classical target tissues as demonstrated in VDR KO mice and men (keratinocytes and hair follicles: alopecia; high renin hypertension; muscle cell maturation defect; differentiation of dendritic/immune cells). Finally all VDR positive cells react to VDR.1,25-(OH) $_2$ D by inhibition of proliferation regulated by a coherent action on cell cycle genes.

Vitamin D deficiency rickets was rapidly eradicated by widespread vitamin D supplementation. Vitamin D deficiency is still widespread in the elderly population and contributes to osteoporotic fractures. Animal studies support human epidemiological data of a possible link between poor vitamin D status and autoimmune diseases (type 1 diabetes, MS, IBD) and major cancers (breast, prostate, colon cancer). The optimal vitamin D status is not well defined but levels <10 ng/ml are certainly deficient, 25OHD above 20 ng/ml are desirable for optimal bone health. Whether higher levels would be beneficial for general health will require large scale intervention studies. Out of more than 3000 synthetic analogues of vitamin D some show selective receptor modulating activity (antiproliferative or immune modulator effects or alternatively by anti-osteoporotic effects, with minimal calcemic activity).

The mode of action of superagonistic and/or selective activation of VDR is not completely understood but involves extra- and intracellular pharmacokinetics, VDR stability and above all selective recruitment of coactivators.

Clinical use of vitamin D analogues is presently limited but likely to expand rapidly.

## Psychological determinants of metabolic risk

S9

### Rhabdomyolysis and other adverse effects of statins: what happens when we inhibit HMG CoA reductase

M Law

London, UK.

HMG CoA reductase, the rate-limiting enzyme in the mevalonate pathway, is 14 steps away from cholesterol synthesis. Statins dramatically reduce the serum concentration of LDL cholesterol by inhibiting this enzyme, but may similarly lower the concentration of many other compounds in the pathway, including ubiquinone (possibly the mechanism of statin-induced rhabdomyolysis though the evidence is far from conclusive). Certain drugs are important co-factors in statin-induced rhabdomyolysis, notably gemfibrozil and inhibitors of cytochrome P450 3A4. Accurate estimates of the incidence of statin-related rhabdomyolysis are now available, and the variation in incidence of rhabdomyolysis is striking. It is rare (1 per 100,000 person-years) in people taking pravastatin, or simvastatin or atorvastatin monotherapy, and more common (20–40 per 100,000 person-years) with certain combinations of statins and other drugs, but it was amazingly high, 10% per person per year, in people taking cerivastatin and gemfibrozil together. It is worrying that so high an incidence of a serious hazard from a combination of two drugs in that any doctor could prescribe for any patient went undetected for so long. In preventing statin-induced rhabdomyolysis, monitoring by measuring serum creatine kinase is worthless. Avoiding certain drug combinations and not using gemfibrozil is most effective. Muscle disorders apart, statins appear remarkably safe drugs. The incidence of liver disease in people taking statins is scarcely elevated, if at all, though peripheral neuropathy is more common.

## Embryonic stem cells

S10

### Stem cells: regeneration in type 1 diabetes

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Type 1 diabetes (T1D) is an autoimmune disease, the clinical onset of which most frequently presents in children and adolescents who are genetically predisposed. T1D is characterized by a T-cell mediated, specific, insulin-producing beta cell destruction. The well-differentiated and specialized islet beta cells seem to physiologically retain the ability to compensate for the cells lost by reproducing themselves, while undifferentiated cell sources may help in generating new ones, even while the autoimmune process takes place. Diabetes clinical onset, i.e., establishment of a detectable, chronic hyperglycemia, occurs at a critical stage when autoimmunity, having acted for a while, supersedes the regenerative effort and reduces the number of beta cells below the physiologic threshold at which the produced insulin is insufficient for the body's needs. Clinical solutions aimed at avoiding cumbersome daily insulin administrations by the re-establishment of physiologic insulin production, like whole pancreas or pancreatic islet allotransplantation, are limited by the scarcity of pancreas donors and by the toxic effects of the immunosuppressive drugs (i.e., calcineurin inhibitors) administered to prevent rejection and autoimmunity recurrence. However, new accumulating evidence suggests that once autoimmunity is abrogated using non-diabetogenic means, the endocrine pancreas properties may be sufficient to allow the physiological regenerative process to restore endogenous insulin production, even after the disease has become clinically manifest. Knowledge of these properties of the endocrine pancreas suggests the testing of reliable and clinically-translatable protocols for obliterating autoimmunity, thus allowing the regeneration of the patient's own endocrine cells. Safe, gene therapy-based approaches can be used to permanently obliterate autoimmunity by restoring central and peripheral tolerance. The safe induction of an autoimmunity-free status might become a new promising therapy for T1D, since by correcting hyperglycemia using conventional insulin administration or an islet allotransplant, "nature" will be left to spontaneously heal the endocrine damage.

# Symposia

## Thyroid and the heart

### S11

#### Cardiac repercussions of thyroid hormones

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The cardiovascular symptoms and signs of overt thyrotoxicosis are well known. These symptoms and signs may persist even after successful restoration of euthyroidism. Long-term, overt hyperthyroidism is associated with increased vascular mortality, from both cardiovascular and cerebrovascular causes, even in patients treated in the last 20 years. This mortality may particularly reflect an effect of thyroid hormone excess on cardiac rhythm, especially risk of atrial fibrillation. Subclinical thyroid hormone excess is also associated with subtle effects on cardiac physiology and increasing evidence suggests that this too translates into increased risk of atrial fibrillation and of vascular death. This in turn raises questions about the need to treat subclinical hyperthyroidism secondary to Graves' disease or toxic nodular goitre.

Overt hypothyroidism also has cardiovascular consequences, although these are less well documented. Debate surrounds the question of whether subclinical hypothyroidism exerts an adverse on vascular risk, mediated by a minor adverse effect on the lipid profile, or via other mechanisms. Evidence from large-scale cohort studies is conflicting, some evidence suggesting an association of subclinical hypothyroidism with presence of atherosclerosis in elderly subjects. Further evidence is thus required to inform decisions about the need to correct subclinical hypothyroidism with thyroid hormone replacement.

### S12

#### The assessment of cardiovascular risk factors in thyroid disease

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The cardiovascular system is one of the major targets of thyroid hormone action, sensitive enough to detect the effects of thyroid hormone excess or deficiency at tissue level. Triiodothyronine (T3) acts on the heart and vascular system by classic genomic as well as non-genomic mechanisms and influences heart rate, systolic and diastolic function and systemic vascular resistance thereby affecting cardiac performance. In human short-term overt hyperthyroidism, the increase in left ventricular performance is predominantly sustained by the increased preload with enhanced left ventricular diastolic function. The combination of reduced systemic vascular resistance, coupled with the increased venous return and preload, increases cardiac output. The cardiovascular risk of subclinical and overt long-term hyperthyroidism is related to short-term effects due to the electrophysiological effects of thyroid hormones, and to the long-term effects resulting from increased left ventricular mass and increased cardiac workload. The frequency of atrial fibrillation is increased to a similar degree in patients with overt and subclinical hyperthyroidism. The significant increase in left ventricular mass represents the most consistent cardiac abnormality reported in patients with long-standing overt and subclinical hyperthyroidism and is responsible for diastolic dysfunction and systolic dysfunction during effort. Long-standing hyperthyroidism exerts many relevant effects on the cardiovascular system and it may induce abnormalities that may lead to more severe cardiovascular disease, thus potentially contributing to the increased risk of cardiovascular morbidity and mortality observed in these patients. The cardiovascular risk in patients with overt and subclinical hypothyroidism results from the changes in cardiovascular function and from accelerated atherosclerosis. The decreased cardiac output in hypothyroid patients at rest depends largely on changes in diastolic relaxation and hemodynamic loading conditions. The reduced cardiac preload, in combination with bradycardia and slightly depressed myocardial contractility, accounts for a subnormal cardiac output in overt hypothyroidism, whereas peripheral vascular resistance are remarkably increased. The most consistent cardiac abnormality recognized in patients with subclinical hypothyroidism is the impairment of left ventricular diastolic function with an impaired left ventricular systolic function on effort. The negative effect induced by subclinical hypothyroidism on the cardiovascular system is reverted, restoring euthyroidism with L-T4 therapy. Several epidemiological studies examined the linkage between SH and atherosclerosis showing conflicting results. Diastolic hypertension, dyslipidemia, endothelial dysfunction, elevated C reactive protein levels and coagulation abnormalities represent the atherosclerotic risk factors associated with subclinical hypothyroidism and may be reversible with euthyroidism after L-T4 therapy.

### S13

#### Iodothyronamines: rapid-acting thyroid hormone metabolites

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Thyroid hormone controls multiple physiological processes in both development and adult homeostasis. The majority of thyroid hormone actions that are understood at the molecular level involve transcriptional regulation of target genes mediated by hormone binding to the nuclear thyroid hormone receptors. However, a large number of thyroid hormone actions occur on a rapid time scale through unknown mechanisms that do not involve gene transcription. We have recently identified a novel series of thyroid hormone metabolites, called thyronamines, which chemically resemble neurotransmitters such as dopamine and serotonin. We have also found that these substances are potent agonists of a family of orphan G protein-coupled receptors, called the trace amine receptors. Moreover, we have found that these substances induce profound physiological changes when administered *in vivo*, including alterations in thermal regulation, cardiac drive, and energy utilization. In this presentation data will be presented to show the current situation with this new aspect of thyroid hormone research.

### S14

#### Role of thyroid hormone receptor isoforms in development and function of heart and other tissues

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Thyroid hormone receptors are ligand dependant transcription factors. They mediate a genomic response initiated by the thyroid hormone triiodothyronine, T3.

In mice and humans two isoforms, TR $\alpha$  and TR $\beta$ , are produced from two different genes, and several isoforms of each isotype are present in the tissues. The knock out of either TR $\alpha$  or TR $\beta$  genes induces different phenotypes and several studies led to the conclusion that the TR $\alpha$  receptor plays a major role in the development and maturation of many tissues during the fetal to neonatal transition.

In the absence of T3 unliganded TR $\alpha$  (so called apo-receptors) work as transcription repressors. Combination of mutations that abrogate either the production of the receptors and/or the production of thyroid hormone allowed dissecting the physiological roles of apo-receptors *in vivo*.

We have shown that during fetal development and in hypothyroid pathological conditions in adults, TR $\alpha$  are under the configuration of apo-receptors and repress development of heart and erythropoietic tissues. At birth the surge of thyroid hormone transforms these apo-receptors into holo-receptors ensuring the normal postnatal development of these tissues.

Then the switch from apo to holo-receptor plays a major role in controlling normal development at the transition between fetal and neonatal life and in hypothyroid pathological conditions in the adult.

## The endocrinology of psychiatric disease

### S15

#### Glucocorticoids, enzymes and memory impairments with ageing

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Chronic elevation of plasma glucocorticoids adversely affects cognitive processes and the structural and functional integrity of the hippocampus. Crucially, inter-individual differences in memory with ageing directly associate with blood glucocorticoid levels in rodents and humans; indeed higher plasma corticosterone in mid-life in rodents predict subsequent memory impairments. However, although keeping glucocorticoid levels low by adrenalectomy in adult life prevents the emergence of memory impairments with age in rats, such 'prophylactic surgery' is not a practical alternative in human therapy, not least because glucocorticoid deficiency is also harmful.

Recent data suggest that pre-receptor metabolism by 11 $\beta$ -hydroxysteroid dehydrogenases (11 $\beta$ -HSDs), which interconvert active glucocorticoids and inert 11-keto metabolites, potentially regulates glucocorticoid action in specific cells *in vivo*, including in CNS. The brain highly expresses 11 $\beta$ -HSD type 1, an 11 $\beta$ -reductase in intact hippocampal neurons and glia



which regenerates active steroids and thus locally amplifies glucocorticoid action. Inhibition of 11 $\beta$ -HSD1 protects hippocampal cells from neurotoxic challenge *in vitro*. Young 11 $\beta$ -HSD1<sup>-/-</sup> mice, despite elevated plasma corticosterone levels, perform as well as young wild-type animals, suggesting they are relatively 'blind' to the tissue effects of elevated plasma glucocorticoids. Strikingly, aged 11 $\beta$ -HSD1 null mice also learn as well as young mice and avoid the cognitive decline seen in the majority of aged wild-type mice. In explanation, despite maintained plasma corticosterone, aged 11 $\beta$ -HSD1<sup>-/-</sup> mice have markedly *reduced* intrahippocampal corticosterone levels. 11 $\beta$ -HSD1 is also expressed in the adult human CNS. In two small, randomised, double-blind, placebo-controlled, cross-over studies, administration of the 11 $\beta$ -HSD inhibitor carbenoxolone improved verbal fluency after four weeks in ten healthy elderly men and improved verbal memory after six weeks in 12 patients with type 2 diabetes. Thus 11 $\beta$ -HSD1 may be a useful therapeutic target in age-related cognitive disorders and is a prototype for tissue-specific manipulation of the effects of steroids in brain and peripheral tissues.

## S16

### Effects of hypothyroidism on brain metabolism and its associations with neuropsychiatric impairments

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#### Background

Hypothyroidism may profoundly alter mental function and influence mood and cognition, but the neural correlates of these impairments and of thyroid hormone treatment are not well understood.

#### Methods

We prospectively studied 24 subjects, 14 previously untreated newly diagnosed hypothyroid patients (age 42  $\pm$  12 yrs; 11 female, 3 men) and 10 euthyroid control subjects (41  $\pm$  12 yrs). All patients underwent comprehensive neuropsychiatric assessment, blood laboratory testing, and positron emission tomography with [<sup>18</sup>F]-fluorodeoxyglucose, before and after achieving euthyroid status with levothyroxine treatment. Regional normalized brain activity was assessed by statistical parametric mapping (SPM2). Results

Before treatment, hypothyroid patients demonstrated significantly decreased glucose metabolism relative to the control group in the anterior (subgenual/perigenual) and posterior cingulate cortex, the prefrontal cortex and subcortical structures including the dorsal and ventral striatum, the putamen and the amygdala. Hypothyroid patients showed a significant increase of regional normalized brain activity after treatment. No significant differences were found between the hypothyroid and control groups after treatment, indicating a normalization of brain metabolism. At baseline decreased prefrontal metabolism was associated with the severity of somatic complaints. Increase of prefrontal metabolism was associated with normalization of TSH and metabolism in the posterior cingulate cortex correlated with improvement in a standardized measure of short-term memory.

#### Conclusion

This is the first PET study demonstrating *in vivo* regionally specific effects of primary hypothyroidism on cerebral glucose metabolism especially in the perigenual anterior cingulate / medial frontal gyrus and its normalization with levothyroxine treatment. Furthermore, associations between abnormalities of regional brain metabolism and neuropsychological impairments can be demonstrated in adults with hypothyroidism.

Ethical approval by IRB University of California Los Angeles (UCLA).

## S17

### Overview of neuroendocrine side-effects

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The neuroendocrine aspects of schizophrenia generally receive little attention. This is in marked contrast to depressive disorders, where neuroendocrine issues are central to discussions of pathophysiology and treatment. Although the nature of neuroendocrine dysfunction is less well characterized in schizophrenia than major depression, a number of

neuroendocrine abnormalities have been described. Hypercortisolemia has been extensively documented in patients with schizophrenia, particularly during acute exacerbations, with persistent hypercortisolemia being associated with ventricular enlargement and poor outcome. Similarly, abnormalities in thyroid function, the hypothalamo-pituitary-gonadal axis, neurotensin, growth hormone, prolactin and other neuroendocrine parameters have also been described in schizophrenia. While the precise neuroendocrine profile of schizophrenia is incompletely characterized, the impact of antipsychotic medications employed in its treatment on various endocrine parameters is better understood. Different first- and second-generation antipsychotics (FGAs and SGAs) variably contribute to hyperprolactinemia, insulin resistance, and other neuroendocrine abnormalities. A critical overview of neuroendocrine abnormalities in schizophrenia will be provided and the differential impact of different antipsychotics in contributing to neuroendocrine dysfunction discussed. All FGAs and some SGAs (risperidone and amisulpride) cause hyperprolactinemia and associated adverse effects; mechanisms underlying hyperprolactinemia associated with antipsychotics and associated clinical problems will be summarized. Weight gain is emerging as the most prominent long-term side effect associated with SGAs; its prevalence, clinical importance, and underlying mechanisms will be reviewed. The prevalence and significance of diabetes mellitus and hyperlipidemia in the context of antipsychotic treatment will be reviewed and clinical measures to address the problem considered. It is important to appreciate the importance of different neuroendocrine adverse effects associated with antipsychotic use, however, and recognize differences in neuroendocrine side-effect profiles associated with agents so that we can use them optimally.

## S18

### Endocrinology of post traumatic stress

Abstract unavailable

## Cancer and the skeleton

### S19

#### Mechanisms of malignant hypercalcaemia

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Malignancy is the most common cause of hypercalcaemia. Hypercalcaemia in solid tumours involves the secretion of humoral factors by tumour cells that act systemically on target organs (bone, kidney) to alter calcium homeostasis. Parathyroid hormone-related protein (PTHrP) is a major humoral factor. It is produced by a variety of solid tumours and acts on PTH receptors to cause increased calcium reabsorption from renal tubules and increased bone resorption. Other humoral tumour-associated factors likely involve cytokines such as IL-1, IL-6 and TNF $\alpha$ . In addition, such factors enhance the hypercalcaemic effects of PTHrP. In bone, PTHrP produced by tumour cells stimulates osteoclast activity by promoting the expression of the cytokine RANK-L (receptor activator of NF $\kappa$ B ligand) by osteoblastic stromal cells. RANK-L by binding to its cell surface receptor RANK in osteoclast precursors promotes their differentiation and the subsequent osteoclastic bone resorption. Bone-residing tumour cells also produce a number of other important factors (IL-6, IL-8, IL-11, M-CSF) that lead to osteolysis. Consequently, growth factors (TGF $\beta$ , IGFs) that are released from resorbed bone cause tumour cell proliferation and further stimulate the production of PTHrP and IL-11 by tumour cells which, in turn, accelerate bone destruction. High concentrations of ionised calcium released from bone may also contribute to the progression of osteolytic lesions by increasing PTHrP production. These findings led to the use of potent bone resorption inhibitors as an effective therapy for malignant hypercalcaemia.

## S20

### Hypercalcaemia of malignancy

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Hypercalcaemia is the commonest endocrine complication of malignant disease. In most cases the underlying cause is a combination of excessive bone resorption and increased tubular reabsorption of calcium brought about by the effect of the onco-feto protein parathyroid hormone related protein

(PTHrP). Other causes of hypercalcaemia of malignancy include direct interaction of malignant cells with bone; this is most commonly seen with haematological malignancies where there is stimulation of the RANK signalling pathway increasing osteoclastic bone resorption. Rare causes include the activation of vitamin D within malignant tissue and the true production of ectopic PTH.

Clinical manifestations of hypercalcaemia in malignant patients are frequently confused with the underlying malignant disease process and clinicians need to be vigilant for hypercalcaemia in patients with malignant disease in whom there is a deterioration in the clinical circumstances. In patients presenting de novo with hypercalcaemia malignancy must be considered the most likely differential diagnosis if PTH levels are suppressed.

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## S21

### Oncogenic osteomalacia: novel insights into the regulation of phosphate homeostasis

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Despite its broad biological importance, the regulation of phosphate homeostasis remains incompletely understood. Important new insights into underlying mechanisms were made by defining the molecular basis of different inherited disorders characterized by an abnormal regulation of phosphate homeostasis. These efforts have led to the identification of three novel regulators of phosphate homeostasis, namely PHEX, FGF23, GALNT3. Additional studies have furthermore revealed heterozygous mutations in NaPi-IIa in some patients with hypophosphatemia and osteoporosis, and homozygous or compound heterozygous mutations in NaPi-IIc appear to be the cause of hereditary hypophosphatemic rickets with hypercalciuria (HHRH).

Using recently developed immunometric assays, intact and C-terminal FGF23 levels are elevated in patients with oncogenic osteomalacia (OOM) and the tumors that cause this disease overexpress FGF23 mRNA. Intact and C-terminal FGF23 levels are furthermore elevated in patients with X-linked hypophosphatemia, a disease caused by inactivating PHEX mutations suggesting that the encoded endopeptidase has a role in degrading intact FGF23. Surprisingly, C-terminal FGF23 levels, but not intact FGF23 levels, were found to be dramatically elevated in patients with two forms of tumoral calcinosis. One form is caused by homozygous, inactivating GALNT3 mutations implying that the encoded enzyme, which is involved in the initiation of O-glycosylation, has important an important role in preventing cleavage of FGF23 into biologically inactive fragments. Consistent with this hypothesis, a second form of this disease was found to be caused by different homozygous FGF23 mutations, which all affect conserved serine residues that may undergo O-glycosylation by GALNT3; these mutations also lead to an abnormal processing of FGF23 and thus increased secretion of C-terminal fragments. Despite these advances, it remains largely unknown how most of the different proteins mentioned above contribute to the regulation of phosphate homeostasis and it is almost certain that additional proteins are involved in this process.

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## S22

### Why does thyroid carcinoma metastasise to bone?

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The question why thyroid carcinoma metastasises to the skeleton can be reformulated into the more general question why bone metastases occur in cancer.

In the development of bone metastases, three phases can be distinguished. First, tumor cells have to be released into the vascular system. The process of vascular invasion and haematogenous spread involves a cascade of events that will not be summarized here, but apparent differences exist between tumors with regard to their tendency to vascular invasion.

The second phase involves the seeding and survival of metastatic cells in the skeleton. Preferential colonization of certain tissues by cancer cells and their subsequent growth are determined by interaction with the tissue-specific microenvironment. Cancer cells express attachment proteins such as integrins that interact with specific extracellular matrix proteins. Thus the match between tumor attachment proteins and the extracellular matrix "make-up" are important for successful attachment and survival.

In addition, bone is continuously remodelled by bone resorption and formation. Growth factors supporting bone metastatic growth are released especially during bone resorption: transforming growth factor- $\beta$  (TGF- $\beta$ ) stimulates the secretion of bone cytokines, which enhance bone resorption but also stimulate tumor growth by interfering in the BMP pathway. On its turn, thyroid tumor cells synthesize factors that enhance bone turnover, such as IGF-I but interestingly also osteoprotegerin. A relevant factor in thyroid cancer may TSH suppressive treatment: high T3 levels and low TSH levels enhance bone turnover, which from a perspective of bone metastasis is not beneficial.

The third phase involves tumor growth and extension. In this phase, tumor growth is self-maintaining by autocrine factors and as such becomes more or less independent from the bone environment. Processes involved are the synthesis of proteolytic, matrix degrading enzymes such as matrix metalloproteinases and the induction of neovascularization by angiogenic factors.

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## Cannabinoid signalling

### S23

#### Cannabinoids as modulators of neuroendocrine signaling

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During the last few years, the endocannabinoid system has emerged as a relevant topic in the scientific community. Many different regulatory actions have been attributed to endocannabinoids, and their involvement in several pathophysiological conditions is under intense scrutiny. Cannabinoid receptors first discovered as the molecular targets of the psychotropic component of the plant *Cannabis sativa*, participate in the physiological modulation of many central and peripheral functions. CB2 receptor is mainly expressed in immune cells, whereas CB1 receptor is the most abundant G protein-coupled receptor expressed in the brain. CB1 receptor is expressed in the hypothalamus and the pituitary gland, and its activation is known to modulate all the endocrine hypothalamic-peripheral endocrine axes. An increasing amount of data highlights the role of the system in the stress response by influencing the hypothalamic-pituitary-adrenal axis and in the control of reproduction by modifying gonadotropin release, fertility and sexual behaviour.

The ability of the endocannabinoid system to control appetite, food intake and energy balance has recently received great attention, particularly in the light of the different modes of action underlying these functions. The endocannabinoid system modulates rewarding properties of food by acting at specific mesolimbic areas in the brain. In the hypothalamus, CB1 receptor and endocannabinoids are integrated components of the networks controlling appetite and food intake. Interestingly, the endocannabinoid system was recently shown to control metabolic functions by acting on peripheral tissues, such as adipocytes, hepatocytes, the gastrointestinal tract and, possibly, skeletal muscle.

The physiological properties of endocannabinoids and CB1 receptor in regulating several endocrine functions will be discussed in the presentation.

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## S24

### Cannabinoids and feeding behaviour

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Mankind has been aware of the appetite-stimulating properties of *Cannabis sativa* for many centuries. Recent research has shown that cannabinoid molecules present within the plant (e.g.,  $\Delta^9$ -THC) exert their pharmacological effects by stimulating specific cannabinoid receptors that are expressed by the central nervous system and a wide range of peripheral tissues, including hepatocytes and adipocytes. These receptors are normally activated by a class of arachidonic acid-derived eicosanoid molecules that are collectively known as endogenous cannabinoids. It has been demonstrated that, like THC, 'endocannabinoids' (e.g., anandamide and 2-arachidonoyl glycerol) can stimulate appetite and induce eating in laboratory species. Endocannabinoid hyperphagia has been shown to involve CB1 cannabinoid receptors in brain regions classically associated with the neural regulation of eating motivation, including the nucleus accumbens and hypothalamic nuclei. Central endocannabinoid systems appear to be directly involved in the mechanisms that actively promote food



seeking and the enhancement of the salience or incentive value of food stimuli. Data also indicate that endocannabinoids have functional relationships with peptide signals that have been associated with the physiological regulation of eating behaviour. Thus, blockade of CB1 receptors can prevent the orexigenic actions of the putative 'hunger signal' ghrelin. Additionally, feeding induced by stimulation of CB1 receptors appears to involve activation of endogenous opioid peptides which are linked to the hedonic evaluation of foods. We hypothesize that the endocannabinoids are crucial to the psychological processes that underlie the anticipation and appreciation of food. In addition, there is growing evidence for cannabinoid involvement in the regulation of energy metabolism and adipogenesis. Consequently, central and peripheral endogenous cannabinoid systems represent potentially important targets for the pharmacological treatment of disorders of appetite and body weight, including obesity and cachexia.

## S25

### Clinical applications of manipulation of cannabinoid receptors

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Cannabinoid (CB) receptor pharmacology is the subject of intense academic and commercial research effort. Whilst cannabinoid ligands are thought to interact primarily with CB<sub>1</sub> and CB<sub>2</sub> receptors, there is considerable pharmacological evidence that the 'endocannabinoid system' may encompass additional targets. For example, two recent patent applications provide the first evidence that certain synthetic and endogenous cannabinoids interact with the orphan receptor, GPR55. In addition, we have recently produced the first evidence that the cannabinoid CB<sub>1</sub> receptor contains an allosteric binding site. Our hypothesis is that the allosteric binding site on the CB<sub>1</sub> receptor constitutes a highly significant new target with implications for both physiology/pathophysiology and drug discovery. Novel compounds targeting these sites thereby herald a new generation of therapeutics to be used, for example, as analgesics (allosteric enhancers) or anti-obesity agents (allosteric inhibitors). In addition, it has emerged that CB<sub>1</sub> and CB<sub>2</sub> receptors are involved in bone metabolism and that endocannabinoids may have a hitherto unrecognized role in the control of bone density. Small molecule ligands for cannabinoid receptors may represent a novel avenue for the treatment of osteoporosis and other bone diseases.

## Novel peptides in reproduction

### S26

#### The Kisspeptin-GPR54 axis in reproduction

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The ligand-receptor kisspeptin/GPR54 system was originally identified in the context of tumor biology. Thus, KiSS-1 was cataloged as metastasis suppressor gene, which encodes a number of related peptides, termed kisspeptins, with ability to activate the previously "orphan" receptor GPR54. Interestingly, the known biological actions of kisspeptins were apparently limited to their ability to inhibit tumor progression and, likely, to control trophoblast invasion. However, by late 2003, the putative role of kisspeptin/GPR54 system in the neuroendocrine control of the reproductive axis was disclosed by the observation that loss-of-function mutations and deletions of the GPR54 gene were associated with absence of puberty onset and hypogonadotropic hypogonadism both in humans and rodents. These observations drew an immediate attention on the previously unsuspected, reproductive facet of kisspeptins, which was thoroughly analyzed thereafter. Indeed, in the last year, the extraordinary potency of kisspeptins in inducing gonadotropin release has been documented in a number of species, such as the rat, mouse, sheep, monkey and, very recently, the human. Such a gonadotropin-releasing action is believed to derive primarily from a direct stimulatory effect upon the hypothalamic GnRH system, as activation of GnRH neurons and GnRH release by kisspeptins has been very recently demonstrated. In addition, hypothalamic expression of KiSS-1 gene, and to a lower extent of GPR54, appeared to be developmentally (maximum at puberty) and hormonally (by sex steroids) regulated, and functional studies have provided solid evidence for a relevant role of KiSS-1 signaling in timing of puberty onset in rodent and primate species. Altogether, these

experimental observations qualify the KiSS-1/GPR54 system as a novel, essential gatekeeper of the GnRH/gonadotropin axis and, hence, of the reproductive function in mammals.

## S27

### Use of recombinant leptin in women with hypothalamic amenorrhea

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Hypothalamic amenorrhea, characterized by suppressed GnRH secretion, occurs when energy output is greater than energy intake. Leptin concentrations, which reflect adipose stores and energy availability, are low in hypothalamic amenorrhea. Our study and others provide evidence that leptin is the critical link between energy stores and normal reproductive function.

Evidence that leptin is critical for reproduction comes from the onset of pubertal development in patients with leptin mutations who are treated with recombinant leptin. Leptin treatment reverses fasting induced decreases in LH pulse frequency in men and women and testosterone levels in men. Finally, leptin replacement improves menstrual function in women with lipodystrophy and low leptin levels.

In our study, women with hypothalamic amenorrhea due to strenuous exercise or low weight were studied before and during treatment with recombinant human leptin for up to 3 months. The study was approved by the Partners Human Research Committee and all subjects gave written informed consent. Mean LH levels ( $2.8 \pm 1.6$  to  $4.8 \pm 1.5$  IU/L,  $P = 0.005$ ) and LH pulse frequency ( $2.4 \pm 1.2$  to  $5.0 \pm 0.9$  pulses/12-hr,  $P = 0.049$ ) increased after 2 weeks of recombinant human leptin. During leptin treatment, maximum follicle diameter, dominant follicle number, ovarian volume, estradiol and inhibin B levels increased (all  $P < 0.01$ ), despite a decrease in weight and % body fat. 43% of subjects had an ovulatory menstrual cycle and an additional 29% had preovulatory follicle development (both  $P = 0.004$ ) during treatment. Thus, physiologic leptin replacement in women with hypothalamic amenorrhea improves gonadotropins and reproductive function. These findings suggest that leptin is the peripheral signal of adequate energy stores required for normal hypothalamic-pituitary-gonadal function in women. They further suggest that leptin could serve as a treatment for hypothalamic amenorrhea.

## S28

### A genomic analysis of polypeptide ligands and receptors: the example of relaxin and LGR families

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The availability of genome sequences from humans and multiple model organisms has facilitated the identification of many human plasma membrane receptor families and allowed the analysis of their phylogeny. We traced the evolution of known families of human plasma membrane receptors for inclusion in the Human Plasma Membrane Receptome database at <http://receptome.stanford.edu> (Science STKE. 2003). Using an evolutionary approach, we identified new polypeptide hormones and receptors. Because of the coevolution of polypeptide ligands and their cognate receptors, analysis of human genomic sequences allows one to predict the pairing of these elements. Initially, we identified a group of five orphan LGRs (leucine-rich repeat-containing G protein-coupled receptors) homologous to LH, FSH, and TSH receptors (Mol Endocrinol 1998, 1990). Matching the polypeptide hormones and receptors led to the finding of a new glycoprotein hormone as an agonistic ligand for the TSH receptor (J Clin Invest., 2002). In addition, the receptor for the classic hormone, relaxin, was identified as LGR7 (Science 2002) whereas INSL3 was found to be the specific ligand for LGR8 (JBC 2002). We investigated the expression of the INSL3 receptor LGR8 in male and female gonads. We found that LH stimulates INSL3 transcripts in ovarian theca and testicular Leydig cells. INSL3, in turn, binds LGR8 expressed in germ cells to initiate meiotic progression of arrested oocytes in preovulatory follicles and to suppress male germ cell apoptosis (PNAS 2004). Thus, genomic analysis of the evolution of polypeptide ligand and receptor gene families provides a new paradigm for future identification and matching of novel ligands and receptors.

S29

**Novel recombinant gonadotropin molecules in human reproductive dysfunction**

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Org 36286 is a sustained follicular stimulant (SFS) inducing multiple follicular development in patients undergoing controlled ovarian stimulation. Due to its long half-life a single dose of Org 36286 may replace the first 7 injections of daily recFSH and after this one week interval, stimulation may be continued with daily recFSH up to the day of triggering ovulation (Org 36286 regimen). Ideally, recFSH should be started as soon as Org 36286 levels become too low to support multiple follicular growth. Observations in the clinical trials with Org 36286 suggested that follicular growth itself is not a good, immediate, marker for lack of follicular stimulation, as follicles (visualized by ultrasound) may still continue to grow when FSH-dependent hormones rapidly decline. Both inhibin-B and E<sub>2</sub> are FSH-induced hormones and sensitive markers of follicular development and their premature decline indicates insufficient stimulation. To predict the pharmacodynamic effects of Org 36286, models have been developed including inhibin-B levels during stimulation. For this model inhibin-B was preferred over E<sub>2</sub> as it is an LH-independent marker and it shows less variability than E<sub>2</sub>. The clinical importance of a decrease in inhibin-B levels was revealed in a subset analysis of subjects who were treated with a too low dose of Org 36286. The analysis revealed that when the time between a decrease in inhibin-B and the continuation of treatment with recFSH exceeded 1.5 days, the cancellation rate increased and the number of oocytes and number of good quality embryos decreased. The extent of this effect of a decrease of inhibin-B on clinical outcome parameters was further quantified in subsequent modeling. With this set of models, simulations of the outcome have been performed for a range of Org 36286 dose regimens. Taking into account the relevant variables, the simulation results could establish the lowest Org 36286 dose to obtain an optimal outcome in the desired one-week regimen.

S30

**Long live the worm: *C. elegans*, insulin and ageing**

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Understanding the ageing process is one of the most formidable challenges to modern biology. Over the last decade, major advances have been made thanks to the application of simple classical genetic techniques. This approach begins with isolating mutants with altered rates of ageing, and cloning the gene affected. Here, the nematode *Caenorhabditis elegans* is ideal, since it has well developed genetics, its genome is sequenced, and its lifespan is a mere 2–3 weeks. Studies of *C. elegans* mutants with 2–3 fold increases in adult lifespan led to the discovery that the nematode insulin/IGF-1 signalling (IIS) pathway is a powerful regulator of ageing.<sup>1,2</sup> Remarkably, reduced IIS also increases lifespan in the fruitfly *Drosophila melanogaster*,<sup>3</sup> and reduced IGF-1 or insulin signalling can increase lifespan in mice.<sup>4</sup> Thus, the role of IIS in control of ageing is evolutionarily conserved, or *public*.

But how does IIS exert such a powerful control on ageing? DNA microarray analysis has shown that IIS regulates a large number of downstream genes, some of which must directly control longevity and ageing.<sup>5</sup> Identifying these genes and the processes they specify is the key to understanding the ageing process. Non-biased analysis of microarray data from long-lived IIS mutants, and long-lived *C. elegans* diapausal dauer larvae implicates certain processes in longevity assurance, particularly drug detoxification (which removes diverse toxic and damaged molecular moieties from the cell) and heat shock protein (which restore misfolded proteins to their working conformation).<sup>6</sup> Of particular interest now is the question: are the genes and biochemical processes through which IIS acts also public, and if so, what are these processes? New studies suggest that the longevity assurance mechanisms via which IIS acts are lineage-specific at the gene level (private), but may be conserved at the process level (semi-public).

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S31

**Dietary restriction and altered insulin/Igf1 signalling: conserved mechanisms regulating lifespan in worms, flies and rodents**

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It has long been recognised that caloric restriction extends lifespan in a broad range of organisms from nematodes to rodents. Furthermore, it has also been demonstrated that manipulation of components of the insulin and Igf1 signalling pathway also increases lifespan. These findings were initially made in *C.elegans* and *Drosophila* but more recently these observations have been extended to rodents. However, the processes underlying the alterations in lifespan seen with caloric restriction and reduced insulin signalling have yet to be fully elucidated. To investigate this question, we have adopted a comparative functional genomic approach utilising microarray gene expression and metabolomic analyses together with manipulation of insulin signalling and dietary intake in *C. elegans*, *Drosophila* and the mouse. Furthermore we are examining lifespan in a number of rodent models with altered insulin signalling. The mechanisms we have identified will be discussed and the use of *C. elegans* and *Drosophila* models to gain insights into mammalian physiology will be presented.

S32

**Giant flies and more: probing the functions and genetics of insulin signalling in *Drosophila***

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Defective signalling by insulin-like molecules is not only a fundamental characteristic of diabetes, but is also associated with the majority of human tumours, where signalling is typically hyperactivated. Although both these diseases have a significant genetic component, we are still some distance from identifying all the genes and cellular mechanisms affected.

We and other groups use the powerful genetics of the fruit fly, *Drosophila melanogaster*, to dissect the insulin signalling pathway, which is highly conserved in all higher eukaryotes. We showed that PTEN, a major human tumour suppressor, functions by restricting cell growth through acting as an antagonist of PI3-kinase signalling in flies. Other groups have shown an important link between the insulin signalling cascade and the local nutrient sensor TOR and demonstrated that this signalling pathway has a range of physiological roles beyond metabolism and growth, including developmental timing and longevity. All these novel functions appear to be conserved in mammals.

Here we discuss our recent identification of several new conserved components in the insulin signalling pathway. We show that surprisingly the proton-assisted amino acid transporters (PATs) play an essential role in regulating TOR-dependent cellular nutrient sensing, potentially via a novel 'transceptor' mechanism that sets the sensitivity of cells to insulin-like molecules. We also demonstrate that subcellularly localised phosphatase activity plays a critical role in modulating the intracellular insulin signalling in different parts of the cell, allowing a single pathway to differentially regulate processes including cellular morphology, growth and metabolism. Our work suggests that many of the diverse cellular effects of insulin-like molecules are regulated by overlapping signalling mechanisms that can to some extent be independently controlled at the subcellular level. This model may have important implications for our understanding of events involved in establishment of cellular defects linked with diabetes and cancer, such as insulin resistance and metastasis.

S33

**Specifying the cellular responses to IGF signalling by IGF binding proteins: new lessons from the zebrafish model**

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The insulin-like growth factor (IGF) signalling pathway plays a fundamental role in regulating growth and development. In extracellular environments, IGFs are present in complexes with high-affinity binding proteins (IGFBPs). Studies using cultured mammalian cell lines and transgenic mice suggest

that IGFs have the ability to inhibit and/or potentiate IGF actions. Some IGFs may even possess intrinsic biological activities that are IGF-independent. The *in vivo* physiological functions of these IGFs, however, are poorly understood. Most research has relied on rodent models, and attempts to have been hampered by the inaccessibility of the mammalian fetus in the uterus and the greater redundancy associated with the mouse model. In recent years, there has been a remarkable acceleration in our understanding of the IGF signaling system in teleost fishes, most notably in zebrafish. To date, genes encoding for zebrafish IGF ligands, receptors, and several IGFs have been characterized. Taking advantage of the free-living and transparent zebrafish embryo, their expression patterns in early development have been mapped and their *in vivo* functions have been studied by loss- and gain-of-functional approaches. The results suggest that different IGFs are expressed in spatially and temporally restricted fashions and they each play distinct roles in early development. IGF-1 plays a key role in mediating hypoxia-caused growth and developmental retardation in zebrafish embryos. IGF-2 not only regulates global growth and development, but also plays a local role in vascular development. IGF-3 is primarily involved in the formation and differentiation of pharyngeal skeleton and inner ear, while IGF-5 regulates muscle and skeletal tissue differentiation. Based on these findings, a conceptual model on the role of secreted binding proteins in regulating growth factor actions is proposed and discussed.

### Clinical lessons from novel aspects of G protein-coupled receptors signalling

S34

#### How the pattern of GPCR co-expression can regulate function

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The pharmacology and function of G protein-coupled receptors (GPCRs) is frequently studied following expression of a single receptor in heterologous cell lines. However, many GPCRs are co-expressed. We wished to investigate how expression of pairs of receptors might modulate their function. Using a Human Embryonic Kidney 293 cell line in which the CB1 cannabinoid receptor was expressed constitutively and in which varying levels of expression of the orexin-1 receptor could be induced by addition of doxycycline we demonstrated that in the presence of the CB1 receptor the orexin-1 receptor adopted a spontaneous, agonist-independent recycling phenotype that was indistinguishable from that of the CB1 receptor. By contrast, when expression of the orexin-1 receptor was induced in the absence of the CB1 receptor the orexin-1 receptor was plasma membrane delineated and required addition of the peptide orexin-A to induce internalisation. Using single cell fluorescence resonance energy transfer imaging, the co-expressed CB1 and orexin-1 receptors were shown to be present within an oligomeric complex. Although CB1 antagonists, including Rimonabant, have no inherent affinity for the orexin-1 receptor, treatment of cells co-expressing the CB1 and orexin-1 receptors resulted in redistribution of both the CB1 receptor and the orexin-1 receptor to the cell surface. Similarly, treatment of these cells with orexin-1 receptor antagonists with no affinity for the CB1 receptor caused both receptors to redistribute to the plasma membrane. These results introduce a novel pharmacological paradigm, due to hetero-oligomerisation, a GPCR can be regulated by receptor ligands that do not actually bind to the receptor in question. This can only occur in physiological cells and system in which relevant pairs of receptors are co-expressed. Such interactions may be relevant to the clinical effects of a number of medicines targeted at GPCRs.

S35

#### Alpha- and beta-adrenoceptor dysfunction

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The family of adrenergic receptors contains nine different subtypes of G protein-coupled receptors which mediate the biological effects of adrenaline and noradrenaline. With few exceptions, the full therapeutic potential of subtype-selective therapy has not yet been explored for the group of adrenergic receptors. In the absence of sufficiently subtype-selective ligands which can distinguish between individual receptor subtypes of the adrenergic family, gene-targeted mouse models with deletions in these receptor genes have recently been generated and characterized. These genetic mouse models have helped to assign specific pharmacological

effects of alpha2-receptor agonists or antagonists to individual receptor subtypes.

Some unexpected and novel functions of alpha2-adrenergic receptors were also uncovered in these mouse models: Presynaptic control of catecholamine release from adrenergic nerves in the central and sympathetic nervous system is regulated by all three different receptor subtypes, alpha2A, alpha2B, and alpha2C. A similar feedback loop also controls the release of catecholamines from the adrenal gland. Alpha2B-receptors are not only involved in regulating vascular tone in the adult organism, but they are essential for the development of the vascular system of the placenta during prenatal development and for angiogenesis in the adult organism. In humans, genetic diversity of the adrenoceptor genes has been intensely studied recently. Among the alpha2-adrenoceptors, a hypofunctional deletion variant of the alpha2C-receptor has been associated with altered disease progression in patients with chronic heart failure. The challenge will now be to translate the results from subtype-selective gene deletions in mice into clinical science and to predict the action of subtype-selective drugs in humans.

S36

#### Molecular pathology of the FSH receptor

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The identification of naturally occurring genetic mutations of the FSH receptor has led to a better understanding of the role of FSH in folliculogenesis and has allowed to identify the cause of several syndromes. Inactivating mutations of the follicle stimulating hormone (FSH) receptor have been described in rare cases of premature ovarian failure (POF). We have studied a new patient presenting a complete POF phenotype, with high plasma FSH levels, very low estrogen and inhibin B levels and complete lack of biological response to high doses of recombinant FSH. A novel homozygous mutation was found in this patient. The mutation totally impairs adenylate cyclase stimulation *in vitro*. Histological studies of the patient's ovaries showed an increase in the density of small follicles when compared to age-matched normal women. A complete block in follicular maturation after the primary stage was also observed. This observation supports the concept that FSH seems mandatory for the initiation of follicular growth in humans only after the primary stage. Complete FSH resistance provokes infertility in our patient, remarkably associated with persistence of a high number of small follicles.

Spontaneous ovarian hyperstimulation (OHSS) is a syndrome occurring during pregnancy. It is a rare event in contrast to iatrogenic OHSS, although mild forms may be underestimated. We describe here a familial case of recurrent spontaneous OHSS. A heterozygous transmembrane mutation of the FSH receptor gene segregated with OHSS in this family. The mutated FSH receptor displayed an abnormal sensitivity to hCG. Broadening of specificity of G Protein-coupled receptors is not a common pathogenic mechanism. Molecular elucidation of this case also enlightens possible pathophysiological mechanisms in iatrogenic OHSS induced by gonadotropins used in assisted reproduction medicine.

S37

#### Therapeutic implications from new aspects in V2 vasopressin receptor dysfunction

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More than 190 disease-causing mutations of V2 vasopressin receptor (AVPR2) have been identified in patients with X-linked nephrogenic diabetes insipidus (NDI). About half of the mutations lead to a loss or truncation of the receptor protein whereas missense mutations present a variety of functional alterations such as reduced binding and signaling abilities and improper cell surface trafficking. To date, the therapy of NDI focuses mainly on treating clinical symptoms with thiazide diuretics often in combination with amiloride and indomethacin. Because current therapeutic protocols are of limited efficiency and are often accompanied by adverse



effects, alternative approaches including application of potent agonists and gene therapeutic approaches are under investigation. For example, several therapeutic approaches aimed at modulating chaperone function. These attempts are based on the findings that most inactivating missense mutations in G-protein-coupled receptors (GPCR) lead to receptor retention in the cell interior. Treatment of cells expressing certain mutant GPCR with ligands, which are known to stabilize the receptor protein in its native conformation, resulted in the correct processing of mutant GPCR proteins and their proper delivery to the plasma membrane, thus restoring GPCR function. About 10% of NDI-causing mutations represent nonsense mutations which lead to the generation of truncated, non-functional receptor proteins. Recent *in vitro* and *in vivo* studies have shown that aminoglycoside antibiotics can suppress premature stop codons in the AVPR2, thereby permitting protein translation to continue to the normal end of the coding sequence. Animal models for NDI are now important tools to investigate the feasibility of new approaches and may help to gain more insights into the NDI pathomechanism. Thus, recent animal data implicate an increased bicarbonate and sodium re-uptake in the kidney of AVPR2-deficient mice and provide new mechanistic views of the development of hypernatremia in NDI patients and of the therapeutic benefit from thiazide diuretics.

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### Stromal cell-matrix interactions

S38

#### Role of heparan sulphates in neuronal stem cell differentiation

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Heparan sulphate (HS) is a highly complex linear polysaccharide found attached to core proteins both on the cell surface and in the extracellular matrix. Newly synthesized HS chains are modified by a family of enzymes, many of which have multiple isoforms with differing substrate preferences. These modifications include the addition of sulphate groups at up to four positions on each disaccharide unit. The pattern of modifications a HS saccharide undergoes alters its structure, affecting the ability of the HS to bind and regulate many different growth factors (such as FGFs and BMPs) known to have roles in stem cell regulation. HSs are thus expected to be pivotal molecules involved in controlling stem cell behaviour.

We have been studying the molecular phenotype of HS biosynthesis and structure during mouse ES cell differentiation in both neural differentiation and embryoid body formation. Embryoid bodies (EBs) are layered, ordered aggregates of cells made up of primitive endoderm cells overlaying an epithelium of epiblast cells, separated by a basement membrane and surrounding a central cavity. The generation of EBs from undifferentiated ES cells has been used as a model for early development and has been shown to require signalling through fibroblast growth factor (FGF) receptor 2, implicating HS which is a necessary co-receptor for the formation of a signalling complex. We have observed dynamic spatiotemporal expression patterns of HS (using a panel of anti-HS antibodies), its biosynthetic enzymes, HS core proteins and FGFRs during ES cell differentiation. Variant HS structures could thus provide a basis for regulation of signalling mechanisms in ES cell differentiation. These studies provide a platform for dissection of the structural requirements for the functional role of HS in regulating critical aspects of stem cell differentiation.

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S39

#### Hormonal regulation of mesenchymal stem cell differentiation in bone

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Mesenchymal Stem Cells (MSC) are the source of regeneration of musculoskeletal tissues. Stem cells (SC) are rare and slowly dividing cells, situated in stem cell niches, regulated by non-stem-cell cap cells. Rare symmetric SC division regulates the SC pool. Asymmetric cell division gives rise to a transient amplifying pool (MSC<sub>TAP</sub>). The MSC niche is poorly characterized in contrast to MSC<sub>TAP</sub> and consecutive events of fate decision, commitment and secretion of chemotactic and angiogenic factors. MSC<sub>TAP</sub> can be isolated from bone marrow, bone chips, synovia, skin and other tissues. MSC<sub>TAP</sub> express steroid hormone receptors which are important in early fate decisions and differentiation like VDR, RAR, GR, TR, ER and PPAR $\gamma$ , the latter being involved in adipose tissue differentiation. GR, VDR and TR regulate factors important in

endochondral ossification and chondrogenesis (e.g. dkk and CCN proteins). Estrogens produce proliferative effects on MSC<sub>TAP</sub> which may be mediated by so called non-genomic effects. Early MSC<sub>TAP</sub> express only low levels of ER but knockout leads to loss of mechanosensitivity of the osteogenic pathway of differentiation which may indicate some effects of ER in bone formation. Receptors for PTH/PTHrP are essentially involved in the regulation of early progenitors. It has been shown that they are important in constituting the hematopoietic stem cell niche rendering the osteoblast a cap cell for the hematopoietic niche. New hormonal factors which regulate renal phosphate metabolism and vitamin D activation as well as early fate decision of MSC<sub>TAP</sub> are the phosphatonins FGF 23 and sFRP4. Osteoblast-derived secreted FGF23 signals through FGFR expressed in MSC<sub>TAP</sub> and the kidney. Constitutively active FGFR mutants cause chondrodysplasia syndromes in humans indicating an important role in MSC<sub>TAP</sub> commitment. In summary MSC may be targets and tools for therapeutic strategies and their hormone receptor pattern provides molecular targets of great potential for osteogenesis and chondrogenesis.

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S40

#### Cell-adhesion molecules and osteoclast function

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Bone is a dynamic tissue, maintained and repaired by the actions of, osteoclasts, osteoblasts and osteocytes, together with a variety of bone marrow cells, stromal cells and endothelium. Osteoblasts lay down bone and this is subsequently remodelled by osteoclasts. Osteocytes act mainly as mechanosensors. Osteoclasts are of haemopoietic origin, whereas osteoblasts are derived from mesenchymal stem cells. During cell differentiation and in mature cells, cell adhesion molecules are important regulators of osteoclast function, allowing adhesion, migration, transmigration through endothelium and they act as true signalling molecules. Osteoclasts express mainly members of the integrin family of cell-matrix adhesion receptors. They express very high numbers of vitronectin receptors (VNR,  $\alpha v\beta 3$  and in immature cells  $\alpha v\beta 5$ ). This receptor can, at least *in vitro*, bind to a wide range of RGD-containing bone matrix proteins, but the true *in vivo* ligand for the receptor has not been identified. VNR is expressed on the plasma membrane. In polarised, bone resorbing osteoclasts, VNR is mainly concentrated on the basolateral membrane and in the ruffled border, areas not in direct contact with the bone surface, suggesting that it has a role beyond facilitating initial cell adhesion. Osteoclasts also express high levels of  $\alpha 2\beta 1$  integrins, which facilitate adhesion to collagen and express  $\alpha v\beta 1$ , which can bind fibronectin. Expression of cadherins is limited, with possibly a role for E-cadherin during cell development. ICAM-1 and V-CAM have been found to be involved in osteoclast development in functional studies, but this is most likely through expression on osteoblasts and interactions with LFA-1 on osteoclast precursors. CD44 is expressed on osteoclast and may allow adhesion to osteopontin and hyaluronan *in vivo*. No major adhesion molecule defects have been found in osteoclast diseases so far, and, surprisingly, gene knockout studies of  $\beta 3$  integrins have not yielded very dramatic bone phenotypes, likely through compensation by  $\beta 1$  integrins. Even so, the high expression level of VNR and its clear importance in osteoclast function has made this a target for therapies aimed at reducing bone resorption.

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S41

#### Wnt signalling in the stem cell niche

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It is well recognised that the intestinal epithelium has a remarkable capacity to regenerate after high levels of DNA damage or injury. Although the kinetics of this 'clonogenic repopulation' whereby a new stem cell repopulates the crypt is understood, little is known about the molecular mechanism underlying this, although upregulation of Musashi-1 is known to mark the process. By conditionally deleting genes that are lethal to the intestinal stem cells we have mimicked this process, with non-recombined stem cells repopulating the crypt, a scenario we define as 'genetic repopulation'. During this repopulation process intestinal crypts become enlarged with an induction of proliferation, villi shorten and paneth cells become mislocalised. All of these traits are remarkably similar to the immediate phenotype following Apc loss within crypts. Consistent with this

we find that Musashi-1 is upregulated in Apc deficient crypts in a c-Myc dependent manner. These observations suggested that activation of Wnt signalling drives the repopulation event, and indeed we observe nuclear relocalisation of nuclear beta-catenin and upregulation of two key wnt target genes, CD44 and C-MYC, in repopulating crypts. Taken together these data argue that activation of beta-catenin is not simply a tumorigenic event within the intestine but is essential for normal intestinal stem cell homeostasis.

## Controversies in male health

### S42

#### Does andropause exist?

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Serum testosterone levels decline progressively with age. An estimated 20% of men over the age of 60 years have testosterone levels below the normal range for young men. Ageing men display symptoms similar to those observed in young men with hypogonadism. These symptoms combined with low serum testosterone comprise the entity often referred to as andropause. However, the clinical significance of this decline in testosterone levels remains uncertain. Whereas the testosterone substitution therapy for young men with hypogonadism appears to be beneficial, there is no clear-cut evidence of clinical benefits of such therapy in healthy elderly men with moderately decreased testosterone levels.

We aimed at evaluating the benefits of androgen replacement therapy in andropausal otherwise healthy men in a randomised placebo-controlled setting. Initially all men between the ages of 40–70 living in the City of Turku (total population 170,000) were sent a questionnaire (Heineman's questionnaire) about the "andropausal symptoms". Those with a high symptom score were invited for serum testosterone measurement and the symptoms were re-evaluated. The association of "andropausal symptoms" and serum testosterone levels was also studied in those individuals with high symptom scores. In order to ensure patient safety and avoid the confounding effect of diseases, men with significant disease or contraindications for androgen replacement therapy were excluded. The very low number of strictly healthy men with "andropausal symptoms" and decreased serum testosterone (<9.8 nmol/l) did not allow the required power for statistical analysis of the possible benefits of testosterone replacement and the study had to be discontinued.

Our findings suggest that the incidence of andropause in otherwise healthy men is presently being overestimated. Furthermore, the association of the so called andropausal symptoms and decreased serum testosterone requires further evaluation before androgen replacement therapy of the elderly can be considered evidence based treatment.

### S43

#### Cardiovascular effects of androgens

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The burgeoning interest in treating androgen deficiency against the background of the age-related decline in circulating testosterone in middle-aged and elderly men has outpaced the accrual of an adequate evidence base on causation, natural history, and accuracy/validity of diagnosis. The relevance of age-related hormonal changes in the somatotrophic and adrenal axes are currently also unclear. Fuelled by the increased choice of androgen preparations and heightened patient expectation, clinicians are put under increasing pressure to prescribe without an assured diagnosis. A number of questionnaires have appeared that claim to improve the detection of symptomatic hypogonadism in elderly men – these have not been validated against clinical outcomes. Best practice interim recommendations have also recently been proffered from many quarters in attempts to rationalise management of ageing men suspected of being androgen deficient. The most important principles underpinning these guidelines include are the pre-requisites of clinical features supported by consistent biochemical evidence of testosterone deficiency and exclusion of recognisable reversible pathologies. How these principles can be applied in practice will be discussed. It must be emphasised that any current practice recommendations should be revised in the light of new and more substantial evidence from future research.

### S44

#### Endocrine regulation of prostatic growth

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Prostate cancer cells show a variable expression of androgen receptor (AR). Its function is influenced by coregulatory proteins that may interact with the N-terminal and/or ligand-binding domain. Cofactor expression and function may be altered in prostate cancer. Studies by my group were focused on the transcriptional integrator CBP and its functional homologue p300 in prostate cancer. We found that CBP selectively potentiates activation of the AR by the non-steroidal anti-androgen hydroxyflutamide. Expression of CBP is inhibited by the synthetic androgen R1881 and interleukin-6 (IL-6). Immunohistochemical studies revealed that most prostate cancers, including therapy-resistant ones, express CBP. It is assumed that a high expression of CBP in tumours from patients who failed endocrine therapy is a consequence of androgen deprivation therapy.

IL-6 is a multifunctional cytokine that regulates proliferation, apoptosis, angiogenesis, and expression of secretory proteins in prostate cells. AR activity is up-regulated by IL-6. Proliferation of androgen-sensitive LNCaP cells is inhibited by IL-6. However, during prolonged treatment of LNCaP cells with IL-6, a subline has been generated (LNCaP-IL-6+). This subline acquires a growth advantage *in vitro* and *in vivo*. These cells show up-regulated expression of cyclin-dependent kinases 2 and 4 and down-regulation of tumour suppressors p27 and pRb. There was no phosphorylation of STAT3 in cells generated in the presence of IL-6. In contrast, inhibition of the mitogen-activated protein kinase pathway resulted in a partial retardation of tumour growth. Interestingly, the AR target gene prostate-specific antigen was induced by p300 in that subline.

Taken together, these results demonstrate that (a) CBP and its functional homologue p300 are important coactivators in advanced prostate cancer and (b) IL-6, a cytokine whose levels are elevated in prostate tumour tissues and patients' sera, is a target for novel therapies in prostate cancer.

### S45

#### Stem cell therapeutic approaches to male infertility

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The testis provides stem cell niches which are populated by spermatogonial stem cells. The seminiferous epithelium is the exclusive site where germline cells are proliferating and entering meiosis in the adult organism. The existence of stem cells offers clinically relevant options for preservation and restoration of male fertility. New approaches based on male germ cell transplantation and testicular tissue grafting can be applied to generate sperm. Germ cell transplantation relies on removal of the stem cell from the donor's testicular niche. The stem cell can then be transferred back into the donor's testis or into different testes which provide the suitable microenvironment to generate sperm from the transplanted stem cell. In contrast, grafting can be considered as a transplantation of the stem cell in conjunction with its niche. Germ cell transplantation of human spermatogonia into mouse testes revealed that the spermatogonial stem cells colonize the mouse testes but are not able to differentiate and complete spermatogenesis. This indicates that stem cell recognition and expansion are conserved throughout evolution but also reveals that the early steps in spermatogenesis differ between rodents and primates. This might be due to the fact that the primate testis contains stem cells and progenitors while a progenitor spermatogonium does not exist in rodents. Monkey experiments showed that germ cell transplantation as an autologous approach can be applied in primates. Ectopic xeno-grafting of testicular tissue was applied to generate fertile sperm from a variety of species. Newborn or juvenile testicular tissue from rodents, domestic animals and primates was grafted into the back skin of immunodeficient mice and developed up to qualitatively complete spermatogenesis. The sperm were successfully used to create progeny. We recently showed that autologous grafting can be applied to stimulate testicular development in marmosets. Xenografting of adult human testes, however, revealed only limited success to maintain testicular tissue. We conclude that the rapid progress in the development of novel experimental strategies to generate sperm from cryopreserved spermatogonial stem cells or testicular tissue will lead to many new options for fertility preservation.

## How hormones get into cells

S46

### Specific thyroid hormone transporters

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Both action and metabolism of thyroid hormone are intracellular events which require the transport of iodothyronines across the plasma membrane. This does not occur by simple diffusion but is facilitated by transport proteins. During the last few years thyroid hormone transporters have been characterized at the molecular level. These include the Na<sup>+</sup>-taurocholate cotransporting polypeptide (NTCP), different members of the Na<sup>+</sup>-independent organic anion transporting polypeptide (OATP) family, the heterodimeric L-type amino acid transporters LAT1, LAT2, and the monocarboxylate transporter 8 (MCT8). Most of these transporters accept a variety of ligands, but OATP1C1 and MCT8 show high specificity towards iodothyronines. OATP1C1 is almost exclusively expressed in brain capillaries, and appears crucial for the transport of the prohormone T4 across the blood-brain barrier. MCT8 is also expressed – among other tissues - in the brain, in particular in neurons. MCT8 appears especially important for the uptake of the active hormone T3 into these cells, which is essential for optimal brain development. This T3 is produced from T4 by type 2 deiodinase in neighbouring astrocytes. The neurons express type 3 deiodinase which inactivates T3. The MCT8 gene is located on chromosome Xq13.2 and has recently been associated with a syndrome combining severe X-linked psychomotor retardation and high serum T3 levels. In over 20 families, where affected males present this syndrome, different mutations have been identified in MCT8. The mechanism of this disease involves a defect in the neuronal entry of T3, and thus in the action and metabolism of T3 in these cells, leading to an impaired neurological development as well as a decrease in T3 clearance.

S47

### The spectrum of MCT8 gene mutations

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So far it has been assumed that thyroid hormones -due to their lipophilicity- enter their target cells just by slipping through the membrane. After the initial description of a specific transport molecule for thyroid hormone in 2003 (Friesema et al. JBC) a new paradigm of thyroid hormone physiology has emerged. It turns out that as everything in nature, also the entering of thyroid hormone in its target cell is well regulated and the gate keeper is the MCT8 transporter, at least in the brain. The identification of patients with mutations in this transporter and their severe neurological phenotype accelerated research on this new field of thyroendocrinology. Based on the neurological phenotype of severe muscular hypotonia and lack of normal development, it is evident that this molecule seems to have a central non-redundant function in brain physiology. A further unusual neurological phenotype associated with MCT8 mutations, namely paroxysmal kinesigenic dyskinesias (PKD), provoked by certain stimuli including changing of their clothes or diapers was described. Further widening of the phenotypical spectrum of MCT8 deficiency was shown by the most recent report of Schwarz et al. in patients with Allan-Herndon-Dudley syndrome in older characterised by muscular hypotonia in the first year of life but who later develop spastic paraplegia, a symptom not recognized in the younger patients so far. As in all examples of fundamental new findings, more questions arise than could have been answered so far: What is the real physiological role of MCT8? Why is its impact for brain function much higher than for other organs, e.g. heart? In addition, who can explain why T3 is elevated and T4 is normal with almost normal TSH in these patients? However, the elevated T3 in these patients will give rise to a rebirth of T3 measurements in clinical practice.

S48

### Multidrug resistance P-glycoprotein (mdr-1) excluding corticosteroids from brain

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In rodents synthetic glucocorticoids such as e.g. dexamethasone poorly penetrate the blood brain barrier because of multidrug resistance P-glycoprotein (mdr-Pgp). Also cortisol, not naturally occurring in rats

and mice, is hampered in uptake. The evidence for this role of the efflux transporter was substantiated with the mdr1a knock out mice. <sup>3</sup>H-cortisol and <sup>3</sup>H-dexamethasone administered to the mdr1a mutants did label brain targets that retained the steroids poorly in the wild types. Also in human brain cortisol rather than corticosterone was hampered in brain penetration as judged from steroid profiles generated with Liquid Chromatography-tandem Mass Spectrometry in post mortem samples. These findings were confirmed with monolayers of epithelial cells stably expressing human mdr-Pgp. Interestingly, in this cell system the glucocorticoid antagonist mifepristone blocked Pgp-mediated cortisol transport suggesting that *in vivo* such antagonists actually facilitate steroid uptake in the brain. From functional perspective the findings reinforce the notion that moderate doses of dexamethasone block stress-induced hypothalamic-pituitary-adrenal (HPA) activation primarily at the level of the anterior pituitary. As a consequence the secretion of endogenous corticosterone is suppressed. While peripherally the lack of corticosterone is substituted for by dexamethasone, this is not the case in the brain. Indeed in animals subchronically treated with dexamethasone CRH mRNA and hnRNA expressions were enhanced in the PVN, while stress-induced c-fos mRNA showed resistance to suppression. The results support the concept that low doses of dexamethasone can create a hypocortisoid state in the brain. Supported by the Royal Netherlands Society for Arts and Sciences.

S49

### Cell surface carrier - mediated transport of steroid hormones

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Experimental data demonstrate interactions of steroids with efflux carriers of the multidrug-resistance transporter family (P-glycoprotein, MRP) as well as the poly-specific uptake carriers of the organic anion transporting polypeptide (OATP), organic cation transporter (OCT), and the organic anion transporter (OAT) families in different tissues.

P-glycoprotein is associated with glucocorticoid-resistance of many diseases including cancer but also modulates androgen responsiveness in prostate cancer cells. Transport of conjugated steroid hormones like estradiol-17 $\beta$ -glucuronide or estrone sulfate and DHEAS by OATPs has been demonstrated in placenta, mammary gland, keratinocytes, small intestinal epithelial cells and hepatocytes. Estrone sulfate and DHEAS also interact with OATs in renal proximal tubule cells and placenta. Cortisol transport by OATs has been shown in kidney, placenta, and the blood-brain barrier. In rat adrenal cortex, *in situ* hybridization localized OAT1 to the outer zona fasciculata, OATP3 to the zona glomerulosa, OATP1 and OATP2 to the inner zona fasciculata and outer zona reticularis and OCT2 to the zona glomerulosa and outer zona fasciculata. RT-PCR detected OAT3 and OAT4 mRNAs in human adrenal tissue and the human adrenal cell line NCI-H295R. When human OAT3 and OAT4 were expressed in *Xenopus laevis* oocytes, only hOAT3 showed [<sup>3</sup>H]cortisol uptake (apparent K<sub>i</sub> = 2.4  $\mu$ M). Uptake of the OAT-substrate [<sup>3</sup>H]PAH (p-aminohippurate) into NCI-H295R cells was *trans*-stimulated in cells preloaded with unlabeled PAH, glutarate, and cortisol. This *trans*-stimulation indicates PAH/PAH, PAH/glutarate, and, most important, PAH/cortisol exchange through OAT3. Glutarate and cortisol in the medium *cis*-inhibited, i.e. competed with, [<sup>3</sup>H]PAH uptake, further documenting the interaction of cortisol with OAT3. Finally, glutarate in the medium *trans*-stimulated cortisol release, indicating glutarate-driven efflux of cortisol. These experiments support the concept of anion exchanger(s) contributing not only to uptake of steroids into cells but also, at least in part, to the secretion from adrenocortical cells.

## Endocrinology in the foetus

S50

### Maternal thyroid function and cognitive impairment in childhood

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Thyroid hormone is required for normal brain development from early fetal life onwards. Maternal transfer of thyroxine (T4) constitutes a major fraction of fetal serum T4 in the first half of pregnancy. Early in gestation thyroid hormone receptors with T3 bound to it have been found in different human tissues, while type II deiodinase activity is present in human cortex. In case of fetal and maternal hypothyroidism, severe mental retardation, combined with hearing deficit and muscle rigidity is the resulting clinical picture.



A more subtle picture has been described in association with increased maternal TSH and/or decreased maternal FT4 values in the first half of pregnancy. In 7–9 year old offspring of women with elevated TSH the full scale IQ was 4 points lower compared with those born from women with normal TSH; if FT4 was decreased in addition, this difference was 7 points. In another study a maternal FT4 value in the 12th week of pregnancy in the lowest 10 percentile range was associated with 8–10 points lower score of the offspring at 1 and 2 years both on the mental and motor domain. Also decreased visual contrast sensitivity was found in 3–6 month old offspring of women with hypothyroidism during pregnancy, as well as increased incidence of attention deficit hyperactivity disorder at the age of 10. These development impairments may be comparable with those of very preterm infants with transient hypothyroxinemia.

In the rat, a mild and transient period of maternal hypothyroxinemia has been found to affect radial distribution of certain neurons in the somatosensory cortex and hippocampus, with alterations of inhibitory circuits. Although these papers all point towards deleterious consequences for cognitive and behavioural development of low maternal FT4 early in pregnancy, the lower normal limit for FT4 in pregnancy is not known presently.

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## S51

### Molecular basis of programming of renin-secreting cells

S Pennings

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Renin is a rate-limiting enzymatic component of the renin-angiotensin pathway controlling blood pressure in vertebrates. Using the zebrafish developmental model organism, we study how renin-secreting cells are established in a small region of the early embryo that gives rise to the developing pronephric kidney: the origin of these cells, the factors that induce the differentiation of this cell lineage, and the function of very early renin expression. This study aims to gain insight into the proposed role of renin in kidney organogenesis observed in mice and humans, and the early development of renin-angiotensin function. Functional assays recording the onset of glomerular filtration are combined with gene expression analysis and gene knockdown to focus on how this cell lineage is specified by developmental signals and epigenetic marks programming the cell nucleus.

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## S52

### Birth weight and later disease: genes and maternal diabetes

RS Lindsay

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Epidemiologic studies support a relationship of lower birth weight with later disease- particularly type 2 diabetes. These findings may reflect either the influence of environmental factors before birth to influence risk of later disease (*in utero* programming) or pleiotropic genetic effects. In high risk populations, notably the Pima Indians of Southern Arizona, both low and high birth weight are associated with later risk of type 2 diabetes. In addition, long term follow up studies support the hypothesis that maternal diabetes results in programming of type 2 diabetes and obesity *in utero*. The mechanisms of *in utero* programming by maternal diabetes remain largely unknown. Recent studies in offspring of mothers with type 1 diabetes allow detailed examination of the hormonal effects of maternal diabetes on the offspring. Future follow up studies will highlight how such hormonal changes- such as the marked hyperleptinaemia and hyperinsulinaemia that are found in offspring of mothers with diabetes- might lead to later disease.

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## S53

### Endocrine therapy of the fetus

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Endocrine fetal therapies is currently performed in congenital adrenal hyperplasia due to 21-hydroxylase and 11 beta-hydroxylase deficiencies (CAH), hypothyroidism accompanied by large goiter and in thyrotoxicosis due to maternal autoantibodies. In CAH dexamethasone is administered to the mother 5–6 weeks p.c. to suppress the fetal hypothalamo-pituitary-adrenal axis. If the diagnosis is confirmed by chorionic villus or amniocentesis therapy is continued, while it is discontinued in all males as well as in unaffected females. Studies of patients with prenatally treated CAH following strict protocols in North America and Europe did not observe short-term adverse effects in the fetus and provided evidence that

virilization of female external genitalia can be prevented or reduced thereby reducing the necessity of genitoplasty. However, animal studies and epidemiological data have shown various adverse effects of glucocorticoids on the developing fetus and it is not known whether glucocorticoids induce fetal programming of metabolic changes that manifest as disease in adult life. Therefore according to international consensus guidelines this treatment should be considered as experimental and only be performed in controlled studies. Intra amniotic administration of thyroid hormone has successfully been used in reducing goiter size in fetuses of mothers with antithyroid drug treatment and in fetuses with congenital hypothyroidism. It is still a matter of controversy if prenatal thyroid hormone treatment is necessary in other patients with congenital hypothyroidism. However the normal outcome in the majority of the cases detected by newborn screening indicates that an early and adequate postnatal therapy seems to be sufficient. There are no reports on prospective treatment studies in fetuses of mothers with autoimmune hyperthyroidism. However, there is increasing evidence, that children of mothers with Grave's disease may present with endocrine problems (fetal thyrotoxicosis, fetal and neonatal thyroidal or central hypothyroidism) and that the diagnosis is frequently missed. Therefore prospective, controlled studies as in the prenatal treatment of CAH are required.

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## Monogenic disorders illuminate metabolic disease

### S54

#### Epidemic obesity in humans

S O'Rahilly

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Implicit in the justification for the enormous increase in investment in bio-molecular research that has occurred over the past 20 years has been the promise that this would provide insights relevant to the understanding of human pathophysiology and the ultimate alleviation of suffering from human disease. To this end, model organisms are enormously attractive as they provide tractable and controllable systems in which precise and "clean" data can be obtained. In contrast the study of complex human disease is fraught with difficulties related to the multifactorial nature of most human illness, and the challenges of controlling for the effects of largely immeasurable confounding factors, both genetic and environmental. While it is critical that research on complex human phenotypes and diseases continues an approach that focuses on extreme human phenotypes has established itself as a useful complementary strategy. Firstly, it is more likely that extreme human phenotypes are caused by tractable monogenic or oligogenic defects. Secondly, the consequences of such defects are not always identical to those seen in animal models. Thirdly, once a link between a major mutation particular gene and a human phenotype is established it is much more likely that subtle variation in those genes is involved in influencing susceptibility to common human diseases. Finally, discoveries in this area may lead to effective mechanism-based therapies which provide justification for the continuation of investment in basic biomedical research. In this presentation I will discuss advances that have come from the studies of two cohorts of humans with extreme phenotypes, namely obesity and insulin resistance.

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## S55

### Specific metabolic features in Prader-Labhart-Willi syndrome (PWS) as indicators of the underlying genetic defect

U Eiholzer

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First described in 1956, Prader-Labhart-Willi syndrome is a rare, complex neurodevelopmental disorder characterised by short stature, disturbed body composition with hyperphagia and hypoactivity, hypogonadism, cryptorchidism, oligophrenia, behaviour problems as well as extreme muscular hypotonia in the neonatal period. Its estimated prevalence ranges from 1:15,000–25,000 and it is caused by an absence of expression of paternally active genes in the PWS critical region on chromosome 15q11.2-q13. Despite in-depth knowledge of the genetic condition in PWS, the final link between the chromosomal disorder and the clinical symptoms remains unclear. Hypothalamic dysfunction appears to underlie many of the features of PWS, including short stature, hypogonadism, abnormal control of appetite and energy expenditure, high pain threshold, and sleep disorders, but no overt structural abnormalities of the hypothalamus have been found yet.

The increased fat accumulation in PWS is caused 1) by a reduction of activity-related energy expenditure, because PWS children engage less in physical activity, and 2) by a decrease in basal metabolic rate by 20–50%, reflecting the decrease in lean - mostly muscle - mass. Additionally, growth hormone deficiency contributes not only to the abnormal growth pattern and osteopenia, but also to the deficit of lean body mass and excess of body fat. Children with PWS display a specific form of combined hypothalamic (low LH) and peripheral (high FSH, low inhibin B) hypogonadism suggesting a primary defect in Sertoli and/or germ cell maturation or an early germ cell loss. Diabetes mellitus occurs in 17–40% of adults with PWS. During adolescence, insulin resistance and fasting insulin levels are still low, in parallel to the relatively low degree of visceral fat, but insulin secretion is delayed after glucose load. Later, a type 2-like diabetes is assumed to be precipitated by the addition of excessive obesity to impaired insulin secretion.

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## S56

### PPAR gamma and human metabolic disease

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We have screened the human PPAR $\gamma$  gene in a cohort of subjects with severe insulin resistance and identified defects in nine unrelated families. Heterozygous mutations in affected individuals cosegregate with a phenotype which includes stereotyped (gluteal, limb) partial lipodystrophy, early-onset hypertension, dyslipidaemia and hepatic steatosis. Pathogenesis of the phenotype involves abnormal metabolism of dietary fat intake, with elevated circulating lipids and fat oxidation.

Mutant receptors are functionally impaired and we have delineated three distinct molecular mechanisms: two missense mutants (P467L, V290M) inhibit wild type PPAR $\gamma$  action in a dominant negative manner via enhanced recruitment of corepressor; a loss-of-function, truncation mutant (FSX) in the DNA binding domain, which lacks dominant negative activity, leads to insulin resistance only in combination with a second unrelated gene (PPP1R3) defect; novel mutants in the DNA (C114R, C131Y, C162W) or ligand binding (R357X, fsV315X) domains also limit WT receptor action in a dominant negative manner but may act by sequestering limiting cofactors. PPAR $\gamma$  target gene responses in primary cells from subjects provide *ex vivo* evidence for differing dominant negative properties of mutant receptors and expression profiling of these cells will further elucidate pathogenetic mechanisms in this disorder.

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## S57

### Learning from monogenic diabetes: insights into the fetal and adult beta-cell

AT Hattersley

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The molecular genetics of monogenic diabetes have been defined in the last decade. Most of these genes result in beta-cell dysfunction. Studying these patients has given new insights into the role of key genes in the fetal and adult beta-cell.

Hepatic Nuclear Factor (HNF)-1 $\beta$  mutations causing renal cysts and diabetes (RCAD). HNF-1 $\beta$  mutations are associated with reduced beta-cell development resulting in diabetes, exocrine dysfunction and pancreatic atrophy and as a result of fetal hypoinsulinaemia - low birth weight. Patients with glucokinase (GCK) mutations have a stable glucose sensing defect while patients with transcription factor mutations show a progressive beta-cell defect. In HNF-1 $\alpha$  MODY, we showed a 4 x greater fall in fasting glucose than in BMI matched type 2 patients. This pharmacogenetics effect reflects the defect in the beta-cell being prior to the K<sub>ATP</sub> channel where sulphonylureas act. Differences are seen in MODY fetal beta-cell with birth weight being reduced in GCK, normal in HNF-1 $\alpha$  and increased in HNF-4 $\alpha$ .

Mutations in Kir6.2 are the major cause of permanent neonatal diabetes. All mutations showed reduced channel closure in response to ATP. Despite being insulin dependent these patients can discontinue insulin injections and show improved glycaemic control on high dose sulphonylureas which act to close the K<sub>ATP</sub> channel by a non-ATP dependent route. The excellent control is achieved without restoration of the first phase insulin response.

A molecular genetic diagnosis (see [www.diabetesgenes.org](http://www.diabetesgenes.org)) helps explain clinical features, predicts prognosis and can improve treatment. Study of monogenic patients gives new insights into the role of these critical proteins in man.

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## Steroid hormone receptors

### S58

#### The ups and downs of nuclear receptor action in fat and fertility

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Nuclear Receptors control many developmental and physiological processes by regulating the expression of networks of genes. Their ability to activate or repress gene transcription depends on the recruitment of coactivators and corepressors that function as scaffolds for the binding of chromatin remodelling enzymes. RIP140 is a ligand dependant corepressor for most, if not all, nuclear receptors with key roles in energy homeostasis and reproduction.

RIP140 regulates carbohydrate and lipid metabolism by regulating metabolic gene programmes in adipose tissue and in muscle. Mice devoid of RIP140 are extremely lean, exhibit resistance to high fat diet-induced obesity and are protected against insulin resistance as they age or are fed high fat diets. It appears that peroxisome proliferator activated receptors and estrogen related receptors repress the expression of gene networks in metabolic tissues that would otherwise disrupt energy homeostasis by recruiting the RIP140 corepressor. As a consequence, chromatin remodelling enzymes are recruited to the promoters of target genes and this leads to epigenetic changes which prevents their transcription.

Mice lacking RIP140 also fail to ovulate following the pre-ovulatory surge of luteinising hormone although the process of luteinisation still occurs. This phenotype closely resembles that of the luteinised unruptured follicle syndrome often associated with infertility in women. The ovulatory failure is due to a primary defect within the ovary itself and our recent studies indicate that it is likely to reflect an alteration in the expression of genes involved in granulosa cell expansion and follicular rupture. We are now in the process of identifying direct RIP140 target genes in the ovary and will address the extent to which the function of RIP140 is conserved in different tissues or varies according to the endocrine response.

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## S59

### Chromatin remodeling

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The Estrogen Receptor (ER) is a well-studied, ligand dependent transcription factor. We have analysed, by kinetic chromatin immunoprecipitation (ChIP) assays, the mechanisms by which the ER modulates the permissiveness and transcriptional activity of the pS2 gene. Sequential ChIP assays were used to define the cyclical association of approximately 50 different proteins to the pS2 promoter. Key results from these studies are (i) that unliganded and liganded ER direct cycling on target promoters, with only liganded ER directing the cyclical association and activation of polymerase II; (ii) that there are two phases to cyclical activity, an association phase where the intermediate and basal transcriptional machinery become recruited to the promoter and a clearance phase in which transcription factors generally regarded as repressive, such as histone deacetylases (HDAC's) and chromatin remodelling complexes, act to reset the promoter to allow a subsequent cycle to commence.

This interplay between the ER and chromatin is further underlined by experiments that evaluate the effects of HDAC inhibitors on estrogen signaling. As suggested by the involvement of HDAC activity in transcriptional cycling, inhibition of HDAC activity induced a profound anti-estrogenic effect on gene expression profiles. This notwithstanding, analysis of promoters repressed in response to inhibition of deacetylase activity showed that local histones were in fact deacetylated. This effect is likely to result from the action of NAD dependent deacetylase activity (the sirtuin class of deacetylases).

We also showed that CpG islands associated with the transcriptional start site become methylated in response to deacetylase inhibition. In summary, ER induces transcriptional cycling and this defines additional targets that can be exploited to modulate estrogen signaling. Proof of concept that this model can indeed be used to inhibit estrogen signaling by novel mechanistic strategies have been obtained using inhibitors of Zn dependent deacetylase activity.



**S60****Cell membrane receptors of steroid hormones**J Brosens<sup>1</sup>, I Huhtaniemi<sup>1</sup> & B Gellersen<sup>2</sup><sup>1</sup> Imperial College London, London, United Kingdom; <sup>2</sup> Endokrinologikum, Hamburg, Germany.

Rapid activation of diverse signal transduction pathways is thought to play an integral role in the cellular responses to many steroid hormones, including progesterone. This sex steroid is essential for normal reproduction in nearly all species. The recent cloning of a G-protein coupled receptor with the properties of a membrane progesterin receptor (mPR) in fish and the subsequent identification of three mammalian homologues (mPR- $\alpha$ , - $\beta$ , and - $\gamma$ ) raised the possibility of defining the relevance of non-genomic progesterone actions in normal reproduction and disease. Phylogenetic and sequence analyses demonstrated that the mPRs belong to a larger, highly conserved family of proteins, termed 'progesterin and adonectin receptors' (PAQRs). Furthermore, a comparison of the expression of mPR transcripts in human reproductive tissues revealed markedly divergent expression levels and profiles. Interestingly, mPR- $\alpha$  is most highly expressed in the placenta and its relative expression in many tissues correlates inversely with that of the nuclear PR. For functional analysis, we transiently and stably transfected a panel of cell lines with expression vectors encoding for either wild-type or tagged human mPRs. Confocal microscopy studies revealed that transfected mPRs localize to the endoplasmic reticulum but not plasma membrane or mitochondria. Most notably, endogenous mPR- $\alpha$  could be demonstrated in the MDA-MB-231 and BeWo cells, displaying the same pattern of distribution. Treatment of MDA-MB-231 cells transfected with the fish mPR has been reported to result in a rapid decrease in cAMP levels and an increase in MAPK (p44/p42) phosphorylation. However, progesterone treatment did not modulate either cAMP content, calcium signalling, or MAPK activation in cell lines transiently or stably transfected with human or mouse mPR- $\alpha$ , - $\beta$ , or - $\gamma$ . Our findings suggest that the role of the fish mPR has not been evolutionarily conserved. The mammalian homologues could be currently considered orphan receptors with as yet undefined roles in reproduction.

**S61****Lysine modifications in transcriptional regulation by steroid receptors**

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Covalent modifications offer an efficient and fast means to modulate protein function. They play a key role in the regulation of steroid receptor-dependent transcription by remodeling the chromatin structural proteins. Steroid receptor coregulator proteins, coactivators and corepressors, which function as signaling intermediates between receptors and chromatin or transcription machinery, often possess or recruit activities that catalyze covalent lysine modifications, such as acetylation, methylation, ubiquitination and sumoylation. Several coactivators possess inherent acetyl- or methyltransferase activity or ability to recruit these activities, whereas the function of corepressors often involves the opposite activities – deacetylation and demethylation. Chromatin immunoprecipitation studies have revealed that transcriptional activation by steroid receptors, such as androgen receptor (AR), is accompanied by a cascade of distinct histone modifications and that agonists and antagonists bring about differential recruitment of histone-modifying protein complexes. In addition to the interaction with coregulator proteins, covalent modifications of steroid receptors appear to play an important modulatory role in steroid signaling. These modifications may influence the receptors' function by altering protein-protein interactions, subcellular trafficking, intranuclear targeting, coactivator recruitment and protein degradation. Steroid receptors are ubiquitinated and degraded in the ubiquitin-proteasome system (UPS). Moreover, components of the UPS are recruited to steroid-regulated promoters, and transcription complex assembly on the promoters is dependent on the proteasome activity. Steroid receptors are also modified by small ubiquitin-related modifier proteins (SUMOs). Sumoylation targets specific lysine residues on the receptors' N-terminal domains and inhibits their transactivation activity, especially on promoters harboring multiple response elements. Our research has recently focused on the role of lysine modifications in steroid receptor-dependent transcription. The significance of these modifications in the context of androgen action will be discussed.

**Anabolic hormones in sport****S62****Overview of anabolic steroids in sport**

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Anabolic-androgenic steroids are synthetic derivatives of testosterone. They are abused by athletes in sport. The goal of individuals who use anabolic steroids in sport is dependent on the activity in which they participate. It has been difficult to show scientifically that the use of anabolic steroids increases physical performance. There has been much less discussion on the psychological and central nervous system (CNS) effects of anabolic steroids and on the significance of the CNS effects on performance. Anabolic steroids may have direct neuroactive effects or they may modify the effects of neuroactive steroids on the CNS. The effects of supraphysiological doses or prolonged use on physical and psychological capacities have not been evaluated. On the other hand, the use of supraphysiological doses for long duration may cause significant adverse health effects. The adverse effects of anabolic steroids on the liver, lipoprotein fraction, serum triglycerides, clotting factors, myocardium, immune function and reproductive system are quite well known. In addition, anabolic steroids have effects on behaviour and on neural circuits for these behaviours. It has been shown that anabolic steroids can alter aggression, sexual behaviour, anxiety, reward, learning and locomotion in animals. Some of the human studies have also found an association between the use of anabolic steroids and irritability, aggression, sexual behaviour, personality disturbance and mental disorders. Acute anabolic steroid withdrawal symptoms may include cravings and depression. It is probable that dependence syndrome according to DSM-IV or ICD-10 can develop also for anabolic steroids.

**S63****Detecting growth hormone abuse in athletes**

C Strasburger

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Performance enhancing substances enjoy considerable popularity among athletes, particularly if deemed undetectable. Doping with growth hormone has been considered undetectable until recently. Two strategies have been pursued to detect GH doping: Pharmacological endpoints and GH isoform composition.

For the former approach the consortium GH 2000/2004 has identified markers of GH action and found a combination of parameters from the IGF-system and collagen markers to provide a suitable combination to detect GH abuse. The latter approach is based on the molecular nature of isoform composition in which GH is released from the pituitary. Monomeric 22 kD hGH in its unbound form accounts only for approximately one quarter of all GH isoforms released into circulation. In contrast, the vast majority of hGH as derived from recombinant DNA technology is in the monomeric 22 kD form. We have therefore developed "differential immunoassay" strategy in which every sample is analysed twofold: The first assay preferentially recognises 22 kDa monomeric hGH while the second assay recognises the bulk of isoforms as released from the pituitary. Both assays are based on monoclonal antibodies selectively screened for their specificity characteristics. The 22 kD preferential antibody as well as the "permissive" antibody are immobilised and allowed to react with the sample before incubation with a common labelled anti-hGH antibody. The approach was tested by blinded samples and found capable of distinguishing pituitary-derived- from recombinant hGH. This test has been introduced in several WADA accredited laboratories and has been applied – without detecting positive cases – at the recent Summer Olympic Games in Athens.

The pharmacological marker test will be capable of detecting GH abuse in athletes for a longer period of time than the isoform approach, but may fail after initial GH injections. It can therefore be speculated, that both strategies will be pursued side by side in the future.

**S64****Gene doping: will the age of gene therapy usher in the age of genetic enhancement?**

HL Sweeney

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Gene therapy and pharmacological therapies that target gene expression have tremendous potential as novel therapeutic agents. In many cases these agents not only have applications in disease states, but also could have

tremendous impact on the physiology of normal individuals. A number of strategies being developed for the treatment of disease have the possibility to eventually be used for genetic enhancement of human performance. For example, genes can be easily transferred into the muscles of laboratory animals using modified viruses, known as adeno-associated virus (AAV). These viruses are now in use in early human clinical trials for genetic diseases. Some of the possible treatments for muscular disorders, which include types of muscular dystrophy, and muscle loss associated with either disuse or aging, could be used in healthy adults to build muscle strength and make muscle more resistant to damage.

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## S65

### Erythropoietin

DA Cowan

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The International Olympic Committee (IOC) banned blood doping in 1988 after the discovery that cyclists were transfusing homologous blood at the Los Angeles Olympic Games in 1984. Recombinant erythropoietin (EPO) was developed for the treatment of anaemia following the cloning of the human EPO gene in 1984. Unfortunately, sports competitors could then misuse EPO to increase circulating haemoglobin and its oxygen carrying capacity in order to gain an advantage in endurance events. The IOC added EPO to its list of prohibited substances in 1990, one year after adding peptide hormones as a new class. However, there was no internationally recognised test to detect administration until 2000 when direct and indirect tests were first conducted for EPO at the Sydney Olympic Games. The direct test, developed by Lasne and colleagues, requires a urine sample and uses isoelectric focussing and a Western double blotting technique with chemiluminescent detection. However, since the terminal half-life of EPO is only about 8.5 hours, this test has very little retrospectivity. Nevertheless, several athletes have been successfully prosecuted for administering EPO based on evidence from this test. The indirect test, which is less widely used, requires a blood sample and is based on a variety of markers stimulated by EPO administration.

Several forms of EPO are now on the market including Novel Erythrocyte Stimulating Protein (NESP), which has a modified amino acid backbone and greater glycosylation than EPO. This results in approximately three times the half-life of EPO while maintaining its biological activity. In 2002, despite media claims that it was undetectable, three long distance skiers were disqualified at the Winter Olympic Games in Salt Lake City for using NESP. This presentation will provide an update on the tests to detect EPO administration and highlight their strengths and weaknesses.

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## Disorders of melanocortin receptor functions

### S66

#### MC1R variants: Mendel is quite complex enough

JL Rees

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It is ten years since we identified the human melanocortin 1 receptor (*MC1R*) as the locus that underpins red hair, freckling and sun sensitivity. The *MC1R* is not the only gene that is important in human pigmentation, but it does seem to play a significant role. A striking aspect of the *MC1R* is the degree of genetic diversity. I review how a combination of laboratory and genetic epidemiological studies has shown how a single locus, that in some instances behaves close to a Mendelian trait can, due to a range of mutations with variable functional effects, produce a graded response. Whereas it is possible to report striking associations between *MC1R* variants and physiological traits – with odds ratios of greater than 30 for red hair – when we relate sequence diversity to disease states, the odds ratios we see are much lower and, in this particular context, are probably of no clinical use. It remains a useful thought experiment to imagine what we would have discovered if we had just used anonymous SNPs.

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## S67

### Identification of the genes causing ACTH insensitivity

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ACTH insensitivity or Familial Glucocorticoid Deficiency (FGD) is an autosomal recessive disorder that presents in early childhood usually with hypoglycaemia and seizures, infection or malaise and skin pigmentation.

Plasma cortisol is low or undetectable and ACTH markedly elevated. Renin and aldosterone are not markedly disturbed. FGD results from mutations of the ACTH receptor in about 25% of cases. We have sought further genetic causes of this disorder using a homozygosity mapping approach which resulted in identification of a small locus on chromosome 21. One gene in this region was expressed in the adrenal cortex and contained a range of mutations in patients with FGD. It encodes a single transmembrane domain protein which co-localises with the ACTH receptor and which directly interacts with it as shown by co-immunoprecipitation. Co-transfection of this gene and the ACTH receptor into cells that do not normally express the transfected receptor results in a functional cell surface receptor for ACTH. We have named this gene melanocortin 2 receptor accessory protein (MRAP). MRAP normally functions in the trafficking and cell surface expression of the ACTH receptor. Genetic defects occur in about 20% of cases of FGD and are likely to entirely account for the development of the disease in these patients. A third locus for FGD on chromosome 8q was identified in one large family following a genome search, and we have now confirmed and refined this locus in other families. Very recent data supports the existence of yet a further (fourth) locus. Neither of these two new loci contain obvious candidate genes, but work is in progress to identify these.

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## S68

### Melanocortin receptor mutations in humans

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The anorexigenic hormone leptin seems to be the principal adiposity indicator and signal of the state of nutrition, as its plasma levels are highly correlated to adipocyte number and fat content. The elucidation of the causes of monogenic forms of obesity has benefited from the positional cloning of a series of mouse obesity genes, including those that encode leptin, the leptin receptor (LEPR). Targeted genetic manipulation has also established the vital regulatory role of molecules such as the melanocortin 4 receptor (MC4R), which is vital in the melanocortin pathway, and the orexigenic protein AGRP. These discoveries were rapidly followed by the identification of rare monogenic recessive forms of human obesity that are caused by mutations in the genes that encode leptin, LEPR, prohormone convertase 1 and POMC, all of which result in a phenotype of excessive energy intake relative to energy expenditure. By contrast, more frequent autosomal-dominant forms of obesity are caused by mutations in the gene that encodes MC4R. MC4R deficiency represents the most common monogenic obesity disorder that has been identified so far. It is present in 1–6% of obese individuals from different ethnic groups, with a higher prevalence in cases with increased severity and earlier age of onset. In children with *MC4R* mutations, the degree of obesity and hyperphagia correlates with the extent of impairment of MC4R signalling, but the correlation disappears in adult carriers who cannot be phenotypically distinguished from obese non-carriers. The characteristic intense feeling of hunger during childhood in patients with MC4R deficiency seems to lessen in adolescence, and levels of hyperinsulinaemia also become less marked. Interestingly, MC4R-deficient mice are not hyperphagic when fed a low-fat diet, whereas hyperphagia is observed after the introduction of diets that have increased fat content, indicating gene–environment interactions. Although difficult to assess in humans, such environmental effects for genes that are involved in the control of food intake might also contribute to explaining the increase in childhood obesity that has occurred in the past 20 years.

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## S69

### Gs-independent signalling of melanocortin-4 receptors

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Melanocortin receptors (MCR), which belong to the superfamily of G protein-coupled receptors (GPCR), are preferentially coupled to Gs proteins and play a major role in the regulation of energy homeostasis. In line with this notion, mutations in the MC4R gene are the most frequent monogenic cause of severe obesity in human beings. Surprisingly, MC4R mutations isolated from obese candidates showed both increased or decreased potency to promote the Gs signalling pathway when overexpressed in HEK-293 cells. Therefore, no clear correlation between the functional alterations found in HEK-293 cells and the dysregulation of the energy homeostasis observed *in vivo* could be drawn. The MC4R-D90N mutation, which has also been isolated from an obese individual, binds agonists with unchanged

high affinity, but promotes no detectable activation of the Gs signalling pathway in HEK-293 cells. Despite of the blunted Gs signalling, agonist binding to the MC4R-D90N mutant induced the recruitment of the adapter protein arrestin when both proteins were overexpressed in HEK-293 cells and agonist-promoted interactions between the MC4R-D90N mutant and arrestin in living cells were monitored by the bioluminescence resonance energy transfer technique. For some GPCRs it has been reported that such arrestin-receptor complexes exhibit the propensity to activate the extracellular-regulated protein kinase (ERK) signalling cascade independent of G protein activity. In line with these observations, HEK-293 cells expressing the MC4R-D90N mutation showed enhanced ERK activity after stimulation with MC4R agonists. MC4R-D90N-mediated activation of the ERK signalling pathway is therefore independent of Gs activity but most possibly depends on the recruitment of arrestin. In summary we conclude that mutations within the MC4R gene lead to the inhibition of the Gs but not of the ERK signalling pathway and that apart from the classical Gs pathway other signalling cascades might contribute to the MC4R-mediated regulation of energy homeostasis.

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## Endocrine oncogenesis and management of hereditary endocrine tumours

### S70

#### RET function and dysfunction

L Fugazzola

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The RET proto-oncogene, located on chromosome 10q11.2 encodes a receptor tyrosine kinase. Its extracellular portion contains four cadherin-like repeats and a cysteine-rich region, while the intracellular portion contains the tyrosine kinase domain. RET is essential for development of the sympathetic, parasympathetic and enteric nervous systems and the kidney. Accordingly, RET disruption by germline mutations causes congenital aganglionic of the gastrointestinal tract (Hirschsprung's disease). The RET protein is a subunit of a multimolecular complex that binds growth factors of the glial-derived neurotrophic factor (GDNF) family, including GDNF, neurturin, artemin and persephin. They bind RET in conjunction with glycosyl-phosphatidylinositol-anchored co-receptors (GFR $\alpha$ 1-4), leading to the receptor dimerization and triggering autophosphorylation and intracellular signalling. RET mutations are responsible for the development of several human diseases, including multiple endocrine neoplasia 2A and 2B (MEN2A and MEN2B), familial medullary thyroid carcinoma (FMTC), papillary thyroid carcinoma and Hirschsprung's disease. Most MEN2A and FMTC mutations affect cysteines in the extracellular cysteine-rich domain of RET. MEN2A is typically associated with mutations of codon 634, whereas FMTC is associated with mutations distributed among the various cysteines or in the RET kinase domain. Most MEN2B patients carry the M918T mutation in the RET kinase domain. Rarely, somatic deletions and germline duplications of variable segments of the gene have been reported in sporadic MTC and in FMTC. The mechanism leading to RET oncogenic activation depends on the site of the mutation. RET cysteine mutants form covalent dimers, due to the creation of intermolecular bonds, that display constitutive kinase activity. Differently, a change in substrate specificity has been implicated in the M918T MEN2B mutation. The high incidence of detectable mutations and the small number of target codons facilitate predictive and diagnostic testing. Indeed, soon after the identification of RET as the disease-gene, the genetic testing was introduced and it is now considered the standard of care for MEN2.

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### S71

#### Novel management strategies in medullary carcinoma of the thyroid

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Medullary thyroid carcinoma (MTC) is a neuroendocrine tumour of the parafollicular cells of the thyroid gland accounting for 5-15% of thyroid carcinomas. Most medullary thyroid carcinomas occur sporadic while up to 25% are familial as part of the multiple endocrine neoplasia type 2 (MEN-2). Most of the carcinomas have already metastasized at the time of detection. Surgical therapy is the treatment of choice in patients with MTC, both for patients with the familial and the sporadic form. All patients should be tested to verify or exclude mutations in the RET-Protooncogene (MEN-2 disease). Total thyroidectomy in affected members of kindreds with MEN-2 is able to

prevent or cure medullary thyroid carcinoma. Persistent or recurrent disease is defined by a pathologic pentagastrin stimulation test performed after primary surgery.

In case of recurrent or metastatic disease surgical therapy is useful but normalisation of calcitonin can be expected only in 25% of the cases. Different new strategies in the treatment of MTC are under investigation. Vaccination with calcitonin and/or CEA peptide-pulsed dendritic cells C results in the induction of a cellular, antigen-specific immune response in patients with MTC, leading to clinical response in some patients.

As most MTC's are not sensitive to chemotherapy, new therapy strategies could include the use of COX-2 inhibitors to sensitize MTC cells for cytotoxic effects of drugs such as doxorubicin via reducing the expression and function of the permeability glycoprotein (P-gp) regulated by the multidrug resistance 1 (MDR1) gene.

Experimental data demonstrate the effect of radioiodine therapy of MTC after tissue-specific sodium-iodide-symporter (NIS) gene transfer to MTC cells using the calcitonin promoter gene. Other approaches include the use of humanized anti-CEA antibodies in combination with chemotherapy, adenovirus-mediated tumour-specific combined gene therapy, the use of tyrosine kinase inhibitor therapy and in somatostatin-receptor positive tumours the use of radiolabeled somatostatin analogues.

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### S72

#### Hypoxia inducible factor (HIF) and pheochromocytoma

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Hypoxia Inducible Factor (HIF) is a master regulator of the transcriptional homeostatic response to oxygen deprivation. It was originally discovered in the context of regulation of erythropoietin gene expression, but subsequently shown to have a widespread role in coordinating gene expression in response to hypoxia. The pathways regulating the activity of HIF in normal tissue and cancers will be reviewed. Events leading to the development of pheochromocytoma and other cancers will be discussed. A common feature of many genetic defects which predispose to pheochromocytoma is that they influence the activity of the HIF pathway.

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### S73

#### Familial pheochromocytomas

PF Plouin

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Pheochromocytomas are catecholamine-secreting tumors that usually arise from the adrenal medulla. Catecholamine-secreting tumors may also develop in retroperitoneal, pelvic or thoracic paraganglia. Here, we use the term pheochromocytoma for catecholamine-secreting adrenal or extra-adrenal tumors. Before 2000, 10 to 15% of the patients with pheochromocytoma were reported to have family or phenotypic evidence of a hereditary disease: multiple endocrine neoplasia type 2 (MEN2; in about 7% of cases), von Hippel-Lindau disease (VHL; 3%) and neurofibromatosis type 1 (NF1; 1%). In 2% of cases a family history of pheochromocytoma was found, but with no evidence of these syndromes. There were also reports of pheochromocytomas in hereditary paraganglioma (HPGL) families, who usually develop non-functional head and neck tumors associated with the parasympathetic nervous system. In 2000-2001, mutations in *SDHB*, *SDHC* and *SDHD* genes, that encode the B, C and D subunits of mitochondrial succinate dehydrogenase, were identified in most HPLG families. In 2002, Neumann reported that 66 of 271 patients (24%) with an apparently sporadic pheochromocytoma had unexpected germline mutations in the *RET*, *VHL* or *SDH* genes. In 2005, our group diagnosed hereditary pheochromocytoma (VHL, MEN2, NF1, HPGL) in 86 of 314 patients (27%) with pheochromocytoma and identified an unexpected germline mutation in 30 of the 258 patients (12%) with no evidence of a syndromic or familial disease. These findings suggested that about one pheochromocytoma carrier in three, including 10 to 15% of the patients with an apparently sporadic presentation, had hereditary disease. Most mutations detected in apparently sporadic cases affected the *SDHB* gene. *SDHB* mutation carriers had larger tumors and a more frequent metastatic evolution than those without. These new findings have important consequences for the management of patients with pheochromocytoma and their families.



## Green over-the-counter endocrinology

S74

### Isoflavonoid effects on lipids, lipoproteins, insulin sensitivity and ghrelin in postmenopausal women

Abstract unavailable

S75

### Regulation of steroid receptor expression and proliferation in prostate cancer by genistein and other flavonoids

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A diet rich in phytoestrogens such as flavonoids has been attributed as one of the reasons for the low incidence of prostate cancer in Asia as opposed to Western countries with a diet rich in fat and meat and the highest incidences of this cancer. In many cellular models of prostate cancer these substances, the prototype of which is genistein, inhibit cell proliferation and cell cycle progression and enhance the rate of apoptosis. The molecular mechanisms involve modulation of steroid receptor expression and steroid hormone metabolism, inhibition of growth factor receptor and survival pathways and depend on concentration and cellular context. Prostate cells express high levels of estrogen receptors, estrogen receptor alpha being predominant in the stroma and estrogen receptor beta in the epithelium. Although the central hormonal regulation in the prostate is elicited through androgens, estrogens seem to play an important role as well and have been implicated in proliferative disorders of the prostate, e.g. cancer and benign hyperplasia. In the hormone-sensitive prostate cancer cell model LNCaP, genistein, an isoflavone downregulated androgen receptor protein expression thus abrogating the androgenic stimulation of growth and survival and expression and secretion of PSA. These cells express estrogen receptor beta and the effective concentrations as well as prevention of the genistein effect by estrogen receptor blockage indicated that the genistein effect was mediated via this estrogen receptor. Regulation of prostate growth involves interaction of different cell types and the prostatic stroma plays a crucial role as a paracrine stimulator. The flavonoid apigenin inhibited the proliferation and cell cycle progression of primary prostate stromal cells suggesting that phytoestrogen action on the prostate gland also involves the stromal compartment of the gland.

S76

### Soy, flavonoids and osteoporosis

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Osteoporosis and the associated low-energy fractures is a major public health problem. Phytoestrogens, especially from soy foods with isoflavones, carry the potential of being a nutritional candidate for prevention of osteoporosis. Several studies have been performed to determine the role of soy intake in preventing bone loss in postmenopausal women. Studies with fracture endpoint are not yet available, but several other lines of evidence have been investigated: epidemiological and clinical studies with either markers of bone turnover or measurement of bone mineral density- and content (BMD/BMC). The presentation will focus on reviewing the clinical trials of at least 1y duration with DEXA endpoint:

Two 12 month Italian studies comparing purified genistein at 54 mg/d with estradiol (one study) and placebo (two studies) demonstrated a similar protective effect of genistein and estradiol, with gains in BMD  $\approx$  3% in both lumbar spine and hip for the two active groups (Morabito 2002, Squadrito 2005).

Two of the four 2y studies completed, from Denmark and South Dakota, has demonstrated a preventive effect on lumbar spine bone loss with isoflavone-rich soy foods, 15 and 40 gram protein providing 80–90 mg isoflavones/d, with only minimal changes occurring in the hip in the control groups, having soy food without isoflavones (Lydeking-Olsen, 2004, Lovrien 2005). The third 2y study found less than 1% bone loss in the whole body over two years regardless of isoflavone content (5, 42 and 58 mg respectively), indicating a calcium sparing effect in the whole body bone mass. Specific-site DXA measurements were not performed in this study, making it difficult to compare with the other 2 y studies (Vitolins, 2001).

A 12 mo study did not show any difference from the placebo group (milk protein), as both groups maintained bone mass (Kreijkamp-Kaspers 2004).

One 2 y study did not demonstrate any difference between soy + isoflavones, soy –isoflavones and casein as all three groups lost bone (Gallagher 2005).

S77

### Aftermath of WHI: the risks or benefits of red clover and soy extracts in postmenopausal OTC HRT

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Soy and red clover extracts contain high concentrations of isoflavones, particularly of genistein, which bind to both estrogen receptors  $\alpha$  and  $\beta$  (ER $\alpha$  and ER $\beta$ ). Lifelong nutrition rich in soy proteins or soy protein isolates appear to exert positive effects on certain health aspects including the development of mammary cancer but the beneficial effects become questionable when soy/isoflavone-rich nutrition starts in later adult life. Stimulatory effects on mammary cancer cell growth *in vitro* and in animal experiments were reported. Mammary gland safety studies in climacteric/postmenopausal women who initiated isoflavone-rich intake for climacteric/postmenopausal complaints/diseases are inconclusive. A 5-year-lasting intake of 150 mg of isoflavones resulted in the development of endometrial hyperplasia in 3.37% of the women in a large placebo-controlled study; such effect was not observed in the placebo group. To the best of our knowledge a total of 28 placebo-controlled studies have been published hitherto in which 22 demonstrated no better effect than placebo on climacteric complaints. On the other hand, most animal experimental and clinical studies indicate a mild antiosteoporotic effect of soy/isoflavones. Taken collectively, soy/red clover/isoflavones appear to have mild estrogenic effects with all advantages and disadvantages of a low dose of estradiol-17 $\beta$ .

Extracts of *Cimicifuga racemosa* (Black cohosh) have since long been successfully tested in clinical trials on menopausal symptoms. So far 5 double-blind placebo-controlled studies have been conducted, 4 of which showed positive results on climacteric complaints. The most recent studies indicate that Black cohosh extracts have no effects in the mammary gland and in the endometrium but mild antiosteoporotic effects have been reported. The active compounds in *Cimicifuga racemosa* are not yet identified.

## Management of complex genital anomalies and the transition from childhood to adulthood

S78

### Management of the infant with ambiguous external genitalia. The pediatric endocrinologist's approach

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Ambiguity of the external genitalia constitutes an emergency requiring evaluation by a medical team. Issues of prime importance to be dealt with are: 1) the pathogenetic mechanism involved, 2) the sex assignment, and 3) the timing of corrective surgery. Non-palpable gonads indicate an XX individual, virilized during fetal life from androgens originating from the mother or the fetal adrenal. In such cases assignment should be on the female sex. Palpable gonads denote an XY undervirilized individual. In such cases the diagnostic problem is more complex: Defects in gonadal differentiation, the synthesis of testosterone or DHT, and the action of androgens (AIS). A hCG stimulation test will facilitate the localization of the defect: A normal rise of testosterone and DHT with normal intermediate metabolites and absent uterus indicate AIS. Lack or poor rise of testosterone and of intermediate metabolites point to either LH receptor, SF1 or StAR defect. Impaired testosterone rise along with a rise of intermediate metabolites could indicate a lyase, 17 $\alpha$  hydroxylase, 17 $\beta$  hydroxysteroid dehydrogenase or oxidoreductase defect. The sex assignment in an XY undervirilized neonate constitutes a real challenge. Neonates with severe AIS are raised as females. In the remaining neonates the decision is individualized using all available information. The parents should be fully informed and take part in the decision. However, the nomenclature to be used should be well selected and such stigmatizing terms as "hermaphroditism or intersex" should be avoided. Unfortunately, the protagonist in this team, namely the newborn, cannot participate in the final decision. Appropriate

genital surgery should be scheduled as soon as possible. In conclusion, although no "ideal" solution exists to this problem, there is the "optimal", and it is our task to uncover it. Finally, a harmonious liaison with the adult endocrinologist is one of the primary duties of the pediatric endocrinologist.

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## S79

### **Surgical management of ambiguous genitalia in the infant**

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The Intersex Society of North America stated in 1993 that "Intersex Genital Surgery in Infancy is a form of child sexual abuse". This peremptory statement based on outcome data judged from outdated medical and surgical procedures, led to an even more accurate surgical management of the intersex baby.

An etiological precise diagnosis of the condition and of the FURTHER post-pubertal sexual abilities of the patient, is mandatory before surgery. The choice of sex of rearing weighs heavily on the surgeon since he performs irreversible acts. This choice depends on:

1. mostly the etiology
2. the age at diagnosis: in the case of a late referred child with inappropriate sex of rearing, surgery ought to be postponed.
3. the anatomy of the genitalia, but in some cases, the anatomy will not be concordant with the post pubertal genital abilities.
4. completed, repeated, patient information of the parents (and the child) about the condition and its unavoidable sequelae.

Our experience of post pubertal results is based on our survey performed in 2001 of 270 genitoplasties; we conclude that early ONESTAGE genitoplasty leads to good results in 70% of cases. In this series, there was no close correlation between the anatomical results and the achievement of a "normal" sexual life.

This surgery has to be performed with a comprehensive knowledge of the embryological development and is only one of the therapeutic actions concerning the intersex child (thorough appropriate information, endocrinological and psychological managements are mandatory).

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## S80

### **Management of complex genital anomalies and the transition from childhood to adulthood: A patient's perspective**

PA Carmichael

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The way in which children with complex genital anomalies are managed is the subject of controversy and in a state of flux. The practice of early childhood genital surgery has been challenged in relation to both consent issues and functional outcomes.

For the past fifty years practice has been informed by the so called optimal gender policy which proposed that in disorders of sexual development (DSD) gender assignment should be that which allows optimal psychosexual and psychosocial functioning later in life. This approach assumes that socialisation is the decisive factor in gender identity development. In practice to support the assigned gender early feminising or masculinising surgery of the external genitalia was recommended, as was secrecy about the diagnosis such that the child could be reared unambiguously and informed gradually in line with their cognitive development. In practice many individuals with DSD were never given full information about their diagnosis and childhood surgery.

The optimal gender policy has been challenged; in particular the underlying assumption that gender identity is socially constructed. This together with adult reports about the damaging effects of secrecy and for some childhood surgery has resulted in some calling for a moratorium on all cosmetic genital surgery without consent. Parents are now routinely advised that children have the right to full details about their diagnosis and the medical and surgical management of this. Evidence suggests that parents have significant fears about the impact of ambiguous genitalia and disclosure of diagnosis on their child's development. They are often uncertain about what to say and fear their child will blame them or develop psychological difficulties. The effects of changing and inconsistent practice across centres will present particular challenges in the future for the timing and content of information and support given to children and young people and requires careful monitoring.

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## **Management of Graves' ophthalmopathy**

### **S81**

#### **Prevention of thyroid eye disease**

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Primary prevention aims at keeping the disease from occurring at all by removing risk factors. Secondary prevention is directed at early detection of disease, when the disease is still asymptomatic and when early treatment can stop the disease from progressing. Tertiary prevention refers to those clinical activities that prevent further deterioration or reduce complications after disease has declared itself. When applying the concept of prevention to TED, there is a problem with secondary prevention because there are no good specific serum markers for TED that would allow an early diagnosis of subclinical TED. As a group, such patients have slightly higher proptosis values than healthy subjects, their intraocular pressure on upgaze is abnormally increased in 61%–82% and their extraocular eye muscles are found to be enlarged on orbital ultrasound or CT scan in 70%–100%. Risk factors for occurrence or progression of TED have been partially identified (**non-preventable:** genetic, age, sex. **Preventable:** cigarette smoking, thyroid dysfunction, radioiodine treatment), but the list is probably much longer, and efforts of future research should be aimed at identifying most of them. For non-preventable risk factors obviously little can be done. However, several reports have documented a close association between cigarette smoking and TED. Furthermore, smokers tend to have more severe ocular involvement than non-smokers, although there is no significant association between the degree of tobacco consumption and severity of the ophthalmopathy. Hyperthyroidism seems to influence the clinical course of eye disease and several studies have shown that careful control of hyperthyroidism may be associated with a more favourable outcome of the ophthalmopathy. Finally, it is widely accepted that radioiodine treatment carries a small but definite risk of causing progression of eye disease. The exacerbation of the ophthalmopathy does not take place in patients with concomitantly treated with glucocorticoids.

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## **S82**

#### **Medical treatment**

C Marcocci

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Graves' ophthalmopathy (GO) in the majority of cases is modest and self-limiting and no treatment is required beside local measures and prompt control of thyroid dysfunction. A minority of patients (3–5%) have severe GO which warrants aggressive treatment to arrest further progression and eventually achieve regression of existing ocular signs and symptoms. Treatment of severe GO is a complex therapeutic challenge and available treatments provide unsatisfactory results in about one third of patients. Glucocorticoids (GC), orbital radiotherapy (RT), or a combination of both, are most frequently used. GC are more effective using the iv route than through the oral route, but particular attention should be paid to the possible liver toxicity of IV GC. The efficacy and safety of RT has been confirmed by recent randomized clinical trials. At variance with previous promising results, recent studies have shown that currently available somatostatin analogs are not very effective. Cytokine antagonists, currently used in other autoimmune diseases, have shown positive results in a small series of GO patients. Antioxidants might also be used, at least in mild form of GO. The possibility that total thyroid ablation might be beneficial is under investigation. Finally, particular attention should be paid to correction of risk factors involved in GO progression, such as cigarette smoking, thyroid dysfunction, radioiodine therapy.

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## **S83**

#### **Alternative immunomodulatory therapies for Graves' ophthalmopathy**

JP Banga

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Advances in understanding the pathogenesis of autoimmune diseases is leading to development of new targeted therapies, with the resultant improvements in the management of the patients. Autoimmunity to the TSH receptor is the main cause of Graves' disease and components of this disorder are the extrathyroidal complications which include Graves'

ophthalmopathy and pretibial dermatopathy. Recent studies in Graves' ophthalmopathy implicate an additional target antigen in the orbital fibroblasts, the IGF-1 receptor whose activation by autoantibodies may lead to the cascade of cytokine and chemokines secretion leading to orbital inflammation and swelling. Thus, potential immunomodulatory approaches that target the immune system by focusing on the autoreactive plasma B cell secreting autoantibodies may have beneficial effects to bring therapeutic benefit to the patient. Such immunomodulatory studies are well advanced in terms of clinical phase trails in a number of B cell autoimmune disorders. This talk will highlight the variety of new targeted therapies that are beginning to come to fruition, such as B cell depleting and modulating monoclonal antibodies, interference with B cell survival factors, anti-cytokine therapies and cellular therapies including regulatory T cells and dendritic cells. Their potential translation to treatment and management of Graves' ophthalmopathy will be also be discussed.

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## S84

### Ophthalmic management of thyroid eye disease

GE Rose

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The ophthalmic management of thyroid eye disease has changed markedly over the last 2 decades: There has been a significant trend towards more surgical involvement in both the active disease phase (with orbital decompression for relief of optic neuropathy, or eyelid surgery for severe exposure keratitis) and in the quiescent phase – with rehabilitative decompression (rather than eyelid surgery) having become the procedure of choice for most patients with established proptosis.

The contemporary ophthalmic management of patients with thyroid eye disease will be presented, with illustrative cases and an outline of the available surgical procedures.

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## In vivo imaging of signalling

### S85

#### OPT: a novel wholemount imaging technology for visualising gene & protein expression

S Wedden

MRC Technology, Edinburgh, United Kingdom.

Optical Projection Tomography (OPT) is a novel, unique and fast whole mount imaging technology which generates stunning 3D images plus virtual sections (in 3 orientations) of small biological specimens (1–15 mm). It is ideal for analysis of patterns of gene expression and protein distribution as it can be used with both visible (e.g. LacZ) and fluorescent stains. OPT can also be used on unstained tissue. The virtual sections give cellular levels of resolution, whilst the 3D images allow a detailed exploration of staining pattern and morphology in a way that has never before been possible. OPT is ideal for early and preclinical studies and is particularly suitable for imaging mouse and rat organs (e.g. brain, lung, heart, pancreas), human biopsy tissue and vertebrate embryos. My presentation will focus on the advances that our laboratory in Edinburgh and other OPT users have made in imaging all of these tissues.

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## S86

#### A mouse reporting on estrogen receptor (ER) transcriptional activity to understand the complexities of female physiology in mammals

A Maggi

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We recently generated a transgenic reporter mouse, ERE-Luc, to study ER transcriptional activity in pathophysiological states. In this model, luciferase synthesis is strictly associated with transcriptional activity of both ER $\alpha$  and ER $\beta$ , independent from the activity of receptors related to ERs. This enables to apply non-invasive imaging technologies to visualize ERs transcriptional activity *in vivo*, allowing to follow ERs activity in time in several organs in physiological or pharmacological settings. Initial studies focussed on ERs activity in sexually mature, adult female mice. By bioluminescence imaging, we showed that ERs activity extent in cycling females is proportional to circulating estradiol levels only in reproductive organs. In non-reproductive organs, other factors appear to be responsible for ER

transcriptional activation. More recently, using pharmacological inhibitors of IGF-1 receptors and mice with ablated liver synthesis of IGF-1, we showed that IGF-1 is involved in the regulation of ER-dependent transcription in several ER-positive tissues, in accordance with previous reports, ours and from other laboratories, showing that intracellular signalling cascades induced by IGF-1 may regulate ERs transcriptional activity *in vitro*. In view of the major role played by IGF-1 in less evolved organisms (nematodes, insects) in metabolic path selection determining proliferation or increased lifespan, it should not surprise that its signalling includes a receptor relevant for sexual behaviour and reproduction. ERE-Luc mouse has also been used to study the consequences of long-term treatments with natural and synthetic ligands on ER transcriptional activity. These *in vivo* studies are carried out by daily measurements of bioluminescence emission of female ERE-Luc mice treated with different doses of 17 $\beta$ -estradiol, Raloxifene and Tamoxifen for up to 21 days. Results reveal that therapeutic efficacy is achieved when drugs are administered at concentrations able to reinstate the oscillatory ER transcriptional pattern of naturally cycling mice. Thus, these initial studies point to the physiological relevance of ERs fluctuating activity and to its need, to maintain the tissue-specific protective effects linked to estrogen action.

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## S87

### Micro PET-ing in mice

Abstract unavailable

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## Contrasting practices in European endocrinology

### S88

#### Diagnosis of hypercortisolism

RA Feelders

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The diagnosis and differential diagnosis of hypercortisolism can be a major clinical challenge and involves several stages. In each stage different approaches are currently followed. There are, however, some general principles. In brief, after critical judgement of clinical features, the first step is to assess whether endogenous hypercortisolism is present. First-line tests to screen for hypercortisolism include measurement of 24 h urinary cortisol excretion, cortisol diurnal rhythm, a 1 mg overnight dexamethasone suppression test and, since recently available, midnight salivary cortisol concentrations. Once hypercortisolism is biochemically established, causes of pseudo-Cushing states (due to e.g. psychiatric disorders or chronic alcoholism) should be excluded. Second-line tests for this purpose include midnight plasma cortisol measurement and dexamethasone-CRF testing. Subsequently, ACTH-dependency of hypercortisolism should be assessed. In case of ACTH-independent hypercortisolism, imaging of the adrenals is performed to examine for adenoma, hyperplasia or carcinoma. When ACTH-dependent hypercortisolism is present, the work-up will be continued with pituitary imaging, eventually followed by bilateral sinus petrosus inferior sampling, since this form of Cushing's syndrome is most frequently caused by a pituitary adenoma. The high-dose intravenous dexamethasone suppression test and CRF test are also used to differentiate between pituitary and ectopic origin of ACTH overproduction. When indicated, imaging is performed to detect an ACTH producing neuroendocrine tumor with CT, somatostatin scintigraphy and/or PET scanning.

In each stage of this diagnostic process controversies can be found. In addition, there are considerable differences between, but also within countries, with respect to which tests are applied, how these tests are performed and how tests results are interpreted. Consensus statements and additional research should lead to an improvement of the evidence-based approach of the (differential) diagnosis of hypercortisolism.

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## S89

#### Diagnosis of hypercortisolism

AB Atkinson

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The diagnosis of Cushing's syndrome firstly involves making or excluding the diagnosis. If the diagnosis is confirmed then an accurate diagnosis is needed as treatment modalities are in general specific to the aetiology.



The diagnosis can be straightforward when symptoms and signs are classical and advanced. In these patients 24 h urinary free cortisol levels are markedly raised, late evening serum and salivary cortisol levels are raised and there is failure to suppress serum cortisol to control levels after low dose dexamethasone suppression. However because the clinical manifestations are often nonspecific the diagnosis can often be challenging. Difficulties also occur in cases with less marked or variable elevations in 24 h urinary cortisol and in those with concomitant conditions such as alcohol dependency, depression. In such cases it can be difficult to exclude or definitively diagnose the syndrome and in all a careful clinical judgment has to be made as to when to stop investigating. In some cases extended collections are required to establish cyclical or episodic unpredictable production and in others an extended time of observation confirms or refutes the diagnosis. The differential diagnosis of adrenal adenoma and carcinoma is usually clear with suppressed ACTH level and unilateral adrenal mass on scanning. Investigations for other causes of ACTH-independent hypercortisolism must be considered when ACTH levels are suppressed and there is no unilateral abnormality. These include abnormal receptor regulation and function. Perhaps the most difficult problem is in the differential diagnosis of ACTH-dependent cases. After a review of our Belfast experience looking at stringent responses to high dose dexamethasone testing and CRH testing, and bilateral inferior petrosal sinus sampling (IPSS) we concluded that the best evidence was from IPSS and that it is still required in a significant proportion of patients. Illustrative cases will be presented and discussed

## S90

### New endocrinology medicines: when are they good value?

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Health care payers (governments or insurance plans) are increasingly challenging new health services, including medicines, to prove their value and several European countries have established agencies to help with this task. The example of NICE in England is one example but the Scottish Medicines Consortium (SMC) offers another.

In its first four years the SMC has considered 14 endocrinology products, accepting eleven for use (79%). Manufacturers submit evidence that is critically appraised by pharmacy and economics reviewers, who advise a committee of doctors, pharmacists, managers and lay-people on the likely effectiveness and cost-effectiveness. Successful endocrinology submissions include teriparatide for osteoporosis and insulin glargine for diabetes, while examples of products not recommended include pegvisomant for acromegaly and cinacalcet for hyperparathyroidism in ESRD. The most common reason for not recommending a product was that the cost-effectiveness evidence submitted was either deeply flawed or gave an unacceptable cost per unit of health benefit. Typically, advice was issued to Scottish prescribers just over three months after receipt of the submission. Many Europeans already access this guidance at [www.scottishmedicines.org](http://www.scottishmedicines.org).

What can European colleagues learn that might benefit their own countries? Lesson 1 – confining assessment to efficacy alone merely repeats the licensing process. Payers want to know about effectiveness compared to current practice and cost-effectiveness.

Lesson 2 – all new medicines should be appraised, avoiding arbitrary criteria for topic selection. Those responsible for supplying the evidence cannot say they were taken by surprise.

Lesson 3 – timing of the appraisal. Evidence accrues after a medicine has been launched but prescribing practices also become habits. Early assessment is essential.

Lesson 4 – independent review of all the available evidence is attractive but time-consuming and expensive. Asking manufacturers of medicines to provide evidence that is then critically assessed is quicker, cheaper and (to date) seems to lead to similar recommendations.

## S91

### Contrasting expenditure on expensive endocrine therapies – e.g. growth hormone

Jens Sandahl Christiansen

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The clinical syndrome associated with profound growth hormone deficiency (GHD) in adults has now been established and recognised for more than ten years. The multiple physiological and metabolic actions of growth hormone

(GH) has been shown in a logical way to translate into recognisable symptoms and signs in individuals lacking the hormone. Normalization of abnormal physiological and metabolic parameters secondary to GH-replacement therapy has been demonstrated beyond reasonable doubt. Yet the clinical practice in the various countries in Europe in terms of replacing adult GHD patients with GH shows huge variation. Part but not all of this variability in clinical practice is due to economic factors.

The present status for GH-replacement therapy in adults in Europe will be reviewed and discussed.

## S92

### Contrasting practices in European endocrinology – radio-iodine therapy

AD Toft

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Iodine-131 is increasingly the treatment of choice of the hyperthyroidism of Graves' disease and nodular goitre and is gaining popularity as a means of achieving significant goitre shrinkage in euthyroid patients. There is, however, anxiety among a significant number of patients about the dangers of radiation in general and it is these same patients who pose the question of whether prolonged treatment with antithyroid drugs is an acceptable alternative. In the past, physicians have argued that there is little to be gained in terms of remission rates in continuing antithyroid drugs longer than 18–24 months. However, a recent small study has demonstrated that there was no disadvantage in treating patients with Graves' disease with an antithyroid drug for 10 years when compared to iodine-131 therapy in terms of cost or complications.<sup>1</sup> At a time when there is no consensus about the correct form or dose of thyroid hormone replacement it is perhaps unwise to champion the more liberal use of iodine-131, at least in the treatment of Graves' disease when the majority will develop thyroid failure within months of receiving a standard dose of 400 MBq. Furthermore, there is some evidence for increased morbidity and mortality from cardiovascular disease in patients with both autoimmune and non-autoimmune forms of thyroid disease, and iodine-131 therapy may be a contributing factor.<sup>2</sup> Perhaps it is time to re-consider the relative roles of antithyroid drugs, iodine-131 and surgery. After all, although none of these treatments is perfect, each is effective and there is evidence that patients have no particular preference.<sup>3</sup>

1. Azizi F *et al.* Eur J Endocrinol 2005; 152: 695.

2. Nyirenda MJ *et al.* Thyroid 2005; 15: 718.

3. Ljunggren JG *et al.* Thyroid 1998; 8: 653.

## S93

### Contrasting practices in European endocrinology. Radioiodine therapy.

#### A Danish view

L Hegedüs

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Radioiodine (131-I)-in use for more than 50 years for hyperthyroidism, with or without goitre, and also more recently for non-toxic goitre- has proven effective in the treatment of hyperthyroidism as well as in reducing goitre size. In hyperthyroidism, one or two 131-I doses will cure nearly all patients. Factors such as: sex, age, morphological goitre type, size of goitre, presence of thyroid autoantibodies, and pre- and post-radioiodine antithyroid drug (ATD) therapy influence the outcome, which is difficult to predict. Therefore, many use fixed (185–800 MBq) instead of calculated (corrected for thyroid size and 131-I uptake) doses of 131-I. Thyroid size reduction of 30–70% can be achieved within one year of therapy. Side effects include insufficient effect, myxoedema, thyroiditis, flare-up in hyperthyroidism and ophthalmopathy, but not thyroid cancer. There is insufficient data comparing long-term ATD therapy, 131-I, and surgery.

In non-toxic goitre-where levothyroxine has little or no goitre reducing effect-surgery is declined by or contraindicated in many patients. In the UK many patients are offered watchful waiting, while 131-I therapy is increasingly used in a number of other European countries (especially in Denmark). 131-I reduces goitre size by 30–70% within 12 months of therapy, depending on factors such as: duration of goitre, thyroid size, thyroid morphology, presence of thyroid antibodies, and pre-treatment with recombinant human TSH (rhTSH). The latter increases thyroid size reduction by 30–50%, in preliminary studies, at the expense of an increase in transient side effects, especially hyperthyroid symptoms, thyroid swelling and pain, and risk of myxoedema. Without rhTSH prestimulation, side

effects of <sup>131</sup>I are fewer but qualitatively the same as when used for treating hyperthyroidism.

In conclusion, <sup>131</sup>I is a qualified alternative to ATD or surgery in hyperthyroidism and surgery or watchful waiting in non-toxic goitre. In my view, it should be used more frequently. When investigated, there is little consensus on preferred therapy (in a standard case), most likely due to lack of studies comparing the options.

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## **Research management workshop**

**S94**

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Abstract unavailable

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**S95**

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### **Impact of EU Clinical Trials Directive**

B Moulton  
Dublin, Ireland.

Abstract unavailable

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**S96**

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### **Funding for commercialisation of research after the biotech bubble**

RN Seabrook  
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It is important that the basic discoveries made by the scientific community are translated into practical innovations that can be utilised directly or indirectly to improve human and animal health. To be effective in translating scientific advances into health products it usually requires engagement with the business and investment community. Bridging the gap between academic research and commercial R & D is difficult because of the risks inherent to early-stage translation. This is a particular problem in the healthcare sector where the technical and regulatory hurdles are a significant challenge on the path to market. In combination, the early-stage scientific and regulatory uncertainty is so significant it has led to a much more cautious approach to investment by both the financial and industrial sectors. Alternative sources of mixed motive, early-stage, funding are emerging via government, certain types of not-for-profit organisations (foundations and charities for example) and also high net worth individuals. There are now examples of healthcare enterprises that have successfully deployed funds from alternative sources to enable the enterprise to reach a pivotal stage in development, such as clinical trial or even product launch, at which the enterprise has become investment attractive to the markets or existing companies. In particular, The Wellcome Trust, through its Translation Award funding seeks to mitigate the risks of early-stage translation and has committed in excess of £50 M to these types of project.

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# Nurses Session

## Metabolic syndrome

S97

### Leptin expands function

H Randevo

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Leptin, a 167 amino acid protein product of the *ob* gene, is an adipocyte-derived hormone that acts as a major regulator for food intake and energy homeostasis. Leptin is expressed mainly in white adipose tissue, but also in stomach and placenta. Leptin circulates in the serum in a free form or bound to leptin-binding proteins. Since its discovery, our understanding of leptin's biological functions has expanded from antiobesity to broad effects on reproduction, hematopoiesis, angiogenesis, blood pressure, bone mass, lymphoid organ homeostasis, and T lymphocyte systems. Leptin orchestrates complex biological effects through its receptors, expressed both centrally and peripherally. Leptin deficiency or resistance can result in profound obesity, diabetes, and infertility in humans.

Leptin concentrations correlate linearly with increasing amounts of fat mass. Although the amount of fat is an important determinant of leptin concentrations, other factors are also relevant, including sex, adipose tissue-specific factors/site, hormones and cytokines. Importantly, women have higher leptin concentrations than men even after adjusting for body-mass index, which may be due to differential body-fat distribution or the effects of sex steroids. However, it has been realised that leptin might be more important at the other end of the energy homeostasis spectrum — energy deprivation rather than obesity. Studies in animals and human beings have shown that low concentrations of leptin are fully or partly responsible for starvation-induced changes in neuroendocrine axes including low reproductive, thyroid, and insulin-like growth factor (IGF) hormones and such as exercise-induced amenorrhoea, non-athletic forms of hypothalamic amenorrhoea, and anorexia nervosa. Interestingly, clinical studies have shown that leptin and its receptors exhibit diurnal variation, are influenced by growth hormone status, and are influenced by insulin and glucocorticoids. These findings support the hypothesis that leptin expands function.

S98

### Ghrelin update

V Popovic

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Ghrelin is the brain-gut peptide with growth hormone (GH)-releasing and appetite-inducing activities. Reviewed experimental evidence confirms the role of endogenous ghrelin in regulating GH secretion while studies in humans still fail to pinpoint the exact role of ghrelin in GH secretion. Accumulating evidence supports that ghrelin/GH secretagogue receptor (GHS-R) axis contributes to the maintenance of body weight. Evidence suggests the role of environmental factors modulating ghrelin's activity, the role of constitutive activation of the GHS-R, polymorphisms in the GHS-R gene together with dysregulation of postprandial ghrelin suppression in obesity. New important psychiatric issue is the role of ghrelin in the weight gain, insulin resistance and diabetes type 2 associated with the use of atypical antipsychotics. The novel role of ghrelin is in the adaptive response to caloric restriction and loss of body fat by inducing tissue-specific changes in lipid metabolism favoring triglyceride deposition in liver over skeletal muscle. New insights into the ghrelin's effects on pancreas and liver, gives enough evidence for its role in glucose metabolism. In summary recent studies confirm the role of ghrelin as a marker of changes in energy balance and GHS-R as a modest amplifier of biological function. A ghrelin antagonist may provide answers in regard to ghrelin physiology or its involvement in the etiology of obesity. One of the crucial questions will be which side effects might be to watch for during blocking endogenous ghrelin action.

S99

### Insulin resistance and type 2 diabetes – insights from the study of extreme human phenotypes

DB Savage

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Insulin resistance, which can be defined as a state of reduced responsiveness to normal circulating levels of insulin, plays a major role in the development

of type 2 diabetes. Whilst standard definitions of insulin resistance still define it in terms of insulin's effects on glucose metabolism, the last decade has seen a shift from the traditional "gluco-centric" view of diabetes to an increasingly acknowledged "lipo-centric" viewpoint. This hypothesis holds that abnormalities in fatty acid metabolism may result in inappropriate accumulation of lipids in muscle, liver and  $\beta$ -cells and that this lipid accumulation is involved in the development of insulin resistance in muscle and liver as well as impairing  $\beta$ -cell function (so called "lipotoxicity"). Interestingly, studies in people with rare forms of extreme insulin resistance have provided a number of significant mechanistic insights pertaining to this "lipo-centric" notion of type 2 diabetes and have also begun to yield novel therapeutic targets.

S100

### Metabolic syndrome

N Finer

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Bjorntorp first coined the term 'metabolic syndrome' (MS) in the 1980's to describe the association between obesity, regional fat distribution, disease endpoints and their risk factors (cardiovascular disease, premature death, stroke, non-insulin-dependent diabetes mellitus and female carcinomas). This description also recognised the potential contribution from adreno-cortical activity and stress. Since that time a plethora of research has highlighted the causative links between visceral fat and insulin resistance in the syndrome. Two differing, but overlapping, definitions developed during the 1990's reflecting the clinical background from which they were developed. The WHO definition (1998) required the presence of insulin resistance, while the National Cholesterol Education Program Expert Panel on the Treatment of High Blood Cholesterol in Adults, Adult Treatment Panel III (2001) focussed on the importance of lipid abnormalities. In 2005 the International Diabetes Federation proposed that central obesity (defined by ethnic-specific waist measurements) was required + two of raised TG level, reduced HDL cholesterol, raised blood pressure, raised fasting plasma were necessary to define presence of the syndrome. They recommended that '...Once a diagnosis of MS is made, management of the condition should be aggressive and uncompromising in its aim to reduce the risk of CVD and type 2 diabetes' and that primary intervention '...for MS is healthy lifestyle promotion (to) include moderate calorie restriction (to achieve a 5–10 per cent loss of bodyweight in the first year), moderate increase in physical activity, change in dietary composition'. It was recognised currently to be necessary to treat the individual components of the syndrome.

The value of 'diagnosing' the syndrome is under debate and the ADA and EASD have suggested (2005) that '... too much critically important information is missing to warrant its designation as a "syndrome." Until much needed research is completed, clinicians should evaluate and treat all CVD risk factors without regard to whether a patient meets the criteria for diagnosis of the "metabolic syndrome".'

#### Conclusion

Improved screening for obesity is a priority for referral into weight management services. This study has shown that clinically effective weight reduction can be achieved in the Primary Care setting.

S101

### Nurse-led weight management: the Counterweight Programme

Counterweight Project Team

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#### Introduction

Weight gain and obesity promote metabolic syndrome, diabetes and other cardiovascular risk factors. Weight loss achieved by lifestyle change can limit progression of cardiovascular risk factors. What is the current approach to obesity in general practice and can a nurse-led programme result in clinically beneficial weight change?

#### Methods

The Counterweight Programme has developed a model of best practice for obesity management in UK primary care. 80 primary care practices from 7 areas of the UK have been recruited. The project provides retrospective audit, practice training and support, protocols and prospective evaluation for an evidence based intervention programme for weight loss and maintenance.

Results: retrospective audit

Women were more likely than men to have a record of their weight (69.2%: 57.0%;  $P < 0.0001$ ) or BMI (70.7%: 58.1%;  $P < 0.0001$ ). In those with a BMI record, 13.6% males and 16.5% females were obese. Previous interventions for obese individuals included practice based diet counselling (20%), dietetic referral (4%), anti-obesity medication (2%) and referral to an obesity centre (1%).

Results: intervention programme

By March 2004 1549 patients had been recruited into the programme. Ratio of males to females was 1:3. Mean BMI was  $37 \text{ kg/m}^2$  and mean age 49 years. 74% had at least one obesity-related co-morbidity. 51% of patients having reached 12 months ( $n = 893$ ) were classified as "completers" in that they had attended the minimum required number of programme appointments (4 in 3 m, 5 in 6 m or 6 in 12 m). Of all those with 12 month data ( $n = 445$ ) 34% lost at least 5%. For "completers" this increased to 40%. Mean weight loss at 12 months was 3.2 kg for the whole group and 4.5 kg for "completers". Including those lost to follow up ITT analysis shows 6 people need to enter the programme to have 1 patient with at least 5% weight loss at 12 months.

Conclusion

Improved screening for obesity is a priority for referral into weight management services. This study has shown that clinically effective weight reduction can be achieved in the Primary Care setting.

# Young Endocrinologists Session

## Presenting your research – getting your work known S102

### Giving talks

A McNeilly

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When you sit in meetings I know you will all have thought at different times that this is the most (tick one of the following boxes) – boring – incomprehensible – ridiculous – interesting – inspirational – fantastic – talk you have ever heard. Now that oral presentations at meetings are rare for many with posters taking centre stage it is really important to make the best of the opportunity to impress potential future employers etc with your abilities not only to do excellent science, but also to present well. You should also remember that answering questions is a very important part of giving a talk as this is when you can display your extensive knowledge of the subject. In this talk I will give some ideas as how, and how not, to present a talk, and illustrate some of the problems that can arise when given the gizmo of powerpoint. This can be wonderful or awful! Most of you now will be giving presentations during the course of your studies, so much of what I may say will be common sense. However, as this sometimes seems to be in very short supply, I hope to give you some pointers as to how to maximise the impression you give in your presentations. This may be to realise that I do not know what I am doing, but then this is also a pointer to the future. And remember that discussions are always good for the soul.

## S103

### Practical tips for a perfect poster

A Mostyn, H Budge & ME Symonds

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The key to a great poster is preparation – as soon as you receive notification of your poster presentation, read the “instructions for authors”. Key points to note are:

The size of the poster boards

Attendance times

Is there a presentation in front of your poster?

Turn-around time of the poster printer.

The shape/size of the poster will be dictated by the size of the poster boards. The format should include: introduction, methods, results, discussion and a conclusion. Text and illustrations on the poster should “flow” in a way that a lone reader can follow. Keep layout simple. Choose a colour scheme that will enhance your work – not overshadow it! University corporate styles look professional if a number of delegates from the same university are present. Use your sponsors’ logo to acknowledge support.

Titles should be short and snappy to encourage viewers. Text throughout the poster should be in short sentences – delegates want to be able to scan posters quickly for the important points and should be readable from 3 feet. A sans serif font is recommended (e.g. Arial or Helvetica), keep bold for special emphasis e.g. titles, and avoid acronyms, abbreviations and jargon. Figures look impressive on posters; include cartoons/flow diagrams of methods, graphs of results and photographic images. Keep the text in figures large and easy to understand.

Show several colleagues the poster before having it printed and get feedback, especially if an oral presentation is required. Prepare an overview of the work and practice; it’s not easy to condense months of work into a 2–3 minute sound bite! An A4 handout of the poster is useful. Preparation, consistency and simplicity are the key to a great poster which will showcase your research in an appealing visual format, to a wide number of delegates.

## S104

### Papers for peer reviewed journals

S Pearce

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The most important part of any research project is getting it published in the best way; this usually will include a peer reviewed journal paper. Choice of journal is an important decision, with international broad readership journals having much greater prestige than regional or subject-restricted publications. You will need to decide whether to put all your relevant research findings into a single large manuscript, or to split your work up into a series of smaller papers. If your work follows a logical sequence of experiments, then the *magnum opus* approach is almost certainly preferable. Before you start to write, get together the data (essentially the tables and figures) for the

paper and think about the most important points that you want to make. Also get a copy of your target journal and think how your work will fit in. Many people like to get going by writing the methods section. When writing methods, you are aiming to give enough detail so that someone can repeat your exact experiment and (hopefully) get the same result. The introduction should give someone who is not an expert in your field a broad idea about why the subject is important, what has been done before (is known or otherwise), and why your question is interesting. Aim to quote as many relevant papers as possible; think who will review your paper and keep them happy. The results section should state your findings in a clear way, so that the sequence of experiments can be followed. Take time to explain or present complicated data. Don’t comment on your data in this section. Aim to make the figure legends and tables self-explanatory. Most papers have only 2 or 3 things worth saying about the results. These should be included in the discussion section, along with more detailed comparisons about how your results are similar and different to those previously published, or about how your results affect the interpretation of previous data. Many discussions are too long. While writing, actively revise your text. Think after each point or paragraph: Does it say what I need it to? Could I have expressed the same more clearly or in less words? Always proof-read a hard copy of your paper and look at it again the next day before passing it on for comment.

## S105

### PhD/MD Thesis: a recipe for success

DRE Abayasekara

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In all probability, a thesis is the longest single document that anyone is likely to write as a scientist. It requires much planning to ensure that the thesis gets written and submitted on time. To begin with, plan the overall outline of the thesis. With some variation, most theses begin with a comprehensive introduction/literature review. A chapter describing the materials and methods invariably follows. The subsequent chapters contain the material you have sweated over i.e. your life’s work to date! The content of these results chapters tends to define the thesis and therefore requires much thought. Most theses usually end with a discussion placing the main findings of the thesis in the context of published work. The order in which you tackle the writing of the chapters does not need to follow the sequence described – in fact it is best to start with the easiest one, which often is the Materials and Methods chapter. The outline of the thesis should be discussed with your supervisor(s). They will make suggestions, which you will ignore at your peril! The next step is to set a realistic timetable, which includes deadlines for submitting draft chapters to your supervisor(s). Getting started is the hardest part of writing a thesis. The fact that you have an outline of what you aim to include in the chapters will help. However, the most important thing is to make sure that you write SOMETHING – it is always easier to improve on it at a later date. The following quote regarding the writing of a thesis captures the very essence of the task awaiting you: “*When you are about to begin, writing a thesis seems a long, difficult task. That is because it is a long, difficult task*” - Joe Wolfe of the University of Sydney.

## S106

### Talking to a lay audience

TR Parkhill

Society for Endocrinology, Bristol, United Kingdom.

The public’s understanding of current scientific matters is mostly communicated through the news media. Scientists and clinicians are often reluctant to comment to the press, largely through a fear of these comments being taken out of context. The news media tries to balance reports by taking comment from different sides of the story; if scientists and clinicians do not put their views forward, this void is often rapidly filled by someone with a stronger opinion, for example pressure groups or commercial interests. This means that not commenting produces exactly the lack of objectivity which scientists are trying to avoid. Scientists and clinicians have a duty to make sure that their work is accurately represented.

Scientists and clinicians need to be conscious of finding the right voice with which to communicate. Too often the fear of inaccurate reporting means that public understanding is sacrificed, and this can be true in both research and clinical practice contexts. If we are to communicate to a wider public, we need to take the initiative in putting forward good science. Being proactive means that scientists can set the agenda, and minimise any potential distortion being presented to the lay audience.

# Oral Communications

## Signal transduction

### OC1 Novartis Oncology Young Investigator Award

#### Stress response after TIF-2 disruption: loss of grip on feedback control or adrenocortical impairment?

AV Patchev<sup>1</sup>, D Fischer<sup>2</sup>, M Herkenham<sup>3</sup>, F Götz<sup>1</sup>, SS Wolf<sup>5</sup>, M Gehin<sup>4</sup>, P Chambon<sup>4</sup>, VK Patchev<sup>3</sup> & OFX Almeida<sup>2</sup>

<sup>1</sup>Institute of Experimental Endocrinology, Humboldt University School of Medicine - Charité Universitätsmedizin Berlin, Berlin, Germany; <sup>2</sup>Max Planck Institute of Psychiatry, Munich, Germany; <sup>3</sup>Section on Functional Neuroanatomy, NIMH, NIH, Bethesda, MD, United States; <sup>4</sup>Institute de Génétique et de Biologie Moléculaire et Cellulaire (IGBMC), Illkirch, France; <sup>5</sup>Corporate Research - Gynecology & Andrology, Schering AG/Jenapharm, Jena, Germany.

Efficiency of glucocorticoid receptor (GR) signaling is modulated by a variety of co-activators, including the transcription intermediary factor 2 (TIF-2). Although TIF-2 has been shown to amplify GR signaling in different cellular models and contexts, its system-physiological relevance has not been comprehensively examined. The present work investigated the role of TIF-2 in GR-mediated regulation of hypothalamo-pituitary-adrenal (HPA) axis activity in mice. Experiments were designed to test the hypothesis that ablation of TIF-2 would disrupt the efficacy of GR-mediated control of basal and stress-induced activity at several levels of the HPA axis. We demonstrate that mice with targeted ablation of TIF-2 show alterations in several mechanisms that control the HPA axis activity under both quiescent conditions and stress. Lack of TIF-2 was associated with increased expression of corticotropin-releasing hormone (CRH) and vasopressin in the hypothalamic paraventricular nucleus and elevated pituitary adrenocorticotropic hormone (ACTH) content, whereas expression of GR mRNA in the hippocampus was decreased. Although these findings are suggestive of impaired HPA axis restraint, TIF-2-deficient animals had lower basal corticosterone levels, and their secretory response to stress was blunted and sluggish. Thus, in TIF-2-knockout mice enhanced central 'drive' of the HPA axis is not conveyed into equivalent adrenocortical response. Decreased cellular densities and signs of cytoarchitectonic disorganization in the zona fasciculata of the adrenal cortex suggest that impairment of central HPA regulation in TIF-2-deficient animals is secondary to insidious adrenocortical insufficiency. These data, together with previously described hypogonadism in TIF-2-deficient mice indicate that, besides amplification of nuclear receptor signaling, TIF-2 also appears to be a pre-requisite for normal function of steroid-producing glands.

### OC2 Novartis Oncology Young Investigator Award

#### Signalling and internalisation characteristics of corticotropin-releasing hormone (CRH) receptors

D Markovic & D Grammatopoulos  
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Corticotropin releasing hormone (CRH), and the urocortins (UCN) play key roles in mammalian pathophysiology. Their actions are mediated through two types of G-protein coupled CRH receptors, (R1 and R2) which exhibit distinct pharmacological characteristics and determine specificity of CRH and UCN actions. Several CRH-R1 mRNA splice variants have been identified in native tissues, encoding receptor isoforms with various aminoacid inserts or deletions, termed R1 $\beta$ , R1c, R1d etc. To obtain more information about the impact of these structural alterations on CRH-R1 functionality we expressed different CRH-R1 subtypes and CRH-R2 $\beta$  in HEK293 cells and we monitored their internalisation characteristics following agonist stimulation by using indirect confocal microscopy. Results showed that both CRH-R1 $\alpha$  (the wild type receptor), and the signaling-impaired subtype CRH-R1 $\beta$  (which contains a 29 aa insert in the 1st IC loop), were expressed in plasma membrane and were internalised within 30–45 min of CRH activation in a  $\beta$ -arrestin dependent mechanism. Interestingly, the CRH-R1d that contains a 14 aa deletion in the 7th TMD which interferes with G-protein coupling, was distributed in the plasma membrane as well as the cytoplasm, suggesting that intact TMDs are essential for adequate receptor stability and localization to the plasma membrane. Nevertheless, this CRH-R1 subtype was also able to internalise normally in response to CRH binding. Studies on the CRH-R2 $\beta$  revealed important differences in receptor internalisation characteristics. In response to UCNI (a CRH-R2 specific agonist), the CRH-R2 $\beta$  internalised within 15 min of UCNI activation whereas the weaker agonist CRH, induced CRH-R2 $\beta$  internalization only after 30–45 min of treatment. These results suggest that intact CRH-R1 signaling potency is not essential for activating

the intracellular machinery involved in receptor internalization endocytosis. In addition, CRH-R2 internalisation pathways might be directly related to individual agonists signalling potency and their ability to induce distinct receptor active conformations.

### OC3

#### Activation of cAMP (cAMP) binding protein (CREB) by gonadotrophin-releasing hormone (GnRH) requires calcium influx and PKC activity in gonadotroph cell lines

KC Jonas<sup>1</sup>, IA Thompson<sup>1</sup>, JM Burrin<sup>2</sup> & RC Fowkes<sup>1</sup>  
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The ubiquitous transcription factor, CREB, is responsible for mediating gene transcription in many endocrine tissues. Recent studies reveal that the activation of several signalling pathways can lead to the phosphorylation of CREB at Ser133, including PKC, MAPKs, CaMKs, and PI3K. In the pituitary gonadotroph, there are consensus cAMP response elements (CREs) in several of the gonadotrophin subunit promoters as well as in the GnRH receptor promoter. We have previously shown that activators of the cAMP pathway can phosphorylate CREB on Ser133 in  $\alpha$ T3-1 cells, and others have shown GnRH to activate CREB in rat pituitaries. To establish which signalling pathways are involved in GnRH-stimulated CREB activation, we have used Western blotting, reporter gene assays and electrophoretic mobility shift assays (EMSA) to examine CREB phosphorylation, transcriptional activity and DNA binding.  $\alpha$ T3-1 and L $\beta$ T2 cells treated with 100 nM GnRH for 15 min showed enhanced phosphorylation of CREB (at Ser133) as well as ATF-2. We transiently transfected  $\alpha$ T3-1 and L $\beta$ T2 cells with a TKCRE-LUC reporter construct and stimulated with GnRH, PMA, PACAP (100 nM) or Forskolin (10  $\mu$ M) for 24 h. GnRH (6.4  $\pm$  0.2-fold, 3.9  $\pm$  0.5-fold) and PMA (3.3  $\pm$  0.9-fold, 6.9  $\pm$  0.1-fold) strongly activated TKCRE activity in  $\alpha$ T3-1 and L $\beta$ T2 cells respectively ( $P < 0.01$ ). Pre-treatment of L $\beta$ T2 cells for 30 min with nifedipine (Ca<sup>2+</sup> channel antagonist) or GF109203X (PKC inhibitor) (both 1  $\mu$ M), but not H-89 (PKA inhibitor), significantly inhibited GnRH-stimulated TKCRE activity ( $P < 0.05$ ). EMSA analysis of nuclear protein extracted from  $\alpha$ T3-1 and L $\beta$ T2 cells treated with GnRH for 15 min revealed enhanced binding of CREB, ATF-2 and other CREB-related proteins to the consensus CRE response element from the glycoprotein hormone  $\alpha$ -subunit promoter. These data suggest GnRH enhances CREB phosphorylation at Ser133, increases CRE-mediated transcription by calcium influx and PKC activity, and enhances CREB binding in pituitary gonadotrophs; effects which are apparently cAMP-independent.

### OC4

#### The role of MRAP in the functional expression of the melanocortin 2 receptor

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Familial Glucocorticoid Deficiency type 2 (patients with normal MC2R) is associated with mutations in the MRAP (Melanocortin Receptor Accessory Protein) gene. In order to investigate the function of this novel single transmembrane domain protein, CHO and SKN-SH cells were transfected with MRAP-FLAG and/or MC2R-GFP constructs and imaged using confocal microscopy. Although the MC2R-GFP failed to be expressed at the cell surface when transfected alone, it was found to be expressed at the cell surface in the presence of MRAP implying that MRAP may be involved in the trafficking or folding and processing of the MC2R. (L A Metherell *et al.*, (2005) *Nature Genetics* 37, 166 – 170) Co-immunoprecipitation studies revealed an interaction between the MC2R and MRAP. Co-transfection of MC2R and MRAP generated a functional cell surface receptor as indicated by the enhanced cAMP response to ACTH in SKN-SH and CHO cells and to a limited extent in HEK293 and HeLa cells. RNA interference studies were carried out using MRAP siRNA transiently transfected into Y1 adrenocortical cells, which endogenously express both a functional MC2R and MRAP. The knockdown of MRAP expression in this cell line resulted in the reduction of the MC2R mediated signalling as determined by the use of a CRE-luciferase reporter assay. The production of cAMP through MC1R, MC3R, MC4R or MC5R was not enhanced in the presence of MRAP when



stimulated with NDP-MSH. However, co-immunoprecipitation studies showed an interaction between MRAP and MC1R. In summary MRAP plays a role in the cell surface expression of a functional MC2R, and may also interact with the other melanocortin receptors.

## OC5

### The overexpression Pit-1 pathway, switch on by RET, get into apoptosis fate

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RET is a tyrosine-kinase receptor having a GPI-linked co-receptor or GFR $\alpha$ . Moreover, RET has four different ligands (GDNF, NTN, ART and PSP) with its respective co-receptor (GFR $\alpha$ -1,-2,-3 and 4). Pituitary cells are differentiated specialized secretory cells. Somatotrophs are responsible for growth during infancy and puberty until achievement of the final adult height. Although genetic factors leading to somatotroph hypoplasia are well known, no much is known about the mechanisms controlling somatotroph cell proliferation.

Recently, we have demonstrated the expression of Ret, GDNF and GFR $\alpha$ 1 from all pituitary secretory cells exclusively on the somatotrophs in both rats and humans (Urbano *et al.*, 2001; Japón *et al.*, 2002). In this work we intend to characterize the role of RET in somatotrophs. Thus we used primary monolayer cultures of rat AP cells as well as the GH4C1 cell line. Transfecting the RET receptor allow us to uncover a pathway where Ret is regulating somatotroph cell function through potently inducing Pit-1 expression, as assessed in terms of both mRNA and protein levels. More importantly, Ret-induced Pit-1 overexpression, led to a Pit-1-dependent p53 increase and apoptosis, both of which were prevented by co-transfecting Pit-1 siRNA. We also found that the pathway mediating these effects of Ret involved activation of PKC $\delta$ , JNK, c/EBP $\alpha$  and CREB. Finally, by transfecting different isoforms and mutated-forms of the RET receptor we found that RET-induced apoptosis in somatotroph cell is unrelated to the kinase activity of the receptor.

## OC6

### Activation of androgen membrane binding sites induce potent regression of prostate cancer cells *in vitro* and *in vivo*

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Recent data indicate that steroid action can be completed through genomic (late) and non-genomic (rapid) mechanisms. Non-genomic androgen effects are initiated at the membrane level and imply specific secretory and signaling mechanisms different from the classical intracellular androgen receptor activation. In previous work we have reported that androgen membrane binding sites (Ambs) are present in LNCaP human prostate cancer cells. Their activation mediates a FAK/PI3K/Cdc42/Rac1 signaling pathway, resulting in rapid actin cytoskeleton rearrangements and controlling cell proliferation, secretion and motility. In the present study we further evaluated the molecular mechanism of the activation of Ambs and its clinical significance in iAR-expressing (LNCaP) or iAR-negative (DU145) human prostate cancer cells *in vitro* and *in vivo*. Here we report that in iAR-negative DU145 cells, activation of Ambs by testosterone-BSA induced a significant actin remodeling followed by the decrease in cell migration, adhesion and invasion. These effects were induced through activation of an alternative pathway involving Rho/ROCK/LIMK2 signaling and leading as well to actin-reorganization. Interestingly, this pathway was not activated in LNCaP cells. Testosterone-BSA induced apoptosis in both LNCaP and DU145 cells, an effect apparently regulated by the actin cytoskeleton reorganization. In addition, *in vivo* experiments in LNCaP-inoculated nude mice revealed that treatment with testosterone-BSA (8 mg/kg weight) for one month resulted in a 60% reduction of tumor-size, compared to control animals. This effect was not affected by the anti-androgen flutamide. No apparent toxic effects were observed in all treated animals. Our findings suggest that activation of Ambs induce

apoptotic regression of prostate cancer cells *in vitro* and *in vivo*. Activators of Ambs may represent a new class of antitumoral agents of prostate cancer without apparent side effects.

## OC7

### The effect of bone marrow stem cell (BMSC) differentiation on growth hormone receptor (GHR)-associated signalling pathways

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GH has diverse direct effects including the induction of lipolysis in adipocytes and mineralisation in bone. Interestingly, pluripotent mesenchymal stem cells can be isolated and cultured from adult bone marrow and induced to differentiate *in vitro* to both adipocytes and osteoblasts. GHR activation can be coupled to JAK2/STAT5 and the ERK (MAPK) pathway. We have therefore studied the evolution of GHR-associated cell signalling in adult rat BMSC's during differentiation along adipocyte and osteoblast lineages.

BMSC's were isolated from rat femur (10wk male Wistar), cultured in control medium (alphaMEM 10% FCS) or adipocyte differentiation medium (containing the PPARgamma agonist pioglitazone) for 3-7 days or osteoblast differentiation medium (containing ascorbate and dexamethasone) for 20 days. Cultures were then stained for lipid with oil red O and for alkaline phosphatase or treated with GH (10 min) and PMA (10 min) to study GH induced activation of ERK and STAT5 in each of the cell types. ERK and STAT5 activation was assessed using western blotting probed with activation state (phospho)-specific antibodies.

Oil red O staining was only detectable in the adipocyte differentiated cells. GH stimulation of the ERK pathway was not detectable in undifferentiated or osteoblast differentiated cells but was detectable in 3 day differentiated adipocytes and at a higher level in the 7 day differentiated adipocytes. PMA was able to produce readily detectable activation of ERK in all cell types, demonstrating the presence of a functional ERK pathway. GH induced activation of STAT5 was detectable in all cell types.

In conclusion, despite the presence of both functional GH receptors as judged by GH-induced STAT5 activation and functional ERK (induced by PMA) in all cell types, GHR-activation is only coupled to the ERK pathway in adipocyte differentiated BMSC's. Thus it is possible that the ERK pathway may be more important in mediating the effects of GH in fat than bone.

## OC8

### Role of the calcium-calmodulin dependent kinase II in oncogenic Ras-induced proliferation

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Activation of the Ras/ERK pathway and stimulation of proliferation by integrins in thyroid cells requires CaMKII activation. To date, whether this role of CaMKII is a general mechanism or is restricted to integrin signalling is unknown. As oncogenic activation of the Ras/ERK pathway by mutated Ras, RET/PTC and Trk is frequent in papillary thyroid carcinoma (PTC), we investigated the expression and activation level of CaMKII in PTC primary culture and in stable cell lines.

The level of activation of CaMKII and ERK was determined by Western blot with anti-phosphorylated antibodies and by *in vitro* kinase activity assay. In 5 primary cultures and in cell lines starved from serum and left in suspension for 30 min, CaMKII and ERK remained activated while were inhibited by calmodulin inhibitors. CaMKII inhibitors blocked ERK phosphorylation. These results indicate that constitutive up-stream signals activate CaMKII and that this kinase is necessary to ERK activation in PTC cells.

To determine whether oncogenic Ras induced CaMKII activation, NIH-3T3 were transfected with plasmids encoding recombinant mutated H- and K-Ras<sup>V12</sup> for transient expression. In NIH-3T3 cells starved from serum, CaMKII was not active. Oncogenic Ras induced CaMKII activation inhibited by calmodulin inhibitors. Stimulation of ERK activation and [<sup>3</sup>H]thymidine incorporation by oncogenic Ras were completely suppressed by CaMKII inhibitors. These results indicate that oncogenic Ras induces CaMKII activation through modulation of intracellular calcium



concentration and that this kinase is necessary to stimulate cell proliferation. As the activation of the Ras/ERK pathway is a major mechanism of sustained proliferation in many tumors, CaMKII might represent a novel pharmacological site of intervention in the treatment of cancer.

## Steroids and reproductive endocrinology

### OC9

#### 11 $\beta$ -HSD1: anti-inflammatory mechanism in acute and chronic inflammation?

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Although the anti-inflammatory effects of exogenous glucocorticoids (GC) upon inflammation are well recognized, the contribution of 11 $\beta$ -hydroxysteroid dehydrogenase 1 (11 $\beta$ -HSD1), an amplifier of GC action *in vivo*, in modulating endogenous GC action during inflammation is unknown. Mouse models of arthritis and lung inflammation were used to explore the role of 11 $\beta$ -HSD1 in acute and chronic inflammation. Arthritis was induced in 11 $\beta$ -HSD1<sup>-/-</sup> (KO) and C57BL/6J (WT) male mice by intraperitoneal injection of arthritogenic serum from K/BxN transgenic mice. Clinical signs of inflammation were monitored over 21d. Lung inflammation was induced by intrapleural injection of carrageenan, and inflammatory cells were recovered at 4h and 24h. The onset of arthritis occurred ~1d earlier in KO than in WT mice, and was slower to resolve in KO (50% reduction in clinical score of inflammation at 16d in KO compared to 11d in WT). Preliminary histological assessment revealed a reactive bone phenotype and intense periarticular inflammation (exostosis and ganglion formation) in KO compared to WT mice. Although both showed high basal (0800h) plasma corticosterone levels 2d following serum injection (253  $\pm$  39nM and 218  $\pm$  61nM, WT and KO, respectively), at 21d plasma corticosterone was still elevated in KO (227  $\pm$  76nM) while WT had returned to normal (86  $\pm$  27nM). Following induction of acute lung inflammation, more cells were recovered in pleural lavages from KO than WT mice at 4h (17  $\times$  10<sup>6</sup> vs 9  $\times$  10<sup>6</sup> cells/ml, KO vs WT respectively,  $P < 0.001$ ) and 24h following carrageenan injection (27  $\times$  10<sup>6</sup> vs 18  $\times$  10<sup>6</sup> cells/ml, KO vs WT respectively,  $P = 0.001$ ). Interestingly, in KO at 4h there was a lower proportion of apoptotic cells (3.7  $\pm$  0.8% vs 11.8  $\pm$  0.8%, KO vs WT respectively,  $P < 0.001$ ) and necrotic cells (5.7  $\pm$  2.0% vs 11.4  $\pm$  0.9% at 4h, KO vs WT respectively,  $P < 0.05$ ), not seen at 24h. Mice lacking 11 $\beta$ -HSD1 exhibit an exaggerated acute and chronic inflammatory response. Therefore, amplification of intracellular GC levels by 11 $\beta$ -HSD1 may be a critical anti-inflammatory mechanism to boost concentrations of endogenous active GC.

### OC10 Novartis Oncology Young Investigator Award

#### Progesterone signaling in human myometrium is mediated through two novel membrane G protein coupled receptors

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One of the hallmarks of parturition in most mammals is a dramatic drop in plasma levels of progesterone (P4), which promotes myometrial relaxation, prior to labor. In humans and some other primates however, the opposite occurs and placental P4 production increases with advancing pregnancy. The expression of nuclear progesterone receptor (PR)-responsive genes is decreased in the primate uterus at term, which suggests "functional" progesterone withdrawal involves repression of PR transcriptional activity. Recently, a novel cDNA was discovered in spotted seatrout ovaries that has all the characteristics of a progesterone membrane receptor (mPR) and is structurally unrelated to nuclear steroid receptors, but instead has features typical of G-protein coupled receptors (GPCRs). We hypothesise therefore that presence of these receptors in the human myometrium might further influence events leading to "functional" progesterone withdrawal. The study was approved by the Local Research Ethics Committee and all patients involved gave their informed consent.

We report the presence of two novel functional membrane progesterone receptors (mPRs), mPR $\alpha$ , and mPR $\beta$ , in human myometrium that are

differentially modulated during labor and by steroids *in vitro*. The mPRs are coupled to inhibitory G-proteins, resulting in a decline in cAMP levels and increased phosphorylation of myosin light chain, both of which facilitate myometrial contraction. Activation of mPRs leads to transactivation of PR-B, the first evidence for cross-talk between membrane and nuclear PRs. Progesterone activation of the mPRs leads also to a decrease of the steroidal co-activator SRC2. Our data indicate the presence of a novel signalling pathway mediated by mPRs that may result in functional progesterone withdrawal, shifting the balance from a quiescent state to one of contraction.

### OC11

#### The adrenal X-zone is involved in progesterone inactivation

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20 $\alpha$ -hydroxysteroid dehydrogenase (20 $\alpha$ HSD) has been initially characterized as a progesterone metabolizing enzyme of the ovary mandatory for the final reduction of progesterone blood levels before parturition. As the exact zonal distribution and regulation of adrenal 20 $\alpha$ HSD has not been defined, adrenal 20 $\alpha$ HSD expression and activity was determined by western blotting, immunohistochemistry and enzymatic assays in wild type BALB/c mice of both genders at different time points and following various hormonal treatments. In addition, to explore possible direct effects of 20 $\alpha$ HSD expression on adrenal function, mice with targeted deletion of 20 $\alpha$ HSD were studied for effects on adrenal morphology. Age related enzymatic activity and protein expression showed a clear gender difference with peak activity in males at 3 weeks and loss of activity thereafter but retained activity in virgin female mice. Interestingly, this time course of adrenal 20 $\alpha$ HSD enzymatic activity is reminiscent of the growth kinetics of the adrenal X-zone. In fact, immunohistochemical staining confirmed X-zone restricted expression of 20 $\alpha$ HSD. Accordingly, induction of X-zone regression in female mice by first pregnancy or testosterone treatment was accompanied by a rapid drop of adrenal 20 $\alpha$ HSD expression and activity. In contrast, induction of pseudo-pregnancy did not affect adrenal 20 $\alpha$ HSD enzyme activity. Moreover, gonadectomy in post-pubertal male mice which is known to induce growth of a secondary X-zone results in the restoration of adrenal 20 $\alpha$ HSD enzyme activity. Adrenal glands from 20 $\alpha$ HSD knock out animals at various time points displayed normal timing of X-zone growth and regression. Taking together, these findings indicate that regulation of 20 $\alpha$ HSD activity differs between the ovary and the adrenal cortex. Moreover, although adrenal 20 $\alpha$ HSD expression is restricted to the X-zone, adrenal 20 $\alpha$ HSD expression is not required for X-zone proliferation and regression. Ongoing *in vitro* experiments aim at the further elucidation of factors involved in 20 $\alpha$ HSD regulation through promoter activation vs. regulation through growth and apoptosis of 20 $\alpha$ HSD expressing X-zone cells.

### OC12

#### Expression profiling of genes in the testis of rhesus macaques during development and aging

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Men show an age-associated decline in 24-hour circulating testosterone levels, but the reason for this decline is unclear. To shed light on the possible cause we first established that male rhesus monkeys undergo a similar decline in testosterone with age. Testosterone from young adult (~10 years) and old (~26 years) unanaesthetized males was measured by RIA from blood samples collected remotely through a vascular catheter, every 30 minutes for 24 hours. In both young and old animals, plasma testosterone showed a robust circadian pattern, with a peak occurring at night and a nadir occurring in the middle of the day. As expected, the peak, nadir, and overall mean testosterone levels were all significantly lower ( $P < 0.001$ ) in the old animals than in the young animals. Next, to help elucidate a possible mechanism for this age-associated decline, we extracted RNA from testicular parenchyma of three immature (~2 years), three young adult (~6 years) and three old (~25 years) animals, and subjected the samples to

GeneChip microarray analysis (Affymetrix HG-U133A). The gene encoding 17- $\beta$  hydroxysteroid dehydrogenase 3 (17- $\beta$  HSD) showed a significant ( $P < 0.05$ ) developmental increase, suggesting that this enzyme may play a key role in activating testosterone biosynthesis during puberty. In contrast, the gene encoding steroidogenic acute regulatory protein (STAR), a key regulatory enzyme for testosterone biosynthesis, showed a significant ( $P < 0.05$ ) age-related decrease, suggesting that it may contribute to the age-associated decline in circulating testosterone. Interestingly, the microarray analysis also revealed the expression of the following clock-mechanism genes in the testis: Clock, Bmal1, Per1, Per2, Cry1, Rev-Erba, and CK1e and showed that CK1, declined significantly ( $P < 0.05$ ) during old age. Taken together, these data emphasize the circadian pattern of testosterone secretion in a nonhuman primate species, and suggest that transcriptional changes within the testis may contribute to the age-associated attenuation of this endocrine rhythm.

## OC13

### Anti-Müllerian hormone (AMH) production by granulosa cells from anovulatory PCO is 100 times higher than from normal ovaries, but is inhibited by metformin

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AMH causes regression of Müllerian ducts during male fetal development. Recently, AMH was detected in the adult ovary, principally in granulosa cells (GCs). Studies have demonstrated a 1.5–5 fold increase in serum AMH in women with PCOS compared to normal ovulatory women. The AMH rise was assumed to be secondary to increased numbers of follicles. Interestingly, the insulin sensitiser metformin, which is in widespread use in PCOS, caused a significant reduction in serum AMH levels after protracted treatment. The aim of this study was to compare AMH production per cell between normal and PCO and to investigate effects of metformin. AMH levels in medium conditioned by GCs from normal, ovulatory (ovPCO) and anovulatory PCO (anovPCO) cultured for 48 hrs at  $5 \times 10^4$  cells/well were measured by ELISA (DSLabs). In a parallel study, cells were incubated +/- metformin ( $10^{-7}$ M) for 48 hrs and the AMH protein and mRNA in cell lysates assessed by ELISA (DSL) and qPCR. AMH levels in GCs from anovPCO were significantly increased compared to normal and ovPCO ( $P < 0.001$ ): mean anovPCO 27.4 ng/ml ( $n = 6$ , range, 17.2–42.7), ovPCO 1.4 ( $n = 12$ , range, 0.025–7.6) and normal ovaries 0.29 ( $n = 14$ , range, 0.025–1.7). FSH (5 ng/ml) significantly reduced AMH levels in GCs from PCO ( $P = 0.008$ ) ( $n = 8$ ), but not from normal ovaries. Both mRNA and protein levels of AMH from granulosa-luteal cell lysates were significantly reduced by metformin ( $n = 4$ ,  $P < 0.05$ ).

In summary, AMH production per cell is greatly increased in anovPCO and was reduced by FSH. In addition, metformin inhibited both mRNA and protein in cell extracts.

In conclusion, as AMH-knockout mice have been shown to have an increased sensitivity to FSH, this data has important implications for a role for AMH in the anovulation associated with PCOS. The ability of metformin to reduce AMH production indicates a further possible mechanism of action for this drug in PCOS.

## OC14

### Endometrial intracrinology: Expression of 3 $\beta$ -hydroxysteroid dehydrogenase (3 $\beta$ HSD), and 17 $\beta$ -hydroxysteroid dehydrogenase type 5 (17 $\beta$ HSD-5)

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#### Introduction

3 $\beta$ HSD-5 catalyses the formation of  $\Delta^4$ -3-ketosteroids including progesterone and androgens. Levonorgestrel (LNG) is an androgenic progestogen used in the LNG intra-uterine system (LNG-IUS). 17 $\beta$ HSD-5 is involved not only in androgen and oestrogen metabolism, but also has 3 $\beta$ -reductase activity.

#### Objective

To determine expression patterns of 3 $\beta$ HSD-1 and -2, and 17 $\beta$ HSD-5 in human endometrium across the normal menstrual cycle and in endometrium exposed to intrauterine delivery of LNG (LNG-IUS).

#### Methods

Local ethical approval and informed consent were obtained. Endometrial biopsies were collected with a pipelle-endometrial-sampling device or at

hysterectomy. RNA expression was studied by quantitative real-time PCR using specific and validated primers and probes for 3 $\beta$ HSD-1, 3 $\beta$ HSD-2 and 17 $\beta$ HSD-5. Protein expression and localisation were detected using antibodies against 3 $\beta$ HSD-1 (rabbit polyclonal, also detects 3 $\beta$ HSD-2) and 17 $\beta$ HSD-5 (mouse monoclonal).

#### Results

Neither 3 $\beta$ HSD-1 or -2 mRNA were detectable in normal endometrium. 3 $\beta$ HSD-1 transcript was expressed in endometrium exposed to intrauterine LNG. 3 $\beta$ HSD protein was observed at low levels in the glandular and surface epithelium of normal endometrium, and at greater levels in glands, stroma and surface epithelium endometrium exposed to LNG. 17 $\beta$ HSD-5 mRNA was expressed across the menstrual cycle, with levels greatest in the early secretory phase. Levels were low in endometrium exposed to LNG. 17 $\beta$ HSD-5 protein was expressed in the glandular and surface epithelium endometrium exposed to LNG, and strongly in endothelial cells of the endometrium throughout the menstrual cycle.

#### Summary

3 $\beta$ HSD-1 mRNA and protein expression is upregulated in endometrium exposed to exogenous LNG, potentially due to the androgenic effects of LNG. On the other hand an intracrine role for 17 $\beta$ HSD-5 is implicated in oestradiol synthesis and subsequent action in the endometrial vascular endothelial cells.

## OC15

### Role of the Hu proteins, HuR and HuD, and $\alpha$ CP1 in the regulation of androgen receptor expression and activity in prostate cancer cells

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The primary treatment for prostate cancer (Pca) involves androgen ablation, halting tumour growth through down-regulation of androgen-regulated proliferative genes.<sup>1</sup> Frequently Pca progresses to an androgen-independent state and untreatable disease. The Androgen receptor (AR) continues to be expressed in many of these tumours, often associated with androgen-independent activation of the AR signalling pathway. Thus, the aim of the study is to understand regulation of AR expression in these cells. MRNA decay plays a critical role in AR regulation in Pca cells.<sup>2</sup> We have identified a specific, UC-rich region in the 3' untranslated region (UTR) of the AR mRNA that binds, *in vitro* and *in vivo*, RNA-binding proteins (HuR, HuD and  $\alpha$ CP1), known to modulate mRNA turnover in other systems.<sup>3,4,5</sup> Mutations in this region abrogate binding of these proteins, and decrease luciferase reporter activity when inserted 3' of the luciferase gene. Significantly, HuD, usually restricted to neurons, is expressed in a range of primary Pca samples. We aimed to determine the role of HuR/D and  $\alpha$ CP1 in the regulation of AR expression and activity in Pca cells. We found that AR protein levels were significantly decreased in cells with levels of HuR reduced by RNAi treatment, and preliminary results indicate that knockdown of HuR reduces half-life of AR mRNA. In addition, alteration of HuR or HuD in LNCaP cells moderated cell proliferation. Taken together, these data implicate the Hu proteins, HuR and HuD, as novel AR mRNA-binding proteins that play an important role as regulators of AR expression and signalling in Pca cells. As such, they may represent potential therapeutic targets.

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## OC16

### 17 $\beta$ -estradiol regulates pituitary Anx1 expression and externalization *in vivo* and *in vitro*

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Annexin A1 (AnxA1) acts as a mediator of glucocorticoid (GC) actions in neuroendocrine systems. In the anterior pituitary Anx-1 is expressed mainly by folliculostellate (FS) cells and mediates the early-delayed feedback inhibition exerted by GCs on release of ACTH and other pituitary hormones. GC cause the externalization of AnxA1. The stress responsiveness of the female rat hypothalamo-pituitary-adrenal system varies with the estrous cycle, with increased CORT release in response to stress at proestrus (Viau 1991). Anterior pituitary Anx-1 levels vary with the estrous cycle in the rat (with a peak at estrus) and re positively regulated by a physiological doses of 17 $\beta$ -estradiol (Davies 2005).

To determine whether increased AnxA1 leads to increased AnxA1 action, anterior pituitary tissue from ovariectomised (low AnxA1) and 17 $\beta$ -estradiol-treated (high AnxA1) animals was exposed to forskolin and/or dexamethasone *in vitro*. The addition of 0.1  $\mu$ M dexamethasone caused a greater inhibition of the forskolin-stimulated ACTH release in tissue with a high AnxA1 content compared to tissue with a low AnxA1 content. The effect of 17 $\beta$ -estradiol on externalization of AnxA1 from FS cells was investigated using a mouse FS cell line, TtT/GF cells. TtT/GF cells externalize Anx1 in response to dexamethasone. 17 $\beta$ -estradiol pretreatment (180 nMol, 24h) increased the quantity of Anx1 externalized in response to 0.1  $\mu$ Mol dexamethasone. This effect of 17 $\beta$ -estradiol was abolished by the estrogen receptor inhibitor ICI182,780. Consistent with this, immunofluorescence studies showed both ER $\alpha$  and ER $\beta$  localized to the nucleus of TtT/GF cells.

These data suggest that the variation in anterior pituitary AnxA1 with the estrous cycle is due to the action of estradiol acting through nuclear estrogen receptors. The estradiol-stimulated increase in total pituitary AnxA1 is associated with an increased GF effect. Estradiol potentiation of GC-stimulated externalization of AnxA1 would also increase the GC effect. Together, these data are consistent with the hypothesis that cyclic variation in anterior pituitary AnxA1 is a component of the change in stress responsiveness during the estrous cycle.

## Clinical endocrinology

### OC17

#### Copeptin, a precursor of vasopressin, in critically ill patients – an observational study

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#### Objective

The response of the hypothalamo-pituitary-adrenal axis to stress is mediated mainly through corticotrophin-releasing hormone and vasopressin. Accordingly, vasopressin levels are increased in septic shock. However, measurement of vasopressin is difficult because of its instability and short half-life. Copeptin is a more stable peptide derived from the same precursor molecule. This study aims to evaluate copeptin levels and its prognostic value in a prospective observational study of 101 consecutive critically ill patients, as compared to 50 healthy controls.

#### Methods

Copeptin was measured in the serum of all patients with a newly developed sandwich immunoassay.

#### Results

On admission, 53 patients had sepsis, severe sepsis or septic shock and 48 had systemic inflammatory response syndrome (SIRS). Copeptin levels correlated with basal cortisol levels ( $r = 0.40$ ,  $P < 0.001$ ). Median (range) copeptin values on admission in pmol/l were in patients with SIRS 27.6 (2.3–297), with sepsis 50.0 (8.5–268), with severe sepsis 73.6 (15.3–317) and in patients with septic shock 171.5 (35.1–504), as compared to 5.0 (1.5–30.3) in healthy controls ( $P$  for all comparisons versus controls  $< 0.001$ ). On admission, circulating copeptin levels in patients with sepsis, severe sepsis or septic shock were higher in non-survivors (171.5, 46.5–504.0) as compared to survivors (86.8, 8.5–386.0,  $P = 0.01$ ). In a receiver operating curve (ROC) analysis for the survival of patients with sepsis, the AUC for copeptin was 0.75 (95%CI 0.64–0.82). In comparison, the AUC for CRP was 0.55 (0.440–0.62), for basal cortisol 0.60 (0.49–0.69,  $P = 0.08$ ) and for the APACHE II score (0.71, 0.61–0.80,  $P = 0.78$ ).

#### Conclusions

Copeptin levels are elevated in sepsis, correlate with stress-induced cortisol levels and might provide a useful new tool for an individual risk assessment of septic patients. The availability of a reliable assay for the measurement of vasopressin could also prove useful for the assessment of fluid status in various diseases.

### OC18

#### Endoscope-assisted pituitary surgery for functioning and non-functioning pituitary adenomas. The experience of the first 80 patients in a single centre

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#### Introduction

We present the outcome for the first 80 patients undergoing neuronavigation-guided, endoscopic transphenoidal surgery in our centre since August 2001. All operations were undertaken by the same neurosurgeon and endoscopic nasal surgeon.

#### Indications for treatment

Indications for treatment may be divided into: Functioning microadenomas, with the aim of surgical cure while protecting residual pituitary function. Functioning macroadenomas, where reduction of hormonal secretion is sought and protection of surrounding structures particularly the optic chiasm is important. Functionless adenomas imposing a risk to surrounding structures.

Rapid expansion of tumour bulk, for example due to pituitary apoplexy, to preserve surrounding structures. Other parasellar tumours including craniopharyngiomas, Rathke cleft cysts and arachnoid cysts.

#### Outcomes

This method of surgery was successful in decompressing pituitary mass lesions with suprasellar extension. In the treatment of functioning tumours, of 14 patients with Cushing's Disease (2 macroadenomas) 64% were cured or improved. Of 11 patients with Acromegaly 55% achieved safe GH/IGF-I levels. The patient population is representative with the ratio of females to males being 1.12:1, average age 50.36 years (range 16 – 85).

#### Complications

Three patients suffered intraoperative bleeding controlled endoscopically and one patient died of bronchopneumonia within one month of surgery. All occurred within the initial 14 months of experience. 10 patients required further surgery for tumour removal/CSF leak repair with half occurring within the first 14 months of experience. 17 patients suffered CSF leaks (9 transient).

#### Conclusions

In skilled hands this method of surgery provides a complementary alternative to conventional transphenoidal microscopic surgery. A learning trend was evident in relation to complication rate.

### OC19

#### Disease remission in long term treated acromegalic patients after somatostatin analog withdrawal

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Long acting somatostatin analogs (SSTa) induce both GH/IGF-I reduction and pituitary tumor volume shrinkage in the majority of acromegalic patients. It is not known if SSTa might also definitively cure the acromegalic disease in at least some patients. Aim of the present study was to evaluate this possibility in acromegalic patients very well responsive to chronic treatment. Thirteen acromegalic patients (4M&9F), 5 newly diagnosed and 8 previously operated but none irradiated, treated with SSTa (6 with octreotide-LAR, 4 with lanreotide-SR and 3 with lanreotide-Autogel) for a median period of 51 months (range: 18–156 months) were studied. All these patients were highly responsive to the treatment on the basis of hormonal (GH  $< 2.5 \mu$ g/l and normal IGF-I levels) and tumor volume response (reduction or absence of tumor mass). None of them underwent radiotherapy during SSTa treatment. In all patients, basal GH, post-glucose GH nadir levels, IGF-I concentrations and symptoms score were studied after short-term drug withdrawal (median: 3 months). During this period, 4 patients relapsed, 9 maintained GH  $< 2.5 \mu$ g/l (mean:  $1.8 \pm 0.6$  vs pre-therapy  $6.0 \pm 3.6 \mu$ g/l,  $P < 0.05$ ), 5 of them with postglucose GH  $< 1 \mu$ g/l. Three of these patients, all previously operated, also had normal IGF-I (mean:  $+0.4 \pm 0.3$  vs pre-therapy  $+4.4 \pm 2.2$  SDS,  $P < 0.05$ ) and absence of acromegaly-related symptoms. These 3 patients had lower IGF-I levels during therapy in respect to the other ones ( $-1.1 \pm 0.8$  vs  $+0.4 \pm 1.0$  SDS,  $P < 0.05$ ). The "cured" patients are currently in drug suspension and still had postglucose GH  $< 1 \mu$ g/l and normal IGF-I levels after 6 ( $n = 2$ ) and 30 months ( $n = 1$ ) from SSTa withdrawal. In conclusion, SSTa therapy seems to be able to induce at least a 6-month disease remission in some very well controlled acromegalic patients, a figure that increases if only postoperative



patients are taken into account and suggests the utility of a periodical drug suspension in these subjects. Moreover, IGF-I appeared the main predictive parameter of hormonal control even after SSTA withdrawal.

## OC20

### A European prospective real-life observational study of Quality of Life in patients with acromegaly

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The primary aim of this study, the largest European, single evaluation, observational trial to date, was to evaluate the impact of acromegaly on health-related quality of life (HRQoL) in patients with biochemically documented active disease, receiving Sandostatin<sup>®</sup> LAR<sup>®</sup> at a dose prescribed by their physician for at least 3 months. Secondary objectives were to investigate the relationships between HRQoL and subpopulations based on exploratory variables [age, gender, education, growth hormone (GH) and insulin-like growth factor (IGF-1)] and to compare the results of the Acromegaly Quality-of-Life questionnaire (ACROQOL) and SF-36. The study was approved by Health Authorities and/or local Ethics Committees, according to local regulations. Patients were invited to participate in the study when they attended a scheduled visit. GH and IGF-1 levels were to be measured within two months of completion of the questionnaires.

A total of 817 patients (55% female, mean age 51 ± 14 years) were recruited in England, France, Germany, Greece, Italy, Portugal, Spain and Turkey. Patients had a diagnosis of acromegaly for an average of 8 years, and had been treated with Sandostatin<sup>®</sup> LAR<sup>®</sup> for an average of 3.4 years (range 0.3 – 18 years) at a median dose of 30 mg (range 10–60 mg). Basal GH and IGF-1 mean levels were 4.2 and 345 ng/ml respectively. Both the ACROQOL and SF-36 recorded poorer HRQoL with advancing age and increasing number of comorbidities. The correlation between the ACROQOL and the SF-36 was good ( $P < 0.001$ ) and overall results suggest that ACROQOL may be more sensitive to the psychological impact of acromegaly than to the physical impact. While some cultural differences were found across the SF-36 domains, the results with the ACROQOL were consistent across all countries, confirming the ACROQOL to be a valid and sensitive tool for measuring the impact of acromegaly on HRQoL.

## OC21

### Adjuvant radiation therapy of the tumor bed prevents local recurrences in adrenocortical carcinoma

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#### Objective

Local tumor recurrence is common in adrenocortical carcinoma (ACC) and the most frequent cause for re-operation. Therefore, a concept for an adjuvant treatment is urgently needed. The role of radiotherapy in ACC has not been well defined and many authors have considered radiotherapy as ineffective. However, two studies with a small number of patients ( $n = 3$  and  $n = 4$ , resp.) have suggested adjuvant radiation as an effective treatment to prevent recurrence in ACC (1,2).

#### Methods

We analyzed all patients from the German ACC Registry who have been treated by tumor bed radiation. Only patients treated with 40–54 Gy in an adjuvant setting (no macroscopic evidence for residual tumor after initial surgery) were included. 16 patients (stage I: 1, stage II: 9, stage III: 6) with median tumor diameter of 12.5 ± 4.2 cm were matched with 16 patients for stage, R-status, adjuvant mitotane treatment, tumor size, and age.

#### Results

Local recurrence occurred in 2/16 patients in the radiation group and in 9/16 control patients ( $P < 0.05$ ). The probability to be free of local recurrence 5 years after surgery differed significantly (82% vs. 39%;  $P < 0.05$ ). However, in this small series overall survival was not significantly different between both groups (5-year survival: 46% in radiation group vs. 64% in controls;  $P = 0.6$ ). Acute adverse effects related to radiation therapy were mostly mild (gastrointestinal grade CTC I + II, erythema CTC I, and fatigue CTC II, wound healing CTC II in 10, 5, 4, and 1 patients, respectively). 1 patient developed a partial Budd-Chiari syndrome and 1 patient impaired renal function CTC I during follow up probably related to radiotherapy.

#### Conclusion

These data from the largest series of ACC patients treated with adjuvant tumor bed radiation suggest that radiation therapy is effective in reducing the high rate of local recurrence in ACC. However, no impact on long-term survival was demonstrated in this small patient sample.

1. King *et al.* Cancer 1979.
2. Markoe *et al.* Am J Clin Oncol 1991.

## OC22

### Detection of pheochromocytoma: The emerging role of plasma metanephrines

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Pheochromocytomas (PCC) are rare neuroendocrine tumours of chromaffin cells that are characterised by autonomous production of catecholamines. Fundamental to the detection and diagnosis is the biochemical confirmation of excessive catecholamine production by the measurement of plasma or urinary catecholamines and metanephrines. Recently, plasma metanephrines have been shown to provide a high diagnostic sensitivity for the detection of catecholamine secreting tumours and unlike plasma catecholamines do not appear to require strict attention to standardised sampling protocols. We have measured plasma metanephrines together with paired urinary catecholamines and fractionated metanephrines in 74 patients with a strong, clinical suspicion of PCC and 20 subjects in whom PCC was subsequently confirmed histologically (11 with sporadic tumours, 3 with familial adrenal tumours, 4 with functional paragangliomas, 2 with metastatic tumour). Urinary catecholamines/metadrenalines were measured by HPLC whilst plasma metadrenalines were determined by immunoassay. Results were compared to groups of healthy normotensive subjects, patients with essential and secondary hypertension, patients with histologically verified non-functioning adrenal masses and with a variety of other endocrine disorders. No statistical differences in plasma metanephrines levels were observed between duplicate blood samples collected by venipuncture at time 0 or after 10 minutes ( $P > 0.53$ ) in any patient. Plasma normetanephrine was elevated in all patients with sporadic tumours but was normal in a patient with MEN2A in contrast to an elevated plasma metanephrine level. Urinary levels were consistently normal in this patient. All 4 patients with paragangliomas displayed a noradrenergic secretory phenotype while the largest values for plasma normetanephrines was observed in the 2 patients with metastatic disease. We conclude that finding normal plasma metadrenalines in patients with equivocal urinary catecholamines effectively rules out a PCC/functional paraganglioma and is the test of choice in detecting these tumours in high-risk patients with familial syndromes.

## OC23

### Improved Prognosis in Midgut Carcinoid patients by treating raised circulating Neurokinin A (NKA)

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Midgut Carcinoid disease (MGC) is uncommon, occurring in approximately 1.4 per 100,000 of the population per year. MGC has an unpredictable disease progression varying from rapid and aggressive to slow and indolent. For this reason it is not appropriate to treat all patients according to the same schedule. As some treatments are not without risk, it is important to identify those patients selected for these options at an appropriate stage of disease. In a retrospective study ( $N = 150$ ) we have shown previously that

circulating NKA is the best independent indicator of poor prognosis in MGC. Patients presenting with NKA > 50 pmol/l showed a 3 year survival of 10% in contrast to 65% for those presenting with NKA < 50 pmol/l. In addition the data from this study has shown that the most recent NKA is the most accurate prognostic indicator.

In the majority of MGC patients circulating NKA concentrations may be reduced by use of somatostatin analogue therapy or by intervention with more aggressive treatments.

We now report preliminary results from a prospective study of patients presenting at the regional centre for neuroendocrine tumours in Northern Ireland, where we have treated consecutive, newly diagnosed MGC patients. All have been treated to address disease bulk, symptom control and to maintain NKA concentrations < 50 pmol/l. Thirty patients have been included in this study to date. Mean circulating NKA was 94.5 pmol/l at presentation and was reduced to 28.5 pmol/l at six months. Six of thirty patients (20%) required additional treatments to control a rising NKA concentration within one year of diagnosis.

Preliminary results indicate that MGC patients have a longer survival than expected from the previous study. Patients presenting with high NKA (> 50 pmol/l) who are treated with a more aggressive regime to maintain circulating NKA, < 50 pmol/l survive significantly longer than previously reported in our retrospective study.

## OC24

### Low testosterone and atherogenic lipid profile in aging men

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#### Introduction

Aging men with low testosterone concentration may be at increased risk for cardiovascular disease. In some studies, but not all, low serum testosterone concentration has been associated with an atherogenic serum lipid profile.

#### Methods

Year 2000 all the men aged 40-70 years ( $N = 28,622$ ) in the city of Turku, Finland, received a questionnaire on andropausal symptoms. Those men who reported high andropausal symptoms were tested for serum testosterone, luteinizing hormone and lipids (triglycerides, total-, LDL- and HDL cholesterol).

#### Results

A total of 15,991 men answered to the questionnaire. From these men 2,700 had high andropausal symptom score and 1,670 underwent the laboratory tests. The mean age of the men with laboratory data was  $55 \pm 8$  years, serum total cholesterol was  $6.2 \pm 1.2$  mmol/l and serum testosterone  $15.3 \pm 5.4$  nmol/l. In bivariate analysis, testosterone correlated directly with HDL-cholesterol ( $r = 0.24$ ,  $P < 0.0001$ ), and inversely with triglycerides ( $r = -0.29$ ,  $P < 0.0001$ ), body mass index (BMI,  $r = -0.34$ ,  $P < 0.0001$ ) and total cholesterol ( $r = -0.05$ ,  $P = 0.016$ ). In multivariate analysis, the significant correlates of triglycerides included testosterone, BMI and age ( $P < 0.0001$  in all). The correlates of HDL cholesterol included testosterone ( $P = 0.015$ ), smoking ( $P = 0.02$ ) and alcohol consumption ( $P = 0.003$ ).

#### Conclusion

We conclude that in aging men, low serum testosterone concentration is associated with a potentially atherogenic lipid profile, characterized by low HDL cholesterol and high triglycerides concentrations. These relations are independent of age and BMI status.

## Diabetes and metabolism

### OC25

#### Cannabinoids increase AMP-activated protein kinase (AMPK) enzyme activity in the hypothalamus and heart via different signalling pathways – studies in CB1 knockout animals

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We have recently reported that cannabinoids can stimulate hypothalamic and heart AMPK activity and can inhibit liver and adipose tissue AMPK activity in rats (Kola *et al.*, JBC, 2005). These data are in accordance with

the known orexigenic and adipogenic cannabinoid effects and also with their beneficial effects on the ischaemic heart. We have studied the effects of cannabinoids on AMPK activity in tissues from wild type (WT) and CB1 knockout (KO) mice to see if the CB1 receptor is involved in these effects. Mice were injected ip. with  $10 \mu\text{g}$   $\Delta^9$ -tetrahydrocannabinol (THC) and their tissues collected 1 hour later. Changes in AMPK activity were detected using a kinase assay and immunoblotting for pAMPK.

THC significantly increased AMPK activity in WT animals (hypothalamus  $183 \pm 41\%$ , heart:  $218 \pm 34\%$  of control). These responses confirm our previous data in rats. In the CB1-KO animals, hypothalamic AMPK activity was not modulated by THC injection ( $85 \pm 16\%$ ) suggesting that these central effects are mediated by the CB1 receptor; however, we observed a significant increase in myocardial AMPK activity ( $303 \pm 64\%$ ) indicating that the heart effect is not mediated by the CB1 receptor.

#### In conclusion

(1) the stimulatory effect of cannabinoids on AMPK activity in the hypothalamus, leading to increased food intake, is mediated by the CB1 receptor, and

(2) the stimulatory effect of cannabinoids on AMPK activity in the heart, which could lead to the well-described improved tissue response after ischaemia, is not mediated via the CB1 receptor. We suggest that AMPK activation plays an important role in cannabinoid effects both in the hypothalamus and in the myocardium. These data provide support for the existence of the previously suggested nonCB1-nonCB2 receptor in the heart and could lead to specifically designed cardio-specific therapies not affecting CB1 receptors and therefore devoid of central psychogenic and orexigenic effects.

## OC26

### Neuropeptide S is a novel peptide which potently stimulates the hypothalamo-pituitary-adrenal axis and inhibits food intake

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Neuropeptide S (NPS) is a recently discovered peptide shown to be involved in the modulation of arousal and fear responses. It has also been shown that lateral ventricle administration of NPS causes a significant decrease in food intake. Neuropeptides involved in the modulation of arousal have been shown to be involved in the regulation of the hypothalamo-pituitary adrenal (HPA) axis and food intake.

#### Objective

To examine the effect of NPS on the regulation of the HPA axis, behaviour and food intake.

#### Methods

ICV cannulated unanaesthetised male rats were injected with NPS and the effects on behaviour and the HPA axis were examined. We then studied the effect of paraventricular nucleus (PVN) administration of NPS on the regulation of the HPA axis, food intake and behaviour. We also examined the effect of NPS on the release of CRH and AVP from hypothalamic explants.

#### Results

ICV administration of NPS significantly increased plasma ACTH and corticosterone 10 and 40 minutes post injection respectively. A single ICV injection of NPS caused a significant increase in rearing activity as well as ambulatory movement for up to 45 minutes post injection. There was a significant increase in plasma ACTH and corticosterone following a single NPS PVN injection. Incubation of hypothalamic explants with increasing concentrations of NPS caused a significant increase in CRH and AVP release. In addition, PVN administration of NPS dose-dependently inhibited food intake in the first hour post-injection although no effect on food intake was seen after this time point. PVN administration of NPS caused a significant increase in rearing activity.

#### Conclusion

This data demonstrates that NPS potently stimulates the HPA axis and dose dependently inhibits food intake following PVN administration.

## OC27

### A selective increase in adipose 11 $\beta$ -HSD1 expression in corticosterone-treated *Pomc* null mice is associated with development of metabolic syndrome

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Inactivating mutations of the POMC gene in humans and mice result in a complex phenotype of hyperphagia, obesity and glucocorticoid (GC) deficiency, but without many features of metabolic syndrome. We have previously shown that replacement of GC in *Pomc*<sup>-/-</sup> mice exacerbates their hyperphagia, increases fat mass and elevates plasma leptin and insulin. We have now examined key determinants of glucocorticoid action, including glucocorticoid receptor (GR) and the glucocorticoid amplifying enzyme, 11 $\beta$ -hydroxysteroid dehydrogenase type 1 (11HSD1), in *Pomc*<sup>-/-</sup> mice given corticosterone supplemented water (25  $\mu$ g/ml) (CORT) for 10d. CORT significantly increased adipose 11HSD1 mRNA levels in all fat depots in both genotypes without affecting hepatic 11HSD1 mRNA levels. The magnitude of the increase was greater in *Pomc*<sup>-/-</sup> (untreated vs CORT, 0.07  $\pm$  0.02 vs 0.51  $\pm$  0.15,  $P < 0.001$  compared to 0.15  $\pm$  0.07 vs 0.31  $\pm$  0.17  $P < 0.001$  in wild type (WT) mice). GR mRNA levels were unaffected by CORT treatment, but were higher in retroperitoneal fat of untreated *Pomc*<sup>-/-</sup> mice compared to WT (WT vs *Pomc*<sup>-/-</sup> 0.714  $\pm$  0.05 vs 0.882  $\pm$  0.044,  $P = 0.05$ ). Lipoprotein lipase mRNA levels were increased in all fat depots in CORT treated *Pomc*<sup>-/-</sup> but were unaffected in WT mice. Despite a significant difference in weight, blood pressure in untreated *Pomc*<sup>-/-</sup> mice was similar to WT. However, CORT in *Pomc*<sup>-/-</sup> mice increased BP (untreated vs CORT, 95.8  $\pm$  5.1 mmHg vs 160.5  $\pm$  4.1 mmHg, respectively,  $P < 0.001$ ) and hepatic angiotensinogen mRNA levels (0.595  $\pm$  0.03 vs 0.76  $\pm$  0.03,  $P < 0.01$ ). *Pomc*<sup>-/-</sup> mice had reduced hepatic PEPCK mRNA levels (WT vs *Pomc*<sup>-/-</sup>, 0.30  $\pm$  0.03 vs 0.150  $\pm$  0.03,  $P < 0.01$ ) but following CORT, PEPCK increased (0.352  $\pm$  0.03,  $P < 0.05$ ) to exceed levels in CORT treated WT mice. These data give insights into why *Pomc*<sup>-/-</sup> mice appear hypersensitive to the adverse metabolic effects of glucocorticoids and suggest that adipose-specific amplification of GC by increased 11HSD1 expression may contribute to the adverse metabolic effects of melanocortin deficiency. They may be of relevance to differential susceptibility to metabolic syndrome in common obesity.

## OC28

### 11 $\beta$ -Hydroxysteroid dehydrogenase type 1 oxo-reductase activity is increased in patients with alcoholic chronic liver disease: the key to the phenotype of the alcoholic pseudo-Cushing's state?

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The pseudo-Cushing's syndrome that accompanies both acute alcohol ingestion and alcohol withdrawal is an important differential diagnosis of hypercortisolism that is poorly understood. Two isozymes of 11 $\beta$ -hydroxysteroid dehydrogenase (11 $\beta$ -HSD) interconvert hormonally active cortisol (F) and inactive cortisone (E). Previously we have shown higher urinary F:E metabolite ratios (a reflection of total body 11 $\beta$ HSD activity) in patients with alcoholic liver disease (ALD) compared to patients with chronic liver disease (CLD) of other etiologies, suggesting that the phenotype of alcoholic pseudo-Cushing's may relate to altered hepatic metabolism of F. We have further evaluated hepatic glucocorticoid metabolism by measuring F and E concentrations in the blood obtained simultaneously by selective venous sampling in the hepatic, portal, renal and peripheral veins. 20 patients with histologically confirmed ALD and 19 patients with CLD of other etiologies were admitted for transjugular liver biopsy or insertion of transjugular intrahepatic portosystemic shunt (TIPS) according to clinical indications. Serum F, and E was measured by in-house RIA in each vascular bed. There was a significant difference in the hepatic F gradient (mean  $\pm$  SEM) between groups, indicating increased F production in the liver in patients with ALD (34.5  $\pm$  21.7 nmol/l) compared to those with CLD ( $\pm$  18.5 nmol/l), ( $P < 0.05$ ). There was no significant difference in the renal E generation between groups (mean  $\pm$  SEM) (ALD, 14.3  $\pm$  3.9, CLD, 19.1  $\pm$  3.9), ( $P = 0.4$ ), eliminating differences in renal 11 $\beta$ HSD2 activity between groups. The hepatic vein F/E ratio was greater than the portal vein ratio ( $P < 0.05$ ) in six patients from both groups. The results indicate increased cortisol and F/E ratio in the hepatic vein in patients with ALD compared with patients with CLD, in keeping with increased hepatic cortisol generation via induction of 11 $\beta$ HSD1 oxo-reductase activity. The mechanism is unknown but might be explained on the basis of alcohol-induced changes in intracellular redox potential. Selective 11 $\beta$ -HSD1 inhibitors may offer a novel therapeutic approach to treat alcohol pseudo-Cushings.

## OC29

### A choline deficient diet in mice prevents high fat diet induced insulin resistance in spite of more severe fatty liver disease

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Fatty liver disease is strongly associated with insulin resistance. In order to elucidate the causality of this complex relationship, we studied insulin resistance in a rodent model of fatty liver disease – the choline deficient diet (CDD) – in which fatty liver occurs without generalised obesity. C57Bl/6 mice were fed a low fat (10% calories as fat, 'Lo') or isocaloric high fat diet (45% calories as fat, 'Hi') for 3 weeks; then half of the animals in each group continued either on standard choline supplementation ('CS') or on an identical diet deficient in choline ('CD') for a further 3 weeks. Insulin resistance was assessed by intraperitoneal glucose tolerance test after an overnight fast. Liver fat was quantified biochemically, and expression of hepatic enzymes involved in lipid metabolism was examined by real-time PCR. CDD induced fatty liver with Lo (liver triglycerides, CS 13.4  $\pm$  2.1, CD 31.6  $\pm$  6.3  $\mu$ mol/g,  $P = 0.03$ ) or Hi diet (triglycerides, CS 36.8  $\pm$  4.8, CD 59.5  $\pm$  2.1  $\mu$ mol/g;  $P = 0.006$ ). There was no change in body weight or fat depot size. Choline deficiency significantly improved measures of insulin resistance induced by a high fat diet, despite greater liver fat accumulation (Table 1). mRNA expression of the rate limiting enzyme of phosphatidylcholine synthesis (Pcyt1a) and of enzymes involved in free fatty acid esterification (Acs11, mGPAM, AGPAT1, DGAT2) was significantly increased. In contrast, the expressions of key enzymes or transcription factors involved in free fatty acid synthesis or oxidation was unaltered. These results suggest that liver fat accumulation per se does not cause insulin resistance. CDD, which increases liver triglyceride accumulation by enhancing free fatty acid esterification, improves insulin resistance, perhaps by shunting potentially toxic FFA towards innocuous storage triglyceride.

**Table 1** Measures of insulin resistance. Results were compared by two way ANOVA with post hoc Tukey test; a  $P < 0.01$  for Lo/CS vs Hi/CS, b  $P < 0.01$  for Hi/CS vs Hi/CD.

	Lo/CS	Lo/CD	Hi/CS	Hi/CD
Fasting glucose (mM)	6.1 $\pm$ 0.2 <sup>a</sup>	5.6 $\pm$ 0.3	7.5 $\pm$ 0.3 <sup>a</sup>	6.8 $\pm$ 0.2
Fasting insulin (pM)	26.9 $\pm$ 3.7 <sup>a</sup>	23.4 $\pm$ 1.8	337.6 $\pm$ 71.6 <sup>a,b</sup>	116.2 $\pm$ 17.4 <sup>b</sup>
QUICKI	0.9 $\pm$ 0.1 <sup>a</sup>	1.0 $\pm$ 0.1	0.5 $\pm$ 0.0 <sup>a</sup>	0.6 $\pm$ 0.0
AUCglucose (min.mM)	622.7 $\pm$ 33.6 <sup>a</sup>	603.0 $\pm$ 56	861.4 $\pm$ 26.8 <sup>a,b</sup>	691.4 $\pm$ 14.0 <sup>b</sup>

## OC30

### The release of the adipocytokine visfatin is regulated by glucose and insulin

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#### Background/aim

The novel insulin-mimetic adipocytokine visfatin has been linked to the metabolic syndrome but how the release of the hormone itself is regulated is not characterized yet. The aim of this study was to investigate the effect of different glucose and insulin concentrations on visfatin plasma levels in humans, and to study the involved pathways *in vitro* in human adipocytes.

#### Methods

Randomized, double-blind, placebo-controlled crossover study. Three different study days were carried out in nine healthy male subjects (age 26  $\pm$  6 years). Subjects gave informed written consent, and the protocol was approved by the local ethical committee. On each day, systemic glucose concentrations of 5.0, 8.3, and 11.1 mmol/L were attained by stepwise increasing intravenous infusions of glucose, representing fasting and postprandial conditions. Visfatin plasma concentrations were studied during concomitant exogenous hyperinsulinemia, inhibition of endogenous insulin production by somatostatin infusion, and placebo time control, respectively. Additionally, human adipocytes were cultured to study visfatin release *in vitro*.



## Results

Glucose concentrations of 8.3 mmol/l and 11.1 mmol/l increased circulating visfatin from baseline concentrations of  $0.5 \pm 0.0$  ng/ml to  $0.9 \pm 0.1$  and  $2.1 \pm 0.3$  ng/ml, respectively ( $P < 0.01$ ). Glucose-induced elevation of visfatin was prevented by co-infusion of insulin or somatostatin ( $P < 0.05$ ). Visfatin release from cultured adipocytes was glucose concentration- and time-dependent and involved phosphatidylinositol-(PI)3-kinase and protein kinase B (AKT) pathways.

## Conclusions

Circulating visfatin concentrations are increased by hyperglycaemia. This effect is suppressed by exogenous hyperinsulinemia or somatostatin infusion. Thus, glucose seems to regulate visfatin plasma concentrations. Glucose also regulates visfatin release from cultured human adipocytes; glucose signaling in adipocytes involves the PI3/AKT pathway.

## OC31

### Comparative analysis of the effects of dehydroepiandrosterone (DHEA) on white and brown pre-adipocyte proliferation/differentiation

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Dehydroepiandrosterone (DHEA) is an adrenal sex steroid whose levels decline during normal aging. Epidemiological studies demonstrate inverse correlation between circulating DHEA-sulphate and body fat mass while DHEA administration in elderly subjects reduces visceral and subcutaneous fat accumulation. Although previous studies have shown that some effects may be mediated via DHEA-induced inhibition of white pre-adipocyte proliferation, mechanisms remain unknown. Furthermore, comparative analyses with brown pre-adipocytes, now recognised to have important thermogenic properties in adipose tissue, are lacking.

We aimed to compare the effects of DHEA on white and brown pre-adipocyte proliferation/differentiation.

3T3-L1 (murine white pre-adipocyte) and PAZ6 (human brown pre-adipocyte) cells were exposed to increasing concentrations of DHEA and cell numbers determined using Coulter counting. Cell cycle analysis was performed using flow Cytometry. Spontaneous and PPAR $\gamma$ -induced pre-adipocyte differentiation was determined via colourimetric measurement of oil red O accumulation.

DHEA caused a concentration-dependent inhibition of 3T3-L1 and PAZ6 proliferation at 24 (1  $\mu$ M DHEA:  $71.55 \pm 1.79\%$  of controls (3T3-L1)  $P < 0.001$ ;  $84.4 \pm 4.7\%$  (PAZ6) non-significant) and 48 hours (1  $\mu$ M DHEA:  $29.3 \pm 1.97\%$  (3T3-L1)  $P < 0.001$ ;  $50.4 \pm 4.2\%$  (PAZ6)  $P < 0.001$ ). Cell cycle analysis demonstrated that inhibition was not mediated by increased apoptosis but was accompanied by an increase in the proportion of cells accumulating in G $_2$ /M. (1  $\mu$ M DHEA for 48 hours:  $18.2 \pm 0.07\%$  (3T3L1),  $14.4 \pm 1.1\%$  (control)  $P < 0.001$ ;  $34.8 \pm 0.76\%$  (PAZ6),  $32.5 \pm 1.42\%$  (control)  $P = 0.0038$ ). In contrast, our results suggest DHEA stimulates spontaneous differentiation in PAZ6 but not 3T3-L1 cells. DHEA inhibits white and brown pre-adipocyte cell proliferation by either blocking cell division or causing cell cycle arrest. It appears to stimulate differentiation of brown adipose cells. These results are potentially important in disorders of adipose tissue excess.

## OC32

### The *in vitro* effects of resistin on the innate immune signalling pathway in isolated human subcutaneous adipocytes

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Obesity-associated inflammation is a major contributory factor in the pathogenesis of type 2 diabetes mellitus (T2DM). However, the mechanisms underlying the progression of T2DM are yet to be fully elucidated. The adipokine, resistin, has recently been implicated as a pro-inflammatory cytokine in obesity-related T2DM. Therefore, the aim of this study was to characterise the role of resistin in the innate immune inflammatory pathway within isolated human abdominal subcutaneous (Abd Sc) adipocytes. In particular, we examined the acute *in vitro* effects of human recombinant (h-r) resistin on different components of the NF- $\kappa$ B and JNK signalling pathways. For this study, isolated human Abd Sc adipocytes were treated for 14 hours with increasing concentrations of h-r resistin (10–50 ng). Innate immunity intracellular protein expression, in response to h-r resistin, was

examined by Western blotting. Results demonstrated that h-r resistin upregulated components of the NF- $\kappa$ B pathway. Protein expression of MyD88 ( $n = 4$ ,  $P < 0.01$ ), I $\kappa$ B $\alpha$  ( $n = 4$ ,  $P < 0.001$ ) and NF- $\kappa$ B ( $n = 4$ ,  $P < 0.05$ ) was increased in response to h-r resistin. Similarly, h-r resistin upregulated central mediators of the insulin signalling pathway; phosphospecific JNK1 and JNK2 (JNK-1:  $n = 6$ ,  $P < 0.05$ ; JNK-2:  $n = 6$ ,  $P < 0.001$ ) were both increased in response to h-r resistin treatment. Furthermore, when examining the IKK complex, the central activator of I $\kappa$ B $\alpha$  in the NF- $\kappa$ B pathway, IKK $\alpha$  was upregulated in response to h-r resistin ( $n = 6$ ,  $P < 0.01$ ). Taken together, these findings suggest that resistin could participate in more than one mechanism for the stimulation of pro-inflammatory cytokine release in human Abd Sc adipocytes; potentially via the integration of NF- $\kappa$ B and JNK intracellular signalling pathways. This study further emphasises crosstalk between insulin signalling and inflammatory pathways, in addition to confirming adipose tissue as an important site for the progression of sub-clinical inflammation and insulin resistance.

## Neuroendocrinology and neoplasia

### OC33

#### Effect of overexpression of GH secretagogue receptor (GHSR) in the pituitary of transgenic mice

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The expression of GHSR, an endogenous Ghrelin receptor, has been reported in pituitary, but the levels of expression of this receptor are low and the role of the GHSR in the normal physiology to directly affect GH or Prolactin release in pituitary remains unclear. We used a line of GH-GHSR transgenic mice to analyse the effects of hGHSR over-expression in the pituitary GH cells. We also crossed these mice with GH-GFP transgenic mice with fluorescent somatotrophs.

#### Objectives

(1) Breeding between GH-hGHS-R and GH-eGFP mice, to obtain double transgenic GH-GFPx hGHS-R mice. We obtained four different groups: wild type, transgenic hGH-hGHS-R, transgenic mice GH -eGFP and double transgenic GH-eGFPxhGHS-R mice. (2) Colocalization studies. (3) Physiological studies.

#### Methods

PCR: genotyping. Radio Immuno assay for GH and prolactin pituitary contents in the transgenic mice. Immunocytochemistry: to study the colocalization between GH, GHSR and prolactin

#### Results

Overexpression of GHSR expression in the pituitary did not affected body weight but the GH pituitary content in transgenic mice was lower than in wild type mice. By immunohistochemistry we found that only a few somatotroph cells detectably coexpressed GH and GHSR, and this was similar in males and females. Despite alterations in GH, pituitary prolactin content was not affected by the transgenes. In wild-type mice we could show: Colocalization between GHSR and prolactin, both in pituitary sections and in isolated pituitary cells. This suggests that the GHSR may have a role in regulating lactotroph function directly, consistent with earlier findings in dwarf rats.

#### Conclusions

Transgenic mice overexpressing GHSR maintain lower GH pituitary content, but this does not affect body weight or prolactin pituitary content. Pituitary cells expressing GHSR are expressing GH and/or prolactin, and the latter may suggest that GHS could affect prolactin physiology.

## OC34

### Transgenic mice overexpressing Urocortin 3 show increased anxiety- and depressive-like behaviours but a hyporesponsive ACTH response to stress

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Urocortin 3 is a CRF-related ligand highly specific for the Corticotropin-Releasing Factor Receptor Type 2 (CRFR2). CRFR2 is implicated in the regulation of stress-related behaviours and the neuroendocrine response to

stressors. Close anatomical association between major Urocortin 3 terminal fields and CRFR2 in the limbic system and hypothalamus indicate this system is well placed to be involved in modulating of the physiological stress response. Transgenic mice ubiquitously overexpressing Urocortin 3 under the control of the ROSA26 promoter were generated as a model of chronic CRFR2 activation. Urocortin 3 peptide was expressed in brain by 40 times wildtype levels as measured by specific radioimmunoassay. Studies were carried out on group-housed adult male mice between 12 and 14 weeks old. Urocortin 3 transgenic mice showed increased anxiety-like behaviour in the elevated plus maze with decreased open arm entries, decreased time spent on the open arms and increased time spent on the closed arms of the maze compared to wildtype littermates. No differences in indices of anxiety were observed in the open field or light dark box apparatus. There were no differences in activity levels between genotypes in behavioural tests. Urocortin 3 overexpressing mice also showed increased immobility in the tail suspension test indicative of increased depressive-like behaviour. ACTH and corticosterone levels under non-stressed conditions were not significantly different between genotypes in the AM or PM. However Urocortin 3 transgenic mice showed an attenuated ACTH response to restraint stress, with ACTH peaking earlier and at lower levels. This incongruity between the behavioural and neuroendocrine response to stress has been reported with repeated stressors or maternal separation in rodents and posttraumatic stress disorder in humans due to desensitisation of CRF receptors and the HPA axis. These data suggest a potential role of the CRFR2/ Urocortin 3 system in regulation of the chronic stress response.

### OC35

#### Immunocytochemical colocalisation of hormones in the human fetal pituitary

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Colocalisation of hormones in the adult human pituitary has been demonstrated only for growth hormone (GH) and prolactin (PL) and only for adenomatous tissue. We have investigated whether colocalization occurs earlier in ontogenesis. Pituitaries from male human fetuses of 16–22 weeks gestational age fixed by immersion in 4% buffered formaldehyde and embedded in LR Gold were processed for immunocytochemical double labelling with GH/FSH or GH/LH anti-rat and anti-human primary antibodies, using both fluorescence and electron immunogold (particles 15, 5 nm) microscopic localization.

In the 16-weeks-old fetus, green-red superposition showed the colocalisation GH and FSH in most of the GH-positive cells. The colocalisation decreased in both intensity and number of colocalized cells from 16 to 20 and 22 weeks. In the 22-weeks-old fetus, islets of cells with either green (GH), or red (FSH) or yellow (GH + FSH) fluorescence were present. The superposition of fluorescence was rarely complete, with many cells showing punctuate green, yellow and red spots. The colocalization of fluorescence indicating GH and FSH was much more intense and frequent than that indicating GH and LH. In all the fetuses there were more GH-fluorescent than FSH- or LH-fluorescent cells. Electron microscopy revealed both 15 nm and 5 nm particles in presumed GH (15 nm) cells, usually in different granules. In the rare "small granule cells", 15 nm (GH) and 5 nm (FSH) gold particles were found, seldom within the same granule. The intercellular spaces were almost free of non-specific labelling.

These data suggest that colocalization occurs in normal pituitary cells early during human ontogenesis and that this phenomenon decreases significantly from 16 to 22 weeks and is largely lost during adulthood. However, the potential for colocalization is preserved and can be reactivated in tumoral transformation.

### OC36

#### Somatostatin analogues in the treatment of acromegaly: correlation between somatostatin receptor subtype expression levels and clinical response

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#### Aim

Somatostatin mediates its effects via five known receptor subtypes *sst1*–*sst5*. The somatostatin analogue octreotide, which binds preferentially to *sst2* and to a lesser extent to *sst3* and *sst5*, may be used prior to surgery of GH-secreting tumors. To investigate the variable response rates of such treatment, we analyzed *sst* expression levels in tumor tissue.

#### Methods

44 patients with acromegaly (20 m, 24 f, 48.7 ± 1.8 years, GH 43.7 ± 1.8 ng/ml, IGF-1 1001 ± 56 ng/ml) underwent pre-operative treatment with octreotide for a period of 7.3 ± 0.5 (3–18) months. Clinical response was evaluated by determination of mean GH levels and IGF-1 levels, as well as tumor size by MRI. After surgery, RNA samples obtained from snap-frozen tumor tissue underwent one-step real-time RT-PCR using subtype specific primers. *Sst* mRNA copy numbers were calculated by parallel amplification of specific cDNAs.

#### Results

During treatment with octreotide, GH and IGF-1 levels were lowered to 28.2 ± 8.0% and 57.0 ± 3.4%, respectively. GH < 2.5 ng/ml and normal IGF-1 were obtained in 34.1% and 43.2% of patients, tumor shrinkage > 20% was observed in 38.6%. In tumors, relevant expression of *sst1*, *sst2*, *sst3*, *sst4*, and *sst5* was observed in 88.6%, 97.7%, 43.2%, 15.9%, and 97.7%, with a wide range of variation. Expression levels of *sst2* were significantly higher in patients with pre-operative normalization of IGF-1 (131 ± 21 vs. 54 ± 11 copies/ng RNA, *P* < 0.005), without any differences for the other *ssts*. ROC analysis suggested an optimal threshold of 70 *sst2*copies/ng RNA with a sensitivity of 74% and a specificity of 76% for normalization of IGF-1. No significant differences in expression levels for any *sst* were observed between tumors with and without tumor shrinkage.

#### Conclusions

Quantitative real-time RT-PCR is a precise method to establish clinically relevant information by investigation of tissue samples. *Sst2* is the relevant receptor eliciting the biochemical response to octreotide. Analysis of *sst2* expression levels may be useful to predict response to treatment with octreotide.

### OC37

#### 90Y-DOTATATE in the treatment of neuroendocrine tumours

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Although neuroendocrine tumours (NET) constitute a very heterogeneous group, they express somatostatin receptors in more than 80% of cases that allows for their effective diagnosis and treatment with somatostatin analogues. Introduction in the recent years of new somatostatin analogues and chelators feasible for labelling with radiometals allowed for development of new therapeutic strategy – radiopeptide therapy. The aim of the work is to present experience with <sup>90</sup>Y-DOTATATE therapy of NET. Material and methods

Patients with advanced tumours, who were not candidates for standard therapy, were recruited to the study. In all cases the diagnosis of NET was based on histopathology or cytological examination and the expression of somatostatin receptors was proved in <sup>111</sup>In-OctreoScan scintigraphy. Other diagnostic procedures included radiological examinations and blood samples evaluations. 60 to 80 mCi of <sup>90</sup>Y-DOTATATE treatment was delivered i.v. Patients were scheduled to 4 cycles of therapy administered in 3 months intervals. Before and after the injections of radiolabelled peptide, aminoacid solution was administered to inhibit tubular reabsorption of radiopeptide.

#### Results

17 patients were recruited into the study until September 2005. Eight of them (47%) received at least two cycles of therapy and were evaluated for efficacy and toxicity of the treatment. <sup>90</sup>Y-DOTATATE application was well tolerated. Two patients suffered from nausea and vomits. There were no grade III blood toxicity. Partial remission was achieved in 2. In both of them radiological response was followed by decrease in chromogranin A. In the other 6 patients stable disease was diagnosed. CgA concentration did not change in 5 of them and in one increased during the therapy.

#### Conclusions

<sup>90</sup>Y-DOTATATE therapy is well tolerated and can result in tumour objective response in patients with somatostatin receptor-positive NET who are not candidates to other therapeutic modalities.



## OC38

### Germline mutations in patients with apparently sporadic pheochromocytomas/paragangliomas

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Paragangliomas (PGLs) and pheochromocytomas (PHEOs) are neural crest-derived tumors (NCD). PGLs can be localized in parasympathetic ganglia (in the head-neck region or in the anterior thorax) or in sympathetic ganglia (in the posterior thorax or in the abdomen). PHEOs can be considered PGLs arising in the adrenal gland.

NCD tumors can present as sporadic or familial. The percentage of hereditary forms is supposed to be 25%. The susceptibility genes predisposing to PGLs/PHEOs are: the proto-oncogene RET, the tumor-suppressor gene VHL, the tumor-suppressor gene NF1, the SDHB/C/D genes encoding three of the four subunits of the mitochondrial complex II.

In this study we evaluated 50 patients (20 males and 30 females) with non-syndromic PGLs/PHEOs and without a positive family history for the diseases. Overall, 71 NCD tumors were found: 39 PHEOs (4 bilateral) and 32 PGLs (20 in the head-neck region, 3 in the thorax and 9 in the abdomen). DNA was extracted from leucocytes and tested for germline mutations of RET, VHL, SDHB, SDHC and SDHD genes.

We found 9 subjects (18%) with SDHD mutations (5 with Q109X, 3 with P81L and 1 with G12S mutations), 1 patient (2%) with a novel SDHB mutation (IVS4 + 1 G > A) and 2 patients (4%) with an undescribed VHL mutation (T152I and L198V). All tumors associated to SDHD mutations were benign while the SDHB mutated patient was affected by malignant bilateral PHEO. NCD tumors were the first lesions in patients with VHL mutations.

In conclusion, in patient with an apparently sporadic PGL/PHEO genetic testing revealed that 24% was affected by germline mutations in the susceptibility genes for NCD tumors. Therefore, DNA analysis is recommended in all subjects affected by PGLs and/or PHEOs.

## OC39

### 11 beta hydroxysteroid dehydrogenase modulation of HPA function – importance of genetic background

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Various psychiatric conditions have been associated with stress and alterations in hypothalamic-pituitary-adrenal (HPA) activity. Yet while stress is a general phenomenon, illness is only seen in a proportion of individuals, suggesting genetic modifiers of ability to cope with stress. 11-beta hydroxysteroid dehydrogenase type 1 (HSD1) regenerates corticosterone from inert 11-dehydrocorticosterone, amplifying the levels of glucocorticoids and is expressed in the brain. To determine potential genetic modifiers of the HPA axis we have investigated the effects of genetic ablation of HSD1 in mice with different genetic backgrounds.

HSD1 null and wild type animals from an MF1/129, or C57Bl6 strain background were subject to 10 minutes restraint, and trunk blood collected at various times after termination of restraint for hormone radioimmunoassay. Brains were analysed for gene expression by in-situ hybridisation.

HSD1 null mice from MF1/129 background had 70% larger adrenals, 3-fold elevated morning basal corticosterone and 2-fold higher adrenocorticotropic hormone (ACTH) compared with wild types. In response to 10 minutes restraint, plasma corticosterone rose to 2-fold higher peak levels, and ACTH remained 2-fold elevated 80 minutes after termination of restraint in the null mice. Glucocorticoid receptor (GR) mRNA expression in the Paraventricular nucleus of the hypothalamus (PVN) was reduced to 55% of wild type levels in null mice, while hippocampal GR expression was not changed. Null animals on a C57Bl6 background had 20% larger adrenals, and 50% increased peak stress response. However basal corticosterone, and stress levels of ACTH, were similar to wild type in null mice on this background. Furthermore, GR mRNA expression was increased over 2-fold in the PVN and by 40% in the hippocampus in null animals.

We conclude that the loss of HSD1 activity alters regulation of the HPA axis, however depending upon strain this can be manifested as either hyper or hypo sensitivity to glucocorticoid negative feedback.

## OC40

### Expression of IGF-I & IGF-IR in tamoxifen sensitive and tamoxifen resistant breast cancer

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#### Background

The growth hormone/ IGF-I axis is important in the pathogenesis of breast cancer and increased IGF-I signalling through the IGF-I receptor (IGF-IR) has been implicated in the development of tamoxifen resistance in breast cancer.

#### Aims

To compare mRNA and protein expression of IGF-I & IGF-IR in (i) wild type (WT) and tamoxifen resistant (TR) MCF-7 breast cancer cells and (ii) breast cancer tissue from women with primary and recurrent, tamoxifen resistant breast cancer.

#### Methods

(i) We have already established a TR cell line from previously obtained WT MCF-7 breast cancer cells (ATCC). RNA was extracted from the cells and mRNA levels assessed using quantitative real time (Taqman) RT-PCR. Western blotting for the IGF-IRβ was carried out using standard protocols. (ii) Specimens of breast cancer were obtained at time of surgery from women with primary and TR breast cancer (informed consent was obtained and local regional ethical approval has been granted). Quantitative real time PCR was performed as above. mRNA levels are expressed as copy number per microgram total RNA and are the mean of 10 samples.

#### Results

(i) Neither WT or TR MCF-7 cells express IGF-I. IGF-IR mRNA is significantly decreased in TR (3.60E + 07) compared to WT cells (2.76E + 08) ( $P < 0.001$ ). There was no significant difference in mRNA levels of either IGF-II (TR 2.59E + 05 vs. WT 6.87E + 05) or IGFBP-3 (TR 2.72E + 08 vs. WT 3.16E + 08). Western blotting confirms reduced IGF-IR protein levels in TR compared to WT cells.

(ii) IGF-I (TR 9.9E + 05 vs. WT 1.1E + 07) and IGF-IR (TR 1.34E + 05 vs. WT 1.28E + 06) levels are significantly reduced in tamoxifen resistant compared to primary cancers ( $P < 0.0001$ ). There is no significant difference in either IGF-II or IGFBP3 expression between the two groups.

#### Conclusion

Development of tamoxifen resistance both *in vivo* and *in vitro* is associated with decreased expression of IGF-I signalling components.

## Endocrine genetics

### OC41

#### Mutations in Cullin 7, a cofactor for Ubiquitination, cause the 3M intra-uterine growth retardation syndrome

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3M syndrome is characterised by severe intra-uterine and post-natal growth failure. Patients have a characteristic triangular facial appearance and disproportionate short stature with tall vertebral bodies and over-tubulation of long bones. The condition bears some resemblance to the Russell Silver syndrome, but is transmitted as an autosomal recessive trait. It has been proposed that heterozygous carriers demonstrate mild phenotypic manifestations of the condition.

3M syndrome has been reported in a wide range of populations. We identified the condition within a very large, highly consanguineous pedigree in North-East Brazil that was under study because of a high incidence of severe isolated GH deficiency due to homozygous mutation in the GHRH-receptor gene. Autozygosity mapping in this and other families with multiple affected sibs identified a locus on chromosome 6p21.1. Genetic analysis refined the interval of interest, and sequencing of individual genes in this region led to the identification of pathogenic mutations within Cullin 7 (Huber *et al.* Nat Genet 2005).

Cullin 7 is one member of a family of proteins involved in cell cycle regulation, including acting as a scaffold for the assembly of the E3 ligase enzyme complex that leads to the ubiquitination of substrate protein as a prelude to their degradation in 26S proteasomes. The Brazilian 3M subjects carry a missense mutation in exon 25 of Cullin 7 creating a premature stop codon (4717C > T, R1573X). This region is necessary for ROC1

recruitment and binding, and thus formation of the E3 ligase complex. These investigations demonstrate that mutations in Cullin 7 cause 3M syndrome and suggest that a defect in ubiquitination generates both pre- and post-natal growth retardation.

## OC42

### Linkage of a fourth gene for familial glucocorticoid deficiency to chromosome 13q

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Expression of the ACTH receptor (MC2R), a 7 transmembrane GPCR, has been difficult to achieve in cell lines that are not of adrenal origin. Heterologous expression of this gene in many cell lines (CHO, Hela, H295R, HEK293) produces a protein that is trapped in the ER, suggesting that an accessory factor(s) might be necessary to traffic MC2R through the cell. We recently identified such an accessory factor, MRAP that rescues MC2R expression in some, but not all, cell lines. Mutations in either MC2R or MRAP lead to familial glucocorticoid deficiency (FGD), but account for less than half of the known cases. This observation, combined with the finding that MRAP cannot rescue MC2R function in all cell lines, leads us to conclude that other genes are involved in the trafficking of MC2R and that studying FGD patients might reveal their nature. A third locus for FGD was identified in 2002 by linkage of the disease to chromosome 8q in one family (LOD 3.00). We have now linked further FGD families to this locus and reduced the interval to 4Mb. However, we have also identified several families in which the MC2R, MRAP and 8q loci are excluded as candidate regions. SNP genotyping of one such family has revealed a fourth locus for FGD on chromosome 13 (LOD = 3.37). The regions on chromosome 8q and 13q are 4 and 10Mb in size and contain 48 and 37 known or predicted genes respectively. The identification of the disease causing genes within these regions will reveal further aspects of the pathway necessary to achieve a fully functional MC2R which may have parallels in other GPCR systems.

## OC43

### Mutation screening of SLC26A4 (PDS) gene in patients with Pendred syndrome and nonsyndromic enlarged vestibular aqueduct

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Enlarged vestibular aqueduct (EVA) is the most common malformation of inner ear associated with sensorineural hearing loss. It can either be found in nonsyndromic or in syndromic forms of hearing loss, such as Pendred syndrome (PS). In PS, sensorineural deafness is associated with thyroid abnormalities: goitre and iodine organification defect detected with perchlorate discharge test. Mutations in SLC26A4 (PDS) gene cause PS and can also be found in a proportion of patients presenting with nonsyndromic hearing loss with EVA. It is not clear yet whether PS and nonsyndromic EVA represent two distinct clinical and genetic entities or whether they are part of a continuum of the same disorder. Mutations of SLC26A4 were screened by denaturing high-performance liquid chromatography (DHPLC) in 15 PS patients and in 21 nonsyndromic EVA patients. Thyroid function evaluation was performed, including morphological evaluation of thyroid gland, a perchlorate discharge test and thyroid function tests. In all 15 PS patients, two SLC26A4 mutated alleles were found. One or two SLC26A4 mutated alleles were found in 10 out of the 21 nonsyndromic EVA patients (48%). One mutant allele was detected in 7 patients and 2 mutant alleles were detected in 3 patients. No mutation was found in the other 11 patients. All nonsyndromic EVA patients revealed normal thyroid evaluation, excepted for 2 patients carrying 2 mutant alleles who showed isolated elevated serum thyroglobulin values. Five novel mutations were described. The results of this study support the fact that SLC26A4 mutations are frequent in nonsyndromic EVA Caucasian patients and that most of them have only one mutated allele, suggesting the involvement of other genetic or

environmental cofactors. We also hypothesise that nonsyndromic EVA patients carrying two SLC26A4 mutant alleles may present a subtle iodine organification defect, revealed by elevated serum thyroglobulin value.

## OC44

### Functional impact of polymorphic variation in the gene encoding 11 $\beta$ -hydroxylase (CYP11B1): reduced adrenal 11-hydroxylase efficiency identifies a key intermediate phenotype in hypertension

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The regulation of aldosterone secretion is altered in essential hypertension: the phenotype of relative aldosterone excess is present in up to 15% of subjects. The gene encoding aldosterone synthase (CYP11B2) offers an obvious candidate to account for this and a polymorphism in its 5' untranslated region (UTR; -344C/T) is associated with increased frequency of hypertension and higher aldosterone levels. However, this variation is more closely associated with an increase in the ratio of deoxycortisol to cortisol (S/F), a classic index of reduced efficiency of the 11 $\beta$ -hydroxylase enzyme encoded by the adjacent CYP11B1 gene which is expressed in zona fasciculata. We propose that this phenotypic association with CYP11B2 reflects variation in CYP11B1 and is accounted for by linkage disequilibrium (LD) between the genes. If so, the CYP11B2 polymorphisms should be linked to causal variants that alter 11 $\beta$ -hydroxylase activity.

Sequencing of the CYP11B locus revealed 83 polymorphisms; these formed 4 common haplotypes, accounting for 68% of chromosomes. Two novel CYP11B1 single nucleotide polymorphisms (SNPs) in the 5' UTR (-1888 G/T, -1858 A/G) were in close LD with the -344C/T polymorphism in CYP11B2. There was a significant association between these polymorphisms and the ratio of urinary tetrahydrodeoxycortisol (THS) to total cortisol (F) in a population of hypertensive patients ( $n = 512$ ), indicating impaired 11 $\beta$ -

SNP	THS/Total F $\pm$ SEM	P
-1888 G	8.89 $\pm$ 0.54	0.025
-1888 T	10.77 $\pm$ 0.67	
-1858 A	9.07 $\pm$ 0.57	0.056
-1858 G	10.70 $\pm$ 0.65	

hydroxylase efficiency (see Table 1).

SNP effects on transcription efficiency were tested *in vitro* using a reporter gene system. Compared to wild type, constructs containing the -1888T and -1858G SNPs halved activity in the presence of ACTH ( $P < 0.001$ ) or forskolin ( $P < 0.025$ ), indicating reduced transcription. We conclude that CYP11B2 polymorphisms are in LD with novel SNPs in CYP11B1 which associate with impaired 11-hydroxylation as a result of reduced CYP11B1 transcription. We propose that this may result in small compensatory increases in ACTH drive which, long term, may lead to adrenal hyperplasia and hypertension associated with mineralocorticoid excess.

## OC45

### Genotype/phenotype correlation of PRKARIA mutations in patients with Carney complex (CNC) and/or sporadic primary pigmented nodular adrenocortical disease (PPNAD) from the CNC network

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CNC is an autosomal dominant multiple neoplasia syndrome, responsible mainly for cardiac myxomas, pigmented skin lesions and endocrine tumors (acromegaly, thyroid and testicular neoplasms and primary pigmented nodular adrenocortical disease: PPNAD). The PRKARIA gene was previously found to be mutated in about 41% of CNC kindreds. Most mutations lead to nonsense mediated mRNA decay and preclude expression of the mutant protein. 102 patients (64 with PPNAD and 38 with CNC) from 72 different kindred have been studied. A total of 68 patients were found to have a PRKARIA inactivating heterozygous mutation (27 different mutations).

Among the 72 index cases a PRKAR1A mutation was found in 9/11 (82%) in the group of sporadic CNC cases with PPNAD and 12/14 (86%) in the group of patients with familial CNC. In the group of patient meeting the diagnostic criteria for CNC but without PPNAD nor familial history the mutation rate was lower: 4/14 (29%). PRKAR1A mutation was found in 18/33 (55%) of patients presenting with isolated and sporadic PPNAD. One mutation (Exon 7 IVS del -7-2) was found in 12 different index cases and associated with isolated PPNAD, suggesting the first clear example of genotype/phenotype correlation for PRKAR1A mutation. Out of the 68 patients with PRKAR1A mutation, 32 had a splice site mutation, 28 a frameshift or non-sens mutation and 8 patients presented a mutation that should give an abnormal protein. Mutations that should give rise to an abnormal protein or a stop codon were more often associated with lentiginosis, cardiac myxomas and thyroid nodes by comparison with splice site mutations. This database demonstrates the very high rate of PRKAR1A mutation in patient with familial CNC or sporadic CNC with PPNAD, shows a genotype/phenotype correlation for some mutations and suggest genetic heterogeneity currently under study for patients with isolated PPNAD.

#### OC46

##### Predictors and prevalence of paraganglioma syndrome associated with mutations of the SDHC-gene

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Paraganglioma and pheochromocytomas are described as sporadic and hereditary conditions. Hereditary are the lesions in the tumor syndromes Multiple endocrine neoplasia type 2 (MEN 2), Von Hippel-Lindau disease (VHL), neurofibromatosis type 1 (NF1), and the paraganglioma syndromes type 1 type 3 and type 4 (PGL1, PGL3, PGL4). The susceptibility genes are for PGL1 SDHD, PGL3 SDHC, and for PGL4 SDHB. In contrast to PGL1 and PGL4 in which numerous carriers have been reported, PGL3 was described only in 4 index cases.

We established registries for head and neck paragangliomas (HNP) and pheochromocytomas and paragangliomas of other sites. After exclusion of NF1, MEN 2 and VHL we tested all subjects for SDHC, SDHB and SDHD germline mutations.

The Pheochromocytoma Registry comprised after exclusion of SDHB and SDHD mutations 369 subjects. None of these had an SDHC mutation. The HNP Registry comprised 121 subjects. Five had an SDHC mutation. Thus, prevalence of PGL 3 was 4%.

We were able to compare 22 SDHC positive subjects with 15 positive for SDHB and 42 positive for SDHB and 88 subjects with sporadic HNP (mutations of RET, VHL, SDHB, SDHC, SDHD excluded). Clinical features of SDHC mutation carriers were similar to sporadic HNP patients, but different to SDHB and SDHD carriers. Frequent location of HNP was the jugular ganglion. 4/5 patients had permanent palsy of at least one cranial nerve.

In the Freiburg HNP Registry, mutations were dominated by such of the SDHD gene followed by SDHB whereas SDHC mutations were the smallest group. Molecular genetic investigation of the SDHC gene should not be offered patients with pheochromocytoma. HNP patients who are negative for SDHD and SDHB mutations should be offered testing for SDHC mutations in order to detect HNP relapse and timely tumor diagnosis and treatment in relatives if shown to be carriers.

#### OC47

##### Frequent involvement of BMP15 gene variants in women with premature ovarian failure

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Premature ovarian failure (POF) is a common cause of female infertility affecting about 1–2% of women under the age of 40. This heterogeneous disorder is characterized by primary or secondary amenorrhea and elevated gonadotropin values. Several defects can cause POF, including autoimmunity, X chromosome abnormalities and gene mutations, but its pathogenesis is

still unknown in the vast majority of women with normal karyotype. We recently described two sisters affected with hypergonadotropic ovarian failure and primary amenorrhea that were carriers of a mutation in the gene encoding Bone Morphogenetic Protein 15 (BMP15) that was inherited from their father. This evidence is consistent with a critical role played by this oocyte-derived growth factor in the progression of folliculogenesis in humans, as previously shown in rodents and sheep. In this study we report the genetic analysis of BMP15 gene in 190 unrelated women with idiopathic POF: 25 cases with primary amenorrhea, 153 with secondary and 11 with "intermittent" POF. With the informed consent of patients, genetic screening was performed by dHPLC, using leukocyte DNA. Any identified variant was confirmed and characterized by automated sequencing. Our investigations showed the presence of 6 variants, the already reported Y235C mutation in one case and 5 new variants in 17 patients: one insertion (262insLeu) in 11 cases and four missense alterations (A180T in 5 cases, L148P, R68W and S5R in 1 case each). Except the 262insLeu, the other 4 variants were not found in the genomic DNA from 95 women who experienced the physiological menopause after 50 years of age and 150 controls from the general population. These novel variants were present in the heterozygous state in women with secondary amenorrhea.

In conclusion, these findings indicate the frequent association of BMP15 gene variants with the POF phenotype in humans (9/190 patients) and are consistent with the critical role played by BMP15 in human folliculogenesis.

#### OC48

##### Progressive osseous heteroplasia : a phenotype associated with mutations of the GNAS1 gene

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Progressive osseous heteroplasia (POH, MIM 166350) is a rare autosomal dominant disorder characterised by extensive dermal ossification during childhood, followed by widespread heterotopic ossification of skeletal muscle and deep connective tissue. Recently, genetic basis was found to be common with Albright's hereditary osteodystrophy (AHO) (Shore *et al.*, 2002): paternally inherited inactivating mutations of the GNAS1 gene were found. GNAS1 is the gene for guanine nucleotide binding protein alpha stimulating (G $\alpha$ s protein) activity polypeptide 1. It maps to 20q13 and belongs to an imprinted locus. Deficiency of G $\alpha$ s has been associated with OHA and resistance to PTH also called pseudohypoparathyroidism (PHP1a). In addition, individuals with isolated OHA, GNAS1 lesions but absence of hormonal resistance are identified (pseudopseudohypoparathyroidism or PPHP). Maternal transmission of the hormonal resistance was demonstrated while paternal transmission was associated with PPHP.

We identified heterozygous inactivating GNAS1 mutations in 41 patients (24 females and 17 males) presenting with AHO or PHP1a. 5 patients (2 girls and 3 boys) from 5 different families were diagnosed with POH. Two patients were diagnosed during the first yr with ossified areas coalescing into plaques. These patients lack features of AHO. Gs activity and hormonal parameters are under investigations.

Segregation analysis indicated that 4 mutations had occurred "de novo" and one was paternally transmitted. Two mutations have been previously described either in POH, 345insT (codon 115 in exon 5) or in PHP1a, Q29X in exon 1, which was carried by two patients. We found two new mutations: 624insT in exon 8 at codon 209 and IVS12 - 1G > A (intron 12). In all cases these mutations cause premature termination of translation and loss of protein function.

This finding extends the range of phenotype derived from haploinsufficiency of GNAS1. Evidence that same mutation can cause either AHO or POH makes genetics counselling not easy.

#### Calcium and bone

##### OC49 Novartis Oncology Young Investigator Award

##### The A990G polymorphism of calcium sensing receptor gene (CASR) is associated with nephrolithiasis in patients with primary hyperparathyroidism (PHPT)

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Primary HPT shows a great variability in its clinical course and severity, which might be related to polymorphic variants of the CASR gene. The aim of the study was to evaluate the frequency of two known CASR single nucleotide polymorphisms (SNPs), i.e. G/T at codon 986 and G/A at codon 990, in a homogenous North-Italian cohort of PHPT patients compared with a sex and age matched healthy population and the possible correlation of these CASR gene variants with the clinical and biochemical characteristics of PHPT patients. SNPs were analyzed by direct sequencing in 94 PHPT unrelated patients (79 F, 15 M; age  $65 \pm 13$  years, plasma ionized calcium  $1.52 \pm 0.15$  mmol/L; serum PTH  $192.7 \pm 130.9$  pg/ml) and in 179 healthy subjects (148 F, 29 M). All PHPT patients were studied for renal and bone PHPT complications. The proportion of CASR variants was similar in PHPT and controls (codon 896: G/G 62% vs 66%, G/T 30% vs 28%, T/T 8% vs 6% and codon 990: A/A 88% vs 91%, A/G 10% vs 7%, G/G 2% vs 2%). The G986T polymorphisms were not associated with any clinical or biochemical PHPT parameters. Patients with 990 A/G and G/G genotype showed lower serum PTH ( $139.9 \pm 62.2$  vs  $199.9 \pm 136.3$  pg/ml;  $P = 0.02$ ) and phosphate levels ( $0.69 \pm 0.12$  vs  $0.81 \pm 0.18$  mmol/L;  $P = 0.03$ ). Twenty-four hour-urine calcium excretion was higher in patients with 990G ( $9.05 \pm 2.05$  vs  $6.77 \pm 4.31$  mmol/24h;  $P = 0.012$ ) and it was associated with higher prevalence of nephrolithiasis (90% vs 44.2%;  $P = 0.007$ ) than patients with A/A. In conclusion, A990G variants might increase CASR sensitivity to extracellular calcium, determining a lower PTH secretion. Moreover, at the kidney level the increased CASR sensitivity might result in the inhibition of renal calcium reabsorption and increase in calcium excretion, this phenomenon favoring nephrolithiasis.

## OC50

### Congenitally hypothyroid mice with (*Pax8*<sup>-/-</sup>) or without (*hyt/hyt*) functional TSH receptors (TSHR) display equivalent skeletal phenotypes

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Studies of TSHR<sup>-/-</sup> mice suggest that TSH inhibits bone turnover, but these mice have congenital hypothyroidism and the actions of TSH cannot be separated from effects of thyroid hormone deficiency. We characterised skeletal development in *hyt/hyt* mice, which have a point mutation in the *Tshr* gene, and *Pax8*<sup>-/-</sup> mice with thyroid gland agenesis. *Hyt/hyt* mice have a 100-fold increase in TSH but inactive TSHRs, whereas *Pax8*<sup>-/-</sup> mice have a 400-fold increase in TSH and functional TSHRs. Bone length was reduced by 16% in 7 week-old *hyt/hyt* mice ( $P < 0.01$ ) but heterozygotes were similar to WT. Growth plates from *hyt/hyt* mice showed an increase in total width ( $P < 0.05$ ), reserve and proliferative zones ( $P < 0.05$ ), disorganised proliferating chondrocytes columns and an indistinct hypertrophic zone. This delay in endochondral ossification was associated with a 35% reduction in cortical bone thickness ( $P < 0.001$ ). DXA bone mineral density (BMD) was reduced by 15% ( $P < 0.05$ ) and bone volume fraction (BVF) by 9% ( $P < 0.05$ ). BMD and BVF in heterozygotes were no different to WT, but quantitative backscattered electron scanning electron microscopy revealed a clear reduction in bone micro-mineralisation density in both *hyt/hyt* and heterozygote mice ( $P < 0.01$ ). Bone length was reduced by 19% at 2 weeks ( $P < 0.01$ ) and 7% at 10 weeks in *Pax8*<sup>-/-</sup> mice ( $P < 0.05$ ). Growth plates from *Pax8*<sup>-/-</sup> mice showed an increase in total width ( $P < 0.001$ ), reserve and proliferative zones ( $P < 0.05$ ) and disorganisation of the proliferating chondrocytes columns and hypertrophic zone. Cortical bone thickness was reduced by 31% ( $P < 0.05$ ). DXA-BMD was reduced by 5% (ns) but micro-mineralisation density was markedly reduced in *Pax8*<sup>-/-</sup> mice ( $P < 0.001$ ) indicating that defects of bone mineralisation were similar in *hyt/hyt* and *Pax8*<sup>-/-</sup> mice. These studies demonstrate that the skeletal phenotype of congenital hypothyroidism is largely independent of TSH action, suggesting that the hypothalamic-pituitary-thyroid axis physiologically regulates endochondral ossification via the actions of T3.

## OC51 Novartis Oncology Young Investigator Award

### TSH receptor action in osteoblasts and osteoclasts *in vitro*

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Recent studies suggest TSH inhibits bone remodeling, indicating that TSH deficiency rather than thyroid hormone excess could cause bone loss in thyrotoxicosis. The findings predict that TSH receptor (TSHR) stimulating antibodies (TSHRab) should inhibit bone turnover, whereas Graves' disease patients exhibit high bone turnover with increased fracture susceptibility. We characterized TSH-action in primary human and mouse osteoblasts and osteoclasts, and explored whether a paracrine pathway involving TSH or thyrostimulin (a novel high-affinity TSHR agonist) could account for TSH-action in bone. TSHR expression was identified by RT-PCR in human and mouse osteoblasts and osteoclasts. TSH-alpha was expressed only in human osteoblasts, but TSH-beta was undetectable in all cells. Thyrostimulin-alpha was expressed in osteoblasts and osteoclasts from both species, whereas thyrostimulin-beta was only expressed in human osteoblasts and osteoclasts. Treatment of osteoblasts with TSH (10–100 U/ml; 7–28 days) did not alter alkaline phosphatase activity or bone nodule mineralization. Treatment of osteoclasts with TSH (100 U/ml; 12 days) resulted in 2.8-fold fewer resorption pits formed on dentine slices (control  $116 \pm 5$  vs TSH-treated  $41 \pm 1.2$  pits/slice,  $P < 0.0001$ ) and 2.7-fold fewer osteoclasts (control  $225 \pm 19$  vs TSH-treated  $84 \pm 9$  TRAP + ve cells/well,  $P < 0.001$ ). Treatment of mature osteoblasts or osteoclasts with TSH (10–100 U/ml) or four monoclonal TSHRab failed to induce cAMP, the canonical mediator of TSH-activated TSHR signaling. Thus, although both osteoblasts and osteoclasts expressed TSHR mRNA, only osteoclasts responded to TSH. There was no evidence of a TSH paracrine pathway indicating that skeletal actions of TSH *in vivo* must result from circulating TSH, although activation of TSHR in human bone may also involve locally produced thyrostimulin. The lack of cAMP response to TSH or TSHRab in mature osteoclasts suggests (a) the TSHR acts via an alternative pathway or (b) TSH effects on osteoclast numbers and resorption pits reflect its early actions on progenitor cells to regulate osteoclast recruitment and differentiation.

## OC52

### In acromegaly bone mineral density depends on gonadal function

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The aim of the study was to evaluate factors determining bone mineral density (BMD) and serum concentrations of osteocalcin (OC) and carboxyterminal telopeptide of type I collagen (ICTP) in acromegaly. Material consisted of 121 patients with active acromegaly, aged 23–80 years. In the whole group BMD, assessed by DXA, was:  $0.35 \pm 0.15$  at L<sub>2</sub>-L<sub>4</sub>,  $0.60 \pm 0.11$  at femoral neck,  $0.05 \pm 0.12$  at Ward's triangle and  $0.59 \pm 0.13$  at trochanter and was higher than normal at all sites excluding Ward's. Mean serum OC was  $31.7 \pm 2.2$  ng/ml, and ICTP  $7.3 \pm 0.5$  µg/l. OC and ICTP were higher than normal and positively correlated with each other and with IGF-I concentration. IGF-I was the main factor determining OC. For ICTP and Z-scores the most important were gonadal status and gender. ICTP negatively correlated with Z-scores. One third of the acromegalics were eugonadal, whereas 2/3 were hypogonadal. Z-scores in eugonadal group were higher than normal. Hypogonadal subjects had significantly lower Z-scores and higher ICTP than eugonadal ones. Of hypogonadal patients: 27% had osteopenia and 7% - osteoporosis. When compared to females, males had lower Z-scores and higher ICTP concentrations. Postmenopausal subjects had lower Z-scores than eugonadal females. In the group of women with gonadal function adequate to age the main factor determining Z-scores was estrogen deficiency. CONCLUSIONS: GH-excess in acromegaly leads to increase of BMD, by stimulation of IGF-I-dependent bone turnover, with prevailing synthesis. In hypogonadal acromegalics (2/3 of the population), high bone resorption may cause a decrease of BMD and osteoporosis. Acromegalic males have relatively higher bone resorption and lower BMD than females. The influence of GH on BMD in acromegalic females with preserved gonadal function is stronger than in postmenopausal acromegalics. This suggests synergic effect of GH and estrogens on bone.

**OC53****Bone involvement in patients with adrenal incidentalomas: role of subclinical hypercortisolism**

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Previous studies suggest that in patients with adrenal incidentalomas (AI) subclinical hypercortisolism (SH) exerts a deleterious effect on bone mineral density (BMD), but scarce data are available about vertebral fractures. We evaluate BMD and prevalence of vertebral fractures in a sample of AI subjects with and without SH. Forty-seven consecutive AI inpatients were evaluated (17M, 30F). The patients were subdivided into two groups: with or without subclinical hypercortisolism (SH + /SH -). The diagnosis of SH was made on the presence of 2 of the following 3 alterations in the pituitary-adrenal axis: urinary free cortisol (UFC) levels higher than 70 mcg/24 hrs, serum cortisol levels after a 1-mg overnight dexamethasone suppression test (F after Dex) higher than 3.0 mcg/dL, and ACTH levels lower than 10 pg/mL. In all patients spinal and femoral BMD was measured by DXA and expressed as Z-score (LS Z-score, FN Z-score respectively). Results are shown in Table 1.

	SH + (n = 14)	SH- (n = 33)
Age (Yrs)	71.6 ± 11.1	67.3 ± 10
Gender (M/F)	6/8	11/22
Diameter of adenoma (cm)	2.04 ± 0.88	2.46 ± 0.42
BMI (Kg/m <sup>2</sup> )	28.1 ± 4.7	27.9 ± 7.1
Prevalence of osteoporosis (%)	20	15
Z-score LS (g/cm <sup>2</sup> )	-0.89 ± 1.092	0.184 ± 0.894
Z-score FN (g/cm <sup>2</sup> )	-0.20 ± 0.602	0.342 ± 0.948
Vertebral Fractures: (%)	88.9*	16.7

Data are mean ± SD; \*P = 0.001. In the logistic regression analysis, significant predictor of vertebral fracture was F-Dex (OR 0.024, CI 95% 0.002–0.315, P = 0.005) but not age and BMI.

In patients with adrenal incidentalomas, SH is associated to a higher prevalence of vertebral fractures related to the degree of cortisol secretion. Bone involvement has to be evaluated in AI patients with SH.

**OC54****Adult mice harbouring a dominant negative R384C mutation of TRalpha1 have a marked increase in trabecular bone and micro-mineralisation density**

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T3-receptor alpha (TRa) is the predominant TR isoform in bone. To investigate its function, we analysed mice harbouring a dominant negative R384C mutation in TRa1 (TRa1<sup>mv+</sup>). The homozygous TRa1<sup>mv/mv</sup> mutation is lethal whereas heterozygotes are euthyroid displaying only transient postnatal hypothyroidism. Critically, dominant negative activity of the mutation is overcome by a 10-fold increase in T3, which is achieved by crossing TRa1<sup>mv+</sup> mutants with TRb<sup>-/-</sup> mice. Thus, TRa1<sup>mv+</sup>b<sup>-/-</sup> mice represent a unique model to study reversibility of the R384C mutation. TRa1<sup>mv+</sup>b<sup>+/+</sup> (euthyroid), TRa1<sup>mv+</sup>b<sup>+/+</sup> (equivalent to TRa1<sup>mv+</sup>) and TRa1<sup>mv+</sup>b<sup>-/-</sup> (10-fold increase in T3) mice were analysed between embryonic day 17.5 and 16 weeks of age. Histology revealed delayed endochondral ossification in TRa1<sup>mv+</sup>b<sup>+/+</sup> mice with a maximum 15% reduction in bone length at 2 weeks (P < 0.05). TRa1<sup>mv+</sup>b<sup>-/-</sup> mice displayed only a minor delay until 4 weeks of age. Intramembranous ossification was also delayed in TRa1<sup>mv+</sup>b<sup>+/+</sup> and TRa1<sup>mv+</sup>b<sup>-/-</sup> mice with a >2-fold increase in cranial fontanelle area at

birth (P < 0.001). Bone micro-architecture and micro-mineralisation densities, analysed by quantitative backscattered electron scanning electron microscopy, revealed a 2.7-fold increase in trabecular bone volume (BVf) in adult TRa1<sup>mv+</sup>b<sup>+/+</sup> mice (P < 0.01) and increased trabecular complexity, thickness and micro-mineralisation density (P < 0.001). Extension of trabecular bone into the diaphysis increased from 10% of total bone length in TRa1<sup>mv+</sup>b<sup>-/-</sup> and TRa1<sup>mv+</sup>b<sup>-/-</sup> mice to 33% in TRa1<sup>mv+</sup>b<sup>+/+</sup> mice (P < 0.001). To compensate for the period of transient hypothyroidism during growth, some TRa1<sup>mv+</sup>b<sup>+/+</sup> mice were treated with T3 between days 10 and 35. T3-treated adult TRa1<sup>mv+</sup>b<sup>+/+</sup> mice had reduced trabecular thickness, complexity and micro-mineralisation density but TRa1<sup>mv+</sup>b<sup>+/+</sup> mice were unaffected (P < 0.001), indicating that a short period of T3 treatment during growth profoundly influences adult bone structure. Crossing TRa1<sup>mv+</sup> mice with TRb<sup>-/-</sup> mice further rescued the adult phenotype. These data demonstrate that TRa plays a critical role in establishing adult bone structure and mineralisation.

**OC55****Thyroid hormones stimulate osteoblast differentiation but inhibit mineralization of bone nodules *in vitro***

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Thyroid hormones stimulate bone formation and linear growth in children. Paradoxically, childhood thyrotoxicosis causes short stature and craniosynostosis due to early closure of the growth plates and skull sutures. In adults, however, thyroid hormone excess increases bone turnover and results in progressive bone loss and osteoporosis. Activating mutations of fibroblast growth factor receptor-3 (FGFR3) cause achondroplastic dwarfism and we have shown that T3 augments FGFR signalling during chondrogenesis. Activating mutations of FGFRs 1–3 also cause discrete craniosynostosis syndromes, and we therefore investigated whether T3 stimulation of osteoblastogenesis correlated with T3 regulation of FGFR expression. Primary mouse osteoblasts undergo an established 28-day programme of differentiation and bone nodule formation *in vitro*. Cells were cultured in the absence or presence of T3 (100 nM) and examined at days 7 (cell proliferation), 14 (osteoblast maturation), 21 (terminal differentiation) and 28 (mineralization). Primary osteoblasts expressed two FGFR1 mRNAs, four FGFR2 mRNAs, two FGFR3 mRNAs and a single FGFR4 isoform at all time points. Maximal T3-induction of FGFR expression occurred at day 14 (FGFR1, 3.43-fold, P < 0.01; FGFR3, 5.45-fold, P < 0.01; FGFR4, 7.92-fold, P < 0.001), and was associated with increased expression of key osteoblast differentiation marker genes (osterix, 11-fold, P < 0.01; Runx-2 1.3-fold, P < 0.05; osteocalcin, 1.5-fold, P < 0.05). Furthermore, T3 stimulated alkaline phosphatase activity from day 14 (day 14, 3.36-fold, P < 0.001; day 21, 1.52-fold, P < 0.01; day 28, 1.45-fold, P < 0.01) but, surprisingly, inhibited bone nodule mineralization at day 28 (P < 0.01). These data demonstrate that T3 stimulation of FGFR1, 3 and 4 expression correlates with induction of osteoblast differentiation and suggest that, like chondrogenesis, T3-induced osteoblastogenesis involves augmentation of FGF signalling by T3. By contrast, T3 inhibited the mineralization activity of mature osteoblasts, suggesting a mechanism by which T3-excess stimulates high bone turnover with net loss of mineralised bone in adults.

**OC56****The Scandinavian investigation of primary hyperparathyroidism (pHPT) – end of inclusion and preliminary results**

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**Background**

From 1999 to the end of June 2005, 186 patients (26 men) with truly asymptomatic pHPT based on the NIH 1990 criteria were included at 11 centres in Scandinavia. The mean age at inclusion was  $63.2 \pm 7.8$  (SD) years. After informed consent the patients were randomised to operation (93) or medical observation (94), in order to investigate the effect of operation on Quality of Life and co-morbidity. At present, 159 patients are ongoing, 3 dead, and 24 have withdrawn. Follow-up data after 1 year were available for 117 patients equally distributed between the two groups.

**Results**

At baseline, mean s-calcium was  $2.69 \pm 0.07$  mM, s-PTH  $10.3 \pm 4.2$  pM and s-creatinine  $80.7 \pm 14.7$   $\mu$ M, equally distributed between the groups. Compared to normative Swedish data, the patient population showed significantly lower SF-36 score in the mental health related domains. Bone mineral density (BMD) at baseline was without difference from normative data. During follow up, calcium and PTH levels were unchanged in the observation group, but normalized in the operated group, as expected. Creatinine level was unchanged in both groups and without difference. BMD in the spine and femoral neck did not change in the observation group, whereas significant increases were found in the operated patients. SF-36 scores did not differ between the groups at baseline or after 1 year of observation. Within the groups only minor changes in SF-36 were observed.

**Conclusion**

One hundred eight-nine truly asymptomatic patients with pHPT were included in the study and 159 are ongoing. At baseline the patients had decreased QoL as judged by SF-36 compared to normative data. The normalization of PTH and Ca-levels following parathyroidectomy was associated with increases in BMD of the spine and femoral neck; however QoL did not improve. The total follow-up time is planned to be 10 years.

**Thyroid****OC57 British Thyroid Association Award****Treatment of experimental hyperthyroid Graves' disease with decoy TNF-family ligand receptors, suggests an essential role for BAFF in production of thyroid stimulating antibodies**

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**Introduction and aims**

Graves' disease (GD) is mediated by thyroid stimulating antibodies (TSAbs) to the TSH receptor (TSHR). Treatments for GD have remained unchanged for more than 40 years with less than 50% of patients achieving long term remission with medical therapy. Experimental animal models have been described which facilitate understanding of disease mechanisms and permit studies of novel immunotherapeutic interventions. However studies aiming to perturb the Th1/Th2 balance of the autoimmune response have failed to modify the course of established GD. Recently identified TNF superfamily ligands; B cell activating factor (BAFF), a proliferation-inducing ligand (APRIL) and their receptors (BAFFR) and B cell maturation antigen (BCMA) have been implicated in auto-antibody production. We investigated the therapeutic effects of blockade of BAFF or BAFF + APRIL in a murine model of GD, using soluble decoy TNF receptor proteins BAFFR-Fc and BCMA-Fc.

**Methods**

BALB/c mice ( $n = 110$ ) were immunized with adenovirus TSHR-A-subunit construct to induce elevated serum levels of TSABs and thyroxine (T4). These mice were randomized to treatment with either BAFFR-Fc, BCMA-Fc, IgG or PBS. Serum TSABs and T4 were measured pre- and post treatment (RIA). Splenic plasma cell populations were assessed using anti-mouse syndecan-1 (CD138).

**Results**

In animals with established hyperthyroidism, blockade of BAFF or BAFF + APRIL with BAFFR-Fc and BCMA-Fc led to significant reductions in serum T4 compared with pre-treatment values (78% and 100% mice per group respectively;  $P < 0.01$ ). Significant reductions in serum T4 were also observed compared with control groups ( $P < 0.01$ ). This was supported by a parallel pattern of declining TSABs in mice treated with BCMA-Fc ( $P < 0.05$ ). However, splenic sections from mice treated with BAFFR-Fc or BCMA-Fc revealed persistence of plasma cells.

**Conclusion**

This is the first report of attenuation of established induced hyperthyroidism in a murine model of GD using immunotherapeutic agents targeting autoreactive B cells.

**OC58****Interleukin 1: the optimal cytokine target in thyroid associated ophthalmopathy?**

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**Introduction**

Anti-cytokine therapy may be useful for the treatment of active thyroid associated ophthalmopathy (TAO). We aimed to establish the effects of selected cytokines on intercellular adhesion molecule 1 (ICAM1) expression and adipogenesis in orbital fibroblasts (OFs) from patients with and without TAO.

**Methods**

Orbital tissue was taken during surgery from 5 patients with TAO and 5 control subjects. Primary cultures of OFs were established and stained for ICAM1 expression by flow cytometry. OFs were exposed to cytokines (TNF $\alpha$ , IL1, IFN $\gamma$ , IL10 and TGF $\beta$ ) and/or anti-cytokine agents (adalimumab, etanercept, infliximab and anakinra) and grown for 48 hours. The concentrations of anti-cytokine agents used were in the range of peak serum levels after therapeutic administration and also 1/100 of peak concentration. OFs were also cultured in adipogenic media in the presence of TNF $\alpha$  or IL1  $\pm$  their antagonists for 10 days, and adipogenesis measured by staining and extraction of oil-red-O, and by visual assessment.

**Results**

TNF $\alpha$ , IL1 and IFN $\gamma$  (0.1 ng/ml) stimulated ICAM1 expression 8 to 10 fold in OFs from patients with TAO. Interleukin 10 (10 ng/ml) and TGF $\beta$  (1 ng/ml) had no effect. Similar responses were seen in OFs from control subjects. Anti-cytokine agents inhibited the ICAM1 responses to 0.1 ng/ml of TNF $\alpha$  or IL1 by 84% to 92% using peak concentrations and by 60% to 88% using 1/100 of peak concentrations ( $P$  values  $< 0.01$ ). IL1 stimulated adipogenesis (+42% and +400%) whilst TNF $\alpha$  inhibited adipogenesis (-41% and -100%), as measured by oil-red-O extraction and visual scoring, respectively ( $P$  values  $< 0.05$ ).

**Conclusion**

TNF $\alpha$  and IL1 have stimulatory effects on ICAM1 expression, but opposite effects on adipogenesis in orbital fibroblasts *in vitro*. Anti-TNF $\alpha$  agents have the potential to worsen TAO, particularly in patients where adipogenesis is prominent. Anti-IL1 agents may be the anti-cytokine agent of choice for clinical trials in active TAO.

**OC59****Tri-iodothyronine improves haemodynamic performance and is associated with improved myocardial protection post on-pump coronary artery bypass grafting**

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**Objectives**

Tri-iodothyronine (T<sub>3</sub>) can improve cardiovascular performance following cardiac surgery. Its effects on myocardial protection are unknown.

**Methods**

We performed two consecutive randomised double-blind placebo-controlled trials (identical management protocols) on patients undergoing first time isolated elective or urgent on-pump coronary artery bypass graft surgery (CABG). Between January 2000 and September 2004, 440 patients were recruited and randomised. Within this study population 160 patients were randomised to receive placebo (5% dextrose) and 63 to T<sub>3</sub> therapy (0.8  $\mu$ g.kg<sup>-1</sup> bolus, followed by 0.113  $\mu$ g.kg<sup>-1</sup>.hr<sup>-1</sup>) ( $n = 63$ ). T<sub>3</sub>/placebo therapy was administered for a 6-hour period from removal of aortic cross clamp (AXC). Serial haemodynamic measurements were performed at baseline and up to 12 hours following removal of AXC along with cardiac troponin I (CTnI) levels.

**Results**

Results are summarised in the accompanying table ( $P$  values correspond to placebo compared with treatment group). Repeated measures ANOVA demonstrated that T<sub>3</sub> therapy increased cardiac index (CI) versus placebo between 6 and 12 hours after AXC removal ( $P = 0.01$ ). CTnI release was significantly lower in all treatment groups at 6 hours following removal of AXC.



	Placebo	T <sub>3</sub>
Baseline CI (l.min <sup>-1</sup> m <sup>-2</sup> )	2.17	2.28 (P = 0.157)
6 h post AXC CI (l.min <sup>-1</sup> m <sup>-2</sup> )	2.7	2.86 (P = 0.08)
12 h post AXC CI (l.min <sup>-1</sup> m <sup>-2</sup> )	2.75	3.02 (P = 0.002)
6 h CTnI (ng. ml <sup>-1</sup> )	8.66	4.56 (P < 0.001)
12 h CTnI (ng.ml <sup>-1</sup> )	6.66	3.37 (P = 0.001)

#### Conclusions

Treatment with T<sub>3</sub> improves haemodynamic performance and results in reduced CTnI release in patients undergoing on-pump CABG surgery. Precise mechanisms for these effects still remain to be fully elucidated.

#### OC60

##### 4-methylbenzylidene-camphor (4MBC) causes pituitary effects comparable to hypothyroidism

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4-methylbenzylidene-camphor (4MBC) is an endocrine active compound used as an UV absorber in sunscreens and other cosmetics such as anti-aging lotions and day care products. 4MBC shows weak estrogenic effects on the reproductive system but strong anti-osteoporotic effects after chronic application (Seidlova-Wuttke D *et al.* 2005 Toxicol Appl Pharmacol. 24:*in press*). Additionally, 4MBC can be resorbed through the skin (Janjua NR *et al.* 2004 J Invest Dermatol. 123:57–61).

Experimental set-up: Two month old female Sprague-Dawley rats in groups of 12 animals were treated per gavage with five concentrations of 4MBC (10, 33, 100, 333, 600 mg/kg b.w./day) over a period of five days on a background of a soy-free diet two weeks after bilateral ovariectomy. We used T<sub>4</sub>, the antithyroidal drug methimazole (MMI), and estradiol benzoate (E2) as controls. Total T<sub>3</sub>, T<sub>4</sub>, and TSH serum levels were measured by RIA; the expression of TSH $\alpha$ , TSH $\beta$ , 5'-deiodinase type I (5'DI) and II (5'DII) transcript levels in the pituitary were detected by real time RT-PCR.

TSH serum levels were significantly elevated from concentrations of 33 mg 4MBC/kg b.w., while T<sub>4</sub> serum levels were slightly decreased and T<sub>3</sub> levels almost unchanged. This serum data are typical for beginning hypothyroidism, when the peripheral organs maintain T<sub>3</sub> serum levels during the initial phase of the disease. In the pituitary TSH $\alpha$  and TSH $\beta$  were markedly increased from concentrations of 33 mg/kg b.w. ( $\geq 2$  fold change). 5'DI mRNA levels decreased proportional to the concentrations of 4MBC applied, while 5'DII transcript levels were elevated. The controls treated with T<sub>4</sub> and MMI behaved as described in the literature. Additionally, the weight of the thyroid glands was remarkably increased after five days of treatment at concentrations exceeding 33 mg/kg b.w.. These results indicate that 4MBC is a potent inhibitor of the pituitary-thyroid-axis.

#### OC61

##### Phosphodiesterase-resistant PKA I agonists inhibit thyroid cancer cell growth

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In thyroid cells TSH/cAMP stimulation is associated with proliferation and differentiation. The principal effector of cAMP is the protein kinase A (PKA) and events downstream PKA activation, signaling specificity and expression of PKA isoforms are largely unexplored. PKA is composed of two regulatory and two catalytic subunits. Two major isoforms of PKA (I and II) are determined by their specific regulatory subunits (RI $\alpha$ , RI $\beta$ , RII $\alpha$  and RII $\beta$ ). A recent gene expression profile of differentiated FRTL5

and de-differentiated FRT rat thyrocytes revealed the lack of RII $\beta$  expression in FRT cells, which was also associated with the loss of TSH-dependent effects. Here, we extended our observation to human thyroid cancer cell lines (ARO, anaplastic; NPA, papillary; WRO, follicular). As in FRT, RII $\beta$  expression was absent in ARO cells. We then performed proliferative assays after stimulation with phosphodiesterase-resistant cAMP analog pair selective for PKA I or II. Only PKA II activation mimicked TSH proliferation in TSH-dependent FRTL5 cells. Conversely, PKA I action resulted in growth inhibition of FRT and human thyroid cancer cell lines, with a more pronounced effect in ARO and NPA (down to 40% of control growth) than in WRO cells. The antiproliferative effect of PKA I agonists was associated with the reduction of ERK phosphorylation. Such PKAI agonist effect on ERK phosphorylation was observed in cells endowed with constitutive MAPK activation through BRAF mutations, as in ARO and NPA. The effect of PKAI agonists was marginal in WRO cells consistently with the different mechanisms that sustain uncontrolled proliferation of these cells. These data indicate that RII $\beta$  can be lost during de-differentiation and carcinogenesis process. In de-differentiated TSH-independent cells, selective PKA I activation has an antiproliferative effect which may open new therapeutic perspectives for thyroid cancer.

#### OC62 Novartis Oncology Young Investigator Award

##### The role of the thyroid hormone transporter monocarboxylate transporter 8 (MCT8) in fetal brain development

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Thyroid hormones play a major role in the metabolic function of mammalian cells and are of particular importance in the development of the fetal brain. The MCT8 gene has recently been shown to encode an active and specific thyroid hormone transporter. Recent reports have identified mutations in the MCT8 gene in several unrelated boys presenting with severe X-linked psychomotor retardation and elevated serum T<sub>3</sub>.

Ontogeny of mRNA encoding MCT8 was examined in 61 human fetal cerebral cortex (from surgical TOP), and 10 normal adult samples (MRC CNS collection, UK). All samples were collected with ethical approval. MCT8 mRNA was detected in the fetal cerebral cortex from as early as 7 weeks gestation and maintained at a similar level through into adulthood.

We transiently transfected N-TERA-2 (NT2) cells (a human embryonic cell line with characteristics of CNS precursor cells) with either wild-type MCT8 or its Leu471Pro mutation, as found in a local patient with severe psychomotor impairment. Immunofluorescence analysis revealed differential localisation of WT and mutant proteins, the mutant mainly being retained intracellularly and WT showing more intense cell-surface expression.

NT2 cells were also grown in T<sub>3</sub> deplete media. Over-expression of both WT and mutant MCT8, alone or in combination, reduced proliferation in the absence of T<sub>3</sub> ( $P < 0.05$  for WT MCT8 transfection,  $P < 0.01$  for mutant or combined transfections). Addition of 10 nM T<sub>3</sub> had a pro-proliferative effect in control cells (8.3% increase,  $P < 0.001$ ,  $N = 36$ ), which was also observed in cells transfected with WT MCT8. However, over-expression of mutant MCT8, alone or in combination with WT, suppressed proliferation in the presence of T<sub>3</sub> (9.8% decrease,  $P < 0.01$ ), indicating a potential alternative role for MCT8 in addition to T<sub>3</sub> transport. We therefore propose that disruption of MCT8 fundamentally alters the proliferation of fetal neural cells in the developing brain.

1. Friesema *et al.*, Lancet, 2004.

#### OC63

##### Gender-specific differences in the expression of deiodinase I (Dio1) in mammals

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Selenium (Se) represents an essential trace element and acts as catalytic entity in thyroid hormone deiodinases (Dio), glutathione peroxidases or thioredoxin reductases. Se-deficiencies worsen a variety of conditions of which some display a sex-bias, including certain forms of cancer, autoimmune- and infectious diseases. Using C57Bl/6 mice as model

organism, we determined enzymatic activities and transcript levels of certain selenoproteins in various tissues. mRNA levels of the Se transporter (SePP) were higher in female liver, but lower in kidney compared to male littermates, but serum Se levels were comparable. Dio1 activities of female mice were lower in liver (to 60% of male values), but higher in kidney ( $14.2 \pm 3.4$  versus  $7.7 \pm 2.1$  pmol/(mg\*min)). Corresponding Dio1 transcript levels were similarly different in kidney of these mice (higher in females by 2-fold compared to male littermates). Unexpectedly, Dio1 transcript levels were also significantly higher in livers of females compared to male littermates (by 1.6-fold). This difference is mirror-image to the measured activities in livers, and argues for differential translational efficiencies of Dio1 mRNA between the sexes, or for different half-lives of the Dio1 protein. Since there are no data supporting the latter theory at present, and because of the similar Se serum levels, we assume that the female hepatic selenoprotein biosynthesis machinery translates Dio1 and SePP mRNA less efficiently.

Therefore, it is unfortunate for endocrinologists, neurologists and oncologists, that large-scale Se supplementation studies Se in the USA (e.g. SELECT, 180 Mio US\$ for 7–12 years and 32400 participants) focus only on male patients.

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## OC64

### Thyroid hormone receptor TR $\beta$ 1 mediates Akt activation by T3 in pancreatic $\beta$ cells

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#### Background

It has recently been recognized that thyroid hormones may rapidly generate biological responses by nongenomic mechanisms that are unaffected by inhibitors of transcription and translation. The signal transduction pathways for these effects are just beginning to be defined.

#### Aim

To examine the specific pathway via which T3 can activate Akt in pancreatic beta cells and to understand further the possible implication of a nongenomic action of T3 in these cellular systems.

#### Results

We demonstrated that thyroid hormone T3 rapidly induces phosphorylation of Akt *in vitro* in pancreatic beta cells rRINm5F and hCM via thyroid hormone receptor  $\beta$ 1. The T3-dependent phosphorylation of Akt was blocked by PI3K inhibitor and by the T3 analog BPA, indicating the effect to be T3 specific and dependent. Coimmunoprecipitation and colocalization experiments revealed that the PI3K p85 $\alpha$  subunit and the thyroid receptor  $\beta$ 1 were able to form a complex at the cytoplasmic level in both the cell lines, suggesting that a “cytoplasmic TR $\beta$ 1” was implicated. The silencing of TR $\beta$ 1 expression through RNAi confirmed this receptor to be crucial for the T3 induced activation of Akt. This action involved a T3-induced cytoplasmic recruitment of Akt and the consequent nuclear translocation of activated Akt as demonstrated by confocal immunofluorescence.

#### Conclusion

T3 is able to specifically activate Akt in the islet beta cell lines rRINm5F and hCM through the interaction of TR $\beta$ 1 and PI3Kp85 $\alpha$ , demonstrating the involvement of TR $\beta$ 1 in this novel T3-nongenomic action in islet beta cells.

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# Poster Presentations

## Bone

### P1

#### An audit of the role of Sestimibi imaging of the parathyroid glands in the management of parathyroid disease

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The requirement for Sestimibi imaging of the parathyroid glands has increased over recent years, partly due to advances in surgical techniques and the need for accurate pre-operative localisation. We audited sestimibi imaging to ensure efficient and appropriate use of this tool in the face of increasing demand. Over two and a half years 128 scans were identified by Nuclear Medicine. We obtained biochemistry and operative status for these using the hospital patient administration system. We examined the notes of a subgroup (68) to obtain more detailed information about the use of the scan in clinical decision-making. Only 70 (52%) of the scans were requested by endocrinology with the largest other users being Rheumatology and Nephrology, jointly requesting 41 (32%) scans. A total of 53 operations were identified, 43% of patients undergoing a scan. A total of 19 (15%) scans were requested with normal calcium of which 4 also had a normal parathyroid hormone (PTH). Those operated on were younger (57.58 years v 63.65 years,  $P = .03$ ) with higher calcium (2.97 mmol/L v 2.67 mmol/L,  $P < .001$ ). The requesting speciality also influenced the likelihood of operation (endocrinology 51%, Renal 45%, Rheumatology 19% and others 33%,  $P = .004$ ). The reasons for these potentially unhelpful scans were examined in our subgroup. These included 12 scans (18%) in patients with secondary hyperparathyroidism and 22 (32%) where a contraindication to surgery was already present. Overall 41 (63%) scans played no role in preoperative decision-making, a significant waste of resources. On this basis each year 30 scans are being performed inappropriately, as a test for isolated PTH or in patients for whom surgery is not appropriate or contraindicated. Scanning needs to be directed at those who require it to reduce delays in those that need surgery. We believe a dedicated parathyroid clinic, run jointly with an endocrine surgeon will achieve this.

### P2

#### Does adrenal function influence bone status in primary hyperparathyroidism? Preliminary results in a large series of patients at the time of the diagnosis

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PTH has been reported to enhance steroid secretion *in vitro* from human adrenocortical cells and some reports indicate an increased cortisol secretion in patients with PHPT that normalizes after surgery. Thus, the possible stimulatory effect of PTH on cortisol secretion could contribute to the impairment of bone status in primary hyperparathyroidism (PHPT). The aim of this study was to evaluate the relationship between parameters of adrenal function, bone metabolism and density as well as biochemical indices of PHPT in a group of consecutive patients at the time of the diagnosis, referred to us from 1993 through 2004. In 191 patients (age, mean  $\pm$  SD, 60.1  $\pm$  13.2 yrs; F/M 145/46, BMI: 25.8  $\pm$  4.8 kg/m<sup>2</sup>, symptomatic/asymptomatic: 91/100, PTH: 235.6  $\pm$  200.1 pg/ml, total Ca<sub>s</sub>: 11.2  $\pm$  0.09 mg/dl, Ca<sub>s</sub><sup>++</sup>: 1.5  $\pm$  0.01 mMol/l) morning and midnight ACTH and cortisol levels, urinary free cortisol (UFC), osteocalcin (OC), total and bone ALP, urinary cross-links (XL), DXA at lumbar spine, total femur and forearm were evaluated. Univariate analysis with Spearman rank correlation test was performed. Mean levels of morning and midnight ACTH and cortisol as well as of UFC were in the normal range. Bone markers were all increased; osteoporosis (T score  $<$  -2.5 DS) was diagnosed in 67.6% of PHPT patients, mostly at lumbar spine and forearm level. PTH negatively correlated with morning ACTH levels ( $P < 0.02$ ) while it did not correlate either with morning or midnight cortisol levels or UFC. Negative correlations ( $P < 0.05$ ) were found: (a) between morning cortisol and femoral BMD, (b) between midnight cortisol and radial as well as femoral BMD. The morning/midnight cortisol ratio showed positive correlation with radial T and Z scores, while negatively correlated with total Ca<sub>s</sub>. In conclusions, our findings indicate some relationship between ACTH and cortisol secretion and PTH/calcium levels in PHPT. They also show a clear association between cortisol levels and bone impairment, particularly at cortical bone. All together these findings suggest a potential role of cortisol secretion in the pathogenesis of bone impairment in PHPT.

### P3

#### The effect of local radiotherapy on osteoprotegerin levels in patients with bone metastases

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Pain due to bone metastases can be relieved by local radiotherapy (RT). The mechanism of pain relief by RT has not been fully elucidated. Inhibition of bone resorption has been proposed as a possible mechanism. Osteoprotegerin (OPG) has been shown to block behaviours indicative of pain in mice with bone metastases and to diminish bone destruction (Honore *et al.*, 2000, *Nature Medicine* 6; 521-8).

The aim of this study was to evaluate serum OPG levels in patients with metastatic bone pain following RT. The hypothesis is that an increase in OPG production following RT might be responsible for inhibition of bone resorption and thus of pain relief.

Sixteen patients with bone metastases were studied after obtaining an ethical committee approval. Blood was collected at baseline (prior to RT), and weekly for 4 weeks after RT for measurement of OPG using ELISA Kits (Biomedica Gruppe). As a bone resorption marker, C-terminal cross-linked telopeptide of type I collagen (CTX) was also measured. Similarly, pain assessment scores were recorded (McGill Pain Questionnaire and Visual Analogue Scale).

Thirteen patients (81%) had pain relief 4 weeks after RT (responders), while 3 (19%) were non-responders. There was no significant difference in mean OPG levels between pre RT and 1, 2, 3 and 4 weeks post RT in both responders (103.7  $\pm$  13.6, 114.1  $\pm$  17.2, 105.7  $\pm$  17, 114.7  $\pm$  21.7, 110.2  $\pm$  17.9 pg/ml) ( $P > 0.05$  for all intervals), and non-responders (113.3  $\pm$  20.67; 115  $\pm$  42; 105.7  $\pm$  18.1, 93.5  $\pm$  7.5 and 84.5  $\pm$  9.5 pg/ml) respectively. Likewise, there was no significant change in mean CTX level before and after RT in both responders and non-responders.

In conclusion, there was no correlation between circulating OPG levels and pain relief following RT.

### P4

#### Clinical aspects of diabetic osteoarthropathy in type 1 and type 2 diabetes mellitus patients

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#### Aims

The clinical and radiological observation of patients with diabetic osteoarthropathy (DOAP) was carried out with the aim of determining the most significant factors in type 1 and type 2 diabetes mellitus (DM) patients. Methods and materials

Anamnesis, clinical and radiological data were analysed in 28 DOAP patients. The patients were divided into two groups according to the type of diabetes: group 1 included 14 patients with type 1 DM (mean age 37.8  $\pm$  8.6 years; disease duration 21.3  $\pm$  11.1 years) and group 2 included 14 patients with type 2 DM (mean age 56.2  $\pm$  5.9 years; disease duration 11.9  $\pm$  9.16 years).

#### Results

Almost 90% of the effected patients were of working age, which is an indication of the socioeconomic consequences of DOAP. 20 patients had a painless foot trauma, 16 patients had a claw toes, 14 patients had neuroparic ulcers, 13 patients had a long loading, 3 patients had minor amputations in anamnesis. 7 patients had different foot deformations before DOAP developing. NDS had not a statistically difference between two groups. Bone density was reduced in type 1 DM (T-score (L2-L4): -1.11  $\pm$  0.59; hip: -1.93  $\pm$  0.83) compared with type 2 DM (T-score (L2-L4): -0.38  $\pm$  2.95; hip: 0.17  $\pm$  3.87;  $P < 0.001$ ). Overweight had a statistically difference ( $P < 0.001$ ) in DM 2 (BMI 31.3  $\pm$  4.9) in comparison with DM1 (25.7  $\pm$  3.04).

#### Conclusions

Our results indicate that foot trauma, claw toes wearing, high mean NDS, duration DM more than 10 years are possible risk factors for DOAP. For type 1 DM patients with DOAP more specifically risk factor is reduced bone mineral density. Overweight is a possible risk factor for the development of orthoarthropathic lesions, in particular in type 2 DM.

## P5

**Osteoporotic fractures in old subjects: role of functional and nutritional status and therapy effects during one year after fracture**F Magri, V Valdes, V Guazzoni, M Cupri, L Chiovato & E Ferrari  
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In elderly subjects the mortality rate after hip fractures ranges from 18 to 33% within one year; among survivors, 40% did not return to the previous level of functional ability. The aim of this study was to evaluate the longitudinal changes of some markers of bone metabolism in relation to functional and nutritional status.

Thirty-seven subjects aged more 70 years with a new vertebral or hip fracture were admitted to the study. Thirty age-matched and not fractured subjects hospitalized for rehabilitation purpose were considered as controls. Fractured subjects were treated with bisphosphonate, calcium and vitamin D, while kinesic therapy was performed in both groups. All subjects underwent cognitive, functional and nutritional assessment and biochemical evaluation (nutritional and bone metabolism parameters). Bone mineral density was measured by calcaneal quantitative ultrasonography.

All measures were repeated 3, 6 and 12 months later.

A very high percentage of osteoporosis and of vitamin D deficiency was detected in both groups (more than 80%). Serum levels of BAP and NTX were higher in fractured subjects than in control group and showed a significant trend towards the recovery in the later phase of follow up (from 6 to 12 months).

In fractured patients the Mini Nutritional Assessment was indicative of malnutrition, but showed a significant improvement during follow up. Both T score and serum vitamin D levels were significantly linked to nutritional status, mood and functional abilities.

Vitamin D deficiency seems to be one of the most important factors involved in osteoporotic fractures. A reduction in functional abilities may play an additive role.

Even in very old subjects treatment with bisphosphonates and calcium and vitamin D supplementation allow a successful osteoporotic fracture recovery.

## P6

**Circulating osteoprotegerin and receptor activator of NF- $\kappa$ B ligand system in patients with beta-thalassemia major**

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**Objectives**

To characterize the possible role of the osteoprotegerin (OPG) and receptor activator of NF- $\kappa$ B ligand (RANKL) system in thalassemia-related bone loss.

**Background**

Osteoporosis represents an important cause of morbidity in patients with  $\beta$ -thalassemia major and its aetiology is multifactorial.

**Methods**

Serum concentrations of OPG, RANKL, markers of bone turnover and lumbar spine bone mineral density (BMD) were measured in random samples of males ( $n = 31$ , mean age 24.4 years, range 13–41) and females ( $n = 35$ , mean age 24.3 years, range 12–35) with  $\beta$ -thalassemia major.

**Results**

There was a significant difference between males and females concerning ferritin levels ( $1873 \pm 214$  ng/ml vs.  $3384 \pm 346$  ng/ml, respectively,  $P < 0.05$ ) and RANKL levels ( $0.5 \pm 0.077$  pmol/l vs  $0.21 \pm 0.042$  pmol/l,  $P < 0.05$ ). Age correlated positively with OPG/RANKL ratio ( $r = 0.38$ ,  $P < 0.05$ ) and negatively with RANKL ( $r = -0.3$ ,  $P < 0.05$ ). Moreover, osteoprotegerin positively correlated with the duration of the interval between the onset of transfusions and chelation therapy ( $r = 0.26$ ,  $P < 0.05$ ). Regarding markers of bone metabolism, plasma values of osteocalcin (OC) correlated positively with RANKL ( $r = 0.43$ ,  $P > 0.05$ ) and negatively with OPG/RANKL ratio ( $r = -0.47$ ,  $P < 0.05$ ). RANKL correlated negatively with age ( $r = -0.68$ ,  $P < 0.001$ ) and ferritin ( $r = -0.60$ ,  $P < 0.001$ ) while OPG correlated positively with ferritin ( $r = 0.36$ ,  $P < 0.05$ ).

**Conclusions**

Although OPG/RANKL system may have some clinical usefulness as a marker of bone turnover in  $\beta$ -thalassemia, only NTX significantly accounted for BMD in multiple regression analysis.

## P7

**The role of genetic individuality and phytoestrogens with minerals in prevention and treatment of Postmenopausal osteoporosis**

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<sup>1</sup>University of Tartu, Tartu, Estonia, Estonia; <sup>2</sup>Tallinn University, Tallinn, Estonia, Estonia.**Aim of the study**

Our study was designed to determine the possible role of phytoestrogens with minerals Ca, Mg, Zn and vitamins D, C, K in the reduction of bone mass lost in postmenopausal women.

**Subjects and methods**

The study population consisted of 86 nonsmoking 60...80 years old women. Their bone mineral density (BMD) was measured by dual energy X-ray absorptiometry (DXA) region L2-L4. The osteoporosis had been diagnosed (T-score by DXA was  $-2.5... -3.2$ ) in all patients. Their diet history, especially daily intake of milk and dairy products was ascertained by questionnaire and analyzed the average daily intake of Ca and Mg. Minerals and isoflavones in 1 tabl. Bonette Comp: soya isoflavones (20 mg), Ca (100 mg), Mg (50 mg), Zn (3 mg), C (12 mg), D (1 mcg), K (14 mcg). VDR gene polymorphism was tested for Ff, ff and FF genotype.

**Results**

After 24 months of treatment we observed the positive changes in BMD from  $+3.2$  to  $+11.8\%$ . The average improvement was  $+8.2\%$ . Before starting the supplementation, an individual variations in the change of BMD were marked, depending on:

- the initial BMD data
- the doses of phytoestrogens
- minerals on supplementation
- VDR-genotype

The best results we observed by 36% of patients (positive changes from  $+7\%...+11.8\%$ ). This group of patients had a good nutritional status and their gastrointestinal tract functioned normally. Appearing of good results in this group can be related with findings that gastrointestinal tract plays a crucial role in the bioavailability and therefore clinical effectiveness of soya isoflavones (K. D. Setchell, 2003). Better benefits effects we tested in patients with VDR-genotype FF.

**Conclusion**

Complex of phytoestrogens and minerals (Bonette comp) have been proven effective in the reduction of postmenopausal osteoporosis. An individual variability was found in positive changes of the BMD. These data support the observation that there is substantial individual variability in isoflavone metabolism and in VDR genotype. In the future studies, a VDR genotype could be tested as potential genetic marker for increased risk of postmenopausal osteoporosis.

## P8

**Is there any combined influence of the N363S polymorphism in the glucocorticoid receptor gene and the BsmI polymorphism in the vitamin D receptor gene on the occurrence of glucocorticoid-induced osteoporosis in patients with bronchial asthma?**U Tworowska<sup>1</sup>, B Bidzinska-Speichert<sup>1</sup>, A Obojski<sup>2</sup>, M Demissie<sup>1</sup>, JW Koper<sup>3</sup> & R Slezak<sup>4</sup>

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Osteoporosis has a strong genetic component and is a common side-effect in patients with long-term glucocorticoid (GC) treatment. Vitamin D plays an important role in bone metabolism, and polymorphisms in its receptor (VDR) gene have been implicated in the pathogenesis of osteoporosis. On the other hand, variability in the sensitivity to GCs, due to polymorphic variants of the glucocorticoid receptor (GR) gene was observed in patients treated with GCs, both with regard to the efficacy of treatment and to the prevalence of side effects. The aim of the study was to analyze the combined influence of polymorphisms in the VDR and GR genes in determining the susceptibility to osteoporosis. Materials and methods: the following groups were studied: 1. asthmatic patients, divided into the subgroups: SS – patients treated with systemic GC ( $n = 38$ , 27 women), IS – patients treated with inhaled GC ( $n = 34$ , 29 women), NS – patients treated with other drugs than GC ( $n = 13$ , 9 women), 2. controls, healthy volunteers ( $n = 31$ , 17 women). BMD was measured using the DXA method. Serum osteocalcin and ICTP were measured using IRMA and RIA methods respectively. GR gene genotypes were determined using custom



designed Allelic Discrimination Assays and VDR genotypes were determined by PCR followed by enzymatic digestion of the products using the BsmI restriction enzyme. Results: Although heterozygotic carriers of the 363S allele showed a tendency towards a lower BMD in the lumbar spine, neither the GR N363S, nor VDR BsmI restriction fragment length polymorphism was associated with BMD or any of the bone-related serum measurements. We identified no significant gene-to-gene interaction effect for the VDR locus and GR N363S polymorphisms, which could impact BMD levels. Conclusions: Neither of these polymorphisms separately, nor their co-occurrence seems to be a marker of glucocorticoid – induced osteoporosis risk, however this should be verified in a larger, population – based study.

The study protocol was approved by Ethical Committee of Wrocław Medical University.

## P9

### Bone mineral density in patients with type 1 diabetes

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#### Background

Type1 diabetes is associated with reduction in Bone mineral density (BMD). It is thought, that metabolic abnormalities associated with type 1 diabetes alter the BMD, but the pathologic mechanisms of reduced bone mass are not fully understood.

#### Objective

The aim of our study was to reveal whether the metabolic control affects bone mass in patients with type 1 diabetes.

#### Materials and methods

We studied 80 patients (45 -male, 35 -female) with type1 diabetes. The inclusion criteria were duration of diabetes 20–25 years.

We measured HbA<sub>1c</sub>, ionized calcium, phosphorus values, parathyroid hormone (PTH), osteocalcin (OC), BMD with ultrasound bone densitometry.

#### Results

The mean value of HbA<sub>1c</sub> was 6.8 mcm/frghb, the mean value of BMD was (-2.1 ± 0.01), Ca++(1.05 ± 0.01 mmol/l) and OC (6.1 ± 0.02 ng/ml) values were near to the lower norm, PTH was at the upper level of the norm (58.3 ± 0.01 pg/ml). We revealed the linear correlation between the BMD and HbA<sub>1c</sub> levels ( $r = 0.42$ ,  $P < 0.05$ ) and the duration of disease ( $r = 0.45$ ,  $P < 0.01$ ).

#### Conclusions

Duration of diabetes and poor metabolic control were the main determinants affecting bone mass. Optimization of metabolic control is required to prevent osteoporosis.

## P10

### Thyroid hormone receptor alpha has a critical negative role in maintenance of the adult skeleton

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In developmental studies of mice lacking T3-receptor alpha (TRa<sup>0/0</sup>) and beta (TRb<sup>-/-</sup>) we demonstrated delayed endochondral ossification, reduced mineralisation and short stature in TRa<sup>0/0</sup> mice, despite euthyroidism. In contrast, TRb<sup>-/-</sup> mice, which display thyroid hormone resistance with elevated T4 and T3 levels, have advanced ossification, increased mineralisation and accelerated growth. T3-target gene studies indicate that TRa<sup>0/0</sup> mice have skeletal hypothyroidism, whereas TRb<sup>-/-</sup> bone is thyrotoxic. To investigate whether these abnormalities influence the adult skeleton, we studied euthyroid and T4-treated 22 week-old TRa<sup>0/0</sup> and TRb<sup>-/-</sup> mice. There was no difference in bone length between WT, TRa<sup>0/0</sup> and TRa<sup>0/0</sup>(+T4) mice, whereas TRb<sup>-/-</sup> and TRb<sup>-/-</sup>(+T4) bones were shorter by 5% and 4%, respectively ( $P < 0.01$ ). Histology revealed an 11% increase in cortical thickness in

TRa<sup>0/0</sup> mice but 16% reduction in TRb<sup>-/-</sup>. T4 treatment reduced cortical thickness by 27% in TRa<sup>0/0</sup> mice ( $P < 0.05$ ) and 29% in TRb<sup>-/-</sup> ( $P < 0.001$ ). Bone micro-architecture and micro-mineralisation densities, analysed by quantitative backscattered electron scanning electron microscopy, demonstrated a 2.8-fold increase in trabecular bone volume (BVf) in TRa<sup>0/0</sup> mice ( $P < 0.01$ ) and increased trabecular thickness and complexity. Extension of trabecular bone into the diaphysis increased from 10% of total bone length in WT to 29% in TRa<sup>0/0</sup> ( $P < 0.01$ ). T4-treatment reduced trabecular thickness and complexity and BVf by 42% ( $P < 0.05$ ), and trabecular bone extent by 20% ( $P < 0.05$ ) in TRa<sup>0/0</sup> mice. In contrast, all trabecular bone parameters were reduced in TRb<sup>-/-</sup> mice and unaffected by T4-treatment. Quantitative micro-mineralisation measurements were similar in TRa<sup>0/0</sup> and WT animals, whereas TRb<sup>-/-</sup> mice had reduced trabecular and cortical mineral concentration ( $P < 0.001$ ). Thus, adult TRa<sup>0/0</sup> mice have increased bone of normal mineralisation density, whilst TRb<sup>-/-</sup> mice are osteoporotic. These data demonstrate that TRalpha plays critical roles in skeletal development, and in the establishment and maintenance of adult bone structure. TRb can partially compensate for TRa in TRa<sup>0/0</sup> mice.

## P11

### PTH and phosphate circadian rhythms are altered in adult growth hormone deficient patients with low bone mineral density

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Adult Growth Hormone Deficiency (AGHD) is associated with osteoporosis. PTH is secreted in a circadian rhythm and temporal fluctuations in PTH concentration, particularly at night, appear to be important in the regulation of bone turnover. Serum phosphate is an important determinant of PTH, with changes in phosphate preceding fluctuations in PTH concentration. We examined the difference in PTH and phosphate circadian rhythmicity in AGHD patients with normal and reduced bone mineral density (BMD).

Forty-three AGHD patients were consented to the study. Twenty-three patients had low BMD (femoral neck or lumbar spine T-score  $< -1.0$ ). Twenty patients had normal BMD. All patients were admitted to hospital for 24h, where 1/2-hourly blood samples were collected for PTH and phosphate. The local ethics committee approved the study. Results are expressed as mean ± SEM.

Each patient and both groups demonstrated significant PTH and phosphate circadian rhythms, as determined by cosinor analysis ( $P < 0.001$ ). The low BMD patients had significantly lower mean nocturnal rise in both PTH ( $4.9 \pm 1.9\%$  versus  $19.5 \pm 3.1\%$ ,  $P < 0.001$ ) and phosphate ( $1.9 \pm 1.3\%$  versus  $9.8 \pm 1.2\%$ ,  $P < 0.001$ ) concentrations, than the normal BMD patients. Low BMD in AGHD is associated with a blunted nocturnal rise in PTH concentration, which may occur as a result of the blunted nocturnal rise in serum phosphate concentration. Therapeutic manipulation of the PTH or phosphate circadian rhythm may be of benefit in improving BMD in patients with AGHD.

## P12

### Oral phosphate therapy used as an adjunct to growth hormone in adult growth hormone deficiency results in greater changes in bone mineral density compared with growth hormone replacement alone

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Adult Growth Hormone Deficiency (AGHD) is associated with reduced bone mineral density (BMD) and bone turnover. Abnormalities in PTH circadian rhythmicity, including blunted nocturnal rise in PTH concentration have been reported in AGHD and may underlie the pathogenesis of osteoporosis. Serum phosphate is an important regulator of PTH, with changes in phosphate concentration preceding fluctuations in PTH. We examined the effect of oral phosphate therapy on PTH circadian rhythmicity, bone turnover and BMD.



Seventeen AGHD patients were consented to the study. Patients were randomised to receive either growth hormone replacement (GHR) alone or GHR plus 1g phosphate-sandoz administered at 2200h. Patients were admitted to hospital for 24 h before and 12 months after initiation of therapy where 1/2-hourly blood samples were collected for PTH, phosphate, CTX (marker of bone resorption) and PINP (marker of bone formation). BMD was measured at baseline and after 12 months. The local ethics committee approved the study. Results are expressed as mean  $\pm$  SEM.

Each patient and both groups had significant PTH and phosphate circadian rhythms at both visits ( $P < 0.001$ ). There was no significant difference in any measured marker at baseline between the groups. The patients receiving oral phosphate therapy had significantly greater increases in mean nocturnal rise in PTH ( $21.4 \pm 1.8\%$  versus  $17.5 \pm 1.6\%$ ,  $P < 0.001$ ) and phosphate ( $15.4 \pm 1.2\%$  versus  $6.3 \pm 1.4\%$ ,  $P < 0.001$ ) at 12 months, compared with patients receiving GHR alone. The percentage increase in bone turnover markers and BMD was significantly higher in patients receiving phosphate (CTX  $79.7 \pm 7.3\%$  versus  $56.4 \pm 6.9\%$ ; PINP  $121.3 \pm 10.4\%$  versus  $93.4 \pm 8.9\%$ ; femoral BMD  $6.8 \pm 0.9\%$  versus  $1.9 \pm 0.8\%$ ; lumbar BMD  $7.1 \pm 1.6\%$  versus  $1.8 \pm 1.2\%$ , all  $P < 0.001$ ).

Oral phosphate therapy appears to potentiate the effect of GHR on bone turnover and BMD. Phosphate therapy administered at 2200h results in a greater nocturnal rise in PTH concentration, which may be important for the stimulation of bone turnover and increase in BMD.

### P13

**Polymorphism in farnesyl pirofosfato sintasi (FDPS) gene and relation with bone mineral density and response to aminobisfosfonate treatment** S Silvestri<sup>1</sup>, S Carbonelli<sup>1</sup>, AB Thomsen<sup>2</sup>, Y Bagger<sup>2</sup>, C Christiansen<sup>2</sup> & ML Brandi<sup>1</sup>

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#### Introduction

Amino-Bisphosphonates (N-BPs) are potent inhibitors of the bone resorption, they inhibit enzymes of the intracellular mevalonate pathway required for prenylation of different important signaling proteins. In order to study the genetic basis of the variability in therapy response we have analysed the polymorphism of farnesyl pyrophosphate-synthase (FDPS) gene in 234 postmenopausal Danish women treated with N-BPs for 1 and 2 years.

#### Methods

The polymorphism of the FDPS was analysed in peripheral blood samples from 234 women, who previously completed treatment with N-BPs for 1 or 2 years in different randomised clinical trials. The PCR product was digested with endonuclease and the digestion products were separated on agarose gel depending on their length, revealing the presence or absence of restriction site. All women had baseline measurements of BMD of spine, femur and distal arm. BMD was followed annually. Bone markers were measured at 6, 12, and 24 months.

#### Results

The genotype distribution followed the Hardy-Weinberger equilibrium. There was no difference in baseline BMD of spine, femur nor arm with the A/C polymorphism. The BMD response to treatment with N-BPs was similar for A/A and A/c genotypes while there was a tendency (borderline significant) for the c/c genotype to have a lower response in BMD in all sites as well as in bone markers after 1 year of treatment. The c/c genotype had a significant ( $P < 0.05$ ) lower response in bone markers after 2 years treatment with N-BPs.

#### Conclusions

The polymorphism of the FDPS gene didn't show relation to baseline BMD in Danish postmenopausal women. There was a tendency that c/c genotype had a lower response to treatment with N-BPs in all skeletal sites examined, confirmed from a significant lower response in bone markers after 2 years of treatment. Even if further studies including larger populations are needed, the prospect of genotyping aiding in the selection of specific treatments for each patient is tempting.

### P14

**Do different generations of PTH assay induce different diagnosis in renal bone disease?**

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#### Background and Aim

Parathyroid hormone (PTH) measurement is critical to evaluate bone status in patients with chronic renal failure as adynamic bone (AB) and bone with a high turnover (HTB) require different therapeutic options. Since the 2nd

generation PTH assays recognise a non 1-84 PTH fragment in addition to the intact 1-84 PTH, a new assay defined as 3rd generation has been commercialised. The aim of this study was to evaluate, in dialysed patients, the rate of false classification of the bone status by using a 2nd generation assay hypothesising that a 3rd generation assay is a gold standard.

#### Patients and methods

120 newly hemodialysed patients (45F 75M, 59 yr old) were recruited during 2 m. PTH was measured by (i) our routine Ntact PTH SP IRMA (DiaSorin) and (ii) duo PTH (Schering/Scantibodies Lab.) which contains 2 PTH assays for total PTH and 1-84 PTH (the active form of PTH also labelled CAP). A ratio CAP/([total PTH] minus [CAP])(i.e. CAP/CIP)  $< 1.3$  is indicative of AB.

#### Results

Among patients with a suspected HTB ([NtactPTH]  $> 300$  pg/ml; 42/120) 66% had a ratio  $< 1.3$  suggesting AB (false negative AB). Among patients with a suspected AB ([NtactPTH]  $< 100$  pg/ml; 27/120) 30% had a ratio  $> 1.3$  suggesting HTB. However, all of these patients had [CAP]  $< 70$  pg/ml suggesting that the elevated ratio was due to an increase of CIP rather than an increase in CAP, the active form of PTH (i.e. no false negative HTB). Among patients with intermediate bone status ( $100 < [NtactPTH] < 300$  pg/ml; 51/120) 43% had a ratio  $> 1.3$ . Of the latter, 27% had [CAP]  $< 100$  pg/ml eliminating a HTB diagnosis.

#### Conclusion

From the data of these unselected patients, it is clear that different generation assays do not classify similarly the patients. One could limit bone biopsy indication to patients with equivocal data.

### P15

**Management of primary hyperparathyroidism – are we following consensus guidelines?**

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To determine our adherence to the Consensus Development Conference on the Management of Asymptomatic Primary Hyperparathyroidism (2002) guidelines, we audited the management of all patients referred to our endocrine unit with the diagnosis of primary hyperparathyroidism during January 2003 to December 2004.

Overall 36 patients were referred. 32 were females and the mean age was 64 years. 19 patients were Caucasians, 8 were Asians and 9 patients were of Afro-Caribbean origin. Mean corrected calcium was 2.84 mmol/L (normal range 2.2–2.6 mmol/L). Mean PTH level was 192 pg/L (normal range 10–65 pg/L). Parathyroidectomy was indicated in 5 patients, criteria being increasing calcium levels in 4 patients and development of symptoms related to hyperparathyroidism in 1 patient. Subsequently they were all referred for parathyroidectomy and 4 patients had their operation done.

At the initial assessment, all patients had their serum calcium, PTH and creatinine checked, 6/36 (16%) patients had their 24 hour urine calcium estimated, 16/36 (44%) had abdominal imaging done and 19/36 (52%) had their bone mineral (BMD) density measured at the hip and spine. None had their BMD measured at the radius. All patients were followed every 6 months and had their calcium and creatinine levels checked every 6 months according to guidelines. None had their bone density measured annually.

In conclusion, more emphasis is needed in measuring bone mineral density both at the initial assessment and annually thereafter (including at the radius). Only 44% of our patients had abdominal imaging (KUB or US) done, with the potential of missing asymptomatic renal calculi.

### P16

**Value of qualitative bone histology assessment in the evaluation of subjects with primary osteoporosis**

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During the past thirty years bone biopsy has been used as an invasive diagnostic and research investigation of bone structure and metabolism. Bone biopsy parameters are used to assess both bone mass and bone quality.

This study aimed to establish the value of routine qualitative bone biopsy evaluation in subjects with unclear primary osteoporosis. One-hundred seventy patients (73 men and 97 women), aged  $54.29 \pm 0.95$  years, were included in the study. The diagnosis was based on clinical data, lumbar spine and hip dual X-ray absorptiometry (DXA) evaluation and routine laboratory measurements. Bone biopsy was performed by horizontal approach, using an electric drill. Of the 170 bone samples, secondary causes of low bone mineral density were identified in 19 patients (mastocytosis, multiple myeloma, myeloproliferative syndrome, sarcoidosis and osteomalacia). Excessive unmineralized osteoid without reduced bone mass was seen in 14 patients. Normal bone histology was obtained in 21 subjects with low bone mineral density. Accelerated bone resorption, as expressed by the daily urinary levels of deoxypyridinoline (D-Pyr), and long-term sodium fluoride therapy were associated with delayed osteoid mineralization. Bone biopsy changes, as assessed by qualitative evaluation, were not related to serum thyroid hormone, parathyroid hormone or 25-hydroxyvitaminD<sub>3</sub> levels. In conclusion, qualitative bone biopsy evaluation may offer valuable information in the diagnosis of metabolic bone diseases in subjects with unexplained causes of low bone mineral density or in non-responders to anti-fracture agents.

### P17

#### Hearing impairment increases risk of bone fractures in women with Turner's syndrome

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#### Aims

To assess factors associated with excess rates of fractures in women with Turner's syndrome.

#### Settings

Adult Turner Clinic.

#### Outcome measures

Bone fracture history, oestrogen, growth hormone, oxandrolone and thyroxine therapies, anthropometry, calcium, vitamin D, parathyroid hormone and karyotype, hearing impairment, spinal bone mineral density (BMD). This study was approved by Ethical Committee.

#### Results

One hundred and seventy-seven consecutive women with Turner's syndrome, aged 19–60 years, were interviewed with respect to bone fracture history, BMD and hearing information were recorded from medical notes. Karyotype was available in 94% of patients (55% monosomy, 45% mosaicism). Subjects had a mean (SD) height of 1.47 (0.07) m and BMI 25.8 (5.2) kg/m<sup>2</sup>. The prevalence of fractures was 32% and hearing impairment 84.2% (17.5% conductive and 66.7% sensorineural, of whom 32% and 16% used hearing aid, respectively). BMD T score was below -1 in 55% of women and below -2.5 in 9%. More subjects with a combination of conductive hearing impairment and low BMD (57%) had a fracture than those with normal hearing and high BMD (25%) ( $P < 0.05$ ). Multivariate logistic regression analysis (adjusted for age and karyotype) showed BMD (OR 3.2, 95% CI: 1.0–10.5) and hearing impairment (conductive: OR 4.8, 95% CI: 1.2–18.9, sensorineural: OR 3.6, 95% CI: 1.1–11.8) were independently associated with increased risk of fractures. Subgroup analysis showed that hearing impairment was associated with fractures only in those with low BMD (OR 9.0, 95% CI: 1.1–73.4). Further adjustments for height, calcium levels, vitamin D supplementation, thyroxin use and oestrogen deficiency, previous use of oxandrolone or growth hormone did not alter these relationships.

#### Conclusions

Women with Turner's syndrome who have low BMD and hearing impairment, particularly conductive type, are at increased risk of bone fractures. Improvement of BMD and hearing ability may help reduce their fracture risk.

### P18

#### Evaluation of bone mineral density and markers of bone turnover in haemodialysis patients

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Recently, since the lives of patients with chronic renal failure have prolonged considerably, the importance of prevention and treatment of metabolic bone disease which inevitably develops in this group, has become better appreciated.

The aim of this study was to evaluate the relation between bone mineral density (BMD) and specific markers of bone turnover in haemodialysis (HD) population.

A cross-sectional study involving 40 HD patients (27 M, mean age  $54 \pm 14$  yrs, and 13 F mean age  $58 \pm 9$  yrs) with no history of bone fractures was performed. Mean period on HD was  $68 \pm 43$  months. Serum levels of osteocalcin, beta-CrossLaps, iPTH and alkaline phosphatase (ALP) were measured before the dialysis session. BMD was estimated in the lumbar spine and in the hip using Dual Energy X-ray Absorptiometry (DXA). Low BMD was found in 67.5% (27/40) of haemodialysis patients. Diagnostic criteria of osteoporosis were fulfilled by 32.5% of subjects, more frequently in women (38.5%) than in men (29.6%). Osteopenia was observed in 35% of patients and was also more frequent in women (42.2%) than in men (29.6%). Mean serum levels of ALP were significantly higher in women than in men ( $135.6 \pm 54$  mIU/mL and  $86 \pm 33$  mIU/mL respectively).

We also observed a positive correlation between serum  $\beta$ -CrossLaps concentrations and PTH (in men  $P < 0.001$ ,  $r = 0.66$ ; in women  $P < 0.006$ ,  $r = 0.71$ ) and between serum osteocalcin levels and PTH (in men  $P < 0.02$ ,  $r = 0.44$ ; in women  $P < 0.01$ ,  $r = 0.65$ ).

ALP levels correlated positively with serum  $\beta$ -CrossLaps concentrations in women  $P < 0.04$ ,  $r = 0.60$ .

#### Conclusions

The frequency of low bone mineral density in haemodialysis patients is high. Low bone density is more common in chronic dialysis women. The levels of bone turnover markers are mainly determined by the severity of hyperparathyroidism.

### P19

#### The effect of ceramide on IGF-1 induced proliferation in growth plate chondrocytes

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Abnormal growth patterns are commonly observed in children suffering from chronic inflammatory diseases. These disorders are associated with the increased production of pro-inflammatory cytokines, which inhibit growth plate chondrocyte dynamics. Ceramide, a sphingosine-based lipid second messenger, mediates many of the actions of pro-inflammatory cytokines. Ceramide inhibits IGF-1 signalling and induces apoptosis in numerous cell types. This study determined the effects of C2-ceramide (C2-c) (40  $\mu$ M, 25  $\mu$ M, 10  $\mu$ M), a cell permeable ceramide analogue, on murine growth plate chondrocytes, using the ATDC5 chondrogenic cell line and cultured fetal metatarsals. In ATDC5 cells, C2-c at 40  $\mu$ M and 25  $\mu$ M significantly induced apoptosis (63%;  $P < 0.05$  and  $x\%$ ) and significantly reduced proliferation (62%;  $P < 0.05$  and  $x\%$ ). C2-c at 40  $\mu$ M significantly reduced fetal metatarsal growth (62%;  $P < 0.05$ ). The effect of C2-c on IGF-1 induced proliferation was examined. In ATDC5 cells, in the presence of IGF-1 (24 h, 100 ng/ml), ceramide (25  $\mu$ M) induced a 68% reduction in proliferation ( $P < 0.001$ ). However, in the absence of IGF-1, ceramide induced a comparable 61% decrease ( $P < 0.001$ ). C2-c (40  $\mu$ M) induced the same 31% reduction in metatarsal growth both in the presence and absence of IGF-1 (8d, 100 ng/ml) (both  $P < 0.001$ ). Therefore, C2-c does not appear to specifically inhibit the pro-proliferative effects of exogenous IGF-1. The effect of C2-c on endogenous IGF-1 induced proliferation was also examined, using AG1024, an IGF-1R and IR blocker (10  $\mu$ M). In the absence of exogenous IGF-1, AG1024 significantly reduced proliferation (28%,  $P < 0.001$ ). C2-c (25  $\mu$ M) significantly reduced proliferation compared to AG1024 treatment (55%,  $P < 0.001$ ). C2-c and AG1024 in combination further reduced proliferation compared to C2-c alone (46%,  $P < 0.01$ ). Further studies are required to confirm whether the inhibitory effects of pro-inflammatory cytokines in growth plate chondrocytes are mediated through ceramide, influencing the growth of children with inflammatory diseases through a local effect at the growth plate.

**P20**

**Treatment and follow up of primary hyperparathyroidism: 50 cases**  
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We report a retrospective study of 50 cases of primary hyperparathyroidism (10 men and 40 women, mean age: 49 years) collected between 1975 and 2004. At the time of diagnosis, 44% of patients had bone disease, renal manifestations were present in 17 cases (34%).

Parathyroidectomy is a safe and effective approach of primary hyperparathyroidism treatment. The management of persistent or recurrent hyperparathyroidism is explained by the fact that parathyroid hyperplasia or carcinoma, ectopic or supernumerary parathyroid tissues are more common in this population. Ultrasonography was performed in 36 cases, it correctly located tumors in normal position in 24 case (66%).

42 patients underwent parathyroidectomy. Surgeons performed unilateral exploration guided by preoperative localization, with contralateral exploration only if hyperplasia is identified.

In 37 cases, only one enlarged gland was removed (36 parathyroid adenomas and a case of parathyroid carcinoma). In 4 patients suspected of having hyperplasia, removal of all enlarged glands (2 in each case) was made. No serious complications of surgery are noted. All symptoms were improved and serum PTH returned to the normal range in 40 successful resections. Persistent disease is noted in 2 unilateral explored cases. Although discordance in results of ultrasonography, magnetic resonance imaging and sestamibi scanning, lesion is located in the two cases of persistent disease. Successful reoperation is performed in one case.

The only opportunity for efficient cure of primary hyperparathyroidism is surgical removal of the abnormal gland or glands. The incidence of failed initial parathyroidectomy can be reduced if bilateral cervical exploration is performed by an experienced surgeon.

**P21**

**Is there any relationship between the BsmI and FokI polymorphisms in the vitamin D receptor gene and bone mineral density in men?**

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**Introduction**

Results of many studies indicate that the BsmI and FokI polymorphisms in the VDR gene may influence bone tissue metabolism and may be useful in means of identifying patients at greater risk of osteoporosis.

**Aim of the study**

To determine frequency of polymorphic variants of VDR gene (BsmI and FokI) and its relationship to phenotypic features characterizing bone status (BMD).

**Materials and methods**

176 randomly selected men, aged 25–65 years (mean 54.16) were studied. VDR gene genotypes were determined using PCR-RFLP method. BMD was measured using DXA method. The study protocol was approved by the local Ethical Committee.

**Results**

Genotype bb was found in 28.7%, BB in 11.4%, and bB in 59.9%. Genotype ff was found in 9.3%, FF in 30.6%, and fF in 60.1%. There was no significant differences regarding BMD between any of genotypes.

**Conclusions**

These data suggest that the VDR genotypes do not seem to be useful for identifying patients at greater risk of osteoporosis, however it awaits to be confirmed by a population-based study.

**P22**

**Mutational Analysis of the PHEX gene in familial and sporadic cases of X-linked hypophosphatemia (XLH)**

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Hypophosphatemic rickets is an X-linked dominant inherited bone disorder, characterized by renal phosphate wasting, inappropriately normal to low vitamin D serum levels and severe skeletal and dental defects from early childhood. Inactivating mutations in the PHEX gene (phosphate regulating

gene with homologies to endopeptidases on the X-chromosome) have been identified as the underlying cause, although the pathomechanism is unknown. The PHEX gene encodes a membrane-bound metalloprotease that is expressed mainly in bone and teeth, but not in the kidney.

We screened 31 patients with suspected XLH by direct sequencing of PCR amplified genomic DNA. In 17 patients (55%) 14 different inactivating mutations in the PHEX gene were identified. The mutations detected include missense, nonsense, splice site and frameshift mutations. In one family a deletion of exon 22 was found. Twelve of these mutations are novel mutations. Among our patients there were four families with hypophosphatemia with pedigrees of two or more consecutive generations, most cases, however, are sporadic. In one of the families, hypophosphatemic rickets was found in four generations. The two year old index patient showed deformities of the lower extremities and low serum phosphate levels which normalized under therapy (phosphate substitution, Vitamin D). Her mother needed surgical corrections of the lower extremities at the age of 13, while the grandmother showed a less severe phenotype. From the grandmother's father severe deformities and a complete loss of mobility are reported. Molecular genetic testing revealed a double mutation in the index patient: Two novel missense mutations were detected, the substitution of cysteine 59 by serine in exon 2 and replacement of alanine 363 by valine in exon 10.

In conclusion, confirmation of the diagnosis of hypophosphatemic rickets by molecular genetic testing has important implications for the clinical management of the patient and affected family members.

**P23**

**The reduction of bone mineral density in post-menopausal women with primary hyperparathyroidism is higher in the presence of concomitant GH hormone secretion impairment**

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Primary hyperparathyroidism (PHP) and growth hormone deficiency (GHD) are both associated with alterations of bone metabolism.

GH secretion is frequently impaired in PHP patients; thus GH/IGF-I system alterations could be involved in the pathogenesis of osteoporosis.

In the present study 50 post-menopausal women with PHP were evaluated by GH response to GH-releasing hormone (GHRH) + arginine (ARG) test and femoral neck bone mineral density (BMD, g/cm<sup>2</sup>) by dual energy X-ray absorptiometry. BMI, serum ionised calcium, PTH and markers of bone remodelling (serum osteocalcin (OC), serum bone-specific alkaline phosphatase (B-AP), serum cross-laps (S-CTX) were evaluated as well. Same data were collected in a group of 60 age-matched controls.

No difference was found between the two groups regarding age, age at menopause, BMI, serum ionised calcium, PTH and IGF-I concentrations.

GH secretion was reduced (mean GH response to GHRH + arg test: 8.8 ± 4.2 µg/liter) in 34 patients and normal (28.7 ± 11.8 µg/liter) in the remaining 16 (P < 0.05), in the control group peak GH was 33.8 ± 10.9 µg/liter.

Osteoporosis (T-score < -2.5) and osteopenia (T-score > -2.5 and < -1) were found in 73.5% and 17.6% of GHD patients, in 37.5% and 43.7% of patients with normal GH secretion and 3.1% and 27% of controls.

The prevalence of osteoporosis in GHD group was significantly different with respect to patients with normal GH secretion (P = 0.02) and to controls (P = 0.02).

T-score was not correlated with ionised calcium, nor with age, age at menopause or BMI, but was correlated with PTH serum levels in both groups and T-score was correlated with markers of bone remodelling only in GHD patients.

In conclusion, impairment of GH secretion could be involved as a pathogenetic factor in bone metabolism alterations of PHP.

**P24**

**Effect of melatonin on gene expression and mineralization in mouse osteoblast culture**

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The physiological and neuroendocrine functions of the pineal gland hormone, melatonin and its therapeutic potential critically depend on the understanding of its target sites and its mechanism of action. There is



evidence that melatonin promoted osteoblast differentiation and mineralization in MC3T3-E1 (mouse preosteoblast) and rat osteoblast-like osteosarcoma 17/2.8 cells. Our group set up an experimental model of mouse osteoblast culture. As a marker specific for osteoblast we used Cbfa1 expression. Cbfa1 is an osteoblast-specific transcription factor positively regulating osteoblast differentiation and function. Its expression and/or transcription activity are thus potential targets for bone anabolic therapies.

#### Objective

The aim of this study was to assess the effect of melatonin treatment on mouse osteoblast in culture by studying the Cbfa1 gene expression, the major regulator of the osteoblast phenotype, and induction of mineralization, expression of differentiated osteoblasts.

#### Material and methods

Mouse osteoblasts were seeded for RNA extraction or von Kossa specific staining for mineralization and treated with melatonin ( $10^{-11}$  –  $10^{-6}$  M). After incubation period cells were harvested and RNA extracted. 1 µg RNA was reverse transcribed to single stranded cDNA and then specifically amplified for Cbfa1. Cells were also stained specifically for alkaline phosphatase and mineralization.

#### Results

Melatonin reduced Cbfa1 expression in both experiments. As regards mineralization, groups treated with melatonin showed high mineralization in an early development stage when normally mineralization does not occur (as could be seen in control sample).

#### Conclusion

The von Kossa positive staining showed that melatonin promotes mineralization in osteoblasts in early development stages probably by its stimulatory effect on BSP, ALP and osteocalcin gene expression. The slight inhibition in Cbfa1 expression needs further studies in order to see if this effect is correlated with the precocious fully differentiation of osteoblasts (mineralizing cells).

## P25

### Effects of growth hormone administration on parathyroid hormone sensitivity and bone mineral metabolism in established osteoporosis

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#### Introduction

The beneficial effect of GH replacement on bone in AGHD patients is mediated by improvements in target organ sensitivity to PTH and phosphocalcium metabolism. In aging women with established osteoporosis, GH and IGF-1 concentrations are lower and administration of GH has been shown to increase bone turnover and BMD, but the mechanisms remain unclear.

#### Methods

Fourteen postmenopausal women ( $63.4 \pm 2.1$  years; mean BMD T score  $\pm$  SEM: lumbar spine (L2–L4)  $-3.3 \pm 0.2$ , femoral neck  $-2.0 \pm 0.2$  were commenced on daily subcutaneous GH. Patients were hospitalised for 24 hours before then 1 and 3 months following GHR. Half-hourly blood and 3-hourly urine samples were collected for PTH, calcium, phosphate, NcAMP (marker of renal PTH activity), CTX (bone resorption marker) and PINP (bone formation marker).  $1,25(\text{OH})_2\text{D}_3$  were measured on 0800 h fasting samples.

#### Results

IGF-1 concentrations increased following 1 and 3 months of GH ( $P < 0.001$ ). The 24-h mean PTH concentration decreased from  $5.41 \pm 0.08$  pmol/L to  $5.19 \pm 0.07$  pmol/L at 1 month and  $4.99 \pm 0.06$  pmol/L at 3 months ( $P < 0.001$ ) and the NcAMP increased from  $17.62 \pm 1.19$  nmol/LGFR to  $25.11 \pm 2.58$  nmol/LGFR ( $P < 0.05$ ) and  $28.62 \pm 1.39$  nmol/L GFR ( $P < 0.01$ ) at 1 and 3 months respectively. The 24-h mean adjusted serum calcium and phosphate concentration increased at 1 and 3 months. An increase in 24h UCa excretion was observed at 3 months ( $P < 0.05$ ) and the  $\text{TmPO}_4/\text{GFR}$  increased following 1 and 3 months ( $P < 0.001$ ).  $1,25(\text{OH})_2\text{D}_3$  concentration increased following 3 months of GH. CTX and PINP concentrations increased at 1 and 3 months ( $P < 0.001$ ) but the percentage increase in PINP was greater than CTX ( $P < 0.01$ ).

#### Conclusion

GH administration to aging osteoporotic women improves PTH sensitivity with increases in serum  $1,25(\text{OH})_2\text{D}_3$ , calcium and phosphate concentration as well as an increase in  $\text{TmPO}_4/\text{GFR}$ . These changes and a greater increase

in bone formation may explain the previously demonstrated increase in BMD following long term administration of GH in aging women with osteoporosis.

## P26

### Is 1 g of calcium and 800 IU of Vitamin D sufficient to replenish Vitamin D stores in postmenopausal women with low bone density?

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Calcium and Vitamin D deficiency is a common cause of falls and fracture and supplementation with 1 g of calcium and 800 IU of vitamin D is commonly recommended.

However, the serum level of 25 OHD that defines deficiency is controversial, and the 25 OHD level required to normalise serum parathyroid hormone (PTH)-in the presence of normal calcium- is often used. In this study we set out to explore how effective is 1 g of calcium and 800 IU of vitamin D in suppressing PTH in an unselected population of postmenopausal women with low bone density and normal serum calcium.

From the osteoporosis clinic computerised database 947 women had recorded values of serum PTH and 25 OHD as well as a biochemical profile including renal function and serum calcium levels. We excluded women with high serum calcium ( $>2.6$  mmol/L) or severe renal impairment (glomerular filtration rate  $<60$  mL/min/1.73 m<sup>2</sup>). A total of 723 women, all white Caucasian females were entered in the analysis.

Receiver Operating Characteristic (ROC) plotting showed that the lowest threshold value for plasma 25 OH vitamin D that prevents elevated PTH levels was 27.7 nmol/L (Sensitivity 90% Specificity 30%) Two-hundred-eleven women has normal serum calcium and raised PTH levels (29%) A total of 298 women were supplemented with 1000 mg of calcium and 800 IU of ergocalciferol, in spite of this 113 (53%) women still had unsuppressed PTH. Serum Vitamin D levels are not very reliable in identifying patients who are vitamin D deficient. Furthermore over 53% of patients in spite of recommended doses of Calcium and Vitamin D supplementation are still Vitamin D deficient. This may compromise skeletal health and explain some recent negative findings on the fracture preventing effect of calcium and vitamin D supplementation.

## P27

### Synergistic induction of osteoblastic local glucocorticoid metabolism by inflammatory cytokines and glucocorticoids: a novel mechanism for glucocorticoid-induced bone disease

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When used to treat inflammatory disease therapeutic glucocorticoids (GCs) cause rapid bone loss. However clinical studies suggest that in patients without inflammation GCs have little impact on the skeleton. The mechanism by which inflammation magnifies the effects of GCs is unknown. We have proposed that intracellular GC generation (inactive cortisone/prednisone to active cortisol/prednisolone conversion) via the 11 beta-hydroxysteroid dehydrogenase type 1 (11b-HSD1) enzyme determines the clinical effects of GCs on bone. In healthy subjects the effect of prednisolone on bone formation markers is predicted by measures of 11b-HSD1 activity. We have now examined the hypothesis that the modifying effect of inflammation is due to increased local GC generation in human osteoblasts. In MG63 osteosarcoma cells and primary osteoblasts IL-1 and TNF $\alpha$  potentially induced 11b-HSD1 expression and activity (100 to 200-fold) whereas DEX treatment only mildly increased expression (10 to 50-fold). However, when combined with IL-1 or TNF $\alpha$ , DEX synergistically induced 11b-HSD1 expression and activity (1500-fold). Similar induction was also seen in primary cultures of human fibroblasts from bone marrow and synovium. This effect was specific for 11b-HSD1 with DEX inhibiting the cytokine induction of other genes such as OPG and COX-2. GC receptor alpha and beta and H6PDH (11b-HSD1 cofactor generating enzyme) expression were unchanged. Time course and actinomycin D studies showed that the synergistic effect of cytokines and GCs on 11b-HSD1 expression is transcriptional. Promoter-reporter analyses indicated that an AP-1 site in the 11b-HSD1 proximal promoter mediated the induction of activity with cytokines. However, DEX and cortisol suppressed IL-1/TNF $\alpha$ -induced luciferase activity, suggesting a novel mechanism for

synergistic induction of 11b-HSD1 expression. In response to inflammation 11b-HSD1 activity increases in osteoblasts. This effect is magnified by GC treatment. Inflammatory cytokine/GC synergism of 11b-HSD1 induction is a potential mechanism to explain the modifying effect of inflammation on GC-induced bone loss.

## P28

### Evidence for high prevalence of hormonal irregularities in male osteoporosis

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#### Introduction

Osteoporosis in males is an underdiagnosed condition. It is considered to be of secondary pathogenesis in up to 60% of cases. In order to establish rational flow charts for the diagnosis and management of osteoporosis in men solid data seem to be necessary about the incidence of certain underlying diseases.

#### Methods

In a prospective screening for hormonal alterations we analysed 65 patients with male osteoporosis according to laboratory parameters which comprised routine laboratory profile and hormonal screening of Estradiol, Testosterone, SHBG, Prolactin, LH, FSH, IGF-1, GH; PTH, 25-OH-Cholecalciferol. Patients were included according to the German guidelines for postmenopausal osteoporosis. Patients already on antiresorptive therapy were also included.

#### Results

All patients had undergone a basal laboratory screen according to guidelines for female osteoporosis and no irregularities had been found. However we found abnormal serum hormone levels of at least one parameter in approx. two thirds of these patients. 28.6% of patients showed alterations in the vitamin D3/parathyroid hormone system, e.g. showed low levels of vitamin D3 and borderline or elevated PTH levels possibly indicating intestinal malabsorption even in those already on calcium/vitamin D3 therapy. 14.3% showed low testosterone levels with or without consecutive elevations of LH and/or FSH. Prolactin was not elevated in any of the cases. SHBG levels were elevated in 39.3% of patients as was the ratio between testosterone and SHBG. Basal IGF-1 was below normal in 4% possibly indicating growth hormone deficiency.

#### Conclusion

We conclude that in male osteoporosis the common screening procedure for secondary osteoporosis is insufficient and that dominant risk factors for osteoporosis can be found in a high percentage of patients. Further screening and consecutive stimulation tests of the somatotrophic axis of the pituitary will foster these preliminary findings and will help to establish diagnostic algorithms. The diagnosis of growth hormone deficiency might allow the intervention by substituting GH.

## P29

### Evaluation of bone markers and structure in subclinical Cushing's syndrome

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Overt hypercortisolism causes reduction in bone mass and density. The effects of subclinical Cushing's syndrome (SCS) on bone markers and structure are still debated. We therefore studied 56 patients with adrenal incidentaloma: group A = 35 (20F 15M, age 43-79, mean  $64.6 \pm 1.5$ ; BMI  $29.9 \pm 0.9 \text{ kg/m}^2$ ) without evidence of hypercortisolism, and group B = 21 (10F 11M, age 51-76, mean  $63.4 \pm 1.9$ , BMI  $28.0 \pm 1.2 \text{ kg/m}^2$ ) with SCS (i.e. with 2 or more alterations in biochemical tests of HPA axis). All patients underwent evaluation of HPA axis (plasma ACTH, plasma cortisol at 8 and 24, 24 h UFC, Nugent test), bone markers (serum BGP and urinary DPD), lumbar spine DEXA and bone ultrasonography of the phalanx. Group B showed lower values compared to group A of DEXA BMD, T-score, Z-score ( $P < 0.05$ ), and bone transmission time (BTT) ( $P < 0.05$ ), an ultrasonographic parameter who reflects bone structure. No significant differences in bone markers were detected in the two groups. We found significant inverse correlations between midnight serum cortisol and indicators of bone density, such as AD-SoS, UBPI ( $P < 0.05$ ) and DEXA T-score ( $P < 0.01$ ). A direct correlation between midnight serum cortisol and urinary DPD ( $P < 0.01$ ) was found. BTT correlated directly with BGP and inversely with urinary DPD ( $P < 0.0001$ ). There was also a significant direct correlation between BTT and DEXA BMD ( $P < 0.001$ ). Taken

together, these data indicate that bone structure is compromised in SCS patients. Moreover, our results suggest that ultrasonographic densitometry is a useful tool in the assessment of bone alterations occurring in SCS. Midnight serum cortisol correlates with parameters of bone structure, providing a highly sensitive marker, that could be used to identify patients with higher risk for osteoporosis.

## P30

### Asymptomatic primary hyperparathyroidism: preliminary results of a prospective randomized study on the effect of parathyroidectomy

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Aim of this 2-year, prospective, randomized study is to compare the effect of parathyroidectomy (PTX) vs no treatment in patients with asymptomatic primary hyperparathyroidism (PHPT), who didn't meet the 1991 NIH criteria for surgery. The study was approved by our local Ethical Committee. The primary endpoint was the change in lumbar spine bone mineral density (BMD); secondary endpoints were BMD changes at femur and distal radius, markers of bone turnover, quality of life and psychosocial well-being evaluation, echocardiographic parameters, complications of surgery and progression of the disease in the untreated group. Forty-nine patients (45 women and 4 men) have been enrolled so far: 24 underwent PTX and 25 were not treated. We report the results in the 39 patients who completed the 1-year follow-up (18 treated with PTX and 21 untreated). At baseline the 2 groups were similar for age, biochemical parameters and BMD. PHPT persisted after surgery in one patient (excluded from the analysis). In the PTX-treated group lumbar spine and total femur BMD increased after one year by 4.3% and 2.5%, respectively, whereas in the untreated group they decreased by 1.3% and 2.3% respectively. The % changes were significantly different between the two groups ( $P < 0.005$  at both sites). No clinically meaningful changes in quality of life, psychosocial well-being and echocardiographic parameters were observed in the two groups. Seven of the 21 untreated patients (33%) had *de novo* appearance of at least one of the NIH criteria for surgery: worsening of hypercalcemia in 1, hypercalciuria in 4, worsening of cortical BMD in 2, kidney stone in 1 and clinical vertebral fracture in 1. Non-complications of surgery were observed. In conclusion our preliminary results suggest that PTX is advantageous in patients with asymptomatic PHPT since treated patients had significantly improved BMD at lumbar spine and femur compared to untreated ones and conservative follow-up was associated with progression of the disease in about one third of patients.

## P31

### Brown tumors in a patient with gluten enteropathy and masked primary (or tertiary) hyperparathyroidism

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A 57 year old Caucasian woman complained of severe back pain and arthralgia of large joints. Her arthralgia gradually got worse and she also noticed proximal muscle weakness. Blood tests showed normal CRP but elevated alkaline phosphatase of 521 iu/l with normal Gamma GT. Phosphate was low at 0.75 mmol/l with normal corrected calcium of 2.6 mmol/l. A DEXA scan suggested osteoporosis (Spine T -2.5). Isotope bone scan revealed increased activity in the right tibia and plain films confirmed multiple lytic lesions within her tibia as well as mid femur raising the suspicion of primary bone neoplasm or metastases. CT scans of chest and abdomen as well as mammogram was normal. Bone biopsies showed intertrabecular fibrosis resorption and adjacent fibrosis in cores of thickened cortical bone. Renal function was normal but parathyroid hormone (PTH) was elevated at 1012 pg/ml and low vitamin D (25OHD 9.0 ng/ml). Anti endomysial antibodies were positive and small bowel biopsy confirmed coeliac disease. A diagnosis of osteomalacia due to gluten enteropathy and brown tumours due to secondary hyperparathyroidism was made. She was given dietary advice as well as Calcium & vitamin D supplements. Subsequently her parathyroid levels remained consistently elevated but now with persistently elevated serum calcium of 3.45 mmol/L. A large left lower parathyroid adenoma was then identified and resected. Post parathyroidectomy her calcium, phosphate, alkaline phosphatase and

PTH are within normal limits. Repeat DEXA suggests improvement in osteoporosis (T score -0.3).

In this case the osteomalacia possibly masked the severe primary (or tertiary) hyperparathyroidism. Interpretation of total plasma-calcium is likely to be unreliable unless the 25-hydroxyvitamin-D levels can be shown or assumed to be normal.

### P32

#### Baseline characteristics of postmenopausal Greek women with osteoporosis who had inadequate clinical response to antiresorptive medications

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#### Aim

The Observational Study of Severe Osteoporosis (OSSO) is a 12-month, European, prospective study aiming to assess the changes in Health Related Quality of Life (HRQoL) of women with osteoporosis (OP) and an inadequate clinical response to antiresorptive (AR) medications.

#### Methods

In Greece, 263 of 271 (97%) patients (pts) with severe OP met the inclusion criteria. The study population was assigned to 1 of 2 groups according to one or more of the following criteria: (a) Pts who sustained a new clinical fragility fracture, despite an AR treatment at least 12 months prior to this fracture (Group A); (b) Pts who discontinued AR therapy because of non compliance and/or side effects (Group B). After applying a Bonferroni correction, p-values less than 0.003 were considered statistically significant.

#### Results

Group A consisted of 143 pts (54.4%) aged 71.4 yrs with BMI 27.4 kg/m<sup>2</sup>. T-score was -3.1 both at the lumbar spine vertebra (LSV) and femoral neck (FN). Group B consisted of 120 pts (45.6%) with aged 64.7 yrs and BMI of 26.4 with T-score -3.1 at LSV and -2.6 at FN. 63% and 42% from groups A and B respectively had  $\geq 1$  fragility fractures (excluding the index fracture that made them eligible for the study) <40 yrs. In group A calcitonin (CAL) was the most common OP treatment taken at study entry (47.6%) followed by bisphosphonates (BPs) (39.2% = Aln + Ris + Eti + Pam = (34 + 16 + 5 + 1)/143) while for group B BPs was 40.8% and CAL was 15.0%. Alendronate was the most frequently discontinued qualifying AR drug (38.2%) due to non compliance. The 2 groups were similar with respect to age at menopause (46.5 yrs), number of live born children, hearing (19.8%) and sight problems (49.0%). Differences were observed between groups A and B in yrs past menopause (25.0 vs 17.9), current (18.9% vs 7.5%) and past (25.9% vs 11.7%) use of glucocorticoids and women who had falls in previous year (51.1% vs 31.7%).

#### Conclusions

Despite the differences observed in baseline characteristics between the 2 groups of postmenopausal women with inadequate response to AR therapy according to the previously mentioned criteria they both had numerous risk factors for a next fracture.

### P33

#### Effect of grape procyanidins on TNF alpha mRNA expression and cell DNA damage in MC3T3-E1 osteoblast-like cells *in vitro*

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#### Aim

This study was to investigate the protective effect of grape procyanidins on ethanol and carbon tetrachloride induced cell damages in MC3T3-E1 osteoblast-like cells.

#### Methods

Normal MC3T3-E1 osteoblast-like cells and cells damaged by ethanol or carbon tetrachloride were incubated with different doses of grape procyanidins for 24 hours. Cell TNF alpha mRNA expression and apoptotic DNA fragmentation were subsequently determined using *in situ* hybridization and cell death ELISA. MTT assay was applied to measure cell proliferation levels in the cells received various treatments.

#### Results

TNF alpha mRNA expression and apoptotic DNA fragmentation of the cells in ethanol and carbon tetrachloride injury groups significantly increased compared to the normal control, but reversed by grape procyanidins at 50

and 100 mg/L ( $P < 0.05$ ). Grape procyanidins significantly stimulated cell growth, with a better effect observed at the dose of 100 mg/L, and the reduction of cell proliferation by ethanol and carbon tetrachloride was significantly reversed by grape procyanidins administration.

#### Conclusion

Our data indicates that grape procyanidins exert preventive effect on cell abnormal gene expression and DNA damages induced by oxidative stress. Clinically this implies that grape procyanidins intake might play a beneficial role in the prevention of bone loss.

### P34

#### The effects of Dexamethasone and Dehydroepiandrosterone (DHEA) on cytokines and receptor expression in a human osteoblastic cell line: potential steroid-sparing role for DHEA

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Osteoporosis and associated fractures are the most common and debilitating complication of glucocorticoid use. The use of alternative anti-inflammatory agents without the catabolic and deleterious skeletal side-effects of glucocorticoids is needed. Dehydroepiandrosterone (DHEA) may have immunomodulatory as well as positive effects on bone. To further our understanding of the mechanisms of action of DHEA, as a steroid-sparing agent, we investigated and compared the effects of Dexamethasone (DEX) and DHEA on the regulation of the downstream effector pathway of osteoclastogenesis; RANKL/OPG and a range of inflammatory/pro-resorbing cytokines and receptors using a human clonal osteoblastic cell line. The cells were treated with DEX, DHEA, and androstenedione (ANDI). The mRNA expression of RANKL and OPG was determined by real-time PCR after overnight incubation. The regulation of a broad spectrum of cytokines by DEX and DHEA was also investigated using a human cytokine/growth factor and receptor gene array consisting of 268 cytokine-related cDNAs. To confirm some of the gene expression changes, protein production was measured by ELISA. RANKL expression and RANKL/OPG ratio were increased by DEX. This effect was reversed by co-treatment with both DHEA or ANDI. Several pro-inflammatory cytokines including IL-6, IL-4, IFN- $\gamma$ , macrophage inhibitory factor (MIF) were down-regulated not only by DEX but also by DHEA. In contrast to DEX, DHEA did not lead to suppression of growth factors including vascular endothelial growth factor (VEGF), fibroblast growth factor-5 (FGF-5), insulin-like growth factor-binding protein3 (IGF-BP3). Several new target genes previously documented to influence bone formation were up-regulated by DHEA such as notch 2, insulin receptor, thrombin receptor (PAR1). The data suggest that DHEA has immunomodulatory properties without the catabolic effects on bone remodeling, observed with glucocorticoid use. DHEA may thus prove useful as a steroid-sparing agent in the management of inflammatory disorders such as SLE or rheumatoid arthritis. Further *in vivo* studies are indicated.

### P35

#### Assessment of bone health and body composition in Glasgow school children

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#### Objective

To examine different methods of analysing bone health and body composition in children and examine the relationships between these methods.

#### Method

In a LREC approved cross-sectional study, DXA was used to assess bone mineral content (BMC) (at total body (TB), lumbar spine (LS), femur (F) and femoral neck (FN)), cross-sectional moment of inertia (CSMI) at the proximal femur and body composition (defined as Lean mass (LM) and Fat mass (FM)) in 60 healthy Glasgow school children (30M:30F) aged between 5 and 16 years (median age:10.5 yrs, 10th, 90th centiles:6.1,14.4). Speed of Sound Quantitative Ultrasound (QUS) was used for assessment of tibia (t) and radius (r), and Maximal Isometric Grip Force (MIGF) of the dominant hand as a surrogate marker of muscle strength. All children were measured for routine anthropometry, hip and waist circumferences.



**Results**

BMC(TB), BMC(LS) and BMC(F) were correlated with LM ( $r, 0.9, P = 0.0001$ ;  $r, 0.6, P = 0.0001$ ;  $r, 0.9, P = 0.0001$  respectively). BMC(TB) and BMC(F) were also independently correlated with FM ( $r, 0.5, P = 0.001$ ;  $r, 0.5, P = 0.008$  respectively). FM was inversely correlated to Hip/Waist ratio ( $r, -0.5, P = 0.001$ ). BMC(TB) and BMC(LS) were correlated with QUS(t) ( $r, 0.3, P = 0.04$ ;  $r, 0.4, P = 0.007$  respectively) and with QUS(r) ( $r, 0.3, P = 0.03$ ;  $r, 0.4, P = 0.04$  respectively). BMC(TB) and BMC(LS) were correlated with MIGF ( $r, 0.8, P = 0.0001$ ;  $r, 0.4, P = 0.004$  respectively). LM was correlated to QUS (t&r), MIGF and CSMI ( $r, 0.4, P = 0.04$ ;  $r, 0.4, P = 0.02$ ;  $r, 0.8, P = 0.0001$ ;  $r, 0.9, P = 0.001$  respectively). MIGF was correlated with CSMI and QUS (r) ( $r, 0.8, P = 0.0001, r, 0.5, P = 0.03$ ). There were no correlations between BMC(F) and BMC(FN) with QUS and MIGF.

**Conclusions**

BMC is associated with both LM and FM at multiple skeletal sites. Hip/waist ratio is a good marker of FM as measured by DXA. BMC and LM measured by DXA are correlated with both QUS and MIGF. DXA, QUS and MIGF are important modalities in the assessment of bone health and body composition in children.

**P36****Assessment of bone mineral density (BMD) in patients with adrenal incidentaloma (ai): comparison between dual-energy x-ray absorptiometry (DEXA) and quantitative ultrasonometry (QUS)**

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The aim of the present study was to compare the assessment of BMD measured either by DEXA or by QUS in patients with AI. We evaluated 40 patients with AI, who underwent an evaluation of hypothalamic-pituitary-adrenal (HPA) axis function and bone densitometry measured either by DEXA (Hologic QDR 4500 W) at lumbar spine and at femur or QUS (Achilles Express) at the right foot. Subclinical Cushing's syndrome (SCS) was defined as abnormal response to at least 2 standard tests of the HPA axis function in the absence of clinical signs of glucocorticoid excess. The measurement of BMD by DEXA either at lumbar spine or at femur was strongly correlated with stiffness value obtained by QUS. The patients classified as osteopenic or osteoporotic (WHO criteria) were 25/40 by using DEXA at lumbar spine and 26/40 by using QUS. These patients showed both lower BMI and DHEAS levels than subjects with normal BMD. The comparison between the two techniques in each subjects demonstrated a concordant result in 77.5% of patients. In a multiple regression model, either lumbar spine BMD or QUS were associated only with DHEAS levels, whereas there was a positive correlation between femur BMD with both DHEAS and BMI and a negative correlation with cortisol levels after 1mg dexamethasone suppression test. The 13 patients with SCS were older than the remainder, but we did not observe any significant differences for BMD value measured either by DEXA or by QUS. In these preliminary data QUS measurement presents a good correlation with DEXA technique and seems to be a reliable tool for the detection of bone alteration in subjects with adrenal incidentaloma also in patients with SCS. Moreover, in patients with AI lower DHEAS levels and BMI values appear to have a main role in the pathogenesis of osteopenia or osteoporosis.

**P37****A comparative study of neck ultrasonography, sestamibi scan and parathyroid surgery findings**

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**Background**

Many Endocrinologists believe that preoperative localisation/lateralization in patients undergoing initial neck exploration for primary hyperparathyroidism is unimportant because of high cure rate in the hands of experienced surgeons. Tcm-MIBI imaging has a sensitivity of 70 to 95% in localizing parathyroid tumors. Ultrasonography has lower sensitivity but is noninvasive and relatively cheap. Unilateral surgical exploration has potential benefits including reduced operative time and reduced operative morbidity such as recurrent laryngeal nerve injuries and hypoparathyroidism.

**Aim**

To assess the accuracy of preoperative imaging studies in primary hyperparathyroidism.

**Methods**

Retrospective casenotes review of all patients treated surgically for primary hyperparathyroidism at North Manchester General Hospital between January 2003 and December 2004. Seventeen patients who had Neck Ultrasonography and SestaMIBI scans as preoperative imaging were included. Results of preoperative imaging studies were compared with surgical and histopathological findings.

**Results**

14 of 17 (82.35%) patients were female. Twelve of Seventeen (70.58%) were over 55 years of age. Ultrasonography correctly localized 8 of 17 (47.06%–95% CI 22.98, 72.19). SestaMIBI scan correctly localized 14 of 17 (82.35%–95% CI 56.57, 96.2). Histology confirmed parathyroid adenoma in 12 of 14 correctly localized by SestaMIBI and the remaining 2 were inconclusive. In 3 patients incorrectly localized by SestaMIBI, one had a moderate sized multinodular goiter, one had inconclusive histology and only one was true negative. Sixteen of Seventeen patient's calcium normalized after surgery. In only one patient surgery was ineffective and was also complicated by recurrent laryngeal nerve palsy.

**Conclusion**

Accurate localization of parathyroid tumors is facilitated by imaging, particularly by SestaMIBI scanning. Localisation/lateralization facilitates unilateral surgical exploration which reduces operative time by 30 minutes and also reduces complications. Localisation/lateralization is of vital importance in our aging population who often have multiple co-morbidities and are often unfit for surgery under general anaesthesia.

**P38****Glucocorticoid metabolism in mouse osteoblasts: a model for the effects of local glucocorticoid generation on bone**

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Human studies have suggested that local glucocorticoid (GC) generation within osteoblasts plays a critical role in bone loss seen during aging, in response to inflammation and treatment with GCs. Human osteoblasts express the enzyme 11 $\beta$ -hydroxysteroid dehydrogenase type 1 (11 $\beta$ -HSD1) that converts inactive GCs (cortisone, dehydrocorticosterone, prednisone) to their active counterparts (cortisol, corticosterone, prednisolone). Enzyme expression increases with age, in response to inflammatory cytokines and with therapeutic GCs. Reports on GC metabolism in mouse osteoblasts have been conflicting however with the expression of the GC inactivating enzyme 11 $\beta$ -HSD2 suggested. To establish whether local GC metabolism differs between humans and mice we have characterised expression, activity and regulation of GC metabolising enzymes in osteoblasts derived from C57BL/6 mice and examined regulation by inflammatory cytokines.

Primary cultures of mouse osteoblasts were derived from calvaria ( $n = 8$  mice) and long bones ( $n = 8$ ) of 14-24 week old mice by outgrowth of collagenase treated bone chips. The osteoblastic character of these cells was confirmed by high basal and GC-inducible alkaline phosphatase activity (2-fold induction with dexamethasone) and expression of bone restricted genes (Cbfa1 and osteocalcin) by RT-PCR. 11 $\beta$ -HSD1 but not 11 $\beta$ -HSD2 mRNA was detected. Enzyme activity studies revealed predominant reductase activity (cortisone to cortisol conversion  $3.8 \pm 2.1$ ; dehydrocorticosterone to corticosterone  $1.7 \pm 0.8$  pmol/mg/hr) further indicating exclusive 11 $\beta$ -HSD1 expression. Enzyme activity was equivalent to that seen in human osteoblasts. As in human osteoblasts 11 $\beta$ -HSD1 expression increased with IL-1 $\beta$  treatment (8.9 + 2.5 fold increase with 10 ng/ml IL-1 $\beta$ ). The cofactor generating enzyme hexose-6-phosphate dehydrogenase was also expressed. These data indicate that GC metabolising enzyme expression in osteoblasts of C57BL/6 mice is similar to that in humans and is regulated in a similar fashion. This supports the use of these mice as a model for the impact of local GC metabolism on bone.

**P39****Long-term growth hormone (GH) replacement in GH deficient adults – favourable effect on bone and body composition**

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Growth hormone deficiency (GHD) in adulthood has been associated with increased central adiposity, reduced lean body mass, increased cardiovascular risk, reduced bone mineral density with increased fracture risk and decreased quality of life. GH replacement might improve these alterations.

We evaluated the long-term metabolic effects of GH replacement on bone metabolism and body composition of 24 GHD patients (20 males, mean age  $38 \pm 11$  years, 20 adult onset). Patients were followed-up yearly for up to five years. Mean GH dose, normalizing IGF-1 was  $0.4 \pm 0.2$  mg/day. IGF-1 levels normalized from  $-2.14 \pm 1.6$  (mean SD  $\pm$  SD) to  $0.11 - 0.87$  mean SD values during five years of treatment. A significant increase in lean body mass and decrease in body fat mass was observed after one year – both 3% of body weight ( $P < 0.05$ ) – and this positive effect persisted. A significant increase in lumbar spine BMD was apparent from 2 years of treatment – after two years  $+4.9 \pm 3.4\%$  (mean  $\pm$  SEM) and after 5 years  $6.1 \pm 6.2$ ,  $P < 0.05$ . Proximal femur BMD increased by  $3.4 \pm 2.1\%$  after 2 years and  $6.3 \pm 2.6\%$  after 5 years ( $P < 0.05$ ). Whole body BMD significantly increased after 3 years by  $3 \pm 2.7\%$  and slowly increased further to  $4.2 \pm 3.7\%$  after 5 years. We observed similar results when data of 56 patients from two centers were analyzed together.

In summary the long-term GH replacement in adults with GHD is effective in restoring IGF-1 levels, favourably changes body composition and increases bone mineral density. These metabolic effects last after five years of treatment.

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#### P40

##### Does parathyroidectomy reduce the risk of fractures and renal stones? Results of a systematic review and meta analysis

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Primary hyperparathyroidism (PHP) is a common endocrine condition. The associated metabolic abnormalities are usually amenable to surgical treatment (PTX). Although it is generally believed that such treatment will reduce the risk of long-term complications this has not been demonstrated in a clinical trial and the current treatment guidelines are based on consensus. In order to assess the effect of parathyroidectomy on two of the most common complications of PHP, renal stones and fractures, we have undertaken a systematic review and meta analysis.

Relevant literature was sought by searching electronic databases supplemented by the reference lists of relevant studies.

Six cohort studies reporting fracture rates in patients who had been subject to PTX were identified. Four of these gave unique data and were included in a meta analysis. This indicated that parathyroidectomy was associated with a 10% reduction in fracture risk. However this change was not statistically significant (RR 0.90 [95% CI 0.78–1.04]).

Four similar cohorts reported renal stone occurrence with and without PTX. Surprisingly meta analysis suggested that PTX was associated with a doubling of stone risk (RR 1.86 [95% CI 1.22–2.85]). 11 studies examined stone occurrence before and after PTX. All of these reported lower rates following surgery than before although the heterogeneity of reporting made it impossible to undertake a formal meta analysis. We believe that the apparent increase inferred from cohort studies reflects the well-known increased risk of recurrent stone in someone who has already suffered a stone episode coupled with the fact that someone with PHP and stones is more likely to be offered PTX than someone who has no stone disease.

We therefore conclude that the literature offers only limited support to the beneficial effects of parathyroidectomy and that a formal assessment using a randomised controlled study would be justified.

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#### P41

##### Brown tumors in hyperparathyroidism may be mistaken for primitive bone neoplasia: report of two cases

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Primary hyperparathyroidism is characterized by abnormal PTH secretion from one or more hyperfunctioning parathyroids and hypercalcemia. PTH exerts a catabolic effect on the cortical bone resulting in the osteitis fibrosa

cystica. Two patients were referred to us after destroyed surgery for brown tumors mistaken for primitive bone tumors.

A 59 years old man was referred to emergency department for a pathologic fracture of the left hip. The X-ray showed a bone diaphysis osteolytic lesion, the calcemia was 15 mg/dl. A biopsy was performed and resection of the bone segment followed. Hystology was: “neoplasm consisting of vascular lacunae bordered by multinucleated giant cells embedded in a fibrillary network (giant cells tumor or aneurysmatic bone cyst)”.

Five months later the patient was referred to the endocrinologist because of persistently high calcemia (14 mg/dl), with serum PTH levels 1638 pg/ml. Parathyroid scan revealed: “hyperfunction parathyroid in the right side” and the patient underwent upper right parathyroidectomy through a minimally invasive technique. The histological examination confirmed the parathyroid adenoma.

A 67 years old women was referred to us following amputation of the left leg for a supposed primitive bone tumor. Serum PTH was 2185 pg/ml and calcemia 13.6 mg/dl indicating primary hyperparathyroidism. Parathyroid scan revealed a hyperfunction parathyroid in the right side. The patient underwent excision of the upper right parathyroid; Hystology revealed a parathyroid carcinoma.

The above two cases suggest that, when X-ray shows an osteolytic lesion, PTH and calcemia should be performed in order to exclude the presence of primary hyperparathyroidism.

Primary hyperparathyroidism can be treated with minimally invasive surgical techniques, thereby sparing the patient invalidating surgery due to wrong diagnosis.

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#### P42

##### The effect of once-weekly Risedronate on biochemical markers of bone turnover after three month of treatment

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The nitrogen-containing bisphosphonate Risedronate have been shown to reduce the risk of both spine and nonspine fractures by reducing bone turnover, increasing bone mass and improving bone strength.

Objective

To study the effects of once-weekly Risedronate on bone turnover markers in osteoporotic patients after three month of treatment.

Patients and methods

Study group included 50 osteoporotic patients (3 males and 47 females), mean age ( $\pm$  DS) of  $62.06 \pm 9.61$  years, without previous medication for osteoporosis. 19 patients (38%) had previous low-impact fracture, 4 patients (8%) had family history of osteoporosis and 6 patients (12%) were smokers. Dual X-ray absorptiometry of the lumbar spine was performed baseline.

We measured serum levels of osteocalcin as a marker of bone formation and beta crosslaps as a marker of bone resorption by immunoassay at the time of diagnosis and after three month of treatment.

Once-weekly Risedronate was administered in all patients; they were also received calcium /vitamin D substitution.

Results

The osteocalcin levels were found to be  $38.8 \pm 2.4$  ng/ml at the baseline and  $16.6 \pm 1.6$  ng/ml after three month of treatment. The beta crosslaps levels were  $0.741 \pm 0.09$  ng/ml at the baseline and  $0.138 \pm 0.04$  ng/ml after three month of treatment. It was shown that Risedronate significantly decreases osteocalcin levels and beta crosslaps after three month of treatment. The medication was well-tolerated with no upper gastrointestinal adverse experiences.

Conclusion

In this study, the use of once-weekly Risedronate was associated with the significant reduction in both formation and resorption biochemical markers of bone turnover after three month of treatment.

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#### P43

##### Quantitative ultrasound at the hand phalanges in patients with acromegaly

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Acromegaly is associated with various skeletal deformities and in some cases with bone deterioration. The aim of the study was to assess the skeletal status using quantitative ultrasound (QUS) in patients suffering from acromegaly. A group of 38 patients with acromegaly (27 women and 11 men) in mean age of  $57.21 \pm 9.85$  y in various gonadal status was compared with a control group matched for sex, age and body mass. QUS measurements at hand phalanges were performed with DBM Sonic 1200 (IGEA, Italy). Amplitude-dependent Speed of Sound (Ad-SoS) at hand phalanges had lower values in patients with acromegaly than in controls, as well in the entire group, as in males and females. The positive correlation between Ad-SoS and height, and a negative one between Ad-SoS and age was found in the entire group of patients with acromegaly. No correlations between Ad-SoS and time from diagnosis or duration of the symptoms were found. No influence of gonadal status and activity of the disease was proven. Our data demonstrated that quality of skeletal status in patients with acromegaly was affected. This could reflect the worsening of the mechanical properties of bones, and the increased risk of fractures in other sites of the skeleton. The changes observed were not related to acromegaly-associated hypogonadism.

#### P44

##### Selenium supplementation restores the antioxidative capacity and prevents cell damage in mesenchymal stem cells *in vitro*

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Mesenchymal stem cells (MSC) are developed for cell based therapeutic strategies and undergo cellular stress during *ex vivo* procedures. Reactive oxygen species (ROS) of environmental and cellular origin are involved in redox signaling, cumulative cell damage, senescence and tumor development. Selenium independent (superoxide dismutases 1 and 2 (SOD1 and SOD2) and catalase (CAT)) and selenium dependent (glutathione peroxidases (GPx), thioredoxin reductases (TrxR)) enzymes regulate cellular ROS steady state levels. SOD process superoxide anion to hydrogen peroxide, which is subsequently neutralized by CAT and GPx. Human primary MSC and telomerase-immortalized human mesenchymal stem cells (hMSC-TERT) express SOD1 and 2, CAT, GPx 1–3 and TrxR1 and 2. We show here that the activity of antioxidative selenium dependent enzymes is impaired in primary MSC and hMSC-TERT in standard cell cultures (5–10% FCS, selenite 5–10 nM). Under these conditions the superoxide anion processing enzyme SOD1 is not sufficiently stimulated by a ROS load. Resulting oxidative burden favors generation of micronuclei in MSC, which is a readout for DNA damage. Supplementing the cell culture medium with selenite (100 nM) restores basal GPx and TrxR activity, rescues basal and ROS-stimulated SOD1 mRNA expression and activity, and reduces ROS accumulation in hMSC-TERT and micronuclei formation in primary MSC.

In conclusion mesenchymal stem cells in routine cell culture have low antioxidative capacity and are subjected to oxidative stress as indicated by the generation of micronuclei. Selenite supplementation of MSC cell cultures appears to be an important countermeasure to restore their antioxidative capacity and to reduce cell damage in the context of tissue engineering and transplantation procedures.

#### P45

##### Expression of the membrane-associated hormone-sensitive protein UO-44 in hMSC-TERT immortalized mesenchymal stem cells

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UO-44 is a novel protein of unknown function which was described in uterus, medium-size ovarian follicles, oviduct and pancreas of different mammalian species. Using differential display analysis in preimplantation rat uteri, Chen et al. 1999 cloned and described it as an estradiol responsive gene named estrogen regulated gene 1 (ERG-1). Subsequent characterization showed that its expression was modified through pregnancy. The human ortholog (HuUO-44) was cloned and characterized by Leong et al. in 2004 and various splice variants were described. Multiple Tissue Northern blots and immunohistochemistry revealed rather specific expression in

pancreas and ovaries. The full length cDNA, when expressed as a GFP-fusion protein, was shown to be associated with the cell membrane.

We prepared total RNA from human fetal osteoblasts (hFOB), telomerase-immortalized human mesenchymal stem cells (hMSC-TERT) and four different strains of primary human mesenchymal stem cells (hMSC). Whole genome microarray (Affymetrix, HG-U133 2.0plus) analysis of hMSC-TERT expression pattern revealed HuUO44 to be expressed in these cells. Results were confirmed by RT-PCR. Using framing primer oligonucleotides for RT-PCR in hMSC-TERT cells, we obtained two distinct full length bands. Cloning and consequent sequence analysis verified presence of two different splice variants of HuUO44 in hMSC-TERT. RT-PCR using cDNA preparations from primary hMSCs and hFOBs depicted expression of HuUO44 in these cells.

In summary we have demonstrated the expression of two splice variants of the estrogen responsive gene of unknown function UO-44 in human cells of mesenchymal origin.

#### P46

##### The effect of once-weekly Risedronate on biochemical markers of bone turnover after three month of treatment

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The nitrogen-containing bisphosphonate Risedronate have been shown to reduce the risk of both spine and nonspine fractures by reducing bone turnover, increasing bone mass and improving bone strength.

##### Objective

To study the effects of once-weekly Risedronate on bone turnover markers in osteoporotic patients after three month of treatment.

##### Patients and methods

Study group included 50 osteoporotic patients (3 males and 47 females), mean age ( $\pm$  DS) of  $62.06 \pm 9.61$  years, without previous medication for osteoporosis. 19 patients (38%) had previous low-impact fracture, 4 patients (8%) had family history of osteoporosis and 6 patients (12%) were smokers. Dual X-ray absorptiometry of the lumbar spine was performed baseline.

We measured serum levels of osteocalcin as a marker of bone formation and beta crosslaps as a marker of bone resorption by immunoassay at the time of diagnosis and after three month of treatment.

Once-weekly Risedronate was administered in all patients; they were also received calcium /vitamin D substitution.

##### Results

The osteocalcin levels were found to be  $38.8 \pm 2.4$  ng/ml at the baseline and  $16.6 \pm 1.6$  ng/ml after three month of treatment. The beta crosslaps levels were  $0.741 \pm 0.09$  ng/ml at the baseline and  $0.138 \pm 0.04$  ng/ml after three month of treatment. It was shown that Risedronate significantly decreases osteocalcin levels and beta cross laps after three month of treatment. The medication was well-tolerated with no upper gastrointestinal adverse experiences.

##### Conclusion

In this study, the use of once-weekly Risedronate was associated with the significant reduction in both formation and resorption biochemical markers of bone turnover after three month of treatment.

#### Clinical case reports

##### P47

##### Forced conservative management of a patient with pituitary tumour apoplexy and reduced conscious level

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Historically, acute neurosurgical decompression has been considered treatment of choice for pituitary apoplexy with some studies advocating a conservative approach. We describe a patient, with reduced consciousness due to apoplexy of a gonadotroph adenoma, in whom there was spontaneous tumour shrinkage. A 68-year-old gentleman presented following a collapse with hypothermia, bradycardia and bitemporal hemianopia. Initial investigations showed low plasma sodium and glucose. Over 24 hours his conscious level deteriorated and he required ventilation. CT-scan revealed a  $20 \times 17$  mm pituitary tumour and subsequent MRI confirmed a pituitary mass lesion with marked sellar expansion and high signal on pre-contrast T1 consistent with recent haemorrhage. He was commenced initially on



steroids and subsequently triiodothyronine. Initial endocrine investigations confirmed the presence of an FSH-oma: FSH 36.2 U/l (1.3–9.2), LH 0.8 U/l (2–12), free T4 5.8 pmol/l (9.0–20.0), TSH 2.14 mIU/l (0.2–3.0), cortisol 153 nmol/l (200–650), prolactin 86 mU/l (40–375), growth hormone 0.3 mU/l, IGF1 7.0 nmol/l (11–30), testosterone 1.6 nmol/l (5.4–30.4). Subsequently the patient developed an aspiration pneumonia and melaena requiring blood transfusion. As a result, acute neurosurgical intervention was prevented and over the course of the following days the patient regained consciousness. Seven weeks after initial presentation the patient's FSH levels had fallen to 0.2 U/l and follow up MRI-scan confirmed marked shrinkage of the pituitary tumour with no optic chiasm compression. The patient continued to require pituitary replacement therapy and the bitemporal hemianopia remained. Acute co-morbidities did not allow neurosurgical decompression, the preferred intervention in this case of pituitary apoplexy with reduced consciousness. However, conservative management (advocated routinely by some) was associated with marked involution of the tumour and resolution of the conscious level. The neurosurgeon commented "Had I operated on this patient I would have been very pleased with the radiological outcome"!

#### P48

##### An unusual presentation of 'fits'

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We report a 29 year old man (staff nurse) who initially presented with a grand mal seizure in October-2003 to the neurologists. A preliminary diagnosis of epilepsy was made, though the subsequent EEG only revealed predisposition to epilepsy. In December 2004 he again presented with a grand mal seizure despite being on Epilim. He also complained of episodes of lethargy, sweating and inability to concentrate towards the end of his shifts at work for a few months prior to this. Before this admission there was a record of increased frequency of seizure like episodes. His blood glucose levels just before admission with the second seizure in December-2004 was 3 mmol/l and the paramedics had reported that his blood glucose was 1.7 mmol/l. He denied any history of sulfonylurea ingestion or exogenous insulin. Further history revealed that he had gained about 9 kgs in weight gain over the previous 12 months, though his appetite remained normal. He consumed ethanol very occasionally and was on treatment with sertraline for depression. Blood tests including thyroid functions and a short synacthen test were normal. MRI brain was essentially normal. A prolonged fast was organized and his initial overnight fasting blood glucose levels were 2.2 mmol/l with corresponding insulin and C-peptide levels being 115 picomoules per liter and 847 picomoules per liter respectively and four hours later blood glucose dropped to 2.0 mmol/l and corresponding levels of insulin and c-peptide levels were 259 picomoules per liter and 1634 picomoules per liter respectively. Urine sulfonylurea screen was negative. CT abdomen revealed a nodule in the tail of the pancreas. He underwent successful distal pancreatectomy and histology was compatible with insulinoma. Subsequent investigations confirmed normal PTH, IGF-1 and 24 hour urinary catecholamines. He now remains free of seizures without any anticonvulsant therapy and running normal blood sugar readings. This demonstrates yet another well described, but less well recognized cause of seizures.

#### P49

##### An unusual cause of confusion

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A 37-year-old female was referred with recurrent episodes of altered consciousness and disorientation. She described a three-year history of recurrent "funny turns". They started with a sensation of warmth accompanied with cold sweat followed by unfamiliarity with the surroundings and cognitive impairment. The attacks increased in frequency gradually over time. There was a tendency for the attacks to occur in the morning, before bed and 3–4 hours after food. The patient didn't gain weight and found that eating more frequent meals minimises symptoms.

Past medical history, family history and drug history were unremarkable. Two random glucose samples 2.7 and 3 mmol/l (at 11:00 and 17:00 hours respectively) were low. Full blood count, urea and electrolytes, thyroid function test, liver function tests, bone profile and short synacthen tests were all normal. Supervised fasting provoked hypoglycaemia (blood glucose 2 mmol/l) with inappropriately high insulin (610 pmol/l), C-peptide (411 pmol/l) and pro-insulin (23 pmol/l) levels with no evidence of ketonemia. Sulphonylurea was not detected in any of the samples. A diagnosis of insulinoma was made. Prolactin levels and gut hormone profile were normal. The patient was started on diazoxide 300 mg/day that resulted in improvement in her symptoms and fasting glucose of 3.6–4.2 mmol/l, but she was developing hypertrichosis. CT scan of the Abdomen was normal. Endoscopic ultrasound failed (EUS) to localise the tumour despite good views of the pancreas. Octreotide scan showed no focus of increased activity. Pancreatic MRI showed a 1.7 × 2.6 cm tumour in the pancreatic tail. Intraoperatively a single pancreatic tumour, consistent with that found on MRI, was found and resected. The histology was consistent with an insulinoma. The patient's symptoms improved after surgery.

Our patient had an insulinoma, which was localised by abdominal MRI preoperatively while EUS failed to localise the tumour. The literature suggests EUS has far higher sensitivity in detecting insulinomas (65–90%) compared to CT (30%) and MRI (as low as 15% in some series). Lesions in the pancreatic tail as in our patient may however be missed. MRI scan of pancreas should be included in the initial assessment of such patients.

#### P50

##### Recurrent congenital neonatal hyperthyroidism in a mother with Graves' disease (post radio-ablation) on thyroxine replacement

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Thyroid dysfunction is not uncommon in pregnancy and is associated with various maternal, foetal and neonatal complications. We report the occurrence of neonatal hyperthyroidism in two successive pregnancies in a post radio-ablation mother with Graves' disease who is on thyroxine replacement.

##### Mother

A 30-year-old lady, treated for Graves' thyrotoxicosis with radioiodine 10 years ago, was on thyroxine 100 mcg replacement for hypothyroidism. She was seen in antenatal clinic during the final trimester of her first pregnancy. Premature delivery occurred prior to initiation of foetal heart monitoring in her first pregnancy. She was biochemically euthyroid but with elevated Thyroid Blocking Inhibiting Immunoglobulin (TBII) levels (Table 1). During her second pregnancy she was treated with Propylthiouracil in the hope of preventing neonatal hyperthyroidism (thyroxine replacement was continued).

Table 1.

	Mother		Baby 1 Day 5	Baby 2 Day 8
	Pregnancy 1	Pregnancy 2		
FT4 pmol/L	17 (10–23)	16	> 100	74
TSH mIU/L	0.1 (0.4–5.5)	0.3	< 0.05	< 0.05
TBII Units	83 (0–15)	89	39	55

##### Baby 1

Male infant had meconium staining at birth and cord around the neck which resulted in premature delivery at 34 weeks gestation and weighed 1.835 kg. He developed thyrotoxicosis postpartum (Table 1) and was treated with block-replace (Carbimazole + Thyroxine) and propranolol for 3 months.

##### Baby 2

Female infant was born at 39 weeks gestation and weighed 2.5 kg. She had neonatal hyperthyroidism (Table 1) and was treated with block and replacement akin to her brother.

Foetal problems caused by transplacental transfer of thyroid blocking (Inhibitory and Stimulating) Immunoglobulins could easily be missed in a euthyroid mother with treated Graves' disease. The bioassay of TBII has been used to predict the occurrence of neonatal hyperthyroidism but early anticipation and recognition remains cornerstone of treatment.

## P51

**Isolated ACTH deficiency following long-term benzodiazepine treatment**

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**Objective**

Benzodiazepines are among the most commonly used symptomatic treatment of insomnia and anxiety. Human and animal studies suggest that benzodiazepines suppress the hypothalamo-pituitary adrenal (HPA) axis. We report on a rare case of isolated ACTH deficiency due to long-term treatment with flunitrazepam.

**Case report**

A 66-year-old man presented to our outpatient department with persistent feelings of physical exhaustion, low vitality, reduced daily activities during the last 2 months. The patient's past medical history revealed a continuous intake of 1 mg flunitrazepam daily over the past 35 years because of sleep disturbances following a severe car accident. According to a psychiatric interview with an experienced clinical psychiatrist there was no present or past history of other psychiatric disorders else than a low-dose dependency of hypnotics (DSM-IV 304.10). Decreased basal concentrations of plasma ACTH, serum cortisol, as well as mean 24-h urinary free cortisol excretion, a blunted response of ACTH and cortisol levels to intravenous application of 100 µg CRH, and concentrations of the other anterior pituitary hormones within the normal range were consistent with secondary adrenal insufficiency due to an isolated ACTH-deficiency. Magnetic resonance imaging of the pituitary gland revealed no abnormal findings. Immunological studies, including CNS specific and thyroid antibodies, were negative. After tapering from the benzodiazepine treatment we observed a stable increase to normal levels of the serum and urinary concentrations of cortisol and plasma levels of ACTH. The patient suffered from a mild benzodiazepine withdrawal syndrome. However, at the end of the tapering period he reported a clear improvement in vitality.

**Conclusion**

Clinicians should be alert of possible inhibiting effects of benzodiazepines on the HPA axis in patients with suspected dysfunctions of the corticotroph function.

**Conclusion**

Cases of 21-hydroxylase deficiency diagnosed in late adulthood are rare; however, clinicians should be alert of this possibility in patients presenting with infertility or unclear testicular masses.

## P53

**Grave's eye disease developing following radioiodine treatment for toxic nodular goitre**

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We report a 49 year-old female patient who was referred to the endocrine clinic with two months history of heat intolerance and weight loss. She denied any symptoms related to her eyes. Past medical history included hypertension, epilepsy and manic-depressive psychosis, and she was on lithium, atenolol, phenobarbitone, doxazosin and phenytoin. On examination, there was a 45 g firm symmetrical multi-nodular goitre (MNG) with normal examination of both eyes. Thyroid function tests (TFTs) showed thyrotoxicosis (TSH < 0.05 µU/L, FT4 36.7 pmol/L). She was started on carbimazole 30 mg once/day and radioiodine (RI) treatment was planned. Thyroid ultrasound showed multinodular goitre. TSH receptor antibodies (TRAb) were positive. Five months after the initial review, the patient received RI therapy after being rendered euthyroid on carbimazole. Four days later, the patient started to complain of pain in both eyes and pressure-like feeling in the left eye particularly. She denied diplopia or any other visual symptoms. On examination, visual acuity was 6/6, minimal exophthalmos and lid lag in the left eye without any restriction in eye movements. MRI of the orbits showed minor thickening of the left inferior rectus muscle. Following the RI, the patient became euthyroid and the goitre regressed in size. Six months after RI, she started to develop mild T3 thyrotoxicosis biochemically but remained asymptomatic and clinically euthyroid. The ophthalmic symptoms are improving gradually. The presence of TRAb has been reported in patients with clinical/ultrasonographic evidence of MNG. RI has also been reported to induce TRAb in patients with undetectable TRAb prior to the RI treatment. This case highlights the importance of recognising patients with thyrotoxicosis and MNG who have positive TRAb as they could develop thyroid eye disease following RI treatment and that these patients should be warned about this possibility.

## P52

**Infertility and bilateral testicular masses due to 21-hydroxylase deficiency**

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**Objective**

Congenital adrenal hyperplasia results from 21-hydroxylase deficiency in more than ninety percent of cases. The classical form of 21-hydroxylase deficiency presents in the neonatal period with virilization or adrenal insufficiency, with or without concurrent salt wasting. We report on a rare case of classic 21-hydroxylase deficiency diagnosed in late adulthood.

**Case report**

A 39-year-old male patient presented for workup of infertility. Urologic investigation revealed small testes, bilateral testicular masses, and asthenozoospermia. The patient's steroid metabolism showed markedly increased levels of adrenal androgens, in particular of 17-hydroxyprogesterone and 21-deoxycortisol. The gas chromatographic-mass spectrometric (GC-MS) urinary steroid profile was dominated by metabolites of 17-hydroxyprogesterone, while the endogenous glucocorticoid production was subnormally low. ACTH levels in plasma were elevated. These hormonal findings were consistent with 21-hydroxylase deficiency. Therapy with dexamethasone was initiated. The CYP21A2 gene analysis revealed the mutation I172N (ATC → AAC) in exon 4 of allele 1 and a large gene deletion in allele 2.

## P54

**Recurrent hypoglycaemia: an unusual association of islet cell hyperplasia and coeliac disease**

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A 72 year old, non-diabetic, woman was referred with recurrent severe hypoglycaemia. Twenty-one years ago she was investigated for hypoglycaemia when inappropriate hyperinsulinaemia had been confirmed. Although a CT of her abdomen could not demonstrate an insulinoma she underwent a distal pancreatectomy and subsequent histology showed islet cell hyperplasia. She was managed with dietary intervention and diazoxide. At the time of the current referral she was suffering daily severe hypoglycaemic episodes requiring prolonged hospital admissions. A repeat 48 hr fast confirmed spontaneous hypoglycaemia and inappropriate hyperinsulinaemia (glucose 1.8 mmol/L, insulin 88 pmol/L, C-peptide 734 pmol/L). A sulphonylurea screen was negative. An abdominal CT showed no pancreatic lesion. Subcutaneous Lanreotide LA was administered with no improvement in symptoms. Further investigations showed evidence of malabsorption: folate 2.1 µg/L (RR 2.5–15.0), ferritin 7 µg/L (RR 12–250), Adjusted calcium 2.20 mmol/L (RR 2.20–2.60), PTH 21 pmol/L (RR 1.5–7.6) and Vitamin D 11.3 µg/L (RR 10–45) although she had no gastrointestinal symptoms. Endomysial antibodies were negative and an OGD was normal but the D2 biopsy showed sub-total villous atrophy consistent with coeliac disease. Within days of starting a gluten free diet her symptoms dramatically improved. She continues on diazoxide and reports only occasional, mild hypoglycaemic episodes.

This woman has a rare cause of spontaneous hypoglycaemia that had been well controlled for 20 years with dietary intervention and diazoxide. Her recent deterioration in symptoms was a result of malabsorption of carbohydrates and diazoxide secondary to coeliac Disease. With

commencement of a gluten free diet she significantly improved and we were able to avoid long-term use of somatostatin analogues or further abdominal surgery.

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### P55

#### Screening of osteoporosis in a small Romanian town

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#### Introduction

In the last decade, after the revolution in December 1989, osteoporosis started to be seen as an important problem for the women in Romania too. This screening that last three years is trying to find the prevalence of osteoporosis and how is she influenced by different factors as: age, body mass index, smoke, coffee, and years from menopause.

#### Method

We have investigated 440 caucasian women with age between 30 and 80 years old divided in three lots: I – 227 women in the evidence of the Endocrinology Clinic, II – 43 women working at a confection factory, III – 70 women working at a pipe factory. The method we used is quantitative ultrasound.

All the participants were asked about: the first period, bone pain, record of fracture, the age of menopause if it was the case, if they smoke or drink coffee, if they have records of osteoporosis in family, there were established the anthropometrically parameters (high and weigh).

#### Results

Following the study we have accede to the next conclusions: T score value are declining with age: 24.57% of women have T score  $> -1$ , 29.38% of women have osteopenia, T score between  $-1$  and  $-2.5$ , 46.05% of women have osteoporosis, T score  $< -2.5$ . T score values are growing up with the rise of the BMI because of the estrogens stored in the adipose tissue. T score values are declining faster at smoking persons to nonsmoking one. At coffee drinkers T score is not declining so fast to those who does not drink coffee, because the noxious effects of the coffee are evolving slower than those of the tobacco. T score values are getting worse with the years from menopause, explained by the decline of the estrogens.

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### P56

#### Acute myocardial infarction and Graves' thyrotoxicosis

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The link between thyrotoxicosis and coronary artery disease is complex with associations between therapy and vasculitis, as well as thyroid hormone excess and coronary artery spasm described. We present a 37-year-old female with a one-year history of Graves thyrotoxicosis treated with Propylthiouracil, who presented acutely with chest pain and a left hemiparesis. Typical electrocardiography changes and elevation of Troponin T confirmed a recent myocardial infarction, and CT scanning demonstrated a right cerebral infarction. Echocardiography demonstrated severely impaired left ventricular function with a suspected left ventricular thrombus. Persistent thyrotoxicosis was also demonstrated with a T4 = 120 pmol/L and TSH  $< 0.02$  mU/L. Beta-blockers and Propylthiouracil, together with Aspirin, Enoxaparin and Simvastatin were commenced. Warfarin was started 10 days later. At that stage coronary angiography demonstrated severe left main stem stenosis.

Following the restoration of euthyroidism, repeat angiography demonstrated complete resolution of the left main stem stenosis. Normal left ventricular function was noted on repeat echocardiography. These findings suggest coronary artery spasm in association with thyrotoxicosis.

Coronary artery spasm can account for myocardial infarction in the absence of atherosclerosis. Abrupt decrease in the diameter of epicardial coronary arteries leads to myocardial ischemia, which if prolonged leads to myocardial infarction. Thyrotoxicosis is a recognised cause of coronary artery spasm and enhanced sympathoadrenal activity is a postulated mechanism. Increased numbers of myocardial beta-adrenergic receptors, increased sensitivity of these receptors and increased myocardial tissue levels of catecholamines are thought to mediate this. This provides a rationale for the successful use of sympatholytic agents here. Our patient responded well to rapid restoration of euthyroidism with beta blockade and Propylthiouracil together with conventional cardiac therapy. Once euthyroid a block and replace regime was employed pending radioactive iodine therapy.

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### P57

#### Acromegaly, pregnancy and diabetes mellitus – a successful maternal and foetal outcome

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There are at least 60 reports of pregnancy and acromegaly. The maternal and foetal morbidity relates to associated diabetes mellitus and hypertension rather than maternal growth hormone excess. A 32-year-old Asian female who spoke no English, was noted to have acromegalic features during a hospital admission for pneumonia. She was 6 weeks pregnant and had been recently diagnosed with type 2 diabetes mellitus. Acromegaly was confirmed with GH nadir 105 mU/l during 75 g OGTT and serum IGF1–115 nmol/l (NR 15–65 nmol/l). A short synacthen test was normal with peak cortisol at 30 minutes of 661 nmol/l. Clinically she was hypothyroid and free T4–11.9 pmol/l (NR 10–25 nmol/l) and TSH  $< 0.05$  mU/l. She was treated with thyroxine 125 mcg daily and her twice daily insulin regimen continued. Visual fields were full on formal testing. Pituitary MRI scanning was delayed because of pregnancy and performed at week 20. A macroadenoma was confirmed (3.6 × 3.1 × 3.0 cm) abutting the optic chiasm. Very close monitoring of visual fields continued without visual deterioration. Glycaemic control remained excellent with mean HbA1c 5.8%. No hypertension observed. Growth and development of the foetus during pregnancy was normal. Elective LSCS occurred at 38 weeks where a 2.72 kg healthy baby was delivered. Transsphenoidal surgery was undertaken 6 months following childbirth. Post operatively GH levels remained elevated with mean GH 30 mU/l and IGF-1 raised at 113 nmol/l. In summary this case illustrates a successful maternal and foetal outcome during pregnancy in a patient with severe growth hormone excess when managed conservatively by a multi-disciplinary approach.

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### P58

#### Surgical management of metastatic pheochromocytoma: review of 2 cases

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We report two cases of MIBG avid secretory pheochromocytoma in 2 middle-aged ladies. Adrenalectomy, after standard preparation, was performed successfully and histology confirmed complete excision of a pheochromocytoma in both cases; however, there was capsular infiltration and metastasis to a neighboring lymph node in the first case. In this case, surveillance CT scans 3 and 6 months later showed left adrenal bed recurrence and rapidly growing lesions in the right lobe of the liver but normal urinary catecholamines. Right hepatic lobectomy was performed successfully and histology confirmed aggressive pheochromocytoma. In view of the MIBG avidity, MIBG therapy was administered 7 weeks after surgery. End of therapy scan showed abnormal uptake in the left lobe of the liver, mid and lower abdomen, pelvis, right hip, neck and the spine. The patient subsequently received palliative radiotherapy for painful metastases in the ribs and 2 cycles of palliative chemotherapy before she died 10 months after the second surgery.

In the second case, repeated radiological surveillance revealed a 14 mm solitary lesion in the lower lobe of the right lung 2 years after surgery. This was MIBG positive. Complete excision of the lung lesion was achieved surgically and histology confirmed a metastatic pheochromocytoma. Adjuvant MIBG therapy was given 2 months later and the end of therapy scan was negative. The patient remains disease free on clinical, biochemical and radiological grounds 4 years after initial surgery.

These cases highlight the importance of routine surveillance imaging after successful initial surgical treatment of pheochromocytoma and the fact that negative biochemical markers do not exclude a recurrence or metastases of a previously secretory pheochromocytoma. In addition, surgical management of a solitary metastasis can be very successful. However, the benefits of using adjuvant MIBG therapy after complete surgical excision of a suspicious MIBG-avid pheochromocytoma or its metastases remain unproven.



## P59

**The coincidence of mucoepidermoid and papillary thyroid carcinoma. A case report**GL Kovacs<sup>1</sup>, F Salamon<sup>2</sup>, Z Gorombey<sup>1</sup>, L Kovacs<sup>1</sup>, E Hubina<sup>1</sup>, MI Goth<sup>1</sup> & I Szabolcs<sup>3</sup><sup>1</sup>National Medical Center, Department of Internal Medicine, Budapest, Hungary; <sup>2</sup>National Medical Center, Department of Pathology, Budapest, Hungary; <sup>3</sup>Semmelweis University, Budapest, Hungary.

We report the case of a 55 years old female patient with parallel appearance of mucoepidermoid carcinoma and papillary thyroid carcinoma. She had autoimmune adrenal insufficiency and a pulmonary adenocarcinoma cured by lobectomy and external irradiation six years before. She had been referred to our department because of a rapidly growing thyroid nodule. Aspiration cytology suspected papillary tumor and a tumor of non-thyroid origin as well. Thyroidectomy and bilateral neck lymph-node dissection were performed. Histopathology and immunohistochemistry revealed a TG + and CEA-follicular variant of papillary carcinoma in the right lobe, mixed with a TG-CEA + mucoepidermoid carcinoma giving bilateral lymph node metastases. A left side papillary microcarcinoma was also found.

Less than 40 cases of thyroid mucoepidermoid cancer were reported so far in a few cases combined with other types of thyroid cancer. The ultimobranchial apparatus or follicular origin is disputed. We assume that the anamnestic external irradiation of the chest and neck region 6 years before might have played a role in the pathogenesis of this rare combination of malignancies.

## P60

**DIre Straits – hypothalamic diabetes insipidus, acute myeloid leukaemia and high risk cytogenetics: 45,XX,t(3;3)(q21q26),-7**

KS Myint, M Besser, J Craig, D Halsall, H Simpson, DF Wood, VKK Chatterjee &amp; M Gurnell

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We report a rare case of acute myeloid leukaemia (AML) with high risk cytogenetics and associated hypothalamic diabetes insipidus (HDI).

A 48-year-old female presented to her GP with a 3-month history of tiredness and lethargy. Full blood count revealed haemoglobin 7.0 g/dl (MCV 103fl), white cell count  $12.1 \times 10^9/l$  (neutrophils  $1.33 \times 10^9/l$ ), platelets  $91 \times 10^9/l$ ; circulating blast cells were evident on the peripheral blood film, and a diagnosis of acute myeloid leukaemia was confirmed by bone marrow biopsy with immunophenotyping demonstrating 'high risk' cytogenetics: 45,XX,t(3;3)(q21q26),-7.

On admission to hospital, the patient was noted to have polydipsia and polyuria: average 24 hr fluid intake 8–10 L, with negative balance of around 2 L/day. Routine chemistry: sodium 146 mmol/l, potassium 3.4 mmol/l, urea 0.6 mmol/l, creatinine 78 micromol/l, calcium 2.25 mmol/l, random glucose 7.1 mmol/l, serum and urine osmolalities 296 (NR 280–300) and 56 mosmol/kg respectively. A water deprivation test confirmed the presence of HDI. MRI of the brain revealed loss of the posterior pituitary 'bright spot', but no macroscopic evidence of tumour infiltration. Anterior pituitary function tests were entirely normal. The patient's symptoms were well controlled on desmopressin nasal spray. Unfortunately, despite receiving three different AML chemotherapy regimes, only a short period of remission was achieved, and the patient died 12 months after her initial presentation. More than seventy cases of HDI in association with AML have been described in the literature, with cytogenetic profiles reported in eighteen subjects: sixteen exhibited chromosome 7 abnormalities (predominantly monosomy 7), eight 3q abnormalities [most commonly: t(3;3)(q21q26) and inv(3;3)(q21q26)] and six combined chromosome 7 and 3 abnormalities as in our case. The pathogenesis of HDI in this setting has not been clearly elucidated: for example, leukaemic infiltration of the hypothalamus/pituitary gland has been observed in only a limited number of cases at post-mortem. Several other molecular mechanisms have been postulated, and these will be discussed in more detail.

## P61

**Metastatic insulinoma or not?**

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The localization of biochemically proven insulinoma remains a challenge despite the advent of more sensitive methods for radiological detection. We report a case with conflicting cross-sectional, angiographic and scintigraphic findings, in which selective angiography combined with intra-arterial calcium stimulation and hepatic venous sampling (ASVS) proved to be the most accurate localizing investigation.

A 17-year-old female with cerebral palsy and learning difficulty, but no prior endocrinopathy, presented with a 6-month history of seizures typically occurring prior to breakfast. She was admitted to hospital for a 72-hour fast, but developed symptomatic hypoglycaemia almost immediately, with biochemical profiling confirming the diagnosis of insulinoma [plasma glucose 1.9 mmol/l, insulin 67 pmol/l (NR 0–60), proinsulin 131 pmol/l (NR 0–7), 32–33 split proinsulin 100 pmol/l (NR 0–13), sulphonylurea screen negative]. Serum corrected calcium (2.3 mmol/l, NR 2.1–2.5) and prolactin (142 mU/l, NR 59–619) levels were normal. High dose diazoxide therapy and a continuous 10% dextrose infusion were required to maintain euglycaemia pre-operatively. CT of the abdomen demonstrated a bulky pancreatic head, but failed to reveal a discrete mass. Two small lesions were also identified within the liver, with appearances suggestive of possible metastases. Endoscopic ultrasound by an experienced radiologist confirmed an enlarged/lobulated pancreatic head, but no other abnormality. Whole body octreotide scintigraphy failed to demonstrate any abnormal uptake. Selective angiography with ASVS revealed a tumour blush and insulin secretory response only in relation to the splenic artery, suggesting functional tumour within the pancreatic body/tail.

At operation, direct visualization and palpation of the pancreas, coupled with intra-operative ultrasound, identified a 11 mm mass within the body. The head of the pancreas was normal. Frozen section biopsy of the liver lesions revealed focal nodular hyperplasia. Accordingly, surgical resection was limited to a distal pancreatectomy. Histology confirmed the presence of a single insulinoma. The patient has suffered no further hypoglycaemic episodes post-operatively.

## P62

**APECED (Autoimmune PolyEndocrinopathy-Candidiasis-Ectodermal Dystrophy) syndrome with atypical or incomplete presentation: report of 4 cases with AIRE mutations in heterozygosity**C Mazza<sup>1</sup>, I Sparapani<sup>2</sup>, L Costa<sup>2</sup>, S Zanola<sup>2</sup>, M Filisetti<sup>2</sup>, A Pilotta<sup>2</sup>, F Lombardi<sup>3</sup>, MI Spagnuolo<sup>3</sup>, G Valerio<sup>3</sup>, A Franzese<sup>3</sup>, G Radetti<sup>4</sup> & F Buzi<sup>2</sup><sup>1</sup>Institute of Molecular Medicine 'A. Nocivelli', Brescia, Italy;<sup>2</sup>Department of Paediatrics, University of Brescia, Brescia, Italy;<sup>3</sup>Department of Paediatrics, University of Naples, Naples, Italy;<sup>4</sup>Department of Paediatrics, General Hospital, Bolzano, Italy.

APECED is an autosomal – recessive syndrome defined by two of the following conditions: chronic mucocutaneous candidiasis (CMC), hypoparathyroidism (HP) or Addison disease (AD). Other autoimmune endocrine or non-endocrine conditions may be associated, like thyroiditis, celiac disease, alopecia and nail dystrophy. APECED is caused by mutations in the *AIRE* gene, mapping to 21q22.3. We report here 4 cases with atypical or incomplete presentation, that have been genetically defined and found to show *AIRE* mutations in heterozygosity. DR presented at 7 years with hypocalcaemia and undetectable PTH levels, and HP was diagnosed. ST presented at 14 years for alopecia aerata and pitted nail dystrophy and goitre. Thyroid function was normal in the presence of thyroid antibodies (Ab). AP at 7 years was found to have celiac disease. At 13 years developed type 1 diabetes, followed by euthyroid thyroiditis. At 15 yr, she also showed oral and vaginal candidiasis. DC was 4.3 when presented with hyponatraemia, hyperkalaemia, metabolic acidosis, low serum cortisol and elevated plasma ACTH. He was diagnosed AD, confirmed by adrenal Abs. Genetic analysis was by PCR amplification and direct sequencing of *AIRE* by ABI Prism 3130 sequencer (Applied Biosystem), and revealed: a typical mutation (R257X) on a single allele in DR; a novel heterozygous mutation (V484M) involving one of the zinc fingers (ZF) of the 2nd plant homeodomain (PHD) of the protein in ST; a novel heterozygous mutation (R316G) involving one of the ZF of the 1st PHD in AP; a novel heterozygous mutation (T441M), involving the ZF of the 2nd PHD in DC. The 3 latter mutations reside in transcription sites (ZFs of PHDs) and are likely to affect the *AIRE* protein function. These cases underline the importance of performing genetic analysis of *AIRE* in the presence of even one only major condition or minor signs and symptoms of APECED.

**P63**

**Relationship between age of onset, natural course and therapeutic approach in patients with acromegaly**

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The degree of hormonal activity and the duration of active phase of disease are the most important factors in the pathogenesis of system complications, decreasing life span in patients with acromegaly. To determine the age-related features of the clinical course of disease 140 patients with active acromegaly were examined. All patients were divided into 3 groups: group 1 included 14 patients at the age of 20–39, group 2 – 40–59 (72) and group 3 – >60 years (52). The mean age of the onset of disease in 1 group was 27, in 2–37, in 3–51 years old. In the 1st group the mean period from the onset to the diagnosis was 3.5, in 2nd – 6.2, in the 3rd – 9.3 years. The mean level of the GH in the 1st group was  $56.8 \pm 15.3$ , in 2nd –  $41.2 \pm 8.3$ , in 3rd –  $22.9 \pm 5.8$  ng/ml. The mean level of the IGF-I substantially exceeds the normal levels. In the 1st group macroadenomas were founded in 50%, in 2 – in 57%, in 3 – in 35%. In patients with the onset at the age younger than 30 years the main factor of the pathogenesis is the increased hormonal activity with the development of skeletal and organ changes. The complications are usually caused by extrasellar increase of the tumor with the development of neurologic disturbances. Therefore surgery is the basic method of treatment. In patients with the onset at the age older than 50 years the main factor of the pathogenesis, decreasing life span, is the duration of active phase. There are revealed the expressed cardiovascular, respiratory, metabolic disturbances, and neoplasms. Because of lesions of many organs the most preferable method of treatment is primary drug therapy (long-active somatostatin analogues). Use of surgical treatment is limited.

**P64**

**Sweet's syndrome and thyroid diseases: is there a link?**

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Sweet syndrome is a febrile dermatosis characterized by painful light red patches of quite different size involving various skin zones associated with flu like symptoms, arthralgias and rarely frank arthritis. Originally considered rare, over 500 cases in the past 10 years have been described. The etiology of Sweet's syndrome is unknown, but a type of hypersensitivity reaction leading to stimulation of a cascade of cytokines has been strongly suggested. In very few cases an association between thyroid disorders and Sweet's syndrome has been reported.

This report describes a case of relapse of Sweet's syndrome concomitant to changes in thyroid autoimmunity. A 50 year old women with a biopsy documented Sweet's syndrome in 2003 was referred to our endocrine unit because of a slight overweight (BMI =  $28.5 \text{ kg/m}^2$ ) that stressed the pain in the knees joints. Our observation occurred 2 months later a relapse of Sweet's syndrome (Februry 2005) following a 2 years glucocorticoid-induced remission. In this occasion we documented anti-TPO and anti-TG positivity (previously negative) and FT4 value within the low normal range ( $0.76 \text{ ng/dL}$ ).

The patient described in this report offers some points of discussion: the cytokines cascade occurring in Sweet's syndrome can stimulate HLA class II expression on immune thyroid epithelial cells, a phenomenon relevant for the amplification and progression of autoimmune thyroid diseases; furthermore, cytokines may play a cytotoxic effect on and induce apoptosis of thyroid cells. Another point of interest is represented by the cross reactivity between antigens from *Yersinia Enterocolitica* (highly positive in our patient) and thyroid-cell-membrane antigens.

In conclusion, many pathogenetic mechanisms proposed for Sweet's syndrome are potentially able to affect thyroid gland, suggesting a careful evaluation of thyroid function and autoantibodies in every patient with Sweet's syndrome.

**P65**

**Bone marrow aplasia in a patient with panhypopituitarism responsive to sex hormone therapy. The importance of compliance with testosterone therapy**

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The integrity of bone marrow and its various haemopoietic cells require several hormones including sex steroids. Sex steroids were used in the past to treat aplastic anaemia. A 27 years old Saudi male who underwent a resection of hypothalamic rocytoma and developed panhypopituitarism was admitted to ICU with shunt related meningitis and noted to have thrombocytopenia of 90,000 anemia of 9.5 g/dl, which progressed over the subsequent week following admission. He recovered from his meningitic illness uneventfully, however he remained with persistent thrombocytopenia and his platelet count dropped to a critical level but there was no bleeding diathesis. He also remained anaemic and showed evidence of leucopenia. Full investigation for the cause didn't reveal any abnormalities, and a bone marrow biopsy showed hypoplastic bone marrow with megakaryocyte dysplasia. Checking the patient compliance with his medication in the past revealed that he never bothered to take sex hormone therapy since he has had his surgery at age 16 years, despite that the family claimed that he was taking thyroxin and hydrocortisone regularly. Daily testosterone decanrate 250 mg IM for 3 days was initiated which resulted in dramatic rise of platelet count to 430,000 and normalization of his leucocyte count Maintenance therapy with testosterone therapy every 3 weeks was initiated. Repeat bone marrow biopsy after 6 weeks of therapy showed normo-cellular marrow with disappearance of megakaryocyte dysplasia. This case highlights the importance of sex hormone replacement therapy in patients with hypopituitarism not only for sexual potency and sense of well being but also for integrity of bone marrow function.

**P66**

**Multiple endocrine neoplasia type 1 (MEN1) in a patient with systemic mastocytosis**

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In 1984 an 18-year-old girl was operated because of primary hyperparathyroidism (PHPT). Recurrence was seen 13 years later and another neck exploration was performed. Histological examination was consistent with both adenoma and hyperplasia.

She had a history of urticaria pigmentosa since the age of one year. In 1991–94 she complained of syncope, weight loss, diarrhoea, abdominal pain and alcohol intolerance. Typical skin lesions were found. Total 24-h urinary methylimidazolacetic acid was markedly elevated ( $16.5\text{--}23.5 \text{ mmol}$ , reference range  $0.4\text{--}2.4$ ) in several samples. Biopsy of the duodenal and gastric mucosa demonstrated increased amount of mast cells and a bone biopsy was consistent with systemic mastocytosis. The diagnosis of SM was evident from the clinical picture and the bio-chemical findings. Treatment with interferon-alpha was instituted with good clinical and biochemical response.

In 2003 the patient was investigated because of a family history of PHPT (mother + two siblings). Very high levels of plasma pancreaspolypeptide were found ( $270\text{--}390 \text{ pmol/L}$ , reference range  $< 40$ ). Plasma chromogranin A concentration was normal, so were all other endocrine parameters. An abdominal CT revealed three cystic tumours in the pancreatic tail. MRI confirmed the tumours with irregular capsules, loading contrast media. A subtotal resection of the pancreas was performed. Histological examination demonstrated a neuroendocrine tumour, staining for chromogranin and synaptophysin. A pituitary MRI demonstrated a microadenoma, without hormonal secretion. Mutation analysis revealed a mutation in the MEN1 gene (deletion in exon 10).

**Discussion**

The findings of MEN1 and systemic mastocytosis in the same patient are very rare (if ever described). It may be a coincidence, but a mutation in the KIT- or platelet-derived-growth factor-(PDGF)-gene is another possibility. Treatment with a tyrosine kinase inhibitor could be a therapeutic option.

**P67****Brown tumour of the maxilla as the presenting feature of parathyroid carcinoma**

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Brown tumours (osteitis fibrosa cystica) are the classic bone disease of hyperparathyroidism. Parathyroid carcinoma is an uncommon cause of PTH dependent hypercalcaemia. We present the case of a 63-year-old man who presented to the ENT department with persistent right-sided nasal obstruction and nasal swelling. He was otherwise asymptomatic. He had a long history of nasal polyps, which had recently been resected. Brown's tumour of the maxilla was demonstrated on nasal biopsy. Subsequent investigations revealed an elevated calcium of 3.64 mmol/l (Normal range 2.12–2.55 mmol/l) and a grossly elevated parathyroid hormone (PTH) of 827 ng/l (Normal range 15–65 ng/l). Referral was made to the endocrine department. Ultrasound scan of the neck revealed a 3.1 × 2.6 × 3.0 cm mass, with cystic and solid components, in the left lower pole of the thyroid. There was intense uptake of Sestamibi in left lower pole of the thyroid and to the right of the midline in the face. Parathyroidectomy, left lobectomy and central node dissection was performed surgically. Histology demonstrated parathyroid carcinoma with clear resection margins. The patient remains well at follow-up after one year. Sestamibi uptake in the region of the right maxilla was less than previously with none within the neck or elsewhere in the body. His PTH however remains elevated.

First described by von Recklinghausen in 1891 Browns tumours are more often found in trabecular portions of the jaw, long bones and the rib. Brown's tumours of the facial bones have only rarely been described. Classic bone manifestations associated with hyperparathyroidism such as osteitis fibrosa cystica, subperiosteal bone resorption, salt and pepper skull and absent lamina dura are more commonly seen in parathyroid carcinoma (44–91%) than primary hyperparathyroidism (<5%). High serum calcium levels, high levels of parathyroid hormone, nephrocalcinosis, pancreatitis, peptic ulcer disease and anaemia should also raise the suspicion of parathyroid carcinoma.

**P68****Unexpected detection of thyroid cancer in a patient with presumed Graves' thyrotoxicosis**

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Thyroid cancer is rarely seen in the hyperthyroid patient.

We present the case of a 59 year-old lady who presented with marked weight loss of more than 30 kg in less than 6 months, lethargy, palpitations and sweats. Clinically she was obese and thyrotoxic with sweaty palms and a tachycardia. No goitre or neck masses were detected. Thyroid function tests confirmed thyrotoxicosis with free T4 89.1 pmol/l (Normal range 9.8–23.1), free T3 20.2 pmol/l (Normal range 1.8–6.8) and suppressed thyroid stimulating hormone (TSH) <0.01 miu/l (Normal range 0.35–5.5). A clinical diagnosis of autoimmune hyperthyroidism was made. The patient opted for treatment with radioactive iodine and a pertechnetate thyroid uptake scan was performed. This demonstrated increased size of the thyroid and homogenous uptake consistent with Grave's disease. However a focal area of increased uptake was also noted 5 cm below the left lobe of the thyroid. This was also demonstrated on whole body iodine scan following a treatment dose of radioactive iodine. In addition a further lesion was identified in the lower mediastinum and within the body of the tenth thoracic vertebrae. Ultrasound of the thyroid identified an abnormal 2 cm nodule within a multinodular gland. Total thyroidectomy and regional lymph node dissection was performed. Histology demonstrated papillary carcinoma of columnar/ tall cell type. A further dose of radioactive iodine was given. In this case thyroid carcinoma was detected serendipitously. The diagnosis of thyroid carcinoma is infrequently considered in the presence of hyperthyroidism. When thyroid carcinoma is identified with concomitant hyperthyroidism there is typically a non-functioning cold nodule on uptake scan. In our patient very high uptake within the gland may have obscured the presence of a cold nodule. Only the presence of uptake in distant metastasis alerted us to the possibility of thyroid cancer in this patient.

**P69****Primary hyperparathyroidism (PHPT): Still a cause of hypertension resistant to therapy**

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High blood pressure is a common problem among patients in a Nephrology ward and outpatient clinic. However in those patients resistant to therapy, we must exclude a secondary cause. The authors present a case of a patient that was referred to our hospital due to complaints of headache, high blood pressure resistant to therapy (BP = 240/130 mmHg), and images in renal ecography, which were compatible with enlarge left adrenal gland and left kidney atrophy. At admission the blood results were: Glucose = 97 mg/dl; PICr = 2.5 mg/dl; BUN = 33 mg/dl; Serum proteins = 6.7 mg/dl; Na = 129 mmol/L; K = 4.7 mmol/L; Ca = 13.8 mg/dl; P = 2.8 mg/dl.

We performed a CAT scan and MRI, both of which showed: Atrophic left kidney, enlarge right kidney; left adrenal with nodular formation. With such a result, a pheochromocytoma was considered, but all studies were negative. A renogram with diuretic and captopril showed a mute left kidney and excretory defect in the right kidney, without any evidence of renovascular pathology. We review the blood results, and found Ca = 13.8 mg/dl, PTH = 480 pg/ml. The parathyroid cintigraphy revealed an adenoma of left inferior parathyroid gland. The diagnosis of PHPT was done and the patient was submitted to a partial parathyroidectomy. Follow-up at 6 months: Calcium = 9.4 mg/dl; PTH 124 pg/ml; BP = 140/80 mmHg with 2 different drugs.

This case is an example of how, sometimes, a disturbance in calcium-phosphorus metabolism can be more than the consequence of chronic renal disease. Such changes can be related to a disorder that has an estimated prevalence on the order of 1% (PHPT). There is no consensus on the association between high levels of PTH and hypertension, although some authors associate hypertension with elevated PTH and hypercalcaemia.

**P70****Spontaneous pregnancy after trans-sphenoidal surgery in a patient with pituitary hypophysitis – a case report**

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Autoimmune hypophysitis is a rare disease with only 28 cases reported in U.K so far. Subsequent pregnancy, especially after pituitary surgery, is even more rare. We report a 34 year old lady who presented 5 weeks after the birth of her second child with visual loss and headache. Subsequent investigations revealed a pituitary macroadenoma with suprasellar extension and chiasmal compression. She underwent transphenoidal adenomectomy with complete recovery of vision. Preoperatively she was hypothyroid and had suboptimal cortisol reserve. Histology revealed Lymphocytic Hypophysitis.

Post-operative evaluation revealed Growth hormone deficiency, cranial diabetes insipidus, borderline cortisol reserve and normal thyroid function. She was commenced on regular desmopressin and prescribed steroids to be taken at times of stress. She continued to have regular periods and three years after her original presentation she got pregnant spontaneously. She had adequate thyroid and cortisol reserve during this pregnancy but continued to require desmopressin. There was no recurrence of her hypophysitis (the exact incidence of autoimmune hypophysitis with future pregnancies is unknown). She delivered spontaneously under steroid cover and her subsequent MRI scan revealed no change to the size of the residuum.

Literature search had revealed that 12% of women became pregnant again, after their initial presentation with autoimmune hypophysitis during their previous pregnancy or post-partum period. Autoimmune hypophysitis should be considered in the differential diagnosis of any pituitary mass especially when presenting during pregnancy or post-partum. A high index of clinical suspicion is required to diagnose this condition as conservative management may eliminate the need for aggressive pituitary surgery.



**P71**

**Adipsic diabetes insipidus following pituitary surgery for a macroprolactinoma**

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Adipsic diabetes insipidus (ADI) is a rare condition which has been reported following clipping of anterior communicating artery aneurysms, craniopharyngioma and brain trauma, but not with pituitary adenoma. We report a case of ADI following surgery for a pituitary macroprolactinoma. A 14-year-old boy presented with bitemporal hemianopia due to a large macroprolactinoma. Two debulking surgeries were performed without the development of diabetes insipidus. Following a third radical surgery, plasma sodium rose in the post-operative period to 157 mmol/l, after a diuresis of nine litres/24 h. He did not complain of thirst. He was resuscitated with intravenous dextrose and oral desmopressin. Hypertonic saline infusion caused a rise in plasma osmolality from 293 to 318 mOsm/kg, but plasma vasopressin levels remained undetectable (<0.3 pmol/l). Thirst ratings, measured by visual analogue scale, remained low (0.3 to 0.8 cm) and he drank only 400 mls of water (normal 1544 + 306 mls) in 30 min after infusion. These results confirmed ADI. A controlled reduction in mean arterial blood pressure from 93 to 66 mmHg using intravenous infusion of trimetaphan camsylate also failed to stimulate vasopressin secretion (<0.3 pmol/l throughout infusion). This boy therefore had absent osmoregulated thirst and vasopressin secretion and absent baroregulated vasopressin release. This combination of defects implies that the final surgery lesioned the osmoreceptors for thirst and vasopressin in the circumventricular organs and the supraoptic and paraventricular nuclei which synthesize vasopressin. This is the first report of ADI with absent osmoregulated and baroregulated vasopressin release following surgery for a pituitary adenoma.

**P72**

**Regression of somatotropinoma in the course of treatment with octreotide in a female patient with acromegaly**

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Long-acting somatostatin analogs are now increasingly being prescribed as adjuvant and primary therapy for the treatment of acromegaly. Many studies have shown them to be both effective and safe, by suppressing GH levels and normalizing serum IGF-1 levels in most cases. The aim of the study was to analyze the case report of a female patient with active acromegaly and evaluate the efficacy of long-acting synthetic analog of somatostatin (Sandostatin LAR) for biochemical control and tumor shrinkage.

A 68-year-old female patient with symptoms of acromegaly in the course of macroadenoma of the pituitary gland was qualified for the treatment with octreotide (Sandostatin LAR) according to the diagnostic-therapeutic scheme. A notable effect of pharmacological treatment with Sandostatin LAR was observed after administration of three injections - serum GH concentration decreased as much as 94% and IGF-1 concentration was significantly lower than prior to therapy. Taking into consideration these results neurosurgical treatment was ruled out and continuation of pharmacological therapy was decided. Before and after treatment with 3, 6, 9 and 12 injections the clinical examination with the patient's subjective assessment was carried out, biochemical and hormonal tests were also performed. After 6 and 12 injections, a control ultrasound examination of the abdominal cavity was performed. After 12 injections magnetic resonance imaging was performed in order to evaluate the pituitary gland.

**Results**

In the presented case report, the clinical symptoms of acromegaly were markedly reduced or completely regressed after therapy with depot somatostatin analog. During a 48-week observation period the patient did not report any side effects. The most remarkable effect was full regression of the observed pathological tumor mass in the glandular part of the pituitary gland.

**Conclusion**

The long-term treatment with depot somatostatin analogs can be a safe and effective method for patients with acromegaly and may be effective in controlling the course of the disease.

**P73**

**Familial hyperparathyroidism with a mutation in the *HRPT2* gene**

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A 72 years old lady was referred to endocrine clinic with raised serum calcium of 2.69 mmol/l and PTH of 99.6 ng/l (3.0–48.0 ng/l) detected during investigations for an abnormal skeletal survey. She was known to have osteoporosis from the age of 59 diagnosed on DEXA scan. Case notes revealed raised serum calcium of 2.52–2.60 mmol/l since year 2000. Her repeat serum calcium was 2.77 mmol/l with phosphate of 1.03 mmol/l and 25 hydroxy vitamin D of 36 nmol/l. She admitted to symptoms of fatigue and increased thirst. There was no history of dyspepsia or renal calculi. She did not have any jaw swellings.

Family history showed several members of her family affected by primary hyperparathyroidism.

A high resolution ultrasound scan of the neck localised an adenoma in right superior parathyroid gland. The patient underwent parathyroidectomy and a single adenoma weighing 476 mg was removed from right superior parathyroid gland.

Hyperparathyroidism – Jaw Tumour (HPT – JT) syndrome is an autosomal dominant condition characterised by parathyroid adenoma/carcinoma and multiple ossifying jaw fibromas. The latter is not a constant finding. HPT – JT syndrome can be caused by mutations in the *HRPT2* gene. Mutation screening of *HRPT2* gene was undertaken in view of family history of hyperparathyroidism, even though the patient did not have any jaw tumours. She was found to have a T > C point mutation at nucleotide c.188 in exon 2 of *HRPT2* gene. This results in the replacement of the amino acid leucine with proline at codon 63. This is a missense mutation in a highly conserved region, not previously reported in the literature. We are currently investigating the other members of this family.

**P74**

**Pendred's syndrome with three mutations**

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This 48 year old with consanguineous parentage presented at the age of 23 in 1980 with congenital bilateral sensorineural deafness and hypothyroidism requiring replacement with 150 mcg thyroxine. There was a family history of deafness and thyroid disease. A perchlorate discharge test was performed and 42% of the radioiodine within the gland was discharged by potassium perchlorate (NR < 10%).

Over the following decade she developed a diffuse goitre which required treatment with subtotal thyroidectomy because of compressive effects. A CT scan of the petrous temporal bone showed dilated vestibular aqueducts bilaterally.

This case provides a classic example of Pendred syndrome; the most common form of syndromic deafness which is characterized by congenital sensorineural hearing loss and goitre. The gene (*SLC26A4*) has been mapped to chromosome 7q and encodes a transmembrane protein called pendrin which functions as an iodide transporter. Mutation of this gene decreases organification of thyroglobulin.

Pendred syndrome is diagnosed on the basis of the association of sensorineural hearing loss, goitre and an abnormal perchlorate discharge test. The hearing loss is variable and goitre may not manifest until the second decade. Abnormalities of the inner ear such as dilatation of the vestibular aqueduct and Mondini malformation are seen from early infancy in a very high proportion of patients with this condition. A CT scan of the petrous temporal bone is a useful diagnostic aid. Genetic analysis, on the other hand, is difficult and time consuming – often requiring a staged approach. At times, changes of uncertain significance may be identified. Three missense mutations were identified in the *SLC26A4* gene in our patient. We are in the process of testing the other affected and unaffected members of the family to identify the pathogenic mutations in this kindred.

**P75****About three cases of Riedel thyroiditis**

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Riedel thyroiditis is an uncommon form of chronic thyroiditis in which the thyroid gland is replaced by fibrous tissue. The etiological mechanism underlying RT are unclear. The prevailing view is that it is part of a generalized fibro-inflammatory process also involving other organs. Therapeutic options are not standardized (surgery, corticosteroids, and more recently tamoxifene). The aim of this work is to present three new cases, and the favourable evolution observed in one of them with cyclosporine. All three patients were female, aged 48 to 61 years old. Patients 1 and 2 complained of rapidly growing compressive stony cervical nodule, that was diagnosed in the follow-up of an oropharyngeal epidermoid cancer treated by radiotherapy and electrocoagulation 10 years before in case 2. Case 3 was first diagnosed Hashimoto thyroiditis in reason of the presence of antithyroperoxidase auto-antibodies, and of cytodiagnostic. Two years later, the patient presented with stony compressive infiltrative neck enlargement. At the time of diagnosis, patients 1 and 3 complained of dysphagia and dyspnea and patient 2 had vocal cord paralysis. The three patients had hypothyroidism, and lack of uptake on I123 thyroid scintigraphy. Ultrasonography showed hypochoic lesion, infiltrating perithyroid organs, inducing carotid sheathing, with adenopathies. Riedel thyroiditis was diagnosed in these 3 women on surgical biopsy. Cases 1 and 3 were treated with tamoxifene and the result was favourable in patient 1 with overt decrease of compressive symptoms one year later. The results of patient 3 are awaited. Patient 2 was first treated with steroids without any results. Cyclosporine, introduced because of severe psoriasis, induced a dramatic decrease of her thyroid volume. To conclude, Riedel thyroiditis is a rare thyroid disease, often resembling malignancy more often encountered in women in the second part of life. Steroids and tamoxifene may improve symptoms. Cyclosporine was found efficient in one of our cases.

**P76****Parathyroid carcinoma in a patient with chronic renal failure on long-term dialysis**

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**Background**

Parathyroid carcinoma (PC) is a rare endocrine tumour, with difficult intraoperative recognition, histological diagnosis and imprevisible evolution. More unusual are the cases appearing at the patients with end-stage renal disease on maintenance hemodialysis (22 observations in medical literature).

**Case report**

A 46 year-old man which suffered of chronic glomerulonephritis from 13 years, being on hemodialysis from three years, complains of asthenia, adynamia, muscle weakness, severe itching and progressive osteoarticular pains. Preoperative iPTH was 71 ng/ml, seric Ca<sup>++</sup>: 1.23 mmol/l, seric Ca: 2.3 mmol/l, seric P: 2.02 mmol/l. Clinical and ultrasonographical examinations revealed a left "thyroid nodule" of 44/32 mm and moderate enlargement of the right sided parathyroids.

**Results**

At operation three parathyroid glands (two from the right side and the superior left one) was easy identified and resected after frozen section. The fourth gland was not found but the ablation of the "nodule" revealed a PC confirmed by the paraffin examination. Postoperative clinical and humoral course was uneventfully and the patient continued his hemodialysis programme, being well after 20 months, without recurrence or metastasis. The recent iPTH was 5 ng/ml.

**Conclusion**

The authors underlined the criteria and difficulties of pathologic diagnosis of PC and the importance of the initial surgical treatment based of their experience of three cases representing 8.1% (!) in a series of 37 observations of primary and renal hyperparathyroidism operated on in the last ten years.

**P77****Should prophylactic thyroidectomy be carried out in mucosal neuroma syndrome?**

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**Background**

Multiple endocrine neoplasia (MEN) type 2B is an autosomal dominant condition characterised by aggressive medullary C cell tumours, pheochromocytoma and a discrete physical appearance. A specific point mutation in the *RET* proto-oncogene is present in 95% cases; prophylactic thyroidectomy is recommended in the mutation carriers. Occasionally cases present with the characteristic physical appearance of MEN2B but no identifiable germline mutation or endocrinopathy (termed 'mucosal neuroma syndrome'; MNS). Management of such cases remains uncertain.

**Case-history**

A 13-year-old girl initially presented with photophobia, when she was found to have characteristic features of MEN2B, including prominent corneal nerve fibres, thick lips, multiple mucosal neuromas, marfanoid habitus, high arched palate, "coast of Maine" café au lait patch and genu valgum. Blood pressure was 120/65 mmHg. Initial investigations showed normal 24-hour urinary catecholamines, serum calcium, parathyroid hormone, basal and alcohol stimulated calcitonin (<0.08 mcg/L), and CT of the adrenal glands. She was followed up with regular urinary catecholamine and pentagastrin stimulated serum calcitonin levels. Pentagastrin stimulation 18 months after presentation gave equivocal results. Baseline calcitonin level was 0.09 mcg/l (normal < 0.08 mcg/L), rising to a peak of 0.14 mcg/l at 2 minutes. Because of the uncertainty surrounding the natural history of patients presenting with this phenotype at a time when the gene was unknown, in association with a rise in basal calcitonin, a decision was made to proceed to prophylactic thyroidectomy. Histology showed no evidence of medullary carcinoma or C cell hyperplasia. Recently sequencing of exons 10, 11, 13, 14, 15 and 16 of the *RET* gene showed no mutation. The patient has been followed up annually for the last 18 years and has remained well.

**Conclusions**

This case suggests that mandatory prophylactic thyroidectomy is unnecessary in patients with MNS without *RET* mutation or endocrinopathy, although they should be screened for endocrinopathy on a regular basis.

**P78****Synchronous malignant para-aortic pheochromocytoma and vagal nerve paraganglioma in a patient with germline SDHB mutation**

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Pheochromocytomas and paragangliomas arising as part of familial syndromes such as multiple endocrine neoplasia (MEN)2 or von-Hippel Lindau syndrome (VHL) are more likely to be malignant and multiple. Recently the role of mutations in the SDH genes (encoding subunits of succinate dehydrogenase) have also been identified as important in the aetiology of such tumours. We report a case where identification of an apparently sporadic metastatic pheochromocytoma was found to be complicated by a synchronous paraganglioma of the vagus nerve in a patient with a pathogenic mutation of the SDHB gene.

**Case**

A 36-year-old man presented to the emergency department due to right iliac fossa pain and fever. Laparotomy and appendicectomy was carried out but no abnormality detected. An abdominal CT scan indicated the presence of a 5.4 × 5.2 × 6.8 cm retroperitoneal para-aortic mass. No lymphadenopathy was noted. 24 hour urinary excretion of noradrenaline 7589 nmol (<780) and adrenaline 282 nmol (<80) and serum chromogranin-A 50U/l (<21.8) were elevated. Excision revealed an extra-adrenal pheochromocytoma with lymphovascular permeation and a lymph node containing metastatic tumour. Catecholamine excretion returned to normal. CT scanning identified a 2.5 × 2 × 3 cm neck mass with cervical lymphadenopathy. MIBG scanning was normal. PET scanning revealed an FDG avid lesion in the right mid-cervical region. Excision of a vagal nerve paraganglioma was carried out. The patient now has swallowing difficulties. None of the common mutations were found on analysis of the VHL gene and RET proto-oncogene. The R90X mutation was found in exon 3 of the SDHB gene. Family members with the SDHB mutation are being screened for further neuroendocrine tumours.

**Discussion**

Genetic screening in patients with extra-adrenal catecholamine secreting tumours is important. SDHB mutations appear to be associated with paragangliomas with malignant potential. Vagal nerve paragangliomas are rare and involve arduous surgical procedures and outcomes.



**P79**

**Ophthalmic presentations of Cushing's syndrome**

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**Introduction**

Central Serous Retinopathy (CSR) is a condition characterized by the accumulation of sub-retinal fluid at the posterior pole of the fundus, creating a circumscribed area of serous retinal detachment. It is associated with increased levels of endogenous or exogenous glucocorticoids and has been described in patients with Cushing's syndrome (CS).

Here we report on two cases we recently managed in our unit, with central serous retinopathy and Cushing's syndrome.

**Case 1**

40-year-old male referred by the ophthalmology service with visual failure on background of central serous retinopathy. MRI of the pituitary revealed the presence of a large pituitary macro adenoma with optic chiasm compression and involvement of the cavernous sinus. Endocrine investigations confirmed Cushing's disease with 9:00am ACTH greater than 1000. He had Trans-sphenoidal decompression surgery, which made an impact on his visual acuity, and his ACTH has declined to 360. He is awaiting further management for his CS.

**Case 2**

39-year-old male diagnosed with bilateral CRS in 1999 for which he had laser treatment to the left eye. The patient conducted his own literature search on the Internet and found an association between CRS & CS and insisted that his GP screened him for CS to account for some of his symptoms. Although he didn't have florid features of CS, endocrine assessment including IPSS confirmed a pituitary micro adenoma causing Cushing's disease. He subsequently underwent Trans-sphenoidal surgery, which cured his Cushing's disease.

**Conclusion**

These cases give additional evidence that glucocorticoids may play a role in the pathogenesis of CSR and patients presenting with this ophthalmic problem should be questioned for features of Cushing's syndrome.

**P80**

**Long-term outcome of untreated Cushing's syndrome due to pituitary microadenoma**

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**Background**

The diagnosis of Cushing's syndrome is based on clinical suspicion and biochemical confirmation. Most cases are caused by an ACTH secreting pituitary microadenoma. In its severe form and when untreated, Cushing's syndrome is associated with high mortality. We report a case of untreated Cushing's syndrome due to pituitary microadenoma 8 years after the earlier manifestations.

**Observation**

A 21-years-old woman was referred for progressive weight gain. Her normal weight of 60 kg with height of 158 cm increased to 79 kg during pregnancy and to 99 kg two years after term delivery. Serum cortisol level was high (952 nmol/l, N:171-575) with loss of normal circadian rhythm and negative low-dose dexamethazone suppression test, but serum ACTH and urinary free cortisol were in normal range. No other clinical manifestation of Cushing's syndrome was observed. CT scan showed a possible pituitary lesion suspected of microadenoma. Pituitary MRI was not carried up because the patient was lost to follow-up. Eight years later, she was admitted in intensive care unit for acute respiratory distress. She had typical clinical features of Cushing's syndrome, diabetes, hypertension and depressive state. Her weight was 136 kg with BMI of 51 kg/m<sup>2</sup>. Serum cortisol was high with loss of normal circadian rhythm, negative low-dexamethazone suppression test. Levels of urinary free cortisol and serum ACTH were high suggestive of pituitary-dependant Cushing's syndrome. MRI was not performed because of centripetal obesity, but thin CT scan showed a pituitary microadenoma successfully removed by transsphenoidal surgery. ACTH secreting tumor was confirmed by histological study. Hormone replacement was not required because there was no hypopituitarism. Weight and other clinical symptoms of Cushing's syndrome slowly decreased. Diabetes was well-controlled with hypoglycemic agents (Metformine and Pioglitazone) after insulin therapy required before surgery intervention.

**Discussion**

The clinical picture of Cushing's syndrome can vary greatly among patients depending on duration and severity of disease. Mortality rate is approximately 50% at five years in published reports. Obesity is the aspect of Cushing's syndrome that patients find most distressing. Weight reduction following successful treatment is slow. Patients need regular exercise and a suitable diet to control weight gain and to restore muscle strength.

**P81**

**Invasive prolactinoma with multiple recurrences: pituitary atypical adenoma or pituitary carcinoma? Report of a case**

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**Background**

Invasive pituitary tumors may behave like some pituitary carcinomas. Although invasiveness is not indicative of malignancy, it probably puts the patient at higher risk of developing a pituitary carcinoma. These are very rare and the diagnosis requires evidence of metastatic disease, either cerebrospinal or extracranial. Although *de novo* development cannot be excluded they usually present as typical pituitary adenomas, which reveal their malignant character only as time progresses.

**Case**

We describe a 35-year-old woman who presented with a prolactin-secreting pituitary macroadenoma with suprasellar extension, left cavernous sinus and sphenoid sinus invasion and without pituitary insufficiency. Five years later, after the diagnosis, the tumor developed an invasive behaviour with pathological (nuclear pleomorphism, mitotic figures, necrosis and bone invasion) and immunohistochemical studies indicating a highly proliferative activity, aggressive growth and malignant potential (ki-67 > 3% and a p53 expression that revealed scarce nuclear immunostaining). She underwent medical therapy during the first years, four pituitary surgeries, all of them with early recurrence, and radiotherapy. She died approximately nine years after the initial diagnosis and four years following the first pituitary surgery. No proven site metastasis was detected.

**Conclusion**

This case highlights the poor prognosis of this type of tumors. The clinicopathological course in itself should alert clinicians for the tumor to be labelled as aggressive and/or potential malignant. Ki-67 labelling indices in combination with p53 nuclear staining have been shown to correlate with invasiveness growth and aggressive/malignant behaviour. It is, therefore, important to perform these markers in invasive macroadenomas in order to predict the subset of tumors with the most aggressive behaviour. All available treatments should be applied, early in the course of the disease, in an attempt to prolong a better survival rate.

**P82**

**A case of primary pigmented micronodular hyperplasia as a cause of Cushing syndrome**

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ACTH-independent micronodular adrenocortical hyperplasia is a rare cause of Cushing syndrome. In some forms of micronodular adrenal disease, darkly pigmented micronodules are seen in the presence of atrophy of the peripheral adrenal tissue. In most cases of pigmented micronodular hyperplasia Carney complex is associated with the disease. Here, we present a case of Cushing syndrome due to sporadic primary pigmented micronodular hyperplasia not accompanied by Carney complex.

A 30-years old female patient was admitted to hospital because of weight gain, swelling of the face, hypertension and muscle weakness. Physical examination revealed classic Cushingoid features. Her basal serum and urinary free cortisol levels were elevated and ACTH levels were suppressed. After overnight (1 mg), low dose (2 mg) and high dose (8 mg) dexametasone tests urinary and serum cortisol levels were not suppressed and even elevated further. On abdominal MRI, micronodular hyperplasia was shown on both adrenal glands. A 2 mm. lesion suggesting microadenoma was also shown on the MRI of the pituitary. Than a petrosal sinus sampling was performed. In the samples obtained from petrosal sinus and periphery, ACTH

levels were all suppressed and did not increase after CRH stimulation. GIP-dependent Cushing was excluded by studying cortisol response to glucose loading. No physical or laboratory finding regarding Carney complex could be demonstrated. Cushing syndrome was concluded to be due to bilateral surrenal hyperplasia and a bilateral adrenalectomy was performed. In pathological examination, multiple, gray-brown colored micronodules, 0.5–1.2 mm. in diameter were detected in both adrenals. After the operation, she was administered steroid replacement therapy and her Cushingoid features regressed progressively during follow up. Primary pigmented nodular hyperplasia is a rare diagnosis which should also be considered in patients with ACTH-independent bilateral adrenal disease not exhibiting features of Carney complex.

## P83

### Endocrine evaluation in a man with restrictive eating behaviour

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Eating disorders are less common in men than in women. Furthermore, in males, a restrictive eating behaviour is frequently secondary to other psychiatric disorders. In case of suspected primary anorexia nervosa (AN), in male patients it is obviously impossible to rely on a typical clinical sign as is amenorrhea.

#### Case description

A 23-yr old man came to our observation because of an important weight loss in the last two years (from 60 to 48 kg, body mass index at the moment of observation 15.6 kg/m<sup>2</sup>). He denied voluntary restriction of food intake or physical hyperactivity, vomiting, as well as diuretic or laxative abuse. Endocrine evaluation showed normal thyroid function, slightly increased urinary free cortisol, low IGF-I, reduced levels of free testosterone and very low concentrations of gonadotropins. MRI did not disclose any abnormalities of the hypothalamic-pituitary region. According to these findings, the patient was evaluated by a psychiatrist and, upon detailed inquiry, he admitted severe reduction of food intake and physical hyperactivity along with diminished libido and impotence. A diagnosis of AN was established. The patient refused psychopharmacological treatment but, following psychotherapy, spontaneously increased food consumption, reduced physical activity and gained 13 kg in the following ten months. A parallel increase in circulating gonadotropins and free testosterone, along with the reinstatement of normal libido and erectile function, was registered.

#### Comment

This case report underlines the importance of assessing pituitary-gonadal function in men with alimentary restriction likely related to psychological disorders. Indeed, in a male patient in whom AN is suspected, the presence of hypogonadotropic hypogonadism may be useful in confirming this diagnosis, representing a marker equivalent of amenorrhea in women.

## P84

### Hypopituitarism in an adult thalassaemic patient. Effects of different replacement therapies

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In a small but not negligible proportion of thalassaemic patients, a true growth hormone deficiency (GHD), not secondary to pubertal delay, can be documented. Among the features of adult GHD syndrome, osteoporosis leads to a 2.5-fold increase in fracture risk. Thalassaemia is characterized by a peculiar bone disease leading to osteopenia. The effectiveness of GH treatment in GHD adult thalassaemic patients has not been explored yet. We report here the case of such a patient, displaying both true GHD and hypogonadism, treated in different periods with GH and testosterone.

#### Case description

A 22 yr-old man affected by Cooley's disease with a height of 155 cm (< 3rd percentile for age), a bone age of 13 yr 6 mo, and pubertal stage P2G1. Hypogonadotropic hypogonadism and GHD (lack of GH response to stimuli after testosterone priming) were documented, together with femoral osteopenia (T score -2.25) and severe lumbar osteopenia (T score -4.75). After one year of GH treatment 0.24 U/kg weekly, dosage usually used in childhood, clear-cut increases in height (+8 cm), femoral BMD

(+4%) and lumbar BMD (+6.5%) were observed. Pubertal status and bone age were unchanged. Following a two month wash-out period, the patient was started on i.m. testosterone enanthate 250 mg monthly. After one year, further increases in height (+5 cm), femoral BMD (+4.5%) and lumbar BMD (+7.1%) were registered. Pubertal status proceeded to P5G5, with an unchanged delay in bone age.

#### Comment

These data, although available so far in a single subject, demonstrate the effectiveness of GH treatment in Cooley's adult patients with GHD. Given the clinical relevance of adult GHD, the potential benefits of GH replacement deserve considering also in thalassaemia major.

## P85

### Langerhans cell histiocytosis: management dilemma for the adult endocrinologist!

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#### Clinical case

24-year-old student nurse referred to the liver unit with a 6-month history of pyrexia, cognitive dysfunction and abnormal liver function tests. ERCP was normal; CT scan suggested multiple liver deposits; histology showed both fatty infiltration and focal cholangitis. Medical history included type 1 diabetes since age 7 years; an unusual skin rash that spontaneously remitted 4 years previously; recurrent vulval ulceration, variously ascribed to candida and/or excoriation; 3 spontaneous pneumothoraces over the past 5 years, eventually necessitating thorascopic bullectomy; new-onset isolated cranial diabetes insipidus with normal anterior pituitary 2 years before, MRI demonstrating pituitary stalk thickening. She was referred for management of increasing obesity and brittle diabetes and was found to have developed thyrotroph and gonadotroph failure. MRI showed an enhancing mass, posterior to the optic chiasm, involving the hypothalamus and pituitary stalk.

Stereotactic biopsy revealed Langerhans Cell Histiocytosis (LCH). CT thorax confirmed pulmonary involvement and skin biopsy showed cutaneous involvement. Although bone marrow examination was negative for markers of LCH, as was re-examination of her liver histology, she nevertheless had rapidly multisystem LCH, losing corticotroph function a few weeks later.

#### Discussion

Although of adult age at eventual diagnosis, the past history and multisystem involvement were consistent with the pattern of "childhood" LCH. In the absence of large clinical trials, the imperative is *primum non nocere*. Radiotherapy had no role given systemic disease pattern and, in view of her obesity and brittle diabetes, we did not proceed to high-dose steroid therapy and instead treated her for 2 months with Imatinib, building up to 400 mg daily. There was, however, no clinical or radiological response, with FDG-PET imaging revealing persistent localised hypermetabolism in the area of the hypothalamus. Some of the classical chemotherapy regimens have carries substantial morbidity and, following extensive worldwide consultation, she will therefore shortly be beginning therapy with Cladribine, for which both side effect profile and evidence base appear relatively promising.

## P86

### A case of bacterial endocarditis in a patient with acromegaly

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#### Objective and importance

Among pathologies associated with acromegaly, have great importance both colon benignant neoformations such polyposis, and cardiovascular complications such as miocardic hypertrophy, arterial hypertension, coronaropathy and valvulopathy.

#### Clinical presentation

We report a case of 74-years-old woman with acromegaly, diabetes mellitus type II and hyperthyroidism, who was admitted at our clinic with symptoms

of chronic febricula with episodes of high fever in excess of 39°C, preceded by shaking chills and associated with profuse sweating and abdominal pains.

#### Interventions

The first diagnostic step excluded as a possible cause of clinical manifestations, both episodes of hypoglycaemia due to oral antidiabetic drugs, or hyperthyroidism breakthrough. Considering the positivity of haemocultures for *Streptococcus Bovis* during fever's spikes and the possible involvement of cardiac valves in acromegalic patients, a focused transthoracic echocardiogram was done. It showed a mitral valve prolapse and an hyperchogenic formation on the posterior limbus. Thus the diagnosis of bacterial endocarditis was concluded. After the resolution of the acute situation and when haemocultures became negative, the patient, due to the presence of anaemia, was further subjected to a colonoscopy, which showed micropolyps of the transverse colon.

#### Conclusions

In literature the association between colon polyposis and bacteraemia by *S. Bovis* was described. This pathogen would in fact provoke the release of pro-inflammatory cytokines (IL-8, PGE-2) responsible for the destruction of capillaries at the level of the newgrowth permitting as well as for the bacteria penetrating into the haematic circulation. Thus the diagnostic hypothesis is that acromegaly, in our patient, has in one hand favoured colon polyposis, allowing the proliferation of *S. Bovis* and its haematic diffusion, and on the other hand it has induced mitralic valvulopathy, rendering possible bacterium take on the valve limbus.

### P87

#### Gitelman's syndrome in pregnant, type 1 diabetic patient presented with foetal growth retardation, and bilateral hydronephrosis and hydroureter

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Gitelman's syndrome (GS) is an autosomal recessive primary renal tubular disorder with hypokalemic metabolic alkalosis, hypocalciuria, and magnesium deficiency. The association of GS and type 1 diabetes is rare<sup>1</sup>, and bilateral hydronephrosis and hydroureter has not been reported.

A 18-year-old female with known GS diagnosed at 17 years of age and type 1 diabetes diagnosed at 15 years of age was admitted to Leicester Royal Infirmary for premature delivery by caesarean section due to foetal growth retardation at 27 weeks. Patient had history of poor control diabetes with HbA1c 11.2% during pregnancy, reduced to 7.8% postpartum, and moderate renal impairment mainly during her pregnancy (creatinine 247 micromol/l, comparing to 115 just before pregnancy). She was noted to be short, and had congenital skin disorder diagnosed previously as *Cutis marmorata telangiectatica congenita*.

Postpartum laboratory profile revealed increased serum creatinine to 342-micromol/l. 24-hour urine collection for protein was 1.52 g/l. Renal ultrasounds scans showed bilateral hydronephrosis and hydroureter.

Laboratory profile of her premature baby showed, low magnesium and hypokalemia, which corrected by TPN feeding. Genetic analysis needed to confirm the diagnosis of Gitelman's syndrome.

This is the first reported case of bilateral hydronephrosis and hydroureter in the setting of GS. Bilateral hydronephrosis and hydroureter secondary to chronic polyurea reported in Bartter's syndrome. In GS, putative loss-of-function mutation in the thiazide-sensitive NaCl cotransporter located in the apical membrane of the distal convoluted tubule leads to polyuria, inappropriate kaliuresis, and hypomagnesaemia.

1. Farhad Zangeneh, Myra Chiang, *et al*, Pitfalls in the Laboratory Diagnosis of Diabetic Ketoacidosis in Gitelman's Syndrome. *Diabetes Care* 26:955, 2003.

### P88

#### The prolonged progressive course of acromegaly, complicated with diabetes mellitus, bilateral gonarthropathy, thyroid pathology and severe papillomatosis (clinical observation)

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A patient, female, 53 years old was admitted for investigation and treatment of active acromegaly. At the age of 33 on the basis of the clinico-hormonal and CT investigations was diagnosed mixed pituitary adenoma - somatotropinoma. GH was 15.4 ng/ml (0–10 in healthy), prolactin (prl)

2000 ng/ml (<580 in healthy). The short course of parolodel therapy had no effect. At the age of 40, she was performed with transsphenoidal adenectomy. Afterwards, diabetes mellitus (DM), severe gonarthropathy and multinodular goiter were revealed. At the age of 48, the relapse of somatotropinoma was diagnosed. The clinical symptoms of acromegaly persisted. The Parlodel treatment was used unconstantly.

On the basis of clinical, hormonal data and MRI we diagnosed somatotropinoma (size ~2 sm). Blood hormones: GH-45.7 µIU (0.16–13 in healthy), IGF-1-518 ng/ml (87–238 the age range), prl - 18 ng/ml (160–500 in healthy). DM was classified as symptomatic (DM was absent among relatives). High daily Insulin doses (>100 U) and Siofor 850 mg 3 tab/day were used because of insulinoreistance. Severe bilateral gonarthrosis with decreased motility and multiple papillomatosis were diagnosed. The multinodular goiter was diagnosed by the thyroid ultrasonic. Thyroid volume was 30 ml. Two nodules (d > 2 sm) nonhomogenic structure and low echogenicity were revealed. TSH achieved normal significance on replacement L-Thyroxin therapy. Somatulin treatment 30 mg/day/two times i/m each 2 weeks was recommended.

In conclusion, we represented a case of acromegaly with multiple severe complications: symptomatic DM, multinodular goiter, with primary hypothyroidism, bilateral gonarthrosis with decreased motility, multiple papillomatosis, developed in result of nonadequate treatment. The Somatulin therapy was started.

### P89

#### A case of adult GH deficit in a woman with Autoimmune Polyendocrine Syndrome type II

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We report a case of a 53 years old woman who presented asthenia, cramps, cutaneous hyperpigmentation, nausea, vomiting, vague and non-specific epigastric abdominal pain, weight loss and hypotension. She was diagnosed with Addison's disease because biochemical data were indicative for autoimmune adrenal failure (positivity for adrenal cortex antibodies). Nevertheless during the following 9 years the patient began to note the progressive reduction of pubic and axillary hair, an increase of the thyroid gland and irregularity of her menstrual cycle; hormonal and immunological values and ultrasound scan demonstrated primitive hypothyroidism with Hashimoto thyrohiditis and premature ovarian failure associated to the presence of antibodies against ovarian tissue. The patient also showed the specific symptoms of acute hyperglycaemic state and the positivity of GAD antibodies that allowed the diagnosis of diabetes mellitus type 1. She was therefore diagnosed with Schmidt's syndrome (Polyglandular Autoimmune Endocrine Insufficiency-PGAI). Despite of an adequate treatment for Addison's disease, hypothyroidism, premature menopause and diabetes mellitus type 1, the patient continued to show fatigue, asthenia, osteoporosis and became overweight (BMI = 27.71 kg/m<sup>2</sup>). Thus we also studied pituitary function with GH-RH + Arginine test in order to evaluate a possible GH-deficit (GHD) due to an autoimmune inflammations of the pituitary gland, because also this gland could be attacked by organ specific autoantibodies, and in relation to the persistence of clinical symptomatology. The patient was diagnosed with GH deficit in adult (GH nadir during GH-RH + Arginine test: 5 ng/ml). The diagnostic hypothesis is that GHD, in this patient, was caused by autoimmune hypophysitis within Autoimmune Polyendocrine Syndrome type II. Nevertheless antipituitary antibodies (APA) were negative, we suppose that their absence was because they became negative during the time elapsed between hypophysitis onset and its diagnosis.

### P90

#### Spontaneous thrombosis of ophthalmic artery aneurysm causing isolated cortisol deficiency

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A 24 year old man complaining of visual disturbances for 7 weeks presented with acute severe headache and bitemporal visual field defects. A high density lesion in the pituitary fossa suggestive of pituitary apoplexy was initially observed on a CT scan but an MRI of the pituitary revealed an irregular structure in the suprasellar cistern and the differential diagnoses of a pituitary adenoma or an aneurysm were proposed. Routine haematological

and biochemical investigations were normal as were prolactin, TSH, FSH, LH, GH, IGF1 concentrations. Random cortisol concentration was low at 15 nmol/l. Transsphenoidal exploration of the pituitary fossa revealed a normal pituitary gland and fossa and the patient underwent a further craniotomy that revealed that the left optic nerve and chiasm were compressed by either a thrombosed infraclenoid ophthalmic artery aneurysm or a suprasellar tumour with haemorrhage. The lesion was excised and histological appearance was that of a haematoma with fibrous capsule consistent with part of an aneurysm. There was no evidence of neoplasia. Post operatively investigations revealed normal LH, FSH response to GnRH stimulation, normal TSH response to TRH stimulation, normal GH response to hypoglycaemia on an Insulin tolerance test, but a reduced cortisol response to hypoglycaemia (peak value 280 nmol/l at 90 minutes), long synacthen test and glucagon stimulation test (peak value 392 nmol/l at 180 minutes). The possible pathophysiological mechanisms by which aneurysms can affect pituitary hormone secretion include direct hypothalmo-pituitary compression, pressure from haemorrhage and damage by toxic effects of extravasated subarachnoid blood, intracranial hypertension or vasospasm after subarachnoid haemorrhage. We report a case of spontaneous thrombosis of an ophthalmic artery aneurysm as a novel cause of isolated cortisol deficiency.

## P91

### Aortic involvement in Turner syndrome

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#### Background

Turner syndrome has well recognised cardiovascular complications that appear in up to 40% of the patients and are more common in monosomy X. Left sided obstructive lesions are relatively more frequent and predispose to aortic root dilatation and life threatening aortic dissection. Patients with bicuspid aortic valve, hypertension, coarctation and aortic stenosis are at high risk of aortic dilatation and dissection. Various follow up strategies are in use but there is no clear guideline regards the best single test for monitoring this progression.

#### Study

Routine MR imaging of aorta was introduced with initiation of a dedicated Turner Syndrome clinic with the aim repeated MRI every two years. It was found that seven patients out of a total of seventeen developed aortic anomalies during the course of their illness that included coarctation as well as dilatation. None of these patients had any cardiovascular symptoms and the vascular abnormalities were detected on MR imaging either at presentation or during the course of their follow up. We found that in patients with previously normal aortic imaging, the time interval for the lesion to be detectable varied between 4 and 6 years, though in one patient there was progression of an established lesion over the two years period.

#### Conclusion

In the few patients presented here, regular imaging every two years would appear to be warranted, though the exact frequency of imaging and by what modality needs still to be ascertained more definitively.

## P92

### Purely adrenaline-secreting pheochromocytoma: a classical presentation of a rare entity

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A provisional diagnosis of paroxysmal atrial fibrillation with normal blood pressure and echocardiogram was made in a 58 years old man. He described "funny turns" on standing during which he would become sweaty, tremulous with palpitations and headache and facial flushing preceded by pallor. The patient was otherwise fit and healthy with a BMI of 27.3 kg/m<sup>2</sup>; he used to drink about 15 pints of beer per week and smoke 11 cigarettes daily for the last 40 years, both stopped a year after initial diagnosis. General and systematic examination was normal and he

had no significant past medical history or relevant family history. Initial treatment with beta-blockers failed with increasing frequency of these episodes (1–2 per month). Flecainide was then introduced, which provided partial symptomatic improvement in terms of severity of the attacks although the frequency increased. In view of this, urinary catecholamines were requested and demonstrated increased adrenaline of 252 and 196 nmol/24 h (normal range, 0–144 nmol/24 h) but persistently normal noradrenaline and dopamine levels. Abdominal CT scanning revealed a 3.4 cm enhancing heterogeneous mass in the left adrenal gland, the radiological characteristics of which were in keeping with pheochromocytoma. An incidental benign looking renal cyst was found but the patient had normal brain MRI and no retinal angiomas, thus excluding VHL syndrome. MIBG scanning showed intense uptake in the left adrenal. Treatment with alpha blockade (phenoxybenzamine 10 mg qds) and beta blockade (propranolol 40 mg tds) produced complete symptomatic improvement with no objective fall in BP. The patient underwent uncomplicated left adrenalectomy following standard preparation. Histology showed a well encapsulated and completely excised pheochromocytoma. The treatment was stopped after surgery and the patient remained very well and symptom-free with normal urinary catecholamines. Pure adrenaline secreting pheochromocytoma is rare. This case highlights the importance of considering this rare entity in patients with paroxysmal symptoms/cardiac dysrhythmias in the absence of hypertension or cardiac pathology.

## P93

### A scary awakening – acute paralysis in a young man

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#### Clinical Case

A 21 year-old Caucasian kitchen porter presented with an episode of acute severe muscle weakness. He was receiving treatment for Graves' disease which had been diagnosed two months previously. He was fully alert, normotensive, tachycardic, with a flaccid quadriplegia, diminished reflexes and flexor plantars. Sensation was not impaired and his muscles were diffusely tender on palpation. Blood tests revealed hypokalaemia (K<sup>+</sup> 1.9 mmol/l), hypomagnesaemia (Mg<sup>2+</sup> 0.64 mmol/l) and modestly elevated creatine kinase (CK 541). He was thyrotoxic (FT4 74 pmol/l, TSH < 0.01 mU/l) despite carbimazole 30 mg daily.

#### Progress and management

A diagnosis of thyrotoxic hypokalaemic periodic paralysis (TPP) was made. He was given 80 mmols of IV potassium over 8 hours by which time his weakness had resolved. He received oral potassium supplements for 48 hours. Beta-blockers were withheld due to asthma.

Following discharge from hospital he was poorly compliant with carbimazole treatment and did not attend for outpatient review. He presented to hospital a further 3 times with less severe paralysis. On the last occasion he admitted to recreational ecstasy use the night prior to each episode. There have been no previous reported cases of ecstasy induced TPP.

#### Discussion

TPP is a rare but potentially life threatening condition that occurs in predominantly Asian males. Hypokalaemia is a hallmark feature and results from massive intracellular shift of K<sup>+</sup> into muscle cells. Potassium enters cells by activation of the sodium potassium ATPase pump. The number of these pumps and their activity increase in thyrotoxicosis. Catecholamines have a direct stimulant effect on the ATPase pump illustrating how ecstasy use could precipitate an attack.

## P94

### Systemic amyloidosis presenting with thyrotoxicosis: a case report.

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Although amyloid deposition can cause a goitre thyrotoxicosis secondary to amyloidosis is rare. We report a case of fatal systemic amyloidosis presenting as thyrotoxicosis.

A 62-year-old previously healthy male presented with heat intolerance, weight loss and tremor. On examination he had moderate sized firm goitre and mild dysthyroid eye disease. Biochemistry confirmed thyrotoxicosis;



serum free T4 47 pmol/l (normal 10–25) freeT3 14.4 pmol/l (normal 2.5–6.6) and TSH < 0.01mU/L (normal 0.3–5.5). He had no family history of thyroid disease and thyroid antibodies were negative. A technetium scan showed evenly distributed activity throughout the gland. He was commenced on block and replace therapy. Thyroid function tests normalised and the patients' symptoms improved.

Because of the bulky goitre and thyroid eye disease a thyroidectomy was performed and histology demonstrated extensive amyloid deposition throughout the thyroid gland. Thyroxine replacement was required. Further investigations revealed no evidence of myeloma with a normal skeletal survey, normal serum electrophoresis, 10% plasma cells in the bone marrow and normal bone marrow trephine. However his serum free light chain sample showed a heavy kappa predominance (kappa 338 mg/l, lambda 25.5 mg/l ratio 13.3). A rectal biopsy did not show amyloid on congo red staining. He developed progressive lethargy and peripheral oedema and investigation revealed anaemia (Hb 88 g/l) and nephrotic syndrome (24 hr urine protein 4.9 grams).

The patient was reviewed at the National Amyloidosis Centre and a SAP scan demonstrated moderate to large amyloid load in the liver, spleen, kidneys and bone marrow, the last of these being pathognomonic of AL amyloidosis. Reduced dose melphalan chemotherapy was instigated. One week later he suffered a severe GI haemorrhage and endoscopies demonstrated multiple petechial lesions throughout the GI tract, he subsequently developed pancytopenia and was supported actively with blood products. He became increasingly unwell and died 18 months after the diagnosis of thyrotoxicosis had been made.

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## P95

### Unusual case of Pseudohypoparathyroidism type Ib

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A 24 year old lady was initially presented with vomiting due to gastroenteritis and muscle cramps in 1993. Investigations revealed corrected calcium 1.65 mmol/L (range 2.2–2.6), phosphate 1.21 mmol/L (range 1.4–2.2) with intact Parathyroid hormone (PTH) 11 pmol/L (range 1.3–8.1) and potassium 3.1 mmol/L (range 3.5–5). She was found to have hypertension but no somatic signs. Ellsworth Howard test displayed a blunted response of cAMP and a normal phosphaturic response to PTH infusion: Urinary cAMP/Creatinine: 0.5 (30 min), 0.49 (60 min), 1.05 (90 min), 0.84 (120 min), 0.47 (180 min), 0.44 (210 min), Urine Phosphate 2.47 mmol/L, consistent with a diagnosis of Pseudohypoparathyroidism (PHP).

Despite stopping her oral contraceptive pills, she remained hypertensive, requiring 3 antihypertensives. Other endocrine and renal causes of hypertension were excluded.

In 2003, she developed severe Osteoarthritis (OA) affecting both hands confirmed by X-rays changes with negative immunology. This affects her quality of life. She has no family history of OA and works as a teacher.

PHP is a heterogeneous disease characterized by PTH resistance and classified as types Ia, Ib, Ic and II, according to its different pathogenesis and phenotype. Because of her absent somatic features with abnormal biochemistry, the diagnosis of PHP-Ib was made. Her offspring are unaffected.

Hypertension is found in 53% of patients with PHP and strongly linked to severe obesity. Surprisingly, our patient who is severely hypertensive has a normal body mass index. It is rather unusual to see severe OA at her age without any risk factors. Association between PHP and OA changes has not been reported so far yet.

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## P96

### A case of Cushing's syndrome and rapid course of illness

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A 68 year old woman presented with hypertension, oedema, weight gain and moon face. She had signs of proximal muscle weakness, thinning of scalp hair and bruising on her legs. Initial tests showed 24 hour urine free cortisol elevated at 946 nmol (0–300 nmol).

Serum cortisol showed no circadian fluctuation (0900: 1094 nmol/L, 2400 hrs: 886 nmol/L) and no suppression by dexamethasone (2 mg/24 h for 48 h: 1067 nmol/L). ACTH levels were slightly raised (99 pg/ml), indicating ACTH-dependent Cushing's syndrome.

While awaiting IPSS, she was admitted from the clinic with abdominal pain and chest infection, and treated with IV fluids and antibiotics. During the admission she underwent inferior petrosal sinus sampling and no central/peripheral gradient was found in ACTH levels, with no response to CRH. MR scan showed a partially empty pituitary fossa with an intrasellar cyst. During her stay she developed severe abdominal pain and back pain and was found to have pubic ramus and vertebral fractures. She rapidly deteriorated, developing septic shock from her chest infection and required ventilation and inotropic support. She developed multi-organ failure and cerebral infarct and died, 3 weeks after her admission, on the ITU.

Post mortem showed a large left adrenal gland mass weighing 506 grams suggesting adrenocortical carcinoma and an atrophic right adrenal gland and pituitary gland. Histology confirmed the diagnosis.

This patient had developed Cushing's syndrome which followed an aggressive course. Investigations suggested that this was ACTH dependent, with an ectopic source of ACTH, but the patient proved to have adrenal carcinoma. Whether there was ACTH secretion from the adrenal carcinoma causing detectable ACTH levels remains to be confirmed.

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## P97

### Primary adrenal insufficiency, gonadal failure and weak legs

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A 36 year old male presented to neurologists with tiredness and clumsy gait. Examination revealed normal cranial nerves, spastic paraparesis and bilateral extensor plantar response, absent vibration sense and ataxia. He was thought to have an inherited form of Spino-Cerebellar ataxia. His mother had developed similar neurological problems in her 50s, and his maternal uncle had Addison's disease.

He was referred to endocrinologists because of the findings of a low testosterone and slightly enlarged pituitary gland on MR imaging of the brain. Testosterone was low (4.2 nmol/L) and gonadotrophins raised (FSH 19.1, LH 29.2 IU/L). A short synacthen test showed a blunted cortisol response (basal 221, 30 minutes 236 nmol/L). ACTH was raised (182 pg/ml). Renin, aldosterone levels were normal. Very long chain fatty acid (VLCFA) measurements were abnormal: C26 fatty acid levels were high (6.78, NR: 0.3–4.0 umol/l), and C24/C22 and C26/C22 ratios were raised.

These results suggest a diagnosis of adult-onset myeloneuropathy variety of adrenoleukodystrophy (ALD). This is supported by the family history of an X-linked inheritance and finding the gene defect in *ABCD1* gene in the patient.

ALD, an X-linked recessive disorder, is caused by mutations in the *ABCD1* gene (Chromosome Xq28) which encodes a peroxisomal membrane protein of the ATP-binding cassette transporter family. This protein transports VLCFAs across the peroxisomal membrane. In ALD, the VLCFA Co A synthetase in peroxisomes are therefore unable to breakdown VLCFAs which accumulate as cytoplasmic inclusions leading to progressive dysfunction in the nervous system, adrenal glands and testes.

Our patient had developed neurological and endocrine manifestations, notably adrenal insufficiency and primary gonadal failure and is being treated with hydrocortisone and testosterone.

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## P98

### Hypocalcemic cardiomyopathy in a patient with primary hypoparathyroidism and Fahr's disease

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Hypocalcemic cardiomyopathy due to hypoparathyroidism is a very rare condition which responds favorably to vitaminocalcic treatment.

We report the case of a 50 years old man who presented to our unit for general fatigue, apathy and cough lasting several years with a worsening during the last months, there was biological evidence of hypoparathyroidism with profound hypocalcemia (21 mg/l) hyperphosphoremia (53 mg/l) and



undetectable PTH levels the CT scan showed coexisting Fahr's disease, the echocardiography revealed a dilated myocardium with an impaired systolic function (ejection fraction = 38%) after treatment with calcium and vitamin D derivatives the clinical status of the patient improved promptly and the cardiac abnormalities normalized within few days.

## P99

### An unusual case of panhypopituitarism associated with positive ANCA: atypical presentation of Wegener's disease or lymphocytic hypophysitis?

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A 60-year old Caucasian male was referred to our Endocrine unit with symptoms suggestive of adrenal failure (malaise and fatigue).

A Synacthen test confirmed the diagnosis (baseline cortisol <5 nmol/L, post Synacthen 74 nmol/L) and indicated secondary adrenal failure (baseline ACTH undetectable). Further hormonal tests showed secondary hypothyroidism (TSH 0.43 mU/ml, FT4 7.1 pmol/L) and hypogonadism (testosterone <0.7 nmol/L, LH 0.6 U/L). Prolactin was undetectable but IGF1 levels were normal. A pituitary MRI scan showed only a slight enlargement of the hypophysitis and the stalk. Our patient was started on triple hormone replacement therapy. A repeated pituitary MRI scan (ten months after the original) showed a comparative improvement with reduction in the size of the pituitary and the stalk.

We reviewed the patient's previous records in order to find a possible explanation for his panhypopituitarism. He had been recently assessed for recurrent episodes of myalgia associated with an evanescent skin rash and he had been found to have high titre of ANCA with a perinuclear pattern. We then considered a diagnosis of Wegener's disease with pituitary involvement. Our patient denied symptoms suggestive of respiratory or ENT pathology. His urine dipstick and serum creatinine were consistently normal. Chest XR and a high-resolution CT scan were both normal. CT guided biopsy of the maxillary sinuses showed inflammation of the capillaries but no granulomas.

#### Conclusions

Although our patient did not meet the criteria for Wegener's disease, the reason for his hypopituitarism and the high titre ANCA levels remains unclear. The final diagnosis remains open and include possible atypical early Wegener's disease, non-specific ANCA-related vasculitis with pituitary involvement or lymphocytic hypophysitis.

## P100

### Isolated secondary hypothyroidism in identical twins

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#### Background

Isolated secondary hypothyroidism is rare. In the majority of cases the causes are genetic (gene mutations) or structural (mass effect or infiltration). Structural causes are usually associated with simultaneous deficiencies of other adenohypophyseal hormones. We present an identical twin with isolated secondary hypothyroidism.

#### Case report

1st Twin – 26 year old obese lady, of Afro-caribbean origin, presented with tiredness, weight gain, cold intolerance and menorrhagia. Clinical examination was unremarkable. Her TSH was 0.01(0.3–6)mU/l, total T4 48(50–150)nmol/l, T3 1.0(1–2.5)nmol/l and freeT4 7.7(10–24)pmol/l. Thyroid auto-antibodies were normal. Dynamic pituitary function tests showed adequate cortisol and growth hormone response to hypoglycaemia and normal LH/FSH response to GnRH stimulation. TRH test showed blunted TSH response. MRI scan showed enlarged but homogenous and uniformly enhancing pituitary. No infiltrative cause was found. Her total T4 normalized on 200 micrograms of thyroxine.

2nd Twin – 26 year old twin sister of the above patient, presented with progressive tiredness, headaches and weight gain. Clinical examination was normal. Her TSH was 1.2 mU/l, totalT4 51 nmol/l, freeT4 7.6 pmol/l and T3 1.1 nmol/l. Thyroid auto-antibodies were normal. Dynamic pituitary function tests showed adequate cortisol, growth hormone and LH/FSH response but blunted TSH response to TRH stimulation. MRI scan showed hyperplasia of the pituitary gland. No infiltrative cause was found. On 200

micrograms of thyroxine her total T4 normalized.

Genetic analysis in both showed no mutations in the exon2 and exon3 Beta subunit of the TSH gene.

#### Discussion

Isolated TSH deficiency has been reported due to 5 different mutations in the coding region of the Beta subunit of the TSH. To our knowledge, these are the first reported cases of isolated secondary hypothyroidism in identical twins without abnormalities in TSH. The possible mechanism may be a genetic defect in the TRH receptor and the search for any new mutations continues.

## P101

### Adrenal-insufficiency manifesting as hyperkalaemic paralysis

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A-35-years old male, automobile factory worker was brought to emergency room with sudden onset rapidly progressive flaccid quadriplegia of 12 hours duration. He was unable to stand or use his upper limbs. There was tingling in distal aspects of all four limbs. There was no loss of sensation, respiratory muscle, cranial nerve, or cerebellar involvement. He reported no other symptoms. There was no history of alcohol use, drug use, or exposure to sexually transmitted diseases. He worked in night shifts. There was no relevant family history.

On examination he was unable to stand or sit, fully conscious, blood pressure 96/68 mm Hg supine, pulse rate 88 beats per minute regular. Cranial nerves, speech, fundi, and sensory examination were normal. Motor system examination revealed flaccid grade I/V (MRC), areflexic, quadriplegia with flexor plantars. There was no diaphragm or respiratory muscle paralysis. Other systems were normal. A diagnosis of Acute Guillain Barre Syndrome was made and he was admitted to intensive care unit. His haemoglobin, full blood counts, plasma glucose, serum ionized calcium, serum phosphate; thyroid function tests were normal. Serum creatinine 160 mmol/L (<133), urea 15 mmol/L (3.6–7.1), chloride 89 mmol/L (98–106), and bicarbonate 16 mmol/L (21–28). Electrocardiogram revealed heart rate of 90 per minute, small P waves, broad QRS complexes, and peaked T waves. The serum potassium levels were 8.0 mmol/L (3.5–5.0), and serum sodium was 125 mmol/L (136–145), confirmed on repeated samples. Cranial MRI and CSF examination was normal. He was managed with intravenous calcium gluconate, dextrose and insulin. In view of persistent hyperkalemia and hyponatremia, an endocrinology consultation was sought. On further questioning he revealed history of weight loss of 3 kgs, unusual tiredness, and repeated common colds in last six months. A clinical diagnosis of chronic primary adrenal insufficiency presenting with hyperkalemic paralysis was made.

## P102

### Hypercalcaemia and hyper eosinophilic syndrome in a patient with cholangiocarcinoma

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#### Background

Hyper eosinophilic syndrome is a rare condition, which is connected with a variety of diseases. Very infrequently, it occurs in association with malignancy denoting widespread metastases and poor prognosis. We describe a patient with hyper eosinophilia and hypercalcaemia as a manifestation of disseminated cholangiocarcinoma.

#### Case description

A 54-year old man with a history of alcoholic hepatitis for the last 10 years was admitted due to severe fatigue, diffuse abdominal pain, flatulent abdomen and dyspnoea. The symptoms had gradually deteriorated over the past 20 days. Examination showed tense ascites, expiratory wheezing and lower extremity oedema. The following laboratory parameters were of note: total white blood cell count of 14010/ $\mu$ l, 12.4% eosinophils (absolute count: 1737/ $\mu$ l), elevated serum calcium (11.13 mg/dl), normal serum phosphate, low parathyroid hormone (PTH) levels (4.40 pg/ml), negative hepatitis B and C virus serology, normal Alpha-Fetoprotein and extremely elevated CA 19.9 (3666 U/ml). Imaging techniques (chest X-rays, CT of the thorax, abdominal ultrasound, CT and MRI) revealed cirrhotic liver with a 13.5 x 12.5 cm lesion in the right lobe, ascites, enlarged intra-abdominal

lymph nodes, peritoneal carcinomatosis, diffuse lung metastases and left-sided pleural effusion. Both diagnostic thoracentesis and abdominal paracentesis yielded bloody effusion with positive cytology for adenocarcinoma. Cholangiocarcinoma was diagnosed by liver biopsy. The hypereosinophilic syndrome responded to corticosteroid treatment. The patient died of acute respiratory failure on the 20<sup>th</sup> day of hospitalization.

#### Discussion

The association of cholangiocarcinoma with hypereosinophilic syndrome and hypercalcaemia is extremely rare and might account for the poor prognosis in this case. Hypercalcaemia combined with low PTH levels could be explained by the PTH-related protein secretion from cancer cells.

### P103

#### Di-George syndrome presenting with hypocalcaemia and personality disorder in adulthood - a case report

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We report the case of a 42 year old transsexual with Di-George syndrome (DGS) presenting in adulthood with profound hypocalcaemia and personality disorder. The patient had multiple hospital admissions with episodes of deliberate self-harm and recurrent seizures. Routine investigations revealed hypocalcaemia secondary to hypoparathyroidism. The diagnosis of DGS was delayed until six years later. The clue to the underlying condition was the finding of DGS in the patient's daughter with learning disability. The diagnosis was confirmed by fluorescent in situ hybridisation (FISH) analysis, which demonstrated micro-deletion of 22q11.2. Echocardiogram demonstrated no cardiac defects. Medical management of the hypocalcaemia was complicated by non-compliance, likely to be related to the patient's personality disorder. Learning difficulties and schizophrenia are well recognised in association with Di-George syndrome, though no definite personality disorders have previously been described. This shows that Di-George syndrome can present late in adulthood with hypocalcaemia without any other typical features and the psychiatric manifestations can be diverse. The awareness of the phenotypic variability is important as presentation of Di-George syndrome can be subtle which may delay the diagnosis. This has enormous implications on health and family planning as subjects with 22q11 deletion have a 50% risk of transmitting the deletion; they should be offered genetic counselling and FISH analysis for pre-natal detection as early as 10–12 weeks of gestation by chorionic villous sampling.

### P104

#### Phaeochromocytoma presenting as gestational diabetes

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A 35 yr old woman was seen at the medical obstetric antenatal clinic with gestational diabetes (GDM) and new-onset hypertension during the second trimester of her first pregnancy. She was well, with no prior medical history. Blood pressure of 120/80 had been recorded at her first antenatal visit. Throughout the pregnancy, GDM was managed successfully with diet alone: HbA1c always <5.8%. Methyl dopa was initiated for persistent hypertension at 18 weeks gestation, and labetalol added at 28 weeks. Symptomatic postural hypotension required substitution of labetalol with nifedipine. Home BP monitoring revealed labile hypertension; she was admitted for further evaluation. Urinary catecholamine excess was found: noradrenaline 2850 nmol/24 hrs (NR 71-505), and normetadrenaline 8.8 micromol/24 hrs (NR < 4.3). Urinary adrenaline and metadrenaline levels were within the normal limits. A phaeochromocytoma was suspected, therefore alpha-blockade with phenoxybenzamine commenced. On MRI, a 3 x 3 x 4 cm pelvic lesion was identified. Plans were made for elective caesarean delivery at 34 weeks, with simultaneous phaeochromocytoma removal. However, the development of proteinuria, rising AST levels and ongoing diastolic hypertension, led to an urgent caesarean section at 32 weeks. A live female infant was delivered, initially requiring NICU care. Pelvic exploration at the time of delivery did not identify a pelvic phaeochromocytoma. Hypertension persisted post-delivery, requiring intravenous nitrate and esmolol whilst on Critical Care, followed by oral alpha and beta-blockade. There was on-going catecholamine excess: urine noradrenaline 3681 nmol/24 hrs. MIBG scanning identified a focal area of increased uptake within the pelvis. Resection was scheduled for 12 weeks post delivery. At laparotomy, the

pelvic tumour was successfully identified and removed. Despite prolonged alpha and beta blockade pre-operatively, during peri-operative tumour handling, systolic BP rose by 50 mmHg. Surgical resection was otherwise uneventful. Subsequent histology confirmed the complete excision of a benign phaeochromocytoma. The patient is now normotensive, off medication, with a healthy infant.

### P105

#### Conn's syndrome associated with hyperprolactinaemia: two case reports. Hypertension was cured by surgery in both cases despite a 10 and 16 year history

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We describe two cases of Conn's syndrome both of whom had hyperprolactinaemia of which one was associated with pituitary adenoma, possibly as part of MEN 1.

The first patient was a man aged 52 who presented with hypertension present for 10 years and a marginally low serum potassium. He was on doxazosin, lisinopril, candesartan and celiprolol. His Aldosterone/ Renin ratio (8500:1) was very high suggestive of primary hyperaldosteronism. The patient was treated effectively with spironolactone. CT scan showed right adrenal adenoma. He also had erectile dysfunction and a very high prolactin level (18019 mU/l; normal: 70–511) and low testosterone. His serum calcium was normal. MRI scan showed a pituitary adenoma (1.2 cm). The patient was treated effectively with cabergoline. The patient underwent adrenal surgery successfully and currently is on no antihypertensive.

The second patient was a woman aged 56 who presented with a 16-year history of hypertension and profound hypokalaemia. She was on atenolol, amlodipine and HRT following hysterectomy. Her blood test showed a very high Aldosterone/ Renin ratio (7075:1) and CT scan showed a right adrenal adenoma. She was successfully treated with spironolactone and other antihypertensives were withdrawn. She was maintained only on 50 mg of spironolactone. A pituitary screen showed a high prolactin level (2992). MRI scan showed a normal pituitary. She also underwent adrenal surgery successfully and currently is on oestradiol 1 mg and is normotensive.

Both patients were cured with adrenal surgery. Rarely, prolactinoma may be associated with Conn's syndrome as part of MEN 1.

### P106

#### Parathyroid carcinoma in multiple endocrine neoplasia (MEN) type 1: two case reports

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Hyperparathyroidism occurs in most patients with MEN Type 1 but the association of MEN type-1 with parathyroid carcinoma has only been described previously in one patient. In this report, we describe two further cases of parathyroid carcinoma presenting in association of MEN type 1 syndrome.

The first patient was a 69 year-old woman who presented with severe hyperparathyroidism and tracheal compression by a mediastinal mass which was shown histologically to be a parathyroid carcinoma with a second similar lesion in the neck. She was treated with total parathyroidectomy followed by resection of the mediastinal mass with resolution of the hypercalcaemia. Remarkably, she also reported primary amenorrhoea and was found to have an invasive lactotroph pituitary adenoma which we treated with cabergoline. MRI of the pancreas revealed a small lesion characteristic of an islet-cell tumour which was clinically and biochemically non-functioning.

The second patient was a 32 year-old man who presented with intractable dyspepsia associated with raised serum gastrin concentration. A tumour was localised to the neck of the pancreas by endoscopic ultrasound and the calcium stimulation catheter suggested the presence of both a gastrinoma and an insulinoma although he had no hypoglycaemic symptoms. He has surgical resection of the pancreatic islet cell tumour with symptomatic improvement. Additional investigations revealed hypercalcaemia and raised serum parathyroid hormone concentration. Neck exploration revealed two parathyroid glands only. One of the parathyroid glands contained a tumour with fibrous banding, extra-capsular extension and moderate Ki 67 staining; features which are highly suggestive of carcinoma. The patient was also

found to have bilateral adrenal hyperplasia with abnormal cortisol dynamics and suppressed serum ACTH suggesting ACTH-independent cortisol secretion. Pituitary MRI was normal.

These two case reports show that parathyroid carcinoma can occur, albeit rarely, in the context of MEN type 1. Interestingly, neither patient demonstrated a classical germ-line mutation in the *menin* gene.

## P107

### Co-existing hypoparathyroidism and vitamin D deficiency causing life-threatening hypocalcaemia

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We present a case of a 76 year old Caucasian woman with combined primary hypoparathyroidism and vitamin D deficiency resulting in life-threatening hypocalcaemia.

She has a history of COPD and initially presented in 2002 with an exacerbation of her airways disease. During treatment for her condition an incidental note was made of hypocalcaemia (calcium 1.62 mmol/l). PTH was minimally elevated at the time but 25-hydroxycholecalciferol was low (4.2 ng/ml). She was commenced on calcium and vitamin D therapy.

She was readmitted in 2005 with a further exacerbation of her COPD. The calcium and vitamin D was not on her current prescription. During her admission she was noted to have intermittent weakness of her limbs with twitching. During these episodes she became drowsy and her oxygen saturations fell. They were initially described as seizures. CT of her brain was normal.

Upon subsequent re-evaluation of these episodes, the possibility of tetany was raised and the previous history of hypocalcaemia was discovered. Calcium level was 0.98 mmol/l (corrected 1.27 mmol/l). She had normal renal function and magnesium level. PTH was inappropriately low at 2.0 pmol/l.

She was commenced on calcium, vitamin D and alfacalcidol with subsequent increase in her calcium levels and resolution of her symptoms. Primary hypoparathyroidism is a rare endocrinopathy. The combination with vitamin D deficiency is rarer still. This demonstrates how rare conditions can co-exist and how re-evaluation is necessary if the aetiology and the clinical picture is inconsistent.

## P108

### A rare case of metastatic choroidal melanoma to the thyroid gland: the importance of cytology

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Metastases in the thyroid gland are very rare and their true incidence has not been clearly established. There are few reports of metastases to this gland and most of them are not detected in clinical practice.

Although detection of metastases to the thyroid gland usually indicates poor prognosis aggressive surgical and medical treatment in isolated thyroid metastases may be effective as described for renal carcinoma.

Malignant melanoma is one of the tumours which may metastasize to the thyroid gland. We describe the case of a man with a thyroid metastasis from a treated choroidal melanoma incidentally discovered by fine-needle aspiration biopsy.

#### Case report

An 82 year old man with an history of choroidal melanoma treated with proton beam radiotherapy four years before, presented with a thyroid nodule of the left lobe, clinically evident since the last two months. Annual hepatic echotomography and semi annual liver enzyme assays evaluated for the oncologic follow up always resulted within the normal range. Thyroid echotomography showed a unique hypoechoic, dishomogeneous richly vascularized nodule of the left lobe of about 2.5 cm in size. No suspected lymphadenopathy was evidenced.

The fine needle aspiration biopsy showed the presence of many epitheliomorphic cells with nuclear atypia and cytoplasmatic pigmentation as for melanin. The lesion was hence consistent with thyroid metastasis from malignant melanoma.

The evaluation of tumour extension with TC showed multiple pulmonary lesions and abdominal lymphadenopathy consistent with metastases.

## Discussion

This case is to focus on the need to consider even rare possibilities in the differential diagnosis of thyroid nodules and to stress that an accurate echographic exam of the thyroid gland and cytology are essentials for the diagnosis. It could be hypothesized that in patients with melanoma a more extended follow up would evidence metastatic lesions thyroid more precociously so that a surgical treatment could be essayed.

## P109

### A complex case of pregnancy and pegvisamont treated acromegaly

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A 29 year old woman with polycystic ovary syndrome was diagnosed with acromegaly having presented with a two year history of secondary amenorrhea, a change in physical appearance and sweating. The diagnosis was confirmed with an oral glucose tolerance test that also demonstrated she had impaired glucose tolerance. Radiological imaging demonstrated a 2.2 cm pituitary tumor that was compressing the optic chiasm and a bi-temporal visual field defect was confirmed on Goldman fields.

Over a 4 year period she underwent four neurosurgical operations and received 53 Gy radiotherapy but failed to ever achieve biochemical cure. Whilst on a somatostatin analogue her acromegaly remained active and in 2004 she was commenced on pegvisamont, a growth hormone antagonist. The latter was discontinued when she conceived after her first cycle of IVF. During her pregnancy she developed gestational diabetes requiring insulin therapy and pituitary gland enlargement was accompanied by deterioration in her visual fields. The patient was, however, treated conservatively and an elective cesarean section was performed at 37 weeks gestation. A healthy boy was delivered in July 2005 and remains developmentally normal.

In summary this is a complex case with an array of medical problems that required intricate care by a multi-disciplinary team and to our knowledge represents the first human pregnancy conceived on pegvisamont therapy for active acromegaly.

## P110

### Post-partum thyroiditis (PPT): colour-doppler ultrasonography evaluation

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Colour-Doppler Ultrasonography (CDU) is applied to the study of many thyroid diseases. In the last one semiquantitative (colour-signal) and quantitative (peak flow velocity at inferior thyroid artery) evaluations are considered. Graves' disease (GD), amiodarone-induced thyrotoxicosis were better characterized, even Hashimoto thyroiditis and De Quervain thyroiditis were studied. We described a case of Silent Thyroiditis (ST), showing the possibility to distinguish it from GD.

A 27year old woman was referred to us, with thyrotoxicosis arisen after 3 months from delivery. Very low TSH (0.05 mUI/ml, n.v.0.30-4.10), high FT4 (23.5 pg/ml, n.v.8-18), high FT3 limits (5.98 pg/ml n.v.2.20-5.50). We have performed a CDU evaluation with an ATL-HDI-5000 sonographic system; a 5-12 MHz linear array probe was used on the power Doppler imaging mode. Echo demonstrates a diffuse hypoechoic, homogeneous ecopattern; CDU shows an absence of colour-signal (class 0). After eight weeks the symptoms remitted, TSH was slightly elevated (21 mUI/ml), FT4 slightly low (7 pg/ml) and FT3 came back into normal limits. At CDU the colour signal reappeared (class 2).

In this study the first observation with CDU about a case of PPT is reported. PPT belongs to the lymphocytic thyroiditis where the autoimmune process is caused by cell-mediated reaction, bearing a destructive process precipitated in post-partum period. We observed the time-course of disease by CDU. During thyrotoxicosis-phase the colour-signal was absent and reappeared in following phase of primary hypothyroidism.

PPT can represent a challenged diagnosis, because it is difficult to differentiate it from GD during post-partum: differential diagnosis is important for different time course and therapy of two forms. On the other hand scintiscan may be hazardous. CDU could show itself an accurate tool to this purpose: showing a pattern of colour signal 0, it can be useful to suggest a form of PPT, instead of GD where the colour signal is very much increased.

**P111**

**Challenges of managing metabolic disturbances**

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We report two cases of seizures resulting from severe electrolyte imbalance. Case 1: A 48-year old lady with CREST syndrome and oesophageal dysmotility. She was admitted with diarrhoea and vomiting. Routine investigations including FBC, U&E, LFT, and CRP were normal. She later complained of pins and needles in her hands and around her mouth. Twenty-four hours later, she developed blurred vision, neck pain and generalised ache, was found drowsy, with possible neck stiffness. She then proceeded to have several self-limiting seizures. Further blood tests revealed corrected calcium of 1.4 mmol/l (2.10–2.70) and magnesium 0.2 mmol/l (0.78–1.03). Intravenous replacement therapy was commenced at this stage which improved the patient's condition and electrolytes.

Case 2 A 42-year old patient with DiGeorge syndrome. She had multiple hospital admissions with overdoses and recurrent self-limiting seizures over the last two years. Routine investigations were normal. However corrected calcium was 1.6 mmol/l (PTH < 3 ng/L; reference range 14–72). Hypocalcaemia persisted despite being on regular oral calcium supplements and she required repeated intravenous replacement. On further testing she was found to have serum magnesium levels of 0.6 mmol/L (0.78–1.03). Oral magnesium supplements were commenced; however in this case it had little effect on her serum calcium. Hypomagnesaemia has not been described in DiGeorge syndrome, whether it was contributory or a co-incidence in these settings remains unclear and debatable. These cases illustrate that although hypomagnesaemia can cause hypocalcaemia which responds to administration of magnesium, the aetiology of hypocalcaemia can be multifactorial. Co-existence of more than one electrolyte disturbance can aggravate the clinical syndrome. The possibility of this association should be considered in the setting of such presentations with electrolyte abnormalities, especially so with the risk of life-threatening cardiac arrhythmias. Electrolyte disturbances are common presentations during acute medical takes and clinicians should have a high index of suspicion for coexistent electrolyte abnormalities in such situations.

**P112**

**Three generations of generalised resistance to thyroid hormone (GRTH)**

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**Background**

Generalised Resistance to Thyroid Hormone (GRTH) is an autosomal dominant condition with impaired tissue responsiveness to thyroid hormones. The majority of patients are asymptomatic and compensate adequately by elevated thyroid hormone production.

**Index case**

A 47-year-old woman was referred to the thyroid clinic in 2000 when routine thyroid function tests (TFT) showed elevated TSH at 4.9 mU/l and fT4 36 pmol/l. A TSH-oma was excluded by normal levels of SHBG and alpha subunit, normal TSH and prolactin responses to a dopamine antagonist, a marked TSH response to TRH (basal 8.1, peak 44 mU/l) and normal pituitary MRI.

**2<sup>nd</sup> Generation**

Her two children then aged 26 and 24 years underwent TFT testing which showed similar results (see Table 1). DNA testing confirmed all three had GRTH (G345S heterozygous mutation).

**3<sup>rd</sup> Generation**

Her daughter's second child born in 2004 was found to have a raised TSH and fT4. DNA testing confirmed a diagnosis of GRTH with the same mutation.

Test (reference range)	Index case	Son	Daughter	Grandson
TSH (0.35–3.3 mU/l)	4–10	2	0.8–2.0	5.5
fT3 (3–7 pmol/l)	9–10.7	11.9	8.2–11.9	15.4
fT4 (10–25 pmol/l)	31–39	41	31–50	45–67
Thyroid peroxidase antibody (0–500 U/l)	1563	24	90	5

All the affected individuals were clinically euthyroid, did not have a significant goitre and were physically and developmentally normal.

**Comment**

To our knowledge this is the first report of GRTH crossing three generations in the UK. Coexistent autoimmune thyroid disease is challenging to treat and may require high doses of thyroxine to reproduce the initial thyroid profiles. GRTH is a rare cause of discordant TFTs and awareness of the diagnosis in the mother resulted in avoidance of inappropriate treatment and investigation in the infant.

**P113**

**Mineral-rich baths – an unusual source of exposure to excess iodine**

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Mineral-rich baths and other spa treatments are popular and are promoted as providing a sense of well-being and detoxification. However, exposure to minerals that are easily absorbed through the skin may have unwanted adverse effects in certain groups of patients. In 2001, a 50-year-old woman was diagnosed with thyrotoxic Graves' disease. Her thyroid peroxidase antibodies were >1000 IU/mL. She was treated with block and replace therapy for 1 year. She remained euthyroid on followup.

In August, 2004, she went to a health spa and spent 1 hour in a flotation tank. The flotation tank contained minerals including iodine and a sign near the tank warned those with thyroid disease to see a supervisor - she did so. She was told that it was alright to go in – presumably because her thyroid disease was in the past. One month later she had a return of hyperthyroid symptoms and her free T4 was 42.1 (normal range 11.5–23.2 pmol/L), free T3 10.56 (3.5–6.5 pmol/L), and TSH <0.01 mU/L. She was started on Carbimazole, initially 15 mg daily, reduced to 5 mg daily in October, 2004, and then stopped in November, 2004, when she became hypothyroid (FT4 3.6, TSH 81). In late December 2004, she was clinically euthyroid (FT4 10.5, TSH 9.17) and has remained so (FT4 11.9, TSH 5.67).

Exposure to excess iodine has been reported with foods, medication, dietary supplements, topical antiseptics, and iodinated contrast media. To the best of our knowledge, the risk of exposure to excess iodine from mineral-rich baths has not been noted as a source of iodine-induced thyroid dysfunction. Patients with a past, as well as present, history of thyroid disease, and their clinicians, should be aware of this possibility.

**P114**

**Biochemical screening for ret negative medullary thyroid carcinoma (MTC): a case report highlighting difficulties in the interpretation of pentagastrin stimulated calcitonin testing**

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In cases of ret-oncogene positive Medullary Thyroid Carcinoma (MTC), national guidelines advocate screening of 1<sup>st</sup> degree relatives with the Pentagastrin Stimulation Test (PST) to identify those with C-cell hyperplasia, and therefore at risk of MTC. Prophylactic thyroidectomy is recommended in those with positive tests. We highlight the difficulties of quantifying risk in the relatives of a ret-negative patient.

A 30 year-old man presented with a tender neck swelling. Ultrasound demonstrated lymphadenopathy and a cystic (16 x 30 mm) thyroid mass. Fine needle aspirate confirmed metastatic medullary carcinoma. There was no family history or features of multiple endocrine neoplasia. Genetic analysis excluded known RET mutations involved in hereditary MTC (exons 8,10–16).

His parents and four siblings were screened using the PST. Parents' tests were normal. Two siblings had repeatedly abnormal tests despite normal thyroid and parathyroid function and underwent thyroidectomy. Histology showed hyperplasia in one and was normal in the other.

**The case raises several questions**

*Should screening have occurred?* The patient's young age (<35 years) together with the aggressiveness of the malignancy made us concerned about the possibility of an unidentified germline mutation, and so screening was offered.

*Does PST aid management in this case?* The parents negative testing, together with the negative genetics in the index case, make it more than 90% probable that this is sporadic MTC. However, the 2 siblings with positive



screening, together with one with histological C-cell hyperplasia complicates the picture, particularly when C- cell hyperplasia occurs in 30% of the background population.

*Should the children of the sibling with positive PST be screened?* C-cell hyperplasia in a sibling raises the possibility of an un-identified mutation (with incomplete penetrance), and so could support continued screening. Our case highlights the need for clearer guidance as to the management of ret-negative MTC.

## P115

### **A long-standing lump in the neck proving troublesome for the surgeons**

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#### Case report

A 68 year old lady was admitted for an elective partial hepatectomy for a solitary metastasis in the liver. Two years ago she had an uneventful colectomy for a colorectal cancer. During induction for her operation she was noted to be hypertensive (blood pressure (BP) 200/120 mm of mercury (Hg)), though had not been observed to be so previously, and hence her partial hepatectomy had to be abandoned. She was commenced on atenolol and operation rescheduled. The second attempt at hepatectomy was aborted as well, as she was again found to be hypertensive (BP 330/220 mm of Hg). On this occasion the anaesthetist detected a lump, in the right side of the neck, which was present for the last twenty years and had never been troublesome. Twentyfour hour urinary catecholamines (mainly noradrenaline) were raised on three occasions. Meta-Iodobenzylguanidine scan showed a hot spot corresponding to the right cervical mass. An MRI scan showed an encapsulated 7 cm × 4 cm mass deep to the right common carotid artery and extending to the pharynx. Her serum calcium, thyroid function, parathyroid hormone, parathyroid hormone related peptide and calcitonin were normal. She was adequately alpha and beta blocked with phenoxybenzamine (80 mg bd) and propranolol (40 mg tds). Her peri-operative period was uneventful and a 77 gram mass was removed from the neck. Biopsy confirmed paraganglioma.

#### Comments

Paragangliomas are tumours arising from extra-adrenal medullary neural crest derivatives- sympathetic ganglia (from neck to bladder), carotid body, vagal body, mediastinum, aorta, organs of Zuckerkendl and pelvis. This patient had the paraganglioma for twenty years but she was never symptomatic. Surprisingly, she did not have any peri-operative hypertensive crisis during her previous colectomy.

## P116

### **A case of isolated acth deficiency who developed autoimmune-mediated hypothyroidism and hepatitis**

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ACTH deficiency is a rare cause of secondary adrenocortical insufficiency and frequently associated autoimmune-mediated hypothyroidism, but there has been no report associated autoimmune-mediated hepatitis. We present a 36-year-old female with generalized weakness, malaise, hypersomnolence. Investigations showed low albumin, prolonged PT, elevated bilirubin level and abdominal ultrasonography and CT revealed liver cirrhosis with ascites. Though serologic test for hepatopathy were all negative and there was no alcoholic history, antibody for autoimmune-mediated hepatitis were positive such as smooth muscle antibody, SS-A/Ro antibody. Primary hypothyroidism was documented by TFT and Thyroid antibody such as antimicrosomal and TSH-R antibody were positive. There was no change on the patient's weakness after thyroid hormone replacement, the pituitary-adrenal axis was examined. No abnormal findings such as tumor, or empty seller were found on a MRI of the pituitary gland. Dynamic test for pituitary hormones secretion in response to combined stimulation with CRH, TRH, LHRH and GRH revealed a blunted response of ACTH, exaggerated response of TSH and normal responses of prolactin, LH, FSH, GH. During rapid ACTH stimulation, secretion of cortisol were blunted while secretion of aldosterone were normal. After replacement with thyroxine and glucocorticoids, she gradually improved and discharged at the hospital. In conclusion, we report the case of isolated ACTH deficiency associated with autoimmune-mediated hypothyroidism and hepatitis.

## P117

### **Cushing's disease presenting as a Schizophreniform psychosis**

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A number of psychiatric conditions are associated with Cushing's disease. Acute psychosis is rare. We describe a patient with sudden onset of florid psychosis who required medical and surgical treatment.

#### Case

A 38 year old woman previously well and employed in the financial sector was admitted to the acute psychiatric unit with delusional, disorganised and paranoid ideation. Despite management with olanzapine, haloperidol and benzodiazepines her mental state deteriorated. She was noted to be centrally obese and hirsute with multiple bruises. DEXA scanning revealed osteopaenia in the lumbar spine, normal BMD in the femoral neck. 9 am serum cortisol 907 nmol/l (138–690), ACTH 55.6 ng/l (9–52), urinary cortisol >2000 nmol/l (97–331). Cortisol failed to suppress during an overnight 1 mg dexamethasone suppression test (DST) (9 am cortisol 503). During an intravenous DST (1 mg/hr for 5 hours) cortisol did not suppress after 24 hours (695 to 852 nmol/l). MRI pituitary revealed a 3 mm right sided pituitary adenoma with no optic chiasm compression. Initiation of ketoconazole led to deranged LFTs. After 3 weeks of aminoglutethimide and metyrapone there was an improvement in symptoms. During this time she became extremely hypertensive and after 6 successive grand-mal seizures was admitted to ICU. ACE-I, B-blockers, Ca channel blockers, nitrates and spironolactone were required. General anaesthesia was required for inferior petrosal sinus sampling and revealed a 4 fold greater central:peripheral ACTH gradient. Cushing's disease was diagnosed. Transphenoidal hypophysectomy was successful with evidence of adenoma. Post-operative transient diabetes insipidus was treated with DDAVP. Repeat urinary free cortisol 32 nmol/l. She currently requires only cortisone acetate 12.5 mg bd and has restarted part-time work.

#### Discussion

Late-onset schizophreniform psychosis associated with hypertension and central obesity raises the suspicion of Cushing's syndrome. Management may be difficult and take months for symptom resolution.

## P118

### **Obesity, hypertension and elevated catecholamines**

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Human obesity is characterized by abnormalities in sympathetic cardiovascular control. We present two cases to demonstrate the relationship between weight, BP and catecholamines (UC).

#### Case 1

A 39 year-old man reported symptoms of flushing and palpitations. His BMI was 50 kg/m<sup>2</sup>, consistent BP of 240/140 mmHg, with normal renal, thyroid function and normal fasting glucose. Urinary noradrenaline (UNA) 820 nmol/24 hrs) and dopamine (UDA-4500 nmol/24 h) were elevated, adrenaline (UA-48 nmol/24 hrs) levels were normal [24 hr normal ranges- UA < 190 nmol, UNA 120–590 nmol, UDA 650–2700 nmol]. A MIBG scan and abdominal CT-scan were normal. Following 6 weeks of dietary modification, graduated exercise programme and  $\alpha$ -blockade, his weight decreased by 12 kgs, BP to 180/110 mmHg and UC decreased (UNA-600 nmol/24 h, UDA-3700 nmol/24 h). At subsequent visits weight and BP improved further. After 4 months, UC had normalised (UNA-270 nmol/24 h, UDA-2100 nmol/24 h).

#### Case 2

A 38-year-old lady was referred with episodes of palpitations and sweating. Her BMI was 41 kg/m<sup>2</sup>, pulse was 72/m, BP 190/110 mmHg on calcium channel blocker and baseline renal and thyroid function normal. 24 hour UA (8 nmol/24 h) and UNA (510 nmol/24 h) were normal but UDA (3900 nmol/24 h) was elevated. MIBG and abdominal CT scans were normal. She was advised on a low-fat diet with  $\alpha$  blocker for BP control. At 9 months follow-up, she had gained 15kgs, UNA excretion had increased (850 nmol/24 h) and BP normalised. Dietary and exercise education was reinforced when a simultaneous reduction in weight and UC (UNA-300 nmol/24 h) was noted.

#### Discussion

Our cases clearly demonstrate the relationship between weight, BP and UC. In the first case, weight reduction was consistently associated with decreases in BP and UC while in the second, weight changes correlated more with UC excretion than BP. Studies examining weight loss have noted a high correlation between BP reduction and a fall in NA, as was seen in our cases. Primary intervention of weight loss following diet and exercise resulted in reduction in BP and UC, thus emphasising the role of weight reduction in the management of hypertension in obese patients.

#### P119

**Cushing's syndrome in an adolescent with MEN1: where is the lesion?**  
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A 14 year old girl, with a family history of MEN1, presented with secondary amenorrhoea and weight gain. Biochemical investigation revealed raised 24-hour urinary free cortisol excretions (1455, 1190, 614 nmol/24 hours; normal <290) and failure of cortisol suppression following 48 hours of low dose (0.5 mg qds) dexamethasone (199 nmol/l to 202 nmol/l). High dose dexamethasone administration (2 mg qds) for 48 hours resulted in further suppression of serum cortisol to 49 nmol/l. ACTH was easily measurable (37.5 pmol/l) but MRI of the pituitary failed to show clear evidence of an adenoma. Bilateral inferior petrosal sinus sampling (IPSS) failed to support a pituitary source for her hypercortisolism, though there was a modest rise in peripheral ACTH levels post-CRH. CT scanning of the thorax and abdomen was normal but endoscopic ultrasound showed a well circumscribed 5 mm lesion in the head of the pancreas. To determine whether this was a source of ectopic ACTH production, ACTH samples were obtained peripherally and at various drainage points from the coeliac axis via the trans-hepatic route. There was no convincing 'step-up' in the ACTH gradient. Subsequent repeat pituitary MRI with dynamic post-contrast image acquisition showed an area of relative delayed enhancement on the left side of the gland consistent with a microadenoma. She underwent transsphenoidal adenomectomy with a postoperative cortisol confirming remission of the disease. Cushing's syndrome is a rare presentation in MEN1 and this case illustrates the difficulties in determining the underlying source of cortisol excess in some of these patients. Furthermore, the data indicate that patients with a central:peripheral ratio suggestive of a non-pituitary source of ACTH in MEN1 may still have Cushing's disease. Transsphenoidal pituitary exploration is indicated in cases of negative IPSS where search for an ectopic source is unrewarding, particularly if peripheral ACTH levels rise with CRH administration.

#### P120

**Glucagonoma: does the clinical expression depend on the presence of inherited disease?**  
F Smeeton, JS Davies, MF Scanlon & DA Rees  
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A 40 year old gentleman presented with dramatic weight loss and a rash in the perineal area spreading to involve his face, trunk, legs and feet. Initial investigations demonstrated a normocytic anaemia with no evidence of thyroid disease or diabetes. Occasional diarrhoea prompted subsequent investigations including upper GI endoscopy, duodenal biopsy, pancreatic exocrine testing and barium follow-through studies which were normal. His 24 hour urinary 5-hydroxyindoleacetic acid levels corrected to normal with adjustment for creatinine. Serum chromogranin A levels were 160 pmol/L (normal <60) and octreotide scintigraphy demonstrated a focal area of increased uptake in the region of the pancreas/duodeno-jejunal flexure, corresponding to a 7 cm mass in the tail of the pancreas on CT scan. Fasting gut hormone profile demonstrated a glucagon value of 375 pmol/l (normal <50) confirming glucagonoma syndrome with necrolytic migratory erythema. In keeping with this there was evidence of significant hypoaminoacidaemia. He underwent distal pancreatectomy with normalisation of glucagon and chromogranin A, and correction of the hypercatabolic protein state. MEN1 mutational screening was negative. A 35 year old gentleman with established MEN1 and previous parathyroidectomy/thymectomy was found to have a raised glucagon level of 52 pmol/l on annual screening. There were no clinical features of the glucagonoma syndrome but subsequent MRI showed a 2 cm tumour at the

body/tail of the pancreas. He underwent distal pancreatectomy with histology showing at least 28 tumour nodules, the largest measuring 17 mm. Immunostaining of this and five others showed strong expression of chromogranin A and synaptophysin, with focal positivity for glucagon and pancreatic polypeptide. These reports illustrate the contrasting modes of presentation of glucagonomas in sporadic and MEN1-associated disease and suggest that glucagonoma syndrome is less apparent in inherited cases. This is likely to relate to earlier recognition by periodic screening in MEN1 though modification of phenotypic expression cannot be excluded.

#### P121

**A rare cause of gynaecomastia**  
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Adrenocortical carcinoma is extremely rare and accounts for 0.05–2% of all malignant neoplasms with an estimated incidence of 0.5–2% per million people per year. Feminising adrenal carcinoma is even rarer. We submit such a rare case presenting with gynaecomastia. A 50 year old man presented with a year history of increasing bilateral gynaecomastia. He had no problems with his sexual function. Initial investigations by the GP showed low testosterone (4.8 nmol/L) with undetectable LH and FSH, TSH 2.2 mU/L, FT<sub>4</sub> 15 pmol/L, prolactin 131 & random cortisol 293. Renal function and liver enzymes were normal. It was initially thought that he had secondary hypogonadism but his 17 β-oestradiol level was elevated at 332 pmol/L (0–130). α-fetoprotein and β-hCG were normal. Whilst awaiting further review, he went to see his GP complaining of right flank pain. An ultrasound scan showed a large mass in the right flank suggestive of a renal cell carcinoma. A CT scan suggested that the mass was more likely to be of adrenal origin. Further investigations showed normal 24 hour urinary catecholamines, androstenedione level > 34 nmol/L (1.0–10.8) and DHEAS 24 nmol/L (2.2–15.7). After a 48 h low dose dexamethasone suppression test, cortisol suppressed to 83 nmol/L, and androstenedione to 5.3 nmol/L but DHEAS showed minimal suppression at 20 nmol/L suggesting a degree of autonomous cortisol secretion by the lesion but demonstrating dexamethasone suppressibility of the markedly elevated androstenedione level. He proceeded to laparotomy and a 1.2 kg adrenal tumour was removed, measuring 190 × 110 × 120 mm. The histology confirmed adrenocortical carcinoma. Androstenedione, DHEAS and 17 β-oestradiol levels returned to normal post-surgery with improvement of his gynaecomastia. At his last review 2 years after his surgery his hormonal profile was normal with no evidence of recurrence of his tumour. This case illustrates the importance of considering an oestrogen-secreting adrenal tumour in cases of gynaecomastia with suppressed gonadotrophins and low testosterone.

#### P122

**Is the continuation of lithium treatment safe during peri-operative periods?**  
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#### Case Report

A 40-year old male with a background history of autism, severe learning difficulties and childhood Hirschsprung's disease had been on long-term lithium carbonate therapy, without any history of polyuria or polydipsia. During admission he underwent a subtotal colectomy for malignant colonic polyps. Pre-operative serum urea and electrolytes, glucose and lithium levels were normal. Pre-operative fluid balance was normal. The post-operative period was turbulent; he required a repeat laparotomy to washout an intra-abdominal collection and was transferred to the intensive care unit. At this stage, urine output rose to six litres per day, serum sodium 165(135–145) mmol/l, serum potassium 2.9(3.5–5.0) mmol/l, urea 4.1(2.5–7.0) mmol/l and creatinine 129(50–130) μmol/l. Further investigations revealed plasma osmolality of 341(288–298) mosmol, urine osmolality 141(250–750) mosmol and urine sodium of 7(50–125) mmol/l. Although nephrogenic diabetes insipidus was suspected, a water deprivation test was impossible because of his poor clinical state. Lithium was stopped and desmopressin was commenced at 750 mcg/day. Urine output increased to approximately 20 litres per day. Desmopressin was gradually titrated up to 3 mg. His serum sodium

dropped to 140 mmol/l but urine output remained between 8 to 9 litres per day. He was commenced on amiloride (5 mg daily), indomethacin (50 mg tds) and hydrochlorothiazide (2.5 mg daily). His condition improved, serum sodium stayed at around 140 mmol/l and daily urine output decreased to 1.8 litres. Desmopressin and indomethacin were stopped. He was discharged on amiloride (5 mg daily) and bendrofluzide (2.5 mg daily).

#### Comments

Lithium is known to cause nephrogenic diabetes insipidus. In patients on lithium treatment post-operative precipitation of nephrogenic diabetes insipidus is not a common phenomenon, although it has been previously reported. Temporary cessation of lithium should be considered during hypovolaemic states.

## P123

### An unusual cause of jaundice

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We report 2 patients with Graves' thyrotoxicosis complicated by jaundice. Case 1

A 36 year old referred to the gastroenterologists with a 3-month history of general malaise, myalgia, jaundice, 4 stone weight loss and diarrhoea. A hepatitis and autoimmune liver screen were negative, bilirubin elevated at 200 umol/l, coagulation screen and ultrasound scan were normal and a liver biopsy showed cholestasis. Thyroid function tests (TFT's) demonstrated FT4 24.8, FT3 9.8 and TSH 0.07, (TSH receptor and TPO antibodies positive). He was initiated on carbimazole his thyroid function normalised with treatment and he improved clinically, his weight increased and liver function tests normalised. He remains on a block and replace regimen and is awaiting radioiodine therapy.

#### Case 2

A 32 year old man presented with relapsed Graves' thyrotoxicosis and jaundice. He had previously been treated with carbimazole and propylthiouracil at his initial presentation both of which he was unable to tolerate. At relapse his TFT's revealed FT4 85.5 pmol/l and TSH < 0.01, bilirubin elevated at 156 umol/l. Routine autoimmune and hepatitis liver screen were unremarkable and an ultrasound scan of liver was normal. On this occasion the patient was managed symptomatically with propranolol without his anti-thyroid drugs. Subsequently his jaundice settled with improvement in both his liver function tests and thyroid function. He is awaiting definitive surgical treatment as he has declined radioiodine therapy.

Thyrotoxicosis is an uncommon cause of profound jaundice although thionamide therapy is more associated with cholestatic hepatitis with cross-reactivity between carbimazole and propylthiouracil. Cholestasis may be secondary to the toxic effects of thyroid hormone directly affecting the liver or due to the systemic effects of excess thyroid hormone having a detrimental effect on hepatic bilirubin metabolism. Further potential autoimmune mechanisms may also be involved with previous strong associations between lymphocytic thyroiditis and primary biliary cirrhosis.

## P124

### Severe thyrotoxicosis and pregnancy

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A 27-year-old woman presented with severely symptomatic Graves and ophthalmopathy (FT4 49.1; TSI 53). Despite 60 mg carbimazole and 240 mg propranolol she required admission to control her symptoms and thyroid function, changing to propylthiouracil 800 mg daily with propranolol (FT4 19.9). Whilst considering thyroidectomy a 6-week pregnancy was confirmed. She relapsed (? compliance) and was admitted at 14 weeks with hyperemesis gravidarum (FT4 > 77.2), which responded to antiemetics, intravenous fluids and 20 mg prednisolone. She was readmitted at 17 weeks to prepare for

thyroidectomy, receiving Lugol's iodine, propylthiouracil, propranolol and withdrawal of prednisolone. She underwent partial thyroidectomy at 19 weeks (FT4 8.0) and was managed post-operatively on HDU without sequelae, with thyroxine 100 mcg being commenced postoperatively.

At 27 weeks propylthiouracil 100 mg was recommended for foetal protection because of persisting high TSI (TSI 44.0; FT4 17.1; TSH 0.1). At 32 weeks she developed premature labour, an antepartum haemorrhage with an abnormal CTG and underwent an emergency caesarean section. A female baby weighing 1.545 kg was delivered without evidence of foetal thyrotoxicosis. Baby remains well and mother euthyroid.

This case demonstrates a series of problems and management issues in severe thyrotoxicosis and pregnancy.

## P125

### Identification of two novel GALNT3 mutations in a patient with familial tumoral calcinosis (FTC)

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FTC (OMIM #211900) is a rare autosomal recessive disorder characterized by the presence of ectopic calcifications in the skin and subcutaneous tissues. These calcified masses look like irregular tumors which usually develop in a periarticular position, causing pain and often necessitating surgical excision. The majority of affected individuals have hyperphosphatemia due to increased renotubular reabsorption of phosphate and elevated levels of serum 1,25-dihydroxyvitamin D (calcitriol), while calcium and parathyroid hormone (PTH) levels are normal. Recently, biallelic mutations in the *GALNT3* (UDP-N-acetyl- $\alpha$ -D-galactosamine/polypeptide N-acetylgalactosaminyl transferase 3) gene have been identified in 3 families with FTC. *GALNT3* encodes a biosynthetic enzyme which initiates mucin-type O-glycosylation. In the present study we performed mutation analysis of the *GALNT3* gene in a subject with FTC and in his relatives. The proband showed the classical manifestations of the disease, including ectopic periarticular calcifications and hyperphosphatemia. Starting from the first decade of life he developed massive semicalcific lesions and underwent numerous surgeries to remove calcified deposits. Direct sequencing of genomic fragments corresponding to all *GALNT3* coding exons, as well as conserved splice junctions, revealed that the proband was a compound heterozygote for two novel nonsense mutations in exon 4 and in exon 7. Both mutations cause premature termination of protein translation. Proband's parents and his sibs are carriers of one of the two mutations. In order to verify cosegregation of the mutations with the disease, PCR-RFLP analysis was performed for this family, confirming the results of the mutation analysis. These are the first mutations identified in the *GALNT3* gene in an Italian family. Additional studies are needed to elucidate the role of this enzyme during O-glycosylation and to clarify why its impairment causes FTC.

## P126

### Megadenoma

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A 44-year-old woman presented to an emergency department with ear pain and worsening of longstanding headache. There was no history of menstrual disturbance, galactorrhoea, tiredness or weight gain and physical examination demonstrated only a left homonymous hemianopia. CT head scan revealed a brightly enhancing, enormous, lobulated mass arising from the pituitary fossa and causing obstructive hydrocephalus through compression of the fourth ventricle. Following collection of blood and administration of dexamethasone the patient was transferred urgently to a neurosurgical centre.

Whole-brain MRI showed the mass arising from the pituitary fossa, encroaching the orbital apices anteriorly, compressing the pons posteriorly, elevating the floors of the lateral and third ventricles superiorly and eroding inferiorly into the left temporal fossa. Serum prolactin was reported as



3567 mIU/l. Right fronto-temporal craniotomy was performed. Histologically the tumour was a chromophobe pituitary adenoma with prolactin and occasional GH immunostaining.

Post-operatively the patient was transferred back to the referring hospital. She was panhypopituitary, hyperphagic and paranoid. A review of the biochemical results from the blood taken prior to transfer to the neurosurgical centre revealed that her pre-operative serum prolactin, which was outside the assay range initially, had been reported as 1045320 mIU/l (without evidence of macroprolactin). Cortisol, thyroid and gonadal results were within appropriate normal limits. Cabergoline was commenced and increased to 1 mg daily. The serum prolactin fell over weeks into the normal range. Serial MRI scans show substantial resolution of the tumour. The patient remains on anterior pituitary replacement and a reduced dose of cabergoline but, apart from a persistent visual field defect, has made a good recovery.

This case provides a useful reminder of the risk of "hook effect" interference in some prolactin assays. It is all the more extraordinary because the patient was eupituitary pre-operatively, despite the enormous tumour.

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### P127

#### Autoimmune hypophysitis causing permanent diabetes insipidus – a case report

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Autoimmune hypophysitis is a rare cause of pituitary failure. Its aetiology and natural history are not well understood. We present a case of pituitary failure that showed spontaneous resolution of radiological and clinical features, consistent with autoimmune hypophysitis.

#### Case report

An 18 year old male presented with a 7 month history of polyuria and headaches. No significant past medical history or family history were noted. A water deprivation test confirmed cranial diabetes insipidus and he was commenced on DDAVP.

Further endocrine testing showed suppressed gonadotrophins and testosterone (1.8 nmol/L) and an elevated prolactin (all of which had been normal on presentation). During insulin hypoglycaemia, growth hormone response was low (<5 mU/L) and cortisol response was borderline. However 12 months later gonadotrophins had risen to within the normal range and testosterone was 10.9 nmol/L. Prolactin was within normal limits. An insulin tolerance test confirmed normal anterior pituitary function, although diabetes insipidus persists. Initial pituitary imaging showed thickening of the infundibulum and pituitary stalk but repeat scanning showed an improvement, with no residual thickening of the pituitary stalk. This case with transient pituitary failure is likely to represent autoimmune hypophysitis that has resulted in permanent diabetes insipidus. The transient hyperprolactinaemia would be consistent with pituitary stalk disconnection syndrome that appears to have resolved. Transient hypophysitis is a rare autoimmune condition that can present with various abnormalities of endocrine function. This may recover spontaneously, so regular review is required. The permanent diabetes insipidus probably reflects permanent loss of secretion of AVP.

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### P128

#### Time course of neuroendocrine dysfunction after aneurysmal subarachnoid hemorrhage – need for reassessment even years after the hemorrhage?

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In recent years there has been increasing evidence for neuroendocrine dysfunction as a sequel of aneurysmal subarachnoid hemorrhage (SAH). One investigation has assessed pituitary function in SAH-survivors in a longitudinal design 3 and 12 months after the hemorrhage, but nothing is known about the dynamics of the hypothalamo-pituitary axis in the years following the bleeding. We present the data of 5 patients who had sustained aneurysmal SAH and had undergone their first endocrinological evaluation at least one year after the acute event and were reassessed on average three years later because of partial pituitary deficiency already determined in the first investigation. The insulin tolerance test (ITT) was used for assessing corticotroph and somatotroph function. Additionally, TSH, fT4, fT3, FSH,

LH, estradiol (females), testosterone (males) and prolactin were measured. Severe growth hormone deficiency (GHD) was diagnosed if peak GH levels were <3 µg/l and secondary hypocortisolism if peak cortisol levels were <500 nmol/l in the ITT. Based on the ITT criteria 5/5 patients were diagnosed with ACTH deficiency and 2/5 patients with severe GHD in the first and also the second test. Interestingly, basal and stimulated cortisol and ACTH levels were lower at follow-up in all investigated patients, reaching statistical significance in the Wilcoxon-test. Maximal stimulated GH levels were also lower at follow-up in all investigated patients, basal GH levels were lower in 4 of 5 patients. Secondary hypogonadism or secondary hypothyroidism was not observed in the patient group. As the result of the second testing 3 of the patients with corticotroph deficiency were offered hydrocortisone substitution because of stimulated cortisol levels below 300 nmol/l. Our data give preliminary evidence that worsening of neuroendocrine function may occur in SAH patients even years after the hemorrhage and that renewed endocrine function testing has therapeutic implications.

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### P129

#### A case of late onset congenital adrenal hyperplasia in a female epileptic patient: implications for clinical practice

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We report the case of a 53 year old woman with a history of refractory focal epilepsy with complex partial and secondary generalised seizures. She has been on multiple anti-epileptic drugs since childhood. At 42 years of age she was referred to the Endocrinology Department complaining of capital hair loss and hirsutism. At this time her epilepsy was controlled on phenytoin and carbamazepine.

A diagnosis of congenital adrenal hyperplasia (CAH) was made, with genetic analysis showing her to be homozygous/hemizygous for the Val281Leu point mutation in the CYP21 (21-hydroxylase) gene, a mutation usually associated with a mild, non-classical form of CAH. Basal 17-hydroxyprogesterone (17OHP) is often only marginally elevated or even within the normal reference range in patients with the non-classical variant of 21-hydroxylase deficiency. In our patient biochemical analysis showed a basal 17OHP of 83 nmol/L (0.6–11.1) with an androstenedione of 28.4 nmol/L (4.0–10.0), more consistent with severe forms of 21-hydroxylase deficiency.

Phenytoin and carbamazepine are known to be potent inducers of hepatic cytochrome P450 enzymes, and will increase the metabolism of glucocorticoids and adrenal androgens. This increased metabolism of cortisol acts to further increase the stimulation of the adrenal cortex by corticotrophin. This results in an increase in cortisol precursors prior to the partial enzyme deficiency, and an increase in biosynthesis of adrenal androgens. Was it therefore possible that concomitant phenytoin and carbamazepine therapy in this patient was exacerbating the clinical phenotype of a mild form of 21-hydroxylase deficiency?

Subsequently, phenytoin was withdrawn and replaced with leviteracetam which does not induce cytochrome P450 enzymes. Following this change in anti-epileptic therapy the patient noted an improvement in her hair loss and less hirsutism. Biochemistry analysis showed a marked improvement in her adrenal androgens, with a 17OHP of 26 nmol/L and an androstenedione of 1.4 nmol/L.

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### P130

#### Adult-onset nesidioblastosis- a rare clinical case

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#### Introduction

Nesidioblastosis is a rare but well recognized disorder of persistent hyperinsulinemic hypoglycemia in infancy, but adult-onset nesidioblastosis associated with hyperinsulinemic hypoglycemia, termed noninsulinoma pancreatogenous hypoglycemia syndrome (NIPHS) has also been reported. These patients experience predominantly postprandial hypoglycaemia instead of fasting hypoglycaemia that characterizes insulinoma. Histological findings are islet hypertrophy islet-cells budding off exocrine ducts.

#### Clinical case

We describe an extremely rare case of NIPHS in a 23 year-old girl. She experienced predominantly postprandial hypoglycaemia with several



lost consciousness episodes. Low plasma glucose level (19 mg/dl) and high insulin level (35.4 µU/ml) with a ratio insulin/glucose of 1.86 were consistent with the possible presence of insulinoma. Factitious causes were excluded. Imaging studies: CT, endoscopy with ultrasonography, selective arteriography, octreotide scanning and surgical exploration with intraoperative ultrasonography were negative. It was performed a selective arterial calcium stimulation with hepatic venous sampling (ASVS) that suggested the presence of insulinoma in the body and tail of the pancreas. It was performed a subtotal pancreatectomy (body and tail). Postoperatively, her hypoglycaemic episodes completely disappeared. Histological examination of the resected pancreas revealed hyperplasia and hypertrophy of beta cells islets- adult nesidioblastosis.

#### Discussion

Nesidioblastosis is a rare cause of hyperinsulinemic hypoglycaemia. Diagnosis and management continue to be a challenging experience of the endocrinologist and surgeon. When imaging studies are inconclusive, the ASVS is a powerful method for the localisation of insulinomas and can also identify diffuse forms of the disease.

### P131

#### Seeking an appropriate sex of rearing in 5 $\alpha$ -reductase deficiency

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5  $\alpha$ -reductase deficiency is a rare autosomal recessive condition of male pseudohermaphroditism, resulting from mutations of the type 2 isoenzyme 5  $\alpha$ -reductase, crucial in conversion of testosterone to dihydrotestosterone. Case report: Baby P is a full term baby, a product of a consanguineous marriage. The baby had ambiguous genitalia with a clitoris, minimal glans tissue at the apex of the labial folds, gonads in the labioscrotal folds and a urethral opening below the clitoris. Pelvic ultrasound demonstrated absence of müllerian structures. Due to the female phenotypic appearance of the genitalia the parents initially opted to raise the baby as a female. Biochemistry revealed a serum testosterone of 11.9 nmol/l and serum dihydrotestosterone was undetectable at <0.125 nmol/l. Karyotype was XY. Urinary steroid profile was normal. A diagnosis of 5  $\alpha$ -reductase deficiency was made. Management was decided based on a consensus between the parent's socio-cultural views and the medical perspectives of the adult and paediatric endocrinologists and the clinical geneticist. Rearing the baby as a female would involve early surgery to remove the gonads thus eliminating the testosterone drive to prevent further virilization and genitoplasty. However, this would result and the impact of androgen exposure to the brain in utero loss resulting in gender identity problems and permanent infertility in the future. Rearing the baby as a male would involve using DHT cream for virilization of the external genitalia and surgical correction of hypospadias, with preservation of fertility. The parents and the medical team agreed that rearing the baby as a male would be the best option. Daily application of DHT cream for 3 months to the external genitalia resulted in virilization in the form of clitoromegaly reaching a phallus length 2 S.D. below the average male baby. Surgical correction of the peno-scrotal hypospadias is the next therapeutic stage. Summary: Our case illustrates an uncommon cause of male pseudohermaphroditism. Management issues are based on social and cultural factors of the parents, the role of in utero androgen exposure to the brain, the importance of androgens in the evolution of male gender identity in man and the preservation of potential fertility.

### P132

#### 49,XXXXY syndrome connected with hypothyroidism

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49,XXXXY syndrome is a rare defect of sex chromosomes frequently labelled as Klinefelter variant. It is associated with more severe dysmorphic features, hypogonadism and mental retardation.

#### The goal

To describe clinical, biochemical, hormonal, radiological and developmental status of the patient with 49,XXXXY karyotype.

#### Report of the patient

12-month-old boy referred because of underdeveloped genitalia. The patient was born by caesarean section because of placenta praevia, had hypotrophy and respiratory distress, weight 2400 g, length 53 cm, Apgar score 6.

Scoliosis was noticed at the age of 6 months. At 10 months a CT scan confirmed mild hydrocephalus with cerebral atrophy.

At the age of 12 months his weight was 9600, length 80 cm. Physical examination revealed: hypotonia, broad forehead and nasal bridge, hypertelorism, epicanthic folds, rotated left ear, lack of teeth, short neck, sloping shoulders, narrow thorax, left side scoliosis, hypoplastic scrotum, small penis and testes, left testis located in inguinal canal, right in scrotum. Psychomotorical development was retarded: he could not sit or stand without support. A chromosome study indicated the 49,XXXXY karyotype. On radiographic examination: delayed bone age adequate to neonatal period, left side scoliosis in the thoracic-lumbar region of the vertebrae. TSH 1.31 µIU/ml, FT4 - 1.2 ng/dl, FT3 - 5.5 pg/ml. During TRH stimulatory test TSH level increased to 35.2 µIU/ml, which indicated hypothyroidism. After GnRH $\alpha$ : FSH rose from 0.53 to 41.7 mIU/ml, LH from 4.5 to 29.2 mIU/ml. Stimulation with CRH caused the change of ACTH from <10 to 34.5 pg/ml. Echocardiography confirmed PFO, urography was normal. L-thyroxin administration was ordered. After 1 month the first tooth appeared. He started walking at 23 months, but still doesn't talk.

#### Conclusion

Children with 49,XXXXY karyotype require hormonal estimations. Early identification of deficit allows starting early treatment intervention, parallel to rehabilitation, to support individual development.

### P133

#### Giant labia majora in familial partial lipodystrophy

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Partial lipodystrophy syndromes are characterised by a selective paucity of subcutaneous adipose tissue on the trunk and limbs but excessive fat on the face, neck, supraclavicular areas and pelvis. Metabolic complications include insulin resistance and polycystic ovary syndrome (PCOS). There have been no previous reports of genital disfigurement in these conditions. We present the case of a 27 years old female, who initially presented to the gynaecology service with secondary amenorrhoea. She was diagnosed with PCOS and commenced on ethinylestradiol/cyproterone (Dianette). She was noted to have vulval swelling, which was thought to be secondary to lymphoedema.

Following referral to the department of endocrinology, she was noted to have acanthosis nigricans, and complete absence of limb fat. Genetic analysis confirmed the diagnosis of the Dunnigan-Kobberling variant of partial lipodystrophy. Laboratory testing revealed increased free testosterone levels, impaired glucose tolerance and dyslipidaemia. Review of magnetic resonance imaging (MRI) scans revealed that her greatly enlarged labia majora were due to excessive adipose deposition.

She is currently being treated with lifestyle measures, Dianette, metformin and rosiglitazone and is metabolically stable. Her genital disfigurement is disabling because of physical discomfort and cosmetic concerns. She has been referred for plastic surgery, although it is likely that the problem will recur. Pelvic fat accumulation in lipodystrophy syndromes has previously been documented in imaging studies<sup>1</sup>, but to our knowledge, this is the first report of a significant clinical outcome resulting from this effect.

1. Garg A, Peshock RM & Fleckenstein JL Adipose tissue distribution pattern in patients with familial partial lipodystrophy (Dunnigan variety). *J Clin Endocrinol Metab* 1999; 84: 170-174.

### P134

#### Pituitary apoplexy and acromegaly: a case report

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Pituitary apoplexy is an acute medical emergency but there are no evidence based management guidelines, especially for neurosurgical intervention.

A 35-year old male presented with sudden onset severe headache. On examination blood pressure was 180/120 without any lateralising neurological signs. He appeared acromegalic with a short history of enlarging hands & feet. Emergency CT scan and a MR scan later confirmed haemorrhagic pituitary adenoma, 3 cm in height, moderately compressing the optic chiasm. Formal perimetry was normal and daily monitoring of visual fields by confrontation did not reveal evolving deficits. Random serum growth hormone (GH) levels were elevated >100 mU/L. He was discharged home on hydrocortisone and antihypertensives.

Formal endocrine investigations were carried out one month later and demonstrated mean GH through a 4-point day profile of 21 mU/L. Octreotide LAR was started and on 20 mg once monthly, GH profile was suppressed to 0.5 mU/L with progressive fall in IGF-1 to 51 nmol/L (reference range 13–37 nmol/L). Basal 9 am serum cortisol was 367 nmol/L, suggesting adequate ACTH reserve. Other anterior pituitary function tests remained consistently normal. Serial MR Scans showed progressive reduction in size of the pituitary adenoma to 9 mm diameter, confined to pituitary fossa.

Many studies advise early decompressive surgery for pituitary apoplexy for better visual and endocrine outcome, although a recent series has suggested that conservative management may be more appropriate in many cases. In this case, despite some evidence of chiasmal compression, conservative management was justified by the lack of actual visual compromise, and allowed good control of the underlying acromegaly with significant tumor shrinkage. This may facilitate surgical treatment for longer-term disease control.

### P135

#### The effects of lanreotide administration vs unilateral adrenalectomy on disease activity in gip-dependent cushing's syndrome

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GIP-dependent Cushing's syndrome (CS) is caused by ectopic expression in adrenal cortex of gastric inhibitory peptide (GIP) receptors coupled to steroidogenesis, which leads to postprandial increases of cortisol (food-dependent CS). In a few cases a transient effect of short-acting octreotide on cortisol levels has been shown, but bilateral adrenalectomy is still thought to be a definitive treatment.

We report on a case of a 45-year old woman with bilateral macronodular adrenal hyperplasia and clinically apparent CS, chronically treated for several years with aminoglutethimide (A). After we had observed that pre-treatment with octreotide s.c. prevents an increase in cortisol levels during OGTT, we administered a single dose (30 mg i.m.) of long-acting lanreotide (Ipsen Biotech) after 4 weeks' withdrawal of A. Plasma pre- and postprandial levels (90 min.) of intact GIP and cortisol (6-point profile), as well as blood glucose and urinary free cortisol (UFC) were measured on day 0, 2 and 8. Intact GIP levels expectedly reflected normal fasting and postprandial values (range 6–55 pmol/L) and were correlated with the corresponding cortisol levels ( $r = 0.59$ ,  $P = 0.004$ ) on day 0. Mean cortisol levels were moderately suppressed on day 2 ( $60.4 \pm 12.7$  vs.  $43.7 \pm 10.2$ ,  $\mu\text{g/dL}$ ;  $P < 0.01$ ), but rose again on day 8 ( $49.8 \pm 10.4 \mu\text{g/dL}$ ), which was accompanied by a significant suppression of postprandial GIP increases. However, lanreotide induced recurrent postprandial hyperglycaemia (up to 11.1 mmol/L) and failed to control clinical symptoms (hypertension, leg oedema). Unilateral adrenalectomy performed 6 months later did not influence pre- and postprandial intact GIP levels, but in turn normalised UFC during the first 4 weeks postoperatively (50.0–55.7  $\mu\text{g}/24$  hrs). After 6 weeks UFC increased again to supra-normal values, which necessitated continuation of treatment with A. Conclusion: Although pathophysiologically justified, a single lanreotide administration in GIP-CS does not seem to effectively control hypercortisolism and it may worsen glucose metabolism. Short-term results of unilateral adrenalectomy are more convincing, but its curative effect may be transient.

### P136

#### Epilepsy at presentation of macroprolactinoma

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#### Objectives

Prolactinomas can extend out of the sella turcica and invade surrounding structures and the brain. The prevalence and natural history of epilepsy at presentation of macroprolactinoma is not clear.

#### Methods

The case records of 62 patients (34 male) with macroprolactinoma attending a neuroendocrine clinic were studied.

#### Results

Four patients (6.5%, 3 males) had experienced epileptic seizures before the diagnosis of macroprolactinoma was made. The details at diagnosis are shown below:

Age	Sex	Prolactin mU/L	MRI appearance	Epilepsy time present
33	M	30,000	Indents 3 <sup>rd</sup> ventricle	Grandmal, 3 months
42	M	> 500,000	Invades temporal lobe	Simple partial, 7 years
51	M	> 750,000	Invades temporal lobe	Complex partial, 23 years
52	F	> 400,000	Invades temporal lobe	Complex partial, 2 years

One patient (42 M) had simple partial seizures which was undiagnosed for 7 years prior to the diagnosis of macroprolactinoma. Another (51 m) had monthly, complex partial seizures and was on antiepileptic treatment for 23 years. A CT scan in 1982 was reported as "normal" but MRI scan in 2004 showed massive, invasive prolactinoma. His seizures stopped with bromocriptine treatment although MRI appearance is unchanged.

#### Conclusion

Patients with macroprolactinomas should be questioned about possible epilepsy especially partial seizures, which may be present for many years before diagnosis. Dopamine agonist treatment may reduce seizure frequency.

### P137

#### Difficulty to distinguish hyperandrogenia due to a tumoral formation or to polycystic ovary syndrome: a case report

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A 24-year-old woman complained about secondary amenorrhoea after stopping oral contraceptive whereas she wanted to be pregnant. Physical examination showed an android obesity with a non-progressive but major hirsutism. Endocrinological investigations eliminated pregnancy but revealed on different samples a high plasma testosterone level (from 2.15 to 2.77 ng/ml), in the tumoral range whereas DHEA-sulfate, delta4-androstendione, sex binding protein and prolactin were normal. Stimulation test eliminated a 21- hydroxylase block. Further investigations suggested polycystic ovaries: LH hyperpulsatility, typical microfollicular ovaries aspect on pelvic MRI, metabolic syndrome with glucose intolerance and hypertriglyceridemia. Tomography scan of the adrenal glands showed a unilateral hypertrophy. In order to prove that ovaries were responsible of hyperandrogenism, a diagnostic treatment with long-acting gonadotrophin releasing hormone (GnRH) was performed. GnRH administration produced only a partial decrease in serum testosterone levels (0.77 ng/ml). Because of this absence of testosterone normalisation under GnRH and the presence of a unilateral adrenal hypertrophy, an adrenal and ovarian venous catheterism was performed. There was no gradient in testosterone secretion to suggest hyperandrogenia was from an ovarian or adrenal origin. We concluded that high testosterone levels were the results of a benign ovarian hyperandrogenism. A glitazone treatment was initiated associated with hypocaloric diet and physical exercise. Evolution of the adrenal morphology will be performed. This case is an example that polycystic ovaries can produce high levels of testosterone that can remind of a

tumoral secretion. Nevertheless, testosterone levels up to 2ng/ml must lead to eliminate a tumoral origin, even when the metabolic and endocrinological context suggests a polycystic ovary syndrome.

### P138

#### Resolution of diagnostic uncertainties with DAX-1 gene mutation analysis

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Mutations in the DAX-1 gene are well described in patients with adrenal hypoplasia congenita (AHC) and hypogonadotropic hypogonadism but can be a useful tool in the elucidation of unusual or difficult cases of adrenal insufficiency. As the clinical presentation of congenital adrenal hyperplasia and AHC can be clinically identical, diagnosis in the neonate often depends on the levels of 17-hydroxyprogesterone (17-OHP). If these are unavailable or non-diagnostic, gene analysis can be important.

We present three patients from two unrelated families in whom genetic evaluation both corrected misdiagnoses and also significantly altered long-term clinical management and genetic counselling.

In the first family, a male infant presented with a salt-losing crisis at 3 weeks of age and was erroneously diagnosed with 21-hydroxylase (21-OH) deficiency. DNA analysis of the 21-OH gene was normal, but DAX-1 mutation analysis revealed a single base pair G deletion in codon 183 resulting in a premature stop codon at codon 263. They have subsequently had a second son without the mutation.

In the second family, DAX-1 mutation analysis was carried out on a mother who had lost two sons in infancy with adrenal crises of undetermined aetiology thirty years previously. Her daughter was considering starting a family so the cases were revisited. Inspection of the boys' medical records revealed post-mortem confirmation of adrenal hypoplasia in one and aplasia in another. Analysis of the mother's DNA revealed a C to G nucleotide substitution at position 458 causing a stop codon at codon 153.

These 2 families illustrate the need for clinicians to be aware of the possibilities of DAX-1 gene mutations in male patients presenting with hypoadrenalism both in the neonatal period and beyond. It is important to make the diagnosis not only for the patient, but also in order to assess accurately the risks to other family members.

### P139

#### Multihormonal disorders in 17 year old girl with congenital hypoplastic anemia and secondary hemochromatosis

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Congenital hypoplastic anemia (Diamond-Blakfan Syndrom) is genetically determined disorder which is manifested in early childhood with selective deficiency of erythrocyte line in bone marrow. Severe anemia usually appears between 2nd and 6<sup>th</sup> month of life. Survival depends on blood transfusions, which in consequence lead to hemosiderosis. Hypopituitarism, hypogonadism and diabetes mellitus and other endocrinopathies are the complications of hemochromatosis.

15 years old girl, born from 7<sup>th</sup> pregnancy at term with 10 points in Apgar scale, body weight 3000 g, length 52 cm. Her parents and siblings are healthy. Hypoplastic anemia was diagnosed at the age of 3 months. She received multiple erythrocyte mass transfusions and Desferal periodically. Hemosiderosis appeared as a consequence. Additionally she was infected with Hepatitis C Virus.

At the time of her admission to our Department physical examination revealed: body weight 25 kg ( $\ll 3$  c.), height 121 cm ( $\ll 3$  c.), her skin was grey-brown, dry and cold, brown teeth enamel, heart rate 60/min, blood pressure 100/50, hepatosplenomegaly, absence of puberty symptoms.

Preliminary hormonal diagnostic revealed primary hypothyroidism (TSH  $> 75.0$  uIU/ml, fT4  $< 0.2$  ng/dl, fT3 1.0 pg/ml) and hypoparathyroidism (PTH 8.3 pg/ml, hypocalcemia, hyperphosphatemia, bone deficiency). Therapy was introduced: L-thyroxin, calcium carbonicum, 1,25(OH)<sub>2</sub>D vitamin. After euthyrosis was established the function of hypophysis was

evaluated. The dynamic tests revealed somatotrophic and gonadotrophic hypopituitarism. The patients was positively qualified to growth hormone therapy.

The case of our patient justifies the need of endocrinological evaluation of patients with hemochromatosis.

### P140

#### Carcinoid tumour: spontaneous regression following pregnancy

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#### Clinical case

An incidental right sided pelvic mass measuring 6 cm  $\times$  7 cm was found in a 35 year-old nulliparous lady during investigations for secondary infertility. This mass was hard, irregular and white in appearance and biopsy confirmed a carcinoid tumour with a low Ki 67 index of 2.7%. She had no symptoms of flushing, diarrhoea or local discomfort. Endocrine screen showed a non-secretory tumour with normal fasting gut hormones and negative urinary 5HIAAs. Other tumour markers (CEA, CA-125, AFP and calcitonin) were also negative.

#### Progress and management

The patient became pregnant soon after the diagnosis. Close monitoring during antenatal period was uneventful. She had elective caesarean section and delivered a healthy baby boy at 38 weeks with uncomplicated post-natal period. A repeat CT imaging (10 months from the first scan) did not show any measurable increase in the extent of the pelvic tumour, and no liver metastases were identified. An Indium<sup>111</sup> octreotide study showed increased tracer uptake in the lower pelvis, correlating to the tumour on CT as well as a focal increase in the region of the terminal ileum suggestive of a possible primary. A subsequent CT scan four months later however showed that the mass was no longer present and this was confirmed on laparoscopy showing no macroscopic pelvic or abdominal abnormalities.

#### Discussion

The presence of a non-secretory carcinoid tumour has been demonstrated in our patient with CT imaging, octreoscan and histology. Furthermore, serial imaging has demonstrated spontaneous regression of the carcinoid suggesting that pregnancy did not worsen the course of the disease but may have resulted in tumour regression. Nonetheless, long-term surveillance will be required.

### P141

#### A rare cause of thyrotoxicosis and hypercalcemia

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A 32 year old lady was admitted with symptoms of palpitations, tremors, insomnia, heat intolerance, weight loss and excessive perspiration for 4 weeks. Worsening of these symptoms with vomiting prompted the admission. She had similar episode 7yrs ago following the birth of her child when a provisional diagnosis of postpartum thyrotoxicosis was made but lost follow up with in 2 weeks. She works as a carer looking after mentally handicapped children. Her mother suffers from hypothyroidism and is on L-Thyroxine. On examination she was restless and fidgety and had a pulse rate of 140 per minute with tremor of the hand. Thyroid functions done on admission and 2 week prior to that showed free T4 was  $> 108$  pmol/L, free T3  $> 44$  pmol/L and TSH suppressed at  $< 0.03$  Miu/L. She was treated with popylthiouracil as she did not tolerate carbimazole. Calcium measured was **high at 3.55** with a normal alkaline phosphatase. Other causes of hypercalcemia were ruled out including hyperparathyroidism and vitamin D intoxication.

After initial improvement associated with fall in thyroid hormone levels she was discharged. But two weeks later she had recurrence of her symptoms when thyroid hormone level showed further rise with recurrence of hypercalcemia. This picture and given her occupation also raised the possibility of factitious thyrotoxicosis. The thyroid scan showed no uptake confirming our suspicion of factitious thyrotoxicosis. When confronted she admitted buying thyroid hormones online. She also admitted to taking thyroid pills during her first episode 7 years ago.



Diagnosing factitious thyrotoxicosis can be difficult and requires high index of suspicion without which misdiagnoses and mismanagement are common. About 20% of hyperthyroid patients exhibit hypercalcemia but are mild and rarely symptomatic. This case is unusual because of the etiology of thyrotoxicosis and unusually high level of hypercalcemia which to our knowledge has never been reported with thyrotoxicosis

#### P142

##### **Maternal virilization and female fetal pseudohermaphroditism caused by exaggerating androgen secretion of pregnancy leuomas**

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##### Objective

Maternal and female fetal virilization is subject to exaggerating androgen secretion of pregnancy luteomas which is associated with the protective capacity of placenta safeguard against hormone conversion and presents with variable extent of female pseudohermaphroditism as exposure in critical gestational stage.

##### Patient(s)

A nulligravida woman suffering from bilateral hydronephrosis and recurrent acute pyelonephritis caused by bilateral solid ovarian tumors presented virilization during the third trimester. The serum testosterone levels revealed as high as 200-fold the levels of the normal female. Without prompt surgical intervention, the maternal hyperandrogenemia returned to a normal level and regression of bilateral ovarian tumors spontaneously until a female fetus with clitoral hypertrophy and temporal hyperandrogenemia was delivered. In spite of lacking histology, the clinical course is compared to that of pregnancy luteomas.

##### Result(s)

Regression of androgen-secreting pregnancy luteomas and hyperandrogenism occurred during the puerperium but apparent female fetal clitoral hypertrophy.

##### Conclusion(s)

During pregnancy, exposure to excessive androgen at the critical 9–14 week period leads to variable masculinization in female infant. It has been hypothesized that the placenta is protective in this regard, by virtue of its high capacity to convert androgens to estrogen. Thus, a female fetus is usually not affected. However, the present observations – where the female fetus had exposed to maternal hyperandrogenemia to yield clitoral hypertrophy without affecting the labioscrotal fold due to beyond the critical 9–14th week for external genitalia differentiation. The maternal and female fetal virilization was caused by exaggerating androgen secretion of bilateral ovarian solid tumors. Spontaneous regression of ovarian tumors and hyperandrogenemia during the puerperium is the nature course of pregnancy luteoma, not true neoplasms.

#### P143

##### **Two unusual cases of extraadrenal pheochromocytoma**

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We present 2 cases of extraadrenal pheochromocytoma with several unusual features: (a) A 54 year-old female presented with dizziness, falls, and palpitations. She was tachycardic with blood pressure of 218/75 supine and 139/107 standing. An abdominal mass was palpable. Urinary catecholamines were markedly raised and a CT scan showed para-aortic mass with avid MIBG uptake confirming the diagnosis of an extraadrenal pheochromocytoma. Persistent hypokalaemia and hyperglycaemia raised suspicion of hypercortisolism. High plasma cortisol (>1750 nmol/L) failed to suppress after low and high doses of dexamethasone and 9am ACTH was high at 913 pmol/L (N < 46 pmol/L). Results were suggestive of ectopic ACTH production and the pheochromocytoma was considered to be the source. Following pre-operative metyrapone and  $\alpha + \beta$  blockade, tumour was resected and histology confirmed the clinical diagnosis. Postoperatively urinary catecholamines normalised and all medications were weaned off over next 4 weeks. Synacthen test showed normal response. ACTH immunostaining was negative suggesting the possibility of ectopic CRH secretion. To our knowledge this is the first report of an ACTH/CRH secreting extraadrenal pheochromocytoma.

(b) A 27-year old man presented with a 14-year history of paroxysmal headaches and palpitations precipitated by micturition. He was hypertensive and tachycardic. Urinary catecholamines were markedly elevated. MRI identified a paravascular mass and pelvic lymphadenopathy with significant MIBG uptake diagnostic of locally metastatic extra-adrenal pheochromocytoma. Following pharmacological preparation he underwent excision of primary lesion, partial cystectomy and nodal clearance. Post-operatively urinary catecholamines remained elevated. MIBG scan failed to confirm residual disease. However a PET scan revealed widespread distant metastases precluding further surgery. MIBG-labelled radiotherapy is being considered. This case illustrates several interesting features including early onset of symptoms in absence of family history or endocrine syndromes, failure of pre- and post-operative MIBG scan to identify distant metastases and usefulness of PET in evaluation of such cases.

In conclusion, pheochromocytomas often have varied presentation requiring high index of clinical suspicion and systematic localisation. Delayed diagnosis can cause severe morbidity and reduced life span.

#### P144

##### **Prolonged hungry bone syndrome in a patient with wolfram syndrome: a case report**

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Hungry bone syndrome (HBS) is associated with recent parathyroid surgery. The Wolfram syndrome is characterised by diabetes insipidus, diabetes mellitus, optic atrophy and deafness. Other anomalies, such as renal outflow tracts and multiple neurological disorders may develop later. We present a Wolfram patient with tertiary hyperparathyroidism due to chronic renal failure associated with bilateral reterohidronephrosis who developed prolonged HBS after parathyroid adenomectomy.

A 21 year-old woman with Type-1 DM, diabetic polyneuropathy, chronic renal failure due to neurological vesica, bilateral hydronephrosis and single parathyroid adenomectomy 3 months ago was hospitalized for glucose regulation. Calcium concentration was 7.7 mg/dl. She was taking 1 µg oral calcitriol, 4000 mg calcium daily. After five days she developed hypocalcaemia symptoms. Serum calcium level decreased to 6.1 mg/dl. Oral calcitriol dosage was elevated up to 7 µg gradually, calcium concentration decreased to 4.8 mg/dl. Continuous intravenous calcium was initiated, 1500 mg Ca<sup>2+</sup> daily. Intravenous calcium was necessary for 2 months. After operation serum alkaline phosphatase levels increased up to 1614 IU/l, but gradually decreased to 795 IU/l 2 months later. Fundoscopic examination revealed bilateral optic atrophy. Voiding urography and renal ultrasonography revealed hydronephrosis. Urodynamics revealed low capacity, low compliance bladder, emptying problem. Audiometry demonstrated bilateral sensoryneural hearing loss. Diagnosis of Wolfram syndrome was made. We report a patient with chronic renal failure that caused prolonged HBS lasting for 6 months postoperatively. Current case was diagnosed with diabetes mellitus, neurogenic bladder, and bilateral hydronephrosis at the age of 12. Since she did not have diabetic retinopathy in ophthalmic examinations, her chronic renal failure was thought to be associated with recurrent pyelonephritis. She developed bilateral optic atrophy. To our knowledge this is the first case with Wolfram syndrome and prolonged hungry bone syndrome following surgery for tertiary hyperparathyroidism are coexisting.

#### P145

##### **Postmenopausal androgen excess: a clinical perspective**

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We present two cases of postmenopausal, gonadotrophin-dependent androgen excess.

##### Case 1

A 59-year-old postmenopausal female presented with a 4-year history of hirsutism and male pattern balding. Past medical history included Type II



diabetes, hypertension, and polycystic ovarian syndrome. Investigations revealed androgen excess (testosterone 8.9 nmol/L [ref range <2.5], free androgen index 31.8 [ref 0–7], androstenedione 13.7 nmol/L [ref <6.8], DHAS normal). Ovarian and adrenal imaging was normal. Venous sampling was inconclusive and she was referred to our unit for further investigation. Repeat MRI showed bilateral ovarian enlargement.

#### Case 2

A 59-year-old woman with previous hysterectomy presented with 1 year of hirsutism, voice deepening and male pattern alopecia. Investigations revealed androgen excess (Testo6.7, A'dione 40.3, FAI 21.6), failing to suppress with low-dose dexamethasone. Adrenal and Ovarian imaging was normal. A 4-week trial of GnRH analogue (Buserilin 300 mcg tid) suppressed circulating androgens in both cases, consistent with gonadotrophin-dependent hyperandrogenism. Both patients proceeded to bilateral oophorectomy. Pathology in case 1 showed diffuse stromal hyperplasia, in case 2 pathology was reported as normal. In both cases hirsutism has regressed postoperatively and serum androgens have normalised. We present 2 cases of severe postmenopausal androgen excess where gonadotrophin suppression led to a clear fall in androgen production, confirming the ovary as the site of androgen production. Stromal cells may contribute to postmenopausal androgen production through a gonadotrophin-driven mechanism, although this hypothesis remains the subject of debate. Case 1 had a history of PCOS and it is unclear whether the pathological findings represent longstanding changes associated with PCOS, a marked cellular proliferation in the peri/postmenopausal period or a combination of both features. Case 2 probably represents less severe stromal hyperplasia. Gonadotrophin suppression offers a useful diagnostic test in patient evaluation with postmenopausal hyperandrogenism.

#### P146

**Using of pulse methylprednisolon therapy in Graves' ophthalmopathy**  
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The aim of the study is to estimate the efficacy of intravenous corticosteroid therapy in patients with active, severe Graves' ophthalmopathy.

#### Material and methods

Sixteen patients with bilateral active, severe Graves' ophthalmopathy were included to the study. Euthyroidism has been provided, mean dose of methimazol therapy was  $9.4 \pm 6.6$  mg a day, median of TSH- 0.3 mIU/l, median of fT4 – 15.3 pmol/l. The median of duration of GO was 6.5 months. All patients were administered methylprednisolone intravenously at a daily dose of 1 g for 3 consecutive days and repeated 3 times for 3 weeks. Disease activity was carried out with Clinical Activity Score (CAS), disease severity – with exophthalmometry, visual acuity and presents of diplopia.

#### Results

Initially the median of CAS was 4 points, the mean proptosis –  $20.8 \pm 4.3$  mm, median of visual acuity – 0.8. 35.2% of patients had lagophthalm and besides more than half of them had bilateral lagophthalm; 52.9% of subjects had diplopia (more than half of them – in primary gaze). In 1 month after treatment the median of CAS became 1 point and it has been continuing the same after 6 months of therapy. In 6 months visual acuity was not changed after treatment; the degree of proptosis became no significantly less (mean level  $18.8 \pm 5.1$  mm); the frequency of lagophthalm was decreased to 5.9% (what's more there were no patients with bilateral lagophthalm already in 1 month after treatment); the frequency of diplopia was significantly decreased to 5.9% to (there were no cases of diplopia in primary gaze already in 1 month after pulse therapy). Thus in 6 months only 1 patient (5.9%) had active process, unilateral lagophthalm, diplopia in extreme gaze and so the course was repeated with positive effect.

#### Conclusion

Pulse therapy with methylprednisolone can decrease symptoms of activity, frequency of lagophthalm and diplopia.

#### P147

**Psycho-biological correlates of free-floating anxiety symptoms in male patients with sexual dysfunctions**  
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Introduction and objectives anxiety has a relevant impact on everyday life, including sexual life, and therefore is considered the final common pathway by which social, psychological and biological stressors negatively affect

sexual functioning. The aim of this study is to define the psycho-biological correlates of free-floating anxiety in a large sample of patients complaining erectile dysfunction (ED) based sexual problems.

#### Methods

We studied a consecutive series of 882 ED-patients using SIEDY, a 13 items structured interview, composed of three scales which identify and quantify organic, relational and intrapsychic domains. MHQ-A scoring from Middlesex Hospital Questionnaire (MHQ) was used as putative marker of free-floating anxiety symptoms (AS). Metabolic and hormonal parameters, nocturnal penile tumescence (NPT) test and penile doppler ultrasound (PDU) examination were also performed.

#### Results

MHQ-A score was significantly higher in patients complaining difficulties in maintaining erection and in those reporting premature ejaculation ( $6.5 \pm 3.3$  vs.  $5.8 \pm 3.3$  and  $6.6 \pm 3.3$  vs.  $6.1 \pm 3.3$  respectively; both  $P < 0.05$ ). Moreover, AS were significantly correlated to life stressors quantified by SIEDY Scale 2 (relational component) and Scale 3 (intra-psychic component) scores, as dissatisfaction at work or within the family or couple relationships. Among physical, biochemical or instrumental parameters tested, only end-diastolic velocity at PDU was significantly ( $P < 0.05$ ) related to AS.

#### Conclusions

In patients with ED based sexual problems, AS are correlated to many relational and life stressors. Conversely, organic problems are not necessarily associated with MHQ-A score.

#### P148

**Challenges in the management of an uncommon cause of diabetes insipidus**

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Histiocytosis X is a rare disorder in which proliferating Langerhans cells can infiltrate virtually any site in the body. Bone is the most commonly affected site but endocrine involvement is frequently seen. Diabetes insipidus is the most common abnormality when there is involvement of the hypothalamic-pituitary axis.

We report the case of a 42 year old lady who presented with diabetes insipidus in 1997. MR scan of the pituitary showed absence of the posterior pituitary bright spot but was otherwise normal. A few months later she had evidence of gonadotrophin deficiency with a normal prolactin. An MR scan in 1998 showed thickening of the pituitary stalk. Dynamic testing demonstrated growth hormone deficiency (peak GH on ITT was 2.2 mU/l) and hyperprolactinaemia (Prolactin 1314 mU/l). A biopsy of the lesion was considered, but during 1999 there was regression of the infundibular lesion and by 2000 the MR scan was felt to be within normal limits. A repeat MR scan in 2002 showed mild expansion of the pituitary infundibulum, and by 2004 imaging was frankly abnormal with evidence of a suprasellar mass involving the hypothalamus and base of the infundibulum. She remained amenorrhoeic despite a trial of dopamine agonist therapy which normalised her prolactin. She then developed TSH and ACTH deficiency. She underwent subtotal resection of the pituitary stalk lesion in March 2005 and histology confirmed Langerhans cell histiocytosis (eosinophilic granuloma) of the hypothalamus/pituitary stalk. To date this lady has no evidence of multifocal disease. Although radiologically her disease showed a relapsing and remitting course her diabetes insipidus did not resolve, and anterior pituitary deficits evolved gradually.

There is a paucity of long-term follow-up studies of this condition in adults, such that there is little consensus regarding optimal treatment. We are currently considering the need for radiotherapy in this patient.

#### P149

**Psychiatric and movement disorders in primary hypoparathyroidism**

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Described here is a series of 3 patients with psychiatric disorders and 4 patients with movement disorders associated with primary hypoparathyroidism. Patients of ages 18 through 55 years and were seen 1998–2004 in a general hospital. Institutional Ethical Committee approval was obtained for the study.

On presentation, all patients had fasting plasma tested for calcium, magnesium, phosphorus, albumin and intact PTH. All had CT scans of the head. All had low calcium (mean:  $1.41 \pm 0.15$  mmol/l) and iPTH (mean: 7.3 pg/ml, normal range: 11.1–79.5 pg/ml) and were on inadequate treatment when seen. Treatment with oral calcium and calcitriol normalised calcium in 10–30 days.

Patients with psychiatric disorders

Patient 1, a 25 yr male had schizophrenia; Patient 2, an 18 yr woman, initially had acute psychosis and later stabilised to an organic delusional disorder; Patient 3, a 29 yr male had bipolar disorder. All had convulsions as well. Normalisation of calcium did not improve the psychiatric condition in any patient.

Patients with movement disorders

Patient 4, a 50 yr female had dementia and dystonia. Dystonia improved on treatment. Patient 5, a 42 yr female had dystonia, orofaciolingual dyskinesia and ballismus. Only mild dystonia persisted after treatment. Patient 6, a 55 yr female had a 10-day history of chorea and a 4-day history of seizures. Both completely disappeared on treatment. Patient 7, a 48 yr male had choreoathetosis for 6 months. It completely disappeared on treatment.

Conclusion

In primary hypoparathyroidism, psychiatric disorders did not improve but movement disorders markedly improved on correction of hypocalcaemia.

## P150

### A case report of malignant insulinoma

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Insulinoma is a rare neuroendocrine tumor (4 cases/1000000/year), less than 10% are malignant. We report a case of rapidly fatal malignant insulinoma, death occurring less than 3 months after the apparition of hypoglycaemic symptoms.

A 79 years old female type 2 diabetic patient, treated with glicazide 120 mg b.i.d and metformine et 850 t.i.d, was hospitalized for recurrent hypoglycaemia. Insulinoma was diagnosed as severe hypoglycaemic episodes occurred more and more frequently despite withdrawal of all hypoglycaemic agents. Concomitantly insulin plasma levels were not adapted to glycaemia: glycaemia 42 mg/dl et insulinemia 60.9  $\mu$ U/ml while sulfonylurea dosages were negative. CT scan found multiple secondary hepatic lesions of 3 to 30 mm all over the liver and 2 cystic tumours of the caudal part of pancreas. Chromogranin A was 4040  $\mu$ g/l ( $N < 100$ ), NSE was 220.8 ng/ml ( $N < 17$ ). Calcitonin, Gastrin, Serotonin, VIP were in the normal range. Hypoglycaemia episodes were dramatically improved by associating diazoxide 500 mg/day to Somatuline LP 30 mg<sup>o</sup>/15 days and dextrose 30% 500 ml/day. Due to liver secondary localisation clinical status of the patient deteriorated rapidly and increasing peripheral oedema associated to renal failure, patient was transferred to ICU. A programmed hepatic chemo-embolization was postponed due to severe renal failure and alteration of general status and death occurred due to hepatic insufficiency. Anatomopathology of the hepatic biopsy showed a marked expression of antibodies against chromogranin A, secondary analysis concluded to a well differentiated endocrine carcinoma without any immunohistochemistry staining against insulin, glucagon, gastrin or somatostatin. Malignant insulinoma prognosis is less than 2 years after surgery. Prognosis of malignant insulinoma is classically worse when hepatic localisations are present at diagnosis. An immunotherapy as proposed in the parathyroid carcinoma could eventually improve the prognosis.

## P151

### Malignant paraganglioma in young patient without symptoms – a case report

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Pheochromocytomas and abdominal paragangliomas are catecholamine-producing tumors of the sympathetic nervous system, while head and neck paragangliomas are non-secreting tumors of parasympathetic origin.

Here we present a case of 20-year-old patient with the metastases of malignant paraganglioma. In 2004 the disease has been detected at accidental abdominal sonography. Patient has not had any symptoms, such as headache, hyperhidrosis, palpitation, weight loss, dysphagia or hoarseness of voice. Blood pressure was normal. Abdominal MRI sc revealed a 4 × 7 cm mass of left adrenal gland and nodes on abdominal

aorta. The initial laboratory tests were normal. However, hypersecretion of catecholamine was observed.

The immunohistochemical analyze of postsurgical sample of tissue revealed malignancy paraganglioma. The post-surgical outcome of patient showed normal level of catecholamine. After surgical treatment (resection of the left suprarenal gland and local lymph nodes on abdominal aorta) diagnostic <sup>131</sup>I-MIBG scintigraphy showed pathological uptake on the right side, but not certainly on the liver. The liver and suprarenal MRI scan showed no pathological change.

There were no signs of tumor recurrence after one year, except the above mentioned pathological MIBG uptake which is still persisting. The treatment by vascular surgeon is recommended.

## P152

### Secondary amenorrhoea induced by dihydrocodeine ingestion resolves following opiate withdrawal

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Objective

Diamorphine and methadone administration are associated with dysfunction of the hypothalamo pituitary gonadal axis in premenopausal women. However, the effects of less potent opioids on this axis remain unclear.

Methods

We report a case of a 42 year old woman referred with secondary amenorrhoea and low oestradiol levels (oestradiol < 50 iu/L). She started menarche aged 14, followed by regular menstrual cycles, two successful pregnancies, two miscarriages and a laparoscopic sterilization aged 35. Her past medical history included hypothyroidism and endogenous depression. She did not participate in any intensive sport activity and her weight remained stable during the past 10 years (54–56 kg, BMI = 22–23 kg/m<sup>2</sup>). Her medication included: thyroxine 150 mcg/day, escitalopram 10 mg/day and zolmitriptan 2.5 mg/day. In addition, she had a history of dihydrocodeine abuse over the preceding two years. This opiate was initially started as an analgesic but she became dependant on it, with a peak ingestion of 1350 mg/day and an average intake between 600–750 mg/day at presentation. Consumption of other illicit substances was denied.

Physical examination was unremarkable. Repeated blood levels were consistent with hypogonadism hypogonadotrophic (LH < 0.2 iu/L, FSH 1.7 iu/L, oestradiol < 50 iu/L). Her ferritin, prolactin, cortisol, IGF-1 and thyroid hormones levels were within normal range. MRI excluded a structural pituitary lesion. During follow-up, she experienced opioid withdrawal symptoms after she discontinued her dihydrocodeine to avoid long-term opioid dependence. These resolved with reintroduction of dihydrocodeine (240 mg/day) and this dose has subsequently been titrated down. Her menses spontaneously returned within 3 months of her dose reduction (< 240 mg/day) remaining cyclical thereafter. Her gonadotrophin and oestradiol levels normalised (LH = 2.6 iu/L, FSH = 4.7 iu/L, oestradiol = 550 pmol/L).

Conclusion

This case illustrates excessive intake of opioid analgesics used to relieve mild to moderate pain, may induce menstrual irregularities characterised by hypogonadotrophic hypogonadism but this seems to be wholly reversible on withdrawal of the opioids.

## P153

### Combined cortisol and aldosterone hypersecretion in two unusual cases of hypertension due to adrenocortical neoplasia

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Malignant neoplasm of the Adrenal gland is rare and carries a poor prognosis. Presentation is with either symptoms of hormone excess; Cushing's Syndrome (Steroids), Conn's Syndrome (Aldosterone), Virilisation (Androgens), Feminisation (Oestrogens), Pheochromocytoma (Catecholamines) or abdominal mass, and patients often present with hypertension. Laboratory investigations confirm clinical suspicion of isolated hormone excess. Surgical resection offers the best chance for long-term survival and is the treatment of choice.

Here we present two unusual cases of Adrenal Carcinoma presenting with hypertension. Laboratory investigation confirmed concurrent excess secretion of both Cortisol and Aldosterone, and imaging revealed the presence of an adrenal mass. Both were treated by laparoscopic adrenalectomy.

Immunohistochemical analyses of surgically excised adrenal neoplasms reveal that tumour cells clinically secreting one hormone in excess often retain the necessary enzymes for the production of other adrenal hormones. Tumours secreting Mineralocorticoids and Corticosteroids in excess are rare. Nevertheless, metabolites of both should be investigated in cases of Cushings Syndrome or Aldosteronism in order to facilitate adequate pre-surgical treatment of hypertension and whole body potassium depletion, and avoidance of post-operative adrenal crisis respectively.

## P154

### Endocrine disorders in a high secure hospital

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#### Objectives

To audit the endocrine referrals from a High Secure Hospital (HSH) to a department of endocrinology.

#### Methods

During the study, the HSH had approximately 400 beds, 75% male, 75% mental illness (mostly schizophrenia), 25% psychopathic personality disorders. The average length of stay in the HSH was 8 years, the majority of patients receive antipsychotic drugs (APD) and obesity and cigarette smoking is common.

#### Results

Over 10 years, 52 patients, median age 41 years, were seen. The endocrine conditions in order of frequency were:

Males ( $n = 31$ ): Thyrotoxic Graves' disease (7), Hypothyroidism (6), Klinefelters syndrome (5), Hyponatremia and overdrinking (3), Miscellaneous (10).

Females ( $n = 21$ ): APD induced hyperprolactinemia, galactorrhoea, amenorrhoea (11), thyrotoxic Graves disease (6), Miscellaneous (4).

#### Conclusions

In this HSH, the commonest endocrine problem in males was thyrotoxic Graves disease, a condition relatively unusual in men. In several of these patients, marked deterioration in behaviour occurred when they were hyperthyroid.

In the female patients, galactorrhoea – amenorrhoea secondary to APD induced hyperprolactinemia was commonest. Treatment with dopamine agonists was usually successful with no deterioration of mental state.

## P155

### Acromegaly with a double pituitary adenoma

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Although double adenomas may be present in 1% of autopsy pituitaries, in patients with pituitary disease they are extremely rare. In previously reported surgical series an incidence of 0.37%–1.64% has been observed. We report a 55 year old lady with features suggestive of acromegaly and a double pituitary adenoma on MRI. The diagnosis of acromegaly was confirmed by an elevated serum IGF-1 and a nadir growth hormone of 10.3 mU/L during a 75 g oral glucose tolerance test. Other baseline pituitary hormones and serum calcium were normal. She had no symptoms suggestive of hypoglycaemia, anaemia or diarrhoea. There was no family history. MRI pituitary revealed two clearly separated and discrete microadenomas – a left sided 8 mm lesion along with a 6 mm right-sided adenoma and no optic chiasm involvement. She is shortly to commence somatostatin analogue therapy.

In previous reported series of double pituitary adenoma, the majority were encountered in the clinical context of acromegaly with both tumours having functional capability, although individual tumours may be from 2 separate adenoma groups (GH-PRL-TSH group and FSH/LH group). Cases of MEN-1 and familial pituitary tumours unrelated to MEN-1 may be over-represented in the series reported to date, suggesting that genetic abnormalities may be involved in their pathogenesis. Management of double adenomas is challenging since there is a higher risk of surgical failure and a high risk of iatrogenic hypopituitarism. In the present case the choice rests between attempted cure via a one step or two step surgical approach or the use of a somatostatin analogue, or even pre-treatment with the latter before surgery. However, the effect of somatostatin analogue therapy in this clinical context has not previously been reported.

## P156

### 17beta-hydroxysteroid dehydrogenase deficiency caused by homozygous h271r mutation

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17β-hydroxysteroid dehydrogenase-3 (17βHSD3) deficiency is an autosomal recessive form of male pseudohermaphroditism due to impaired testicular conversion of androstenedione to testosterone. 46,XY homozygotes or compound heterozygotes for mutations of the HSD17B3 gene have testes and normally developed Wolffian duct derivatives, but they present with undervirilization of the external genitalia, which are often female. About twenty different mutations of the 17βHSD3 gene, located on chromosome 9q22, have been described up to now. In the current study, we describe an additional mutation of the HSD17B3 gene in a 36-yr old Algerian woman. This subject was born with apparently normal female genitalia, from non-consanguineous parents. Breast development occurred at the age of 12 yrs. At the age of 17 yrs, she presented with primary amenorrhea, a clitoris hypertrophy, a blind-ending vagina and bilateral inguinal hernia. The gonads were removed and histological examination concluded to the presence of testis. Karyotype was 46,XY. As an adult, the hormonal biology was not relevant because of the previous bilateral gonadectomy and pelvic ultrasonography showed neither uterus, deferens, nor prostate. The presumed diagnosis of androgen insensitivity syndrome (AIS) was raised but molecular biology showed no mutation of the androgen receptor (AR) gene. We then sequenced the 17βHSD3 gene, and found a homozygous H271R mutation in exon 10, which is for the first time described. The diagnosis of 17βHSD3 deficiency should then be considered in prepubertal or gonadectomized patients with male pseudohermaphroditism in the absence of AR mutation because it is clinically indistinguishable from AIS. Functional studies of this new mutation are under current evaluation.

## P157

### Small intragenic somatic mutation associated with a germline mutation of HRPT2 gene in a patient with sporadic primary hyperparathyroidism

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We describe a 39-yr-old man with recurrent sporadic primary hyperparathyroidism (PHPT). In 1987, at the age of 21 yr, a severe form of PHPT was diagnosed [serum calcium 17.3 mg/dl; C-PTH 3.17 ng/ml (<0.88 ng/ml), cortical thinning and erosion] and a right parathyroid adenoma was removed. Three years later recurrence of PHPT was diagnosed but no treatment was initially advised. In 1993 the patient underwent cervical exploration and a right parathyroid adenoma was excised. Serum calcium and PTH remained normal for up to 1997 when a further recurrence of PHPT was documented and the patient was referred to our Department. The patient was in good health. Serum calcium and PTH were moderately elevated (10.6 mg/dl and 72 pg/ml, respectively). There was no evidence of MEN1-associated neoplasia. No kidney lesions or jaw tumor were detected. PTx was advised but the patient was lost to follow-up until 2004 when he was submitted to surgery with removal of the superior and inferior left parathyroid glands. Histological examination showed chief cells hyperplasia. After a transient hypocalcemia serum calcium and PTH remained normal up to September 2005. Serum calcium and PTH were normal in 13 family members. Studies of *MEN1* and *HRPT2* genes were undertaken. Matched samples of DNA from patient's blood and abnormal parathyroid tissue were used. Loss of heterozygosity at 11q13 and *HRPT2* genes, using microsatellite polymorphisms PYGM and DS11S449 and intragenic polymorphisms of intron 10 and 14, respectively, was negative. Coding regions and splice junctions of *MEN1* and *HRPT2* genes were sequenced for mutations. Sequence analysis of *HRPT2* gene revealed a somatic stop codon mutation in exon 2 (Y54X) and a germline missense mutation in exon 3 (R91P). The germline R91P mutation was absent in the 13 families members. No mutation of *MEN1* gene was found. In conclusion, we describe a patient with recurrent PHPT with a biallelic inactivation of *HRPT2* gene due to a germline mutation as "first hit" and a small intragenic somatic mutation as "second hit", consistent with the Knudson "two hits" hypothesis.



## P158

### Plasma exchange for the prevention of severe thyrotoxic exacerbation following radioactive iodine therapy for Graves hyperthyroidism

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We present 2 patients who developed severe thyrotoxic exacerbation following radioiodine. The first treated by conventional antithyroid therapy, the second was plasma exchanged.

Patient A aged 39 male with FT3 = 28.2 pmol/l (2.5–5.3) FT4 = 49.0 pmol/l (9.1–23.8) TSH ≤ 0.01 mU/l (0.32–5.0), a large diffuse goitre, thyroid eye disease and asthmatic on inhaled ventolin. He received 3.5 years of carbimazole 40 mg and Thyroxine 100 mcg. Thyroxine was stopped 15 months before radioiodine. Prednisolone 20 mg was started and Carbimazole 60 mg was stopped 4 days prior to 531 MBq Iodine -131. Within 7 days he developed severe thyrotoxic symptoms. Day 12 FT3 = 40.8, FT4 = 67.5, and TSH ≤ 0.05. He was started on Propylthiouracil 200 mg tds, cholestyramine 4gm qds and prednisolone continued. He remained thyrotoxic for 57 days.

Patient B aged 52 male with FT3 = 42.4 pmol/l, FT4 = 61.3 pmol/l, TSH = 0.01 mU/l, a large diffuse goitre and pretibial myxoedema. He received carbimazole 60 mg and Thyroxine 125 mcg daily for 9 months. Thyroxine was stopped after 4 months and carbimazole 2 days before Iodine-131 527 MBq. Toxic symptoms started day 4. On day 7 FT3 ≥ 50 pmol/l, FT4 = 54.6 pmol/l, TSH = 0.01. On day 9 Propylthiouracil 200 mg qds, cholestyramine 1 Gm qds and propranolol 40 mg tds were started and because of previous experience with patient A he was referred for plasma exchange receiving 2 exchanges of 4 L with 4.5% albumin on days 10 and 12. He became biochemically euthyroid on day 10 FT3 = 6.4 pmol/l, FT4 8.5 pmol/l, TSH 0.01, and clinically euthyroid and asymptomatic day 13. Propylthiouracil continues in decreasing doses.

We have shown avoidance of a serious post radiation thyrotoxic exacerbation by the frequently used renal technique of plasma exchange, and advocate its use in potential as well as actual thyroid storm.

## P159

### Not-so-silent, silent corticotroph adenomas

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Late onset Cushing's disease, occurring years following the diagnosis of a silent corticotroph adenoma (SCA) is rare, with very few previously reported cases. We present a series of 5 subjects with SCA, aggressive tumour recurrences and late onset Cushing's disease.

The mean age of subjects at initial presentation was 41 yrs (35–52), sex 3:2 (M:F). There were no clinical features of hypercortisolism at diagnosis. Two subjects had hypocortisolism requiring steroid replacement. Three patients had aggressive tumours radiologically and clinically (visual field defects) at presentation. Initially all patients had trans-sphenoidal surgery, and four subjects had post-operative radiotherapy.

Histologically 80% of tumours had predominantly positive ACTH immunostaining, and one subject had a plurihormonal tumour (<1% ACTH, 5% TSH).

On average each subject had 3 recurrences (range 2–7) requiring surgery or radiotherapy. Mean time to first recurrence was 8.6 yrs (range 2–16 yrs).

Tumour recurrences had a similar immunophenotype to the original tumour. Mitotic index as measured by Ki-67 at presentation was low (0.3–2%), and remained low in two subjects. However recurrent tumours in 3 subjects had unusually high rates of mitosis (>10%).

Cushing's disease occurred rapidly over a few weeks, and required medical treatment in all subjects. This was confirmed by urinary free cortisol measurements and dexamethasone suppression testing. Cushing's disease occurred on average 10.8 yrs (2–19) following the initial diagnosis of SCA. Progress

Two subjects died very rapidly, following aggressive tumour recurrence, one of whom had a spinal metastasis (thus a pituitary carcinoma). The 3 remaining subjects continue to have progressive disease.

## Conclusion

We report a series of five young subjects with a rare particularly aggressive subset of silent corticotroph adenomas, with multiple recurrences and late-onset Cushing's disease occurring up to 19 years following initial diagnosis. This would suggest careful biochemical as well as radiological monitoring of subjects with silent corticotroph adenomas is required.

## P160

### Cushing's syndrome presenting with palpitations

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A 36 year old man presented with a six month history of palpitations. An ECG in a different hospital had showed a sinus tachycardia. An echocardiogram had also been performed and reported as normal. There were no apparent clinical features to suggest Cushing's Syndrome (BMI 26 kg/m<sup>2</sup>). Resting pulse was 86/min. BP was 140/90. Examination was otherwise unremarkable.

Baseline biochemical and haematological investigations including TFT, FBC were normal. Twenty-four hour ECG confirmed sinus tachycardia throughout with a mean heart rate of 90 BPM (range 60–167). Urine VMA was normal at 5.4 mg/24 hours (0–6.3). Adrenal CT (while awaiting urine catecholamines) was requested and showed a left adrenal mass measuring 14 × 11 × 14 cm. MIBG scan was negative.

Serum cortisol was high and failed to suppress with Dexamethasone. Urine HIAA was normal. The patient underwent laparoscopic adrenalectomy. Histology of the adrenal mass was reported as adrenocortical carcinoma. His tachycardia resolved.

This case illustrates an unusual presentation with atypical symptoms. Symptoms of stress due to cortisol excess may be presenting features of Cushing syndrome. This diagnosis should be considered in such patients.

## P161

### An unusual case of PTHrPoma with coexistent secondary hyperparathyroidism

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A 76 year-old man presented with back pain anorexia and cachexia. CT scan confirmed a pancreatic mass with liver metastasis. Liver biopsy confirmed a neuroendocrine tumour. Because of left ventricular failure he was unsuitable for tumour reduction therapy. He was hypercalcaemic (2.54–3.1 mmol/l), PTH and PTHrP were elevated [2.5 pmol/l (1.9–6.9), 7.1 pmol/l (<1) respectively]. A diagnosis of PTHrP secreting neuroendocrine tumour with coexisting primary hyperparathyroidism was made. He was treated with pamidronate infusions and hydration remained well for two years.

He was referred for an endocrinology opinion in October 2004. Investigations revealed serum PTH concentration 2.5 pmol/l adjusted calcium 2.7 mmol/l. 25 (OH)-Vitamin D was <17 nmol/l. Serum PTHrP was markedly elevated at 8.0 pmol/l suggesting the PTHrP- secreting neuroendocrine tumour as a cause of hypercalcaemia. Chromogranin A and B were raised but fasting gut peptides normal. Sestamibi and bone scanning were unremarkable. A radiolabelled octreotide scan demonstrated abnormal uptake in the liver corresponding to hepatic metastasis. These results suggest the hypercalcaemia was due to PTHrP secretion but the raised PTH was due to secondary hyperparathyroidism.

Pamidronate was discontinued and Octreotide LAR initiated. On octreotide therapy calcium levels remained stable at 2.3–2.4 mmol/l and Vitamin D rose to 47 nmol/l whilst PTH concentration decreased to 1.0 pmol/l without vitamin D replacement. Despite stable calcium levels the patient deteriorated and died from carcinomatosis after 6 months of octreotide therapy.

This case is unusual as the patient survived for 40 months when the average life expectancy in PTHrPoma is 2 months, suggesting that in the elderly this marker may not be associated with poor prognosis. The other interesting point is that normally the hypercalcaemia produced by PTHrP suppresses PTH levels. Here Vitamin D deficiency appears to prevent this. Octreotide has been demonstrated to increase plasma vitamin D levels in acromegaly and may have raised them here.



## P162

**False positive newborn screen for congenital hypothyroidism due to a TSH-IgG (macro-TSH) complex**DJ Halsall<sup>1</sup>, SK Hall<sup>2</sup>, P Barker<sup>1</sup>, J Anderson<sup>3</sup>, M Fahie-Wilson<sup>4</sup>, R Gama<sup>3</sup> & VK Chatterjee<sup>1</sup><sup>1</sup>Addenbrooke's Hospital NHS Trust, Cambridge, United Kingdom; <sup>2</sup>Birmingham Children's Hospital, Birmingham, United Kingdom; <sup>3</sup>Royal Wolverhampton Hospitals NHS Trust, Wolverhampton, United Kingdom; <sup>4</sup>Southend Hospital, Southend, United Kingdom.

We report a falsely elevated blood spot thyrotrophin (TSH) concentration caused by a TSH-IgG complex. A routine blood spot screen returned a whole blood TSH of 213 mU/L from a one week-old neonate using the Wallac DELFIA method. Measurement in serum confirmed elevated TSH (826 mU/L, Roche Elecsys assay) but free thyroxine (17.2 pmol/L) was normal. The baby's mother was clinically euthyroid but also showed discordant high serum TSH (287 mU/L) with normal free thyroxine (13.5 pmol/L). Low recovery of immunoreactive TSH following PEG precipitation and Protein A- Sepharose absorption of the maternal serum suggested the presence of an interfering antibody. Gel-filtration chromatography of serum from mother and child showed the presence of high molecular weight TSH immuno-reactivity consistent with a TSH-IgG complex. Incubation of maternal serum with serum from an unrelated hypothyroid patient (TSH 103 mU/L - Roche Elecsys) removed low molecular weight TSH from the hypothyroid serum confirming interference due to a TSH binding immunoglobulin rather than a heterophilic antibody. Serum TSH in the infant decreased to 210 mU/L after 14 days but remained elevated 3 months later (>100) suggesting a TSH-IgG complex with long plasma half-life and an IgG component possibly derived from placental transfer of maternal IgG. Interference was assay platform dependent; TSH on mother's serum was 4.0 mU/L using the Bayer Centaur, 16 mU/L using the DPC Immulite 2000 and 287 mU/L using the Roche Elecsys. Elevated TSH levels detected by newborn bloodspot screening should be interpreted with caution and we recommend that follow up routinely includes checking mother's thyroid hormone status. Furthermore this case demonstrates that macro-TSH should also be considered when elevated serum TSH levels are discordant with free thyroxine or clinical findings.

## P163

**Familial Hypocalcaemic Hypercalcaemia (FHH) caused by P748L mutation in the calcium sensing receptor (CaSR) gene**R Mahto<sup>1</sup>, A Tahrani<sup>2</sup>, A Macleod<sup>3</sup> & RV Thakker<sup>4</sup>  
<sup>1</sup>New Cross Hospital, Wolverhampton, WV10 0QP, United Kingdom; <sup>2</sup>University Hospital of North Staffordshire, Stoke-On-Trent, ST4 6QG, United Kingdom; <sup>3</sup>Royal Shrewsbury Hospital, Shrewsbury, SY3 8XQ, United Kingdom; <sup>4</sup>Nuffield Department of Medicine, University of Oxford, Oxford, United Kingdom.**Objective**

To describe a novel mutation of the calcium sensing receptor gene (CaSR) in a family diagnosed with familial hypocalcaemic hypercalcaemia (FHH).

**Case history**

A 62 year old man being investigated for short term memory loss was referred to us by GP for hypercalcaemia found on routine blood tests. He was asymptomatic otherwise. Repeat blood tests revealed serum calcium high at 2.88 mmol/l, inorganic phosphate of 0.75 mmol/l, and normal serum PTH of 2.9 pmol/l. Calcium creatinine clearance ratio performed on 24 hour urine collection of urine was 0.01 mmol/l. On the basis of these results, a diagnosis of FHH was entertained. Genetic testing revealed the patient to be heterozygous for P748L mutation in CaSR gene.

On our request, the patient's daughter who was 36 weeks pregnant then agreed to be screened for this disorder and was found to be hypercalcaemic. A healthy baby boy delivered by patient's daughter had mild hypercalcaemia as well. Genetic analysis of CaSR of both daughter and her son was found to be heterozygous for P748L mutation. As all the three members of the family showed same mutation in CaSR on gene analysis, we assume this mutation to be the likely cause for FHH in this family.

**Conclusion**

We describe a novel mutation in exon 7 of CaSR gene in a family with FHH. Identification of patients with FHH saves them from undergoing extensive investigations and parathyroidectomy. Continued identification and characterisation of naturally occurring CaSR mutations is critical to enhance our understanding of the workings of this important receptor.

## P164

**Importance of complex evaluation of the changes in ST-segment in ECG: case report of the patient with Addison disease and Brugada syndrome**

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Authors present a case report of the 25-year patient with suspicion of adrenal insufficiency (AI). Based on the clinical picture and plasma cortisol levels in low-dose 1 µg ACTH test (32,00–65,40 nmol/l, normal response >500 nmol/l) AI was confirmed. On ECG record was present deep s-wave in leads I, II, III, aVF, V5–V6 and rs'-shaped QRS complex in leads aVR and V1 – finding consistent with incomplete right bundle-branch block. Moreover horizontal elevation ST in V1, saddleback elevation in V2 and ascendent elevation in V3 were present.

Although mineral changes (hyperkalemia, hypercalcemia) which are typical findings in patients with untreated AI are possible causes of the ST-segment elevations, the patient's mineral levels were in normal values (K 4.0 mmol/l, Ca 2.45 mmol/l). Event in negative family and personal history regarding sudden cardiac death, arrhythmias or need for resuscitation the suspicion for Brugada syndrome (BS) was expressed. Ajmaline test (50 mg i.v. 10 mg/min.) was indicated and performed, in which saddleback ST-elevations (type 2) changed to typical pattern of elevations (type 1) pathognomic for BS. Presently we are waiting for result of the molecular analysis of the gene coding SCN5A sodium channel. Mutations in this gene are at the present time the only underlying cause of malignant arrhythmias in patients with BS. This case presents urge for careful interdisciplinary endocrinological-cardiological differential diagnosis in evaluation of ECG changes in patients with endocrine disorders.

## P165

**Metformin-associated lactic acidosis in a caucasian woman precipitated by acute renal failure treated with bicarbonate haemodialysis**T González Losada, L Del Olmo García, P Martínez de Icaya, V Alcázar Lázaro, T López del Val, M Pérez Pelayo & S Herranz Antolín  
Hospital, Leganés, Spain.**Clinical case**

A 51 year-old caucasian woman was admitted in the emergency room of our hospital with psicomotor agitation and confusion after four days of a profuse watery diarrhea, with 5 stools per day, abdominal cramps and vomiting. She started 500 mg of aspirin on her own initiative. Her medical profile included a type 2 diabetes treated with metformin 850 mg/12 hours and glyburide 7.5 mg/day, and hypertension treated with enalapril 20 mg/day. No renal impairment was documented in annual checkup.

Clinical examination showed an agitated and confused woman, with a Glasgow coma scale of 8/15, apirexial, with dry mouth, pulse 72 bpm, blood pressure 159/75.

**Progress and management**

Her biochemical profile revealed a blood glucose of 41 mg/dL, creatinine 9.06 mg/dL, sodium 140 meq/L, potassium 6.8 meq/L, chlorine 93 meq/L. Venous blood gases were: pH 6.966, bicarbonate 5.3 mmol/l, base excess – 25.9 mmol/l.

Calculated anion gap 41.7 mmol/l with a lactate concentration of 15.40 mmol/L (normal range 0.5–2.2 mmol/L). A diagnosis of metformin-associated lactic acidosis with acute renal failure was made and she was transferred to the Intensive Care Unit. The association of diarrhea, ACE-inhibitors and non-steroidal anti-inflammatory drugs was responsible of her acute renal failure. Aggressive volume expansion and intravenous sodium bicarbonate failed and underwent just one session of continuous veno-venous haemodialysis using bicarbonate dialysate. After dialysis, renal function and acidosis started to improve. She also required inotropic support with vasoactive drugs. Three months after this episode she is dialysis independent and her renal function has stabilized with a creatinine of 1.48 mg/dL.

**Discussion**

Lactic acidosis is a rare but severe complication of type 2 diabetes treated with metformin. Metformin should be stopped when acute renal failure occurs or is anticipated. Early haemodialysis is an adequate treatment to correct acidosis and eliminate accumulated metformin in acute renal failure situations.

**P166**

**Dysgerminoma and gonadoblastoma in children with complete androgen insensibility**

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Children with androgen insensibility and female sexual external organs usually are classified as girls. In the puberty observed is the development of female secondary sexual features without menstruation. The treatment of patients with androgen insensibility, because of the risk of neoplastic transformation must be preceded by castration. Case report: a 14 year old girl was admitted in 2003 to the Clinic with the following anamnesis: birth from the secondary gravidity, normal delivery. In 2002 admitted to the Pediatric Clinic of Surgery because of a dramatic increase of the abdomen circumference. On the 23.06.2002 a surgical intervention was performed during this a tumor of 4000 g, with was grooving up from the right gonad. The histopathological examination ascertained a dysgerminoma partim necroticans.

The examined karyotype proved an XY [24]/ 87 XXYY, dic(1;?10;)(p13;?p15), dic(1;7;6)(pterp13;p15q13pter), dic(12;?20)(p24.3;?),mar[1] In 12. 10. 2002 admitted to the Surgery Clinic because of a neoplastic process in the left gonad, which was removed. After the first surgery admitted to the Pediatric Clinic of Hematology and Oncology where she was treated until 2003. The histopathological examination ascertained gonadoblastoma. The laboratory results at the time of admission: LH-48.0 mIU/ml, FSH-107.0 mIU/ml, TTE-0.24 ng/ml, Estradiole < 20.0 pg/ml, DHS-60.6 ug/ dl. The results now: LH- 155.0 mIU/ml, FSH- 62.5 mIU/ml, TTE-0.34 ng/ml, Estradiole < 20.0 pg/ml, DHS-122.0ug/ dl. Bone age 12.5/12 years, TSH-2.24 uIU/ml, fT3-3.6 pg/ ml, fT4-1.4 ng/dl, B HCG-4.1 mIU/ml (n-0-2.7), densitometry - 24% decrease of the bone density in comparison with the calendar age (2 score). Body mass 51.7 kg (50 cent) height 151 cm (10 cent). Gynecological examination: labia maius end minus pudenda developed normal. Clitoris of normal size. The vaginal admittance normal, the vagina blindly ended. The status after bilateral castrations.

**P167**

**Chronic autoimmune thyroid disease in children and adolescents in Lower Silesia in the years 1999-2004**

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Prader-Willi is a complex genetic syndrome with characteristic phenotype, obesity, hyperphagia, and endocrine hypothalamic dysfunctions. We present particular features of a case with confirmed Prader Willi syndrome (PWS). Case report

EP, only child of a young non-consanguine couple, was born in 1994 at 34 weeks of amenorrhoea, with a low Apgar score (7) and a weight of 2200 g. She presented important hypotonia in the first 6 weeks of life, needing gavage, development retardation, and onset of hyperphagia at the age of 1, with rapid and important weight gain. At 10 years old she presented high stature (+2 SD), and important obesity (+10 SD) with 34% total body fat (electrical impedance). Bone age concordant with the chronological age. She had the typical somatic aspect: narrow forehead, almond-shaped palpebral fissures, and acromicria. Besides incipient telarche (B2-3) she had no other puberty signs. Genetic examination: cariotype 46, XX, FISH test: del (15) (q11.2-q13). Important mental retardation (IQ = 48). Biological investigations: normal thyroid and adrenal function, pre-pubertal values of oestradiol, concordant with gonadotropes values. Despite the height, GH was low (0.2 mcg/l), with insufficient stimulation to clonidine test (5.6 mcg/l), and low normal value of IGF1. The clinical and biological criteria for the PWS diagnosis were fulfilled, with a score of 10.5. A rigorous diet was started and the patient loosed 5 kg in 6 months.

**Discussions**

Short stature (- SD) is common in PWS. Surprisingly, our patient presented high stature, despite the partial GH deficiency. Obesity can only partially explain patient's height. GH treatment, recommended in PWS, remain controversial and probably unnecessary in this case.

**Conclusion**

In spite of some particular aspects, our case presented one of the main features of PWS, important obesity with compulsive food intake. Rigorous control of food intake has proved its efficacy.

**P168**

**Three extreme cases of electrolyte imbalance-induced seizures**

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**Introduction**

We present 3 cases of the same severe electrolyte imbalance presenting with acute neurological symptoms.

**Case descriptions**

Case A, a 48 year old lady with severe CREST syndrome presented with prolonged vomiting and diarrhoea. Initial investigations showed: Sodium 141 mmol/L, Potassium 3.2 mmol/L, Creatinine 53 mmol/L, Glucose 4.3 mmol/L, Haemoglobin 13.9 g/dL, Albumin 38 g/L. 2 days after admission she developed seizures. Case B, a 75 year old residential home resident with Addison's disease who was admitted with an infection-precipitated Addisonian crisis, with increasing frailty during her 3 week in-patient stay, became acutely confused, drowsy and developed a seizure with temporary respiratory arrest. Baseline investigations: Sodium 143 mmol/L, Potassium 3.2 mmol/L, Creatinine 124 mmol/L, Glucose 5.3 mmol/L, Haemoglobin 11 g/dL, Albumin 30 g/L. Case C, a 42 year old man with hypoparathyroidism and poor adherence to alfacalcidol was admitted with several self-limiting seizures. Baseline investigations: Sodium 140 mmol/L, Potassium 3.3 mmol/L, Creatinine 110 mmol/L, Glucose 4.8 mmol/L.

All 3 cases were found to have low calcium and magnesium levels.

Case A: 1.4 mmol/L and 0.2 mmol/L, Case B: 1.63 mmol/L and 0.28 mmol/L, Case C: 1.5 mmol/L and 0.25 mmol/L, respectively.

**Discussion**

Interestingly, the baseline blood tests were virtually normal, apart from the slightly low potassium. Despite these reassuringly normal baseline tests, the history of prolonged vomiting and diarrhoea (A) and prolonged poor nutrition (B) could have prompted an earlier full electrolyte screen. The history of poor adherence to medication (C) allowed prompt diagnosis of the hypocalcaemia and hypomagnesaemia.

**Conclusion**

These cases demonstrate the need to consider metabolic causes in acute neurological disturbances. They emphasise that patients with prolonged hospital stays with poor oral intake and patients with a prolonged history of possible decreased gastro-intestinal absorption and excess gut losses, as in severe diarrhoea, warrant a full electrolyte screen, including calcium and magnesium.

**P169**

**Two cases of idiopathic primary hypoparathyroidism**

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The clinical spectrum of chronic hypocalcaemia mimics various neurological and psychiatric pathologies. Although infrequently encountered, the diagnosis of non-iatrogenic primary hypoparathyroidism has to be considered in order to avoid severe complications or at least to improve neurological manifestations.

We present two unrelated cases of primary hypoparathyroidism clinically manifested in adulthood (case 1) and childhood (case 2). Very low-levels of PTH (3.1 and 0.11 ng/ml) during concomitantly hypocalcaemia (6 and 6.6 mg/dl) with hyperphosphatemia (6.39 and 6.51 mg/dl) made for the diagnosis. However, the diagnosis was obvious only after developing known complications due to chronic hypocalcaemia (subcapsular cataracts, cerebral calcifications). In one case, the long history of tetany crises was misattributed to a conversion neurosis despite repeated low serum calcium levels. Association with oral candidiasis not retractable after correction of hypocalcaemia in the first case suggests the presence of polyglandular autoimmune syndrome type I. Early onset of symptoms and high calcium excretion levels in the second case raised the suspicion of a familial (autosomal dominant hypocalcaemia) or sporadic mutation in the calcium sensing receptor. Remission of symptoms was achieved using calcium and 1alpha-hydroxyvitamin D as treatment, since PTH replacement therapy is not yet available in current medical practice. However, check-up revealed very high calcium excretion levels in the first case, calling for dose management and association of thiazide diuretics.

**P170****Cushing's syndrome due to medullary thyroid carcinoma – a case report**

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Ectopic ACTH secretion due to malignant tumours is a rare cause of hypercortisolism. Induced metabolic disturbances are often serious and the management of such patients may be difficult. We report the case of a 50-yr-old man who had a sporadic medullary thyroid carcinoma (MTC) removed 2 years ago (Dec/2003). Calcitonin and CEA levels remained high postoperatively and a CT scan revealed liver metastases. In August 2005 he was referred to Endocrinology with symptoms of muscular weakness of lower limbs, easy bruising, peripheral oedema, polyuria and polydipsia. These symptoms had begun 2 months earlier and at that time he was diagnosed with diabetes mellitus. He had a Cushingoid appearance, with truncal obesity, moon facies and plethora. Laboratory studies revealed an elevated 24 h urine free cortisol (3209.6 µg/dl). Baseline serum cortisol was 77.8 µg/dl (6.2–19.4) and changed to 80.6 and 56.8 after 2 mg and 8 mg dexamethasone suppression, respectively. Plasma ACTH was 122.0 pg/ml (<46) and changed to 153.0 and 79.0 after dexamethasone. An adrenal CT scan showed no changes and an octreoscan didn't reveal any anomalous fixation areas. However, a PET scan demonstrated metastatic disease to the liver and lymph nodes and adrenal hyperplasia. Liver biopsy confirmed metastatic MTC with positive immunohistochemical stain for calcitonin and ACTH. He started treatment with metyrapone (achieving 3 g/day) with partial regression of symptoms and diminished urinary cortisol levels. In conclusion: The clinical presentation was significant by the severity and the rapidity of the hypercortisolism. The differential diagnosis of ACTH-dependent Cushing's syndrome in this case included pituitary disease or ectopic ACTH secretion. Metastatic MTC was confirmed as the source of ectopic ACTH, by positive immunohistochemical ACTH stain on liver metastasis. This is an extremely rare association with a poor prognosis, which treatment isn't clearly defined. Removal of the source of ACTH is the treatment of choice, but often not feasible. If adrenalectomy fails to control the disease bilateral adrenalectomy should be considered.

**P171****Polymyalgia rheumatica and Hashimoto thyroiditis**

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Polymyalgia rheumatica is a chronic autoimmune inflammatory rheumatic disorder of unknown aetiology. Hashimoto thyroiditis is an autoimmune thyroid disorder characterised by the presence of antithyroid antibodies. Hashimoto thyroiditis has been described in patients with systemic autoimmune disorders such as systemic lupus erythematosus. The aim of the study was to describe the case of a patient who had polymyalgia rheumatica and developed Hashimoto thyroiditis.

A female nurse, aged 52, had polymyalgia rheumatica. She was given corticosteroids for the control of the disease with significant improvement. During follow-up she complained of non-specific musculoskeletal symptoms and a thyroid evaluation was performed. The presence of antithyroglobulin and antimicrosomal antibodies was observed. Thyroid function, however, was normal. On ultrasonography the thyroid was found to be normal in size with a micronodular consistency. The dose of the corticosteroids was tapered and thyroid function is being follow-up.

Autoimmune thyroid disease, Hashimoto thyroiditis and Graves' disease, have been described in patients with systemic autoimmune disorders such as systemic lupus erythematosus. Rheumatoid arthritis patients have been found to suffer also from autoimmune thyroid disease more than a control population. The presence of Hashimoto thyroiditis in a patient with polymyalgia rheumatica is a rare occurrence, as only a few cases have been previously described in the literature. The coexistence may be fortuitous, as the occurrence of autoimmune disorders is common in women. Alternatively, the genetic background of the patient that led to the development of polymyalgia rheumatica may have led to the development of autoimmune thyroiditis.

**P172****Addison's disease: Soy Sauce - a lifesaving concoction**

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**Background**

Before synthetic cortisone was introduced, patients with Addison's disease prolonged their lives by maintaining a high salt intake and taking plant based containing preparations affecting steroid metabolism. We report the case of someone who discovered this regime for herself.

**Case report**

A 42 year old lady presented with a four week history of decreased energy, malaise, and postural dizziness. She was hyponatraemic (plasma sodium 126 mmol/l) with potassium 4.6 mmol/l and random glucose 4.9 mmol/l. Five years previously she was diagnosed with SIADH when working as a teacher in Brunei. Over subsequent years she developed a penchant for liquorice sticks and for 'Dragon' Soy sauce, consuming two 250 ml bottles each week. A family history of Type 1 diabetes was reported. Examination revealed no stigmata of Addison's disease. Lying BP was 120/80 mmHg: no postural drop.

**Investigations**

Short Synacthen Test failed: Basal cortisol 271 nmol/l; 30 min cortisol 298 nmol (should rise to 550 nmol/l or more). Adrenal antibody highly positive. ACTH raised 76 ng/l (0–47). TFTs: Free T4 14 pmol/l (10–23); TSH raised 8.4 mu/l (0.4–5.5). Oestradiol (542 pmol/l) and gonadotrophins (LH 3.5 mu/l, FSH 4.4 mu/l) were in the pre-menopausal range.

**Management**

Started on Hydrocortisone/Fludrocortisone with resolution of symptoms. TSH normalised to 2.9 mu/l; Free T4 14 pmol/l.

**Discussion points**

Our patient avoided any major crisis until 2005 thanks to ingesting 2 moles of sodium each week (analysis of the Soy sauce revealed: Na 4130 mmol/l, Cl 3137 mmol/l, K 20.8 mmol/l, Glucose 77.4 mmol/l) and bolstering mineralocorticoid levels by eating liquorice which contains glycyrrhizic acid, an inhibitor of steroid breakdown. This highlights the value of a dietary history in such cases.

**P173****Pretibial Myxoedema: Is this a marker for occult thyroid ophthalmopathy?**

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**Introduction**

Thyroid eye disease affects an estimated 400,000 people in the UK and for a sizeable minority this is an extremely unpleasant condition. Radioactive iodine treatment with I131 for thyrotoxicosis can cause sight-threatening flare-up of dysthyroid eye disease. Patients with dysthyroid eye signs should undergo specialist ophthalmology assessment prior to administration of I131.

**Case history**

A 56 year old gentleman presented with symptoms of thyrotoxicosis, atrial fibrillation and marked pretibial myxoedema but there were no dysthyroid eye signs or goitre. Free T4 was raised at 50.6 pmol/l (9–23) with TSH suppressed. Thyroid binding inhibitory immunoglobulin was 86 (0–10).

Treatment with Carbimazole restored normal thyroid function and he reverted to sinus rhythm. Elective treatment with 400 MBq I131 was given to minimise the risk of relapse.

Three weeks after radioactive treatment the patient developed ophthalmoplegia, conjunctival injection and chemosis, though at no point was he hypothyroid. He was given high dose oral Prednisolone. He ultimately required radiotherapy to the left and right orbital areas given as 20 Gy in 10 fractions and subsequently corrective upper lid surgery. The eyes are now quiescent and his vision has returned to normal.

**Discussion points**

In the light of this case we suggest that pretibial myxoedema may be a marker of occult orbitopathy. If pretibial myxoedema is present, ophthalmological assessment prior to I131 therapy is recommended.



**P174**

**An unusual carcinoid tumor in a case of Cushing syndrome**

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The overall incidence of carcinoid tumors has been estimated to be 1 to 2 cases /100000 people in USA. Carcinoid tumors habitually accounts for 1% of all cases of Cushing's syndrome. The authors report an unusual case of ectopic secretion of corticotropin from pulmonary carcinoid tumor.

A. F. is a 29 years old patient who has been referred for hypercorticism with patent features including trophical signs and severe depression.

Laboratories findings revealed an elevated ACTH (ACTH = 322 ng/ml) and an abnormal response during the low dose Dexamethasone suppression test (cortisolemia after the test = 89.1 nmol/l). The MRI of the sella was normal. An ectopic secretion of corticotropin was suspected. The CT Scan of the chest and the abdomen showed a left basal pulmonary nodule and hilar lymph nodes.

The Patient underwent to surgical resection of the tumor. Histopathologic examination of the tumor defined it as a typical bronchopulmonary carcinoid with metastatic lymph nodes.

After surgery the evolution was marked by the disappearance of the Cushing syndrome. But the psychiatric signs persisted. A Treatment by chemotherapy and radiation therapy are envisaged.

This observation presents several atypies. The patient is young whereas the disease usually presents in the fifth decade of life. The majority of the tumors are perihilar in location. Atypical carcinoids have an aggressive clinical course, metastasizing to mediastinal lymph nodes, in 30 to 50% of cases. The five year survival rate is between 40 and 60%.

**Clinical practise and governance**

**P175**

**Audit on management of chronic adrenal insufficiency**

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**Background**

Glucocorticoid replacement is life saving in adrenal insufficiency. There are established guidelines for management of adrenal insufficiency. This audit was under taken to evaluate whether we are implementing these guidelines in our day to day management of patients with adrenal insufficiency.

**Method**

A retrospective study was performed on 18 patients. Data was collected from clinical records on the proforma and were evaluated against the recommendations from 2004 Endocrine Society Annual meeting, UK. The age range varied from 33 to 90 years. There was equal number of patients in both sexes. Aetiological causes of adrenal insufficiency were autoimmune, metastasis and pituitary surgery.

**Results**

Symptoms were documented in a generalized manner. Specific symptoms of glucocorticoid over/under replacement were documented in 39%. There was clear documentation of stress related steroid dose adjustment in 33%. Postural drop of BP was documented in 61%. Peripheral oedema was documented in 27%. TSH was measured periodically in 89%. Only in 11% of patients, serum Renin levels were measured. Serum electrolytes were monitored in all the patients.

**Conclusions**

Symptoms of glucocorticoid over/under replacement should be specifically enquired and documented. Detailed account of stress related glucocorticoid dose adjustment should be specifically enquired during each visit and documented. Reinstruction of stress related glucocorticoid dose adjustment should be done in each visit. Emergency bracelet/steroid card should be verified and documented. Peripheral oedema and postural drop of blood pressure should be actively looked for in every patient with chronic adrenal insufficiency. If there are any concerns regarding mineralocorticoid dose adjustment, plasma renin activity should be measured.

**P176**

**Salivary cortisol daycurve and Quality of Life assessment in optimizing glucocorticoid replacement therapy in patients with Addison's disease**

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**Background**

Many patients with Addison's disease experience decreased general wellbeing, mood and physical activity. This decrease in Quality of Life (QoL) is probably related to suboptimal glucocorticoid replacement therapy (GRT). Overreplacement frequently occurs and this can lead to side effects on the long term. The objective of this study was to investigate the effect on QoL of individual adjustment of GRT in order to approach normal cortisol levels as closely as possible.

**Methods**

Nineteen patients with Addison's disease on a stable replacement therapy with either twice or thrice daily hydrocortisone or cortisone acetate and without intercurrent disease were included in this prospective study. At the start of the study a salivary cortisol daycurve (SCDC) was obtained and compared to normal controls; general and disease specific QoL-questionnaires were completed. Based on SCDC assessment of over- and underreplacement (calculated as duration (h) × magnitude (nmol/L)) on different time points, glucocorticoid dose and regime were adjusted. After 4–6 weeks SCDC and QoL assessments were repeated and the effect of adjusting GRT was analysed.

**Results**

At baseline underreplacement was only present in 1 patient, but overreplacement in 16 patients; total calculated overreplacement was 31.8 h.nmol/L. There was no significant correlation between degree of suboptimal replacement and QoL. Overreplacement significantly ( $P = 0.029$ ) decreased to 18.2 h.nmol/L after adjustment of GRT, whereas QoL did not decrease after the changes in dosage and regime.

**Conclusion**

Individual adjustment of GRT based on SCDC to approach normal cortisol concentrations during the day can reduce overreplacement without leading to a decrease in QoL. This can prevent long-term complications of mild cortisol excess. A salivary cortisol daycurve is a simple and patient friendly tool for optimizing glucocorticoid replacement therapy and can be useful in the follow-up of patients with Addison's disease.

**P177**

**Symptom-specific thresholds for testosterone deficiency modulate complaints and metabolic risk in 434 aging male patients**

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**Objective**

The structure of psychological and somatic complaints of aging male patients in relation to sex hormone patterns and metabolism has not been fully elucidated, especially in regard to late-onset hypogonadism.

**Methods**

We investigated the nature of complaints in 434 consecutive patients aged 50–86 years attending our andrology unit and their association with physical characteristics, life style habits and sex hormone levels.

**Results**

Three independent patient profiles were identified by two-step cluster analysis: 1. psychosomatic complaints, 2. somatic disorders and 3. complaints restricted to sexual problems. Age and BMI were higher in cluster 2 while total testosterone (T) concentrations were lower in cluster 1 and higher in cluster 3 compared to the total cohort. The relation of complaints and metabolic risk factors to T was non-linear and symptom-specific, forming a plateau in patient groups with higher levels of T and causing respective prevalence to increase below specific cut-offs: <8 nmol/L: hot flushes, erectile dysfunction; <10 nmol/L: feeling depressed, lacking concentration, diabetes mellitus type 2; <12 nmol/L: obesity (Body Mass Index  $\geq 30 \text{ kg} \times \text{m}^{-2}$ , 15 nmol/L: loss of libido, loss of vigor. Also within the subgroup of non-obese men, diabetes mellitus type 2 was more likely to be present in case of T levels below 10 nmol/L. Erectile dysfunction was a major complaint in 59% of all patients and independently caused by cigarette smoking ( $P < 0.001$ ) as well as  $T < 8 \text{ nmol/L}$  ( $P < 0.001$ ) in binomial regression models.

**Conclusion**

Older men seeking andrological advice form 3 major patient groups in regard to psychosomatic complaints, metabolic risk and sexual function. Symptoms relate to T in a non-linear, symptom-specific manner. In individual patients, occurrence of symptoms related to late-onset hypogonadism may have specific thresholds of T levels.



## P178

**Longterm experience of more than 8 years with a novel formulation of testosterone undecanoate (Nebido) in substitution therapy of hypogonadal men**M Zitzmann<sup>1</sup>, F Saad<sup>2</sup> & E Nieschlag<sup>2</sup><sup>1</sup>Institute of Reproductive Medicine of the University, Muenster, Germany; <sup>2</sup>Schering AG, Berlin, Germany.**Objective**

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).

**Design**

We report data from 22 patients (15 with primary and 7 with secondary hypogonadism) aged 30 to 65 years (mean 43.8 ± 8 years) who received injections of 1000 mg of TU (4 ml - ampoules) for over 8 years.

**Results**

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. If this was the case, it was within the last 2 weeks before the next injection as loss of androgenic psychotropic effects. Hemoglobin concentrations and hematocrit were markedly elevated under treatment but remained within the normal range. Prostate size as assessed by transrectal ultrasound remained below 30 ml in all patients and PSA concentrations did not exceed 2.0 µg/L. Bone density as determined by quantitative computer tomography of the lumbar spine or phalangeal ultrasound generally improved in all patients.

**Conclusion**

In summary, intramuscular injections of testosterone undecanoate represent a feasible, safe and well tolerated modality of androgen substitution in hypogonadal men.

## P179

**Improvements for patients and nurses using 2.5 ml prefilled syringes as the vehicle solution for suspension of Sandostatin LAR<sup>®</sup> microspheres**KT maher<sup>1</sup>, WM Drake<sup>1</sup>, GM Besser<sup>2</sup>, AB Grossman<sup>1</sup>, SL Chew<sup>1</sup>, PJ Jenkins<sup>1</sup>, LA Kalingag<sup>1</sup>, FK Fode<sup>1</sup>, MT O'Sullivan-Hawketts<sup>1</sup>, DM Walker<sup>1</sup> & JP Monson<sup>1</sup><sup>1</sup>Centre for Clinical Endocrinology, William Harvey Institute, St Bartholomew's Hospital, London, United Kingdom; <sup>2</sup>London Clinic Hospital, Centre for Endocrinology, London, United Kingdom.

The preparation of Sandostatin LAR<sup>®</sup> injections using a 2 ml ampoule of vehicle solution may be associated with some technical difficulties of administration, with adverse consequences for patients. The development of a 2.5 ml prefilled syringe may alleviate some of these problems. We have compared these two methods of Sandostatin LAR<sup>®</sup> administration in 17 patients with acromegaly and 5 patients with neuro-endocrine tumours, (6 drug naïve, 13 women, median age 55, range 26 to 83 years). All patients received Sandostatin LAR<sup>®</sup> by both methods in random allocation on two consecutive months by the same nurse, after which both patient and nurse completed a questionnaire assessing pain/discomfort and convenience of administration respectively. Pain was similar on a self-rating scale for both preparations for drug naïve patients, but was worse with the ampoule vehicle in some patients previously established on therapy, (3/22 reporting pain levels as more severe). Patient waiting time was generally shorter with the prefilled syringe, although difficult to record for all patients. Diluent preparation was generally easier and faster for nurses with the prefilled syringe, (6/22 recorded scores of quite easy with the 2.5 ml prefilled syringe vs 3/22 with the ampoule, and 11/22 recorded scores of very easy with the prefilled syringe vs 1/22 with the ampoule ( $P = 0.002$ ). Reconstitution and drug administration were no different between preparations. Other clear advantages of the prefilled syringe include less wastage of diluent and less chance of contamination of diluent and scratches by broken glass. However clear volume markings on the prefilled syringe would be a major improvement. We conclude that the 2.5 ml prefilled syringe of Sandostatin LAR<sup>®</sup> has advantages over conventional diluent in the therapy of neuroendocrine tumours and acromegaly.

## P180

**Impaired thirst and AVP release due to a reset osmostat in a patient with partial cranial diabetes insipidus (CDI) and subtle pituitary disease**

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An asymptomatic 66-year-old man was referred for investigation of chronic hypernatraemia. Plasma sodium varied between 146–152 mmol/l and potassium was normal over several years. There was no relevant past, family or drug history. There were no symptoms or signs of hypercortisolism or other endocrinopathy either. Clinical examination was normal.

Initial investigations were as follows – sodium 149 mmol/l, potassium 4.3 mmol/l, urea 7.9 mmol/l, creatinine 117 micromol/l, serum osmolality 317 mmol/kg and urine osmolality 236 mmol/kg. Appropriate screening tests did not reveal hyperaldosteronism, hypercortisolism or major anterior pituitary dysfunction. However, the cortisol response to ACTH was suboptimal (465 nmol/l at 30 minutes). A water deprivation test confirmed partial CDI and low AVP activity to increasing plasma osmolality. A hypertonic saline infusion test revealed an upwardly reset osmostat (increased thresholds for thirst and AVP release). A MRI scan showed a small pituitary gland, partial empty sella and an absent posterior pituitary "bright signal." Tests to elucidate a cause for his endocrine dysfunction were negative.

**Table 1** Hypertonic saline infusion test.

Time (min)	Sodium (mmol/l)	Serum osmolality (mmol/kg)	Plasma AVP (pg/ml)	Subjective thirst scale (0–100)
0	146	307	0.23	12
30	147	312	0.29	24
60	150	318	0.53	39
90	152	321	0.84	58
100	154	327	1.83	89

The likely cause of these abnormalities is hypothalamic vascular disease. We have advised a daily fixed fluid intake (with attention to variations in weight and serum sodium) and steroid cover at times of stress. When last reviewed he was still asymptomatic but normonatremic.

## P181

**Reactive hypoglycaemia – can measuring insulin concentrations help understand the pathophysiology and support diagnosis?**RB Poole, AJ Brooks & JSW Li Voon Chong  
Royal Hampshire County Hospital, Winchester, Hampshire, United Kingdom.**Objective**

To investigate the insulin response during a 5 hour Oral Glucose tolerance test (OGTT) in subjects with and without symptoms of reactive hypoglycaemia.

**Methods**

12 patients with suspected reactive hypoglycaemia were studied. After fasting from midnight, they were given a 75 g glucose drink at 9 am the following morning. Plasma glucose and insulin levels were measured at baseline and at 30 minute intervals for 5 hours. Relative insulin increase, shape of insulin response curve, time difference in peak insulin and glucose and basal insulin sensitivity using the HOMA method were measured.

**Results**

Patients with reactive hypoglycaemia were defined as those with symptoms of hypoglycaemia and a blood glucose  $\leq 3.5$  mmol/l. 7 of the 12 patients were confirmed to have reactive hypoglycaemia. The other 5 patients were asymptomatic and had glucose levels  $\geq 3.6$ . All patients with reactive hypoglycaemia had a peak of insulin  $\geq 5$  times basal concentration while patients without had peak insulin  $\leq 5$  times basal. For patients in both groups, peak insulin concentration coincided with peak glucose. Patients without reactive hypoglycaemia had a peak insulin response at 30 minutes. Those with reactive hypoglycaemia appeared to have lost their first phase insulin response with a more gradual increase in insulin concentration and a peak insulin level at 60 minutes. HOMA values showed that the patients with reactive hypoglycaemia were more insulin sensitive than those without.

#### Conclusions

Patients with reactive hypoglycaemia are more insulin sensitive and have an abnormal insulin response to an oral glucose challenge with a more gradual increase and delayed peak in insulin concentration. In patients where the diagnosis of reactive hypoglycaemia is not clear, an insulin level at 60 minutes  $\geq 5$  times baseline may help confirm the diagnosis.

#### P182

##### Impaired quality of life in patients with adrenal insufficiency – evidence that improved glucocorticoid replacement strategies are needed

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A recent study has reported impaired subjective health status (SHS) in 79 patients with primary adrenal insufficiency (AI) despite routine steroid replacement<sup>1</sup>. Here we have performed a survey in a large cohort of patients with primary and secondary AI. 348 patients (148 outpatients and 200 registered participants of the self-help network for adrenal and pituitary diseases) were contacted by mail. 256 (74%) agreed to participate and received a disease specific and 3 standardized questionnaires (SF-36, GBB24, HADS) assessing SHS. 216 completed sets (primary AI  $n = 137$ ; secondary AI  $n = 79$ ) were available for analysis. SHS was significantly impaired in AI compared to age- and sex-matched controls from a representative random sample of the general German population ( $n = 2076-7124$ , for respective questionnaires). AI patients had significantly lower scores in 7 of 8 SF-36 dimensions (all  $P < 0.001$ ). Interestingly, scores for bodily pain were higher in patients with AI ( $74.2 \pm 29.5$  vs  $65.4 \pm 26.3$ ;  $P < 0.001$ ), indicating lower subjective pain perception. Similar to SF-36, GBB24-scores indicated significant impairment of well-being (e.g. global score:  $26 \pm 16$  vs  $15 \pm 13$ ;  $P < 0.001$ ). Anxiety and depression as assessed by HADS were also higher in AI (anxiety:  $6.6 \pm 4.1$  vs  $4.4 \pm 3.1$ , depression:  $5.2 \pm 4.1$  vs  $4.2 \pm 3.5$ ;  $P < 0.001$ ). 57 patients receiving DHEA also had similarly significant impairment in SHS. In patients with secondary AI, SHS was not significantly more reduced than in patients with primary AI. To better define the impact of AI per se further subgroup analysis was performed, which revealed that SHS in isolated Addison's disease and APS was similarly impaired. In conclusion, health-related quality of life is significantly impaired in both primary and secondary AI, which holds true independent from contributing factors such as polyendocrinopathy. Failure of DHEA to fully restore well-being in the subgroup of patients with DHEA indicates the need of further improvement of glucocorticoid replacement strategies.

1. Lovas *et al.* 2002

#### P183

##### Long-acting, intramuscular Testosterone undecanoate (TU, Nebido®) in the treatment of female-to-male transgender individuals

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#### Introduction

Intramuscular Testosterone Undecanoate (TU, Nebido®) has become available in November 2004 in Europe for the treatment of male hypogonadism. Continuous physiological hormone replacement is necessary for transgender patients, both prior to the gender adjustment operation and for the remainder of the individual's lifetime. The administration of long-acting TU therefore stood to reason as part of cross-gender testosterone therapy for female-to-male transgender patients.

#### Patients and methods

Beginning in November 2004, a total of 13 transsexual patients (median age 33 years  $\pm 6$  x  $\pm$  SE) have been treated with TU. The first injection interval was six weeks. Subsequent injections were administered in 12-weekly intervals. The diagnosis was made in close cooperation with the sexual therapists in compliance with applicable standards. Close-meshed laboratory controls (Hb, Hk, total testosterone, SHBG, dihydrotestosterone, FSH, estriol, estradiol) were carried out both before and during therapy.

#### Results

12 out of 13 patients are continuing TU therapy to date. In the case of one patient, a pronounced needle phobia led to discontinuation of TU treatment. Total testosterone levels increased from  $0.5$  ng/ml  $\pm 0.2$  prior initiation of cross-gender therapy to  $4.5$  ng/ml ( $\pm 1$ ; x  $\pm$  SE) after 6 weeks and to  $6.5$  ng/ml ( $\pm 1.2$ ; x  $\pm$  SE) after 18 weeks of treatment with TU [normal male reference range  $2.4-8.3$  ng/ml]. Undesirable side effects have not been observed. We were able to prolong the application time period for one patient from 12 to 14 weeks.

#### Conclusions

TU is a safe and effective therapy for female-to-male transgender individuals, maintaining testosterone levels within physiological limits. The three-month intervals make TU a convenient option for the patient within the framework of a cross-gender hormone therapy. Our results confirm the positive experiences that have been reported previously for the application of TU in hypogonadal men. Monitoring and individualisation of the application interval under therapy are necessary.

#### P184

##### Long-acting intramuscular testosterone undecanoate (TU, Nebido®) in treatment of hypogonadal aging males

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#### Introduction

Over the last five years testosterone therapy in men became more safe, efficient and 'patient-friendly' with the introduction of new testosterone preparations. Testosterone gels are in widespread use, but they are associated with the risk of interpersonal transfer and need daily application. Recently, injectable testosterone undecanoate (TU, Nebido®) has become available in Europe.

Long term data of aging males with long-acting testosterone esters are limited. Specific risk data on the prostate and haematological parameters are not available.

#### Patients and methods

33 hypogonadal men with primary, secondary or late-onset hypogonadism between the age 45-79 years (mean:  $59.2 \pm 7.3$  years; x  $\pm$  SE) were treated with TU.

29 patients had been pre-treated, 17 patients with T-gel, 12 patients with intramuscular testosterone enanthate. Two patients assessed tolerability of intramuscular injection as "poor" and dropped out.

21 patients received TU for more than 6 months. Patients were assessed before the first injection and in 6-weekly intervals over the treatment period of 30 weeks. At each consultation, sexual function, mood, quality of life and skin reactions were monitored. Hematology, clinical chemistry, Total Testosterone, SHBG, Dihydrotestosterone (DHT), Estradiol, LH, FSH and prostate specific antigen (PSA) were measured prior the next injection.

#### Results

Testosterone levels increased from  $2.6$  ng/ml ( $\pm 1.09$ ; x  $\pm$  SE) [range  $2.3-6.00$  ng/ml] at baseline to  $3.9$  ng/ml ( $\pm 1.35$ ; x  $\pm$  SE) after 6 weeks and to  $4.73$  ng/ml ( $\pm 1.85$ ; x  $\pm$  SE) after 30 weeks of treatment.

DHT levels increased from  $286$  pg/ml ( $\pm 141$ ; x  $\pm$  SE) [range  $310-1463$  pg/ml] to  $905$  pg/ml ( $\pm 299$ ; x  $\pm$  SE). PSA levels fluctuated minimally within the normal range. In two patients the length between two injections could be prolonged from 12 to 14 weeks.

#### Conclusion

Treatment with TU is a safe and efficacious option for the hypogonadal aging male. Regular clinical and laboratory control is mandatory.

#### P185

##### Screening in primary care using fasting glucose uncovers significant hypoglycaemia including an asymptomatic insulinoma

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Contracts with primary care encourage screening for diabetes and as a result a fasting blood glucose is frequently performed on asymptomatic patients. During the last year three such patients were found to have significant hypoglycaemia with elevated c-peptide levels and were referred for further investigation.

The first, a 50 year old woman, developed significant hypoglycaemia 22 hours into a fast with elevated c-peptide and insulin levels. A CT scan of her pancreas failed to demonstrate a lesion but a 15 mm by 8 mm lesion in the pancreas was demonstrated on endoscopic ultrasound. She was completely asymptomatic prior to the discovery of her insulinoma but later became aware when her blood sugar was low.

The second and third cases were a 36 year old man and a 73 year old man. Both had a low screening blood glucose and a high c-peptide (case 2: glucose 2.7 mmol/l; c-peptide 1344 pmol/l, case 3: glucose 2.2 mmol/l; c-peptide 3069 pmol/l). Both were asymptomatic throughout a 72 hour fast and the bloods at 72 hours revealed hypoglycaemia with ketotic hypoinsulinaemia.

Screening for diabetes has resulted in asymptomatic hypoglycaemia being detected. Experience from case 1 has taught us that this cannot be ignored and should be formally investigated. Insulinoma presenting in this way is very unusual.

## P186

### Comparison of the effect of sibutramine monotherapy with sibutramine-metformin combination therapy in nondiabetic obese women

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#### Aim

Sibutramine, orlistat and metformin appear beneficial for the treatment of adult with obesity. Several investigators have suggested that larger weight losses might be achieved by combining weight loss agents. The aim of this study was to compare the effectiveness of sibutramine monotherapy versus sibutramine plus metformin combination therapy in obese women with normal glucose tolerance.

#### Material-method

This is a 6 month, prospective and randomized study. Forty obese women (age:  $44.1 \pm 8.6$  years) with a body mass index (BMI)  $\geq 30$  kg/m<sup>2</sup> were consecutively enrolled in the study and were randomly assigned to receive either 10 mg/day sibutramine (group 1; *n*: 20) or 1700 mg/day metformin in addition to 10 mg/day sibutramine (group 2; *n*: 20). Dietary counselling was offered to each patient. Body mass index (BMI), waist circumference and insulin resistance by homeostasis model assessment model (HOMA) were measured at baseline, third month and sixth month. OGTT with insulin response was repeated at the end of the study.

#### Results

Baseline characteristics were similar in both treatment groups. Three patients in group 2 were dropped out of the study in the first month because of gastrointestinal discomfort. BMI values decreased significantly in both groups at the end of the study compared with baseline (group 1,  $39.6 \pm 5.7$  vs.  $35.5 \pm 6.6$  kg/m<sup>2</sup>,  $P < 0.001$  and group 2,  $40.1 \pm 4.8$  vs.  $37.3 \pm 4.5$  kg/m<sup>2</sup>,  $P < 0.001$ ) but there was not a statistically significant difference between treatment groups according to BMI at the end of the study. Waist circumference and HOMA values of both groups were significantly decreased at 6th month, but there was not a significant difference initially and at the end between groups either.

#### Conclusion

The combination of sibutramine with metformin or sibutramine alone treatment protocols are effective in maintaining weight loss, reduction in waist circumference and insulin resistance parameters. But still combination treatment of sibutramine and metformin does not seem to be superior to sibutramine alone.

## P187

### Torbay adrenal and pituitary project

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An audit examining self-management skills and pre hospital management of adrenal crisis. This audit was performed following a clinical incident involving an Addison's patient who suffered and survived a cardiac arrest as a direct result of an adrenal crisis. The patient involved in the initial incident lived close to a major A&E.

The paramedic arm of the audit came about after consideration of the geographical nature of this locality. Even if the Air Ambulance is used there

is still the potential for transfer times of more than 60 minutes. There is also the issue of management of steroid impaired patients during concurrent illness or incident such as RTA.

The audit was performed in two parts. Patients and Paramedics

#### Patients

The patients were asked what they understood by their illness, what steroid they are taking, how they manage illness and if they have an emergency injection kit at home.

Understanding was variable and highlighted the need for consistent education at the diagnosis stage. Self-management during illness needed addressing as well as emergency injection.

A working group of patients, paramedics and the endocrine specialist nurse has been set up to develop hand held records and identify areas for further development.

#### Paramedics

The paramedics were asked what they understood by specific illnesses such as Addison's disease, whether they had experience of the disorders mentioned and whether further education and policy change would support a change in practice.

The paramedics demonstrated very little understanding but there was unanimous enthusiasm for further education and expansion of practice.

Discussion with paramedic training officers and Westcountry Ambulance (WAST) management has resulted in a pilot of a new treatment pathway. The audit has also meant that education on pituitary and adrenal problems has now been included in the WAST pre qualifying programme.

## P188

### Autoimmune hypoadrenalism: the profile of related conditions

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Autoimmune hypoadrenalism (Addison's disease) is a relatively rare condition; recent research has found the European prevalence to be up to 140 per million [Lovas & Husebye 2002]. It frequently occurs in association with other organ-specific autoimmune diseases, both endocrine and non-endocrine. Previous studies identified a considerable range in the prevalence of associated conditions and, because of the rarity of the disease, were often drawn from relatively small samples. In 2003 we conducted the largest international survey of autoimmune hypoadrenalism to date [*N* = 613]. We asked patients to list all health conditions, including any apparently unrelated to their adrenal failure. Responses were compared to a well-matched control group [*N* = 612]. There were some unexpected findings.

Autoimmune hypoadrenalism patients reported significant rates of asthma [13% compared to 7% of controls,  $P \ll 0.01$ ], in addition to the anticipated associations of vitiligo, vitamin B12 deficiency, coeliac disease, alopecia and Sjogren's syndrome [ $P \ll 0.01$ ]. Arthritic conditions such as rheumatoid arthritis and ankylosing spondylitis were reported at a similar frequency to controls; multiple sclerosis occurred at the same frequency [0.33%, consistent with Caucasian epidemiological studies eg Nielsen at al 2005]. There was a significant rate of primary biliary cirrhosis among hypoadrenalism patients [ $P = 0.02$ ]. The rate of thyroid disease associated with autoimmune adrenal failure was, at 49%, higher than that found in previous studies [Wilson 1998]. Type 1 diabetes, Type 2 diabetes and premature ovarian failure were reported at lower rates than found in previous studies but were statistically significant compared to controls [5%, 6%, 12%,  $P \ll 0.01$ ]. Male testicular failure was reported at the same level as controls [2%].

These findings, drawn from the largest international survey of Addison's disease to date, have significant implications for managing autoimmune hypoadrenalism patients and screening them for the development of associated autoimmune conditions.

## P189

### Recombinant hGH therapy in males with organic GHD: should we trust in total testosterone levels for diagnosis of central hypogonadism?

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Previous evidences have suggested that in adults with organic hypopituitarism the condition of GH deficiency (GHD) could mask the presence of other pituitary deficits. In our experience, both central hypothyroidism and hypoadrenalism were unmasked during rhGH therapy in adults with GHD due to central organic lesions. Few and conflicting information are available about the relationship among GHD, rhGH therapy and gonadal function. Aim of the present study was to investigate the hypothalamic-pituitary-gonadal axis (HPG) in 12 adult males (mean age  $47 \pm 8$ (SD) yrs) with organic GHD and normal HPG axis. The gonadal function was evaluated by measuring of serum testosterone, LH and FSH (basal and after GnRH challenge) and SHBG levels, before and during rhGH (mean dose  $0.3 \pm 0.1$  mg/day for  $13 \pm 4$  months). Careful clinical evaluation of symptoms of hypogonadism was also performed. To check the efficacy and adequacy of rhGH, serum IGF-I levels and percent of body fat (BF%) were measured too. Serum IGF-I levels normalized during rhGH and BF significantly decreased by 8%. Serum testosterone levels significantly decreased on rhGH (from  $18.1 \pm 5.8$  to  $14.2 \pm 5.4$  nmol/L,  $P = 0.01$ ), along with a parallel and significant decrease of SHBG (from  $31.1 \pm 12.6$  to  $24.3 \pm 8.2$  nmol/L,  $P < 0.05$ ). Thus, FAI (Free Androgen Index: Testosterone/SHBG) did not change ( $0.61 \pm 0.2$  and  $0.63 \pm 0.3$  at baseline and during rhGH, respectively). No difference was found in either basal or stimulated gonadotropins levels. Notably, on rhGH 2 of 12 patients showed low total testosterone levels ( $9.7$  and  $6.7$  nmol/L, n.v.  $10-35$ ) with neither change of FAI nor signs or symptoms of hypogonadism. In conclusion, re-evaluation of HPG axis during substitutive rhGH replacement showed a significant decrease in serum testosterone levels strictly related to variations of transport proteins and not accompanied by signs and symptoms of hypogonadism. Thus, during rhGH, the monitoring of HPG function cannot be based on the measurement of total testosterone levels, but should mainly rely on a careful clinical evaluation, in order to avoid unnecessary replacement therapy.

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## P190

### Survey of steroid replacement therapy

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Research suggests regular assessment of steroid replacement therapy (SRT) is important to avoid over replacement. Also patients need regular educational updates to optimise management of SRT during illness. Over a three-month period (2004) we surveyed SRT and knowledge in patients with primary or secondary hypoadrenalism. Patients were given a questionnaire when attending clinic. We assessed type of SRT, dose and timing; compliance with medication; patient's knowledge of action/intervention during times of illness/vomiting and patient's use of steroid cards and medical alerts.

#### Results

80 patients were given the questionnaire and we had 56 (71%) replies. Hydrocortisone was the main SRT, with 20mgs as the total daily dose for 18 (35%) patients, 30mgs for 22 (43%) patients and 11 (22%) patients on doses ranging from 5mgs to 100mgs. 25 (49%) patients took evening Hydrocortisone after 6pm with 9 (18%) taking it after 8pm. Compliance was good with 54 (97%) either never or only occasionally missing medication. 43 (77%) patients knew to increase their SRT when ill. When vomiting 19 (34%) stated they needed injected steroids and a further 23 (39%) said they would call a doctor. This suggests 37 (66%) of patients on SRT may not get the appropriate treatment when vomiting. 20 (36%) patients either did not have or did not answer the question about possessing a sick day rules leaflet. 33 (59%) of patients owned and carried a steroid card; 25 (45%) owned and carried a medical alert; 42 (75%) owned and carried at least one or the other. Patient's knowledge of who should know they were on SRT varied. These results suggest that there is a need for regular assessment of dosage and timing of SRT. Results indicate that patient's knowledge of managing SRT during illness should be regularly reviewed and reinforced with appropriate education.

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## P191

### Concordance between GH determination and IGF-I in acromegaly using two IGF-I methods

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#### Introduction and aims

Following treatment for acromegaly, both growth hormone (GH) and insulin-like growth factor-I (IGF-I) levels are predictive of mortality. These data are derived from studies of either a single GH or the mean circadian GH, with a threshold of 2mcg/L. However, consensus target criteria (Giustina *et al.*, 2000) require: a nadir GH of  $< 1$ mcg/L on OGTT and IGF-I within the age/sex-adjusted normal range. We aimed to determine the degree to which normalized IGF-I was concordant with basal GH ( $< 2$ mcg/L), OGTT mean GH ( $< 2$ mcg/L) or nadir GH ( $< 1$ mcg/L). In addition, we have examined the performance of two IGF-I assays in their degree of concordance with GH parameters.

#### Methods

Basal, mean and nadir GH values were determined in 75 consecutive GH/OGTT evaluations amongst 55 acromegalic subjects. Serum IGF-I ( $n = 37$ ) was measured using a DSL immunoradiometric assay before Nov 2004 and subsequently ( $n = 38$ ) using a Nichols immunoassay. Comparison of IGF-I (expressed as % of upper limit of the age/sex-adjusted reference range) and GH determinations were made by log-linear regression. Contingency tables were derived to establish the concordance between IGF-I and GH remission.

#### Results

Strong correlations were found between IGF-I and basal, mean and nadir GH with the strongest for nadir GH ( $r = 0.65$ ,  $P < 0.0001$ ). The regression coefficients were equivalent for IGF-I values determined by both assays. However, when a contingency was performed there was a discrepancy between the two assays. The Nichols assay had improved concordance between IGF-I and nadir GH (3/38 had high IGF-I but nadir GH  $< 1$ mcg/L). In contrast, the DSL assay produced 9/37 similarly discordant results.

#### Conclusions

A nadir GH  $< 1$ mcg/L on OGTT showed the greatest correlation with IGF-I. However, although IGF-I results from two assays were concordant over the assay range, at the threshold between active disease and remission there was a magnified discordance between assays.

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## P192

### Adult turner syndrome clinic: what are the patients' perceptions of this specialist service?

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Women with Turner Syndrome (TS) often feel well but require detailed screening for potential sequelae, which may impact on their quality of life, morbidity and mortality. They may also have psychological issues around living with the condition. It is our opinion that women with TS benefit from attending a specialised clinic and consequently an Adult TS Clinic (ATSC) was set up, however the opinion of the women attending such a service is not known. A survey was therefore conducted of the women attending our ATSC to seek their opinion of the service provided.

A questionnaire was designed to allow the individual to rate their perception of the need/importance of a specialised clinic and benefits from this service. This was handed to all attending patients over one year and was returned anonymously. 35 questionnaires were distributed with 100% response rate. 100% of women felt it was important to attend this clinic and 88% appreciated that it provided an overall general check. 81% of women believed that they gained more information from this clinic, 74% felt that it provided a better opportunity to talk to the doctors and nurses and 57% felt more reassured about their condition. Only 34% of women indicated that it gave them a greater opportunity to meet other people with the same condition.

In summary the majority of women perceived the specialised clinic offered an overall general check, provided more opportunity to discuss with medical staff and felt more informed and reassured about the condition. Most importantly all participants recognised the importance of attending this clinic. In conclusion from a patient perspective the setting up of an ATSC has improved delivery of care to women with TS.

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## P193

### Adequacy of post-glucose gh nadir $< 1$ $\mu$ g/L to define biochemical and neuroradiological remission of acromegaly

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We previously demonstrated that acromegalic patients with normal IGF-I levels after surgery also met the current criteria for cure (i.e. postglucose GH nadir  $< 1 \mu\text{g/L}$ ) after long term monitoring. Since some Authors recently proposed to even lower the present GH nadir cut off value, the aim of this study was to confirm its adequacy to define long lasting disease remission. A group of 24 acromegalic patients (9 M&15 F, age  $54.2 \pm 9.6$  yrs) normal IGF-I levels and postglucose GH nadir  $< 1 \mu\text{g/L}$  after 3 months from surgery were reevaluated after a median period of 8.5 yrs (range: 4–19). In all patients, basal and postglucose GH nadir levels, IGF-I concentrations, symptoms score, metabolic parameters such as BMI, fasting and post-OGTT glucose and insulin levels, insulin resistance by HOMA-IR, lipid profile and blood pressure, antero-pituitary functions and magnetic resonance imaging were evaluated. An OGTT was also performed in 29 healthy controls to define the “normal” GH nadir limit (mean + 2SD =  $0.19 \mu\text{g/L}$ ). In the long term follow-up, IGF-I levels remained in the normal range and postglucose GH nadir  $< 1 \mu\text{g/L}$  in all the patients. In particular, GH nadir was  $< 0.19 \mu\text{g/L}$  in 14 patients and above that limit in 10. The 2 groups showed similar interval from surgery ( $11.3 \pm 4.2$  vs  $8.3 \pm 4.3$  yrs,  $P$  NS), IGF-I concentrations ( $-0.8 \pm 0.6$  vs  $-0.2 \pm 1.1$  SDS,  $P$  NS), metabolic parameters except for BMI ( $30 \pm 6$  vs  $26 \pm 4 \text{ Kg/m}^2$ ,  $P = 0.08$ ), fasting insulin levels ( $12.4 \pm 6.3$  vs  $6.6 \pm 4.0 \text{ mU/L}$ ,  $P < 0.05$ ) and HOMA-IR ( $3.1 \pm 2.0$  vs  $1.5 \pm 0.9$ ,  $P < 0.05$ ), number of patients with hypopituitarism and/or secondary empty sella. Finally, none of the patients showed any clinical or neuroradiological evidence of disease recurrence. In conclusion, the current GH nadir cut off is still adequate to define long lasting remission of acromegaly in postoperative patients. The occurrence of obesity and insulin resistance in patients with lower GH nadir might imply the presence of a relative GH deficiency in these subjects or suggest that postglucose GH levels are reduced by progressive BMI increase.

## P194

### Patients with serum prolactin $> 20,000 \text{ mU/L}$ – review of presentation, management and outcome

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#### Introduction

Large prolactinomas are uncommon and there is limited information on their presentation and progression. In this study we reviewed the presenting features, associated endocrinopathy and the response to treatment.

#### Methods

16 patients (aged 20–80 years, 75% male) were identified from our endocrine database as presenting with prolactin  $> 20,000 \text{ mU/L}$  between 1985 and 2005.

#### Results

75% of patients presented with mass effects (10 visual impairment, 5 headaches, 1 dysphagia). 50% initially presented to Ophthalmology and a further 25% to Neurology/Neurosurgery. Initial median prolactin was  $99,000 \text{ mU/L}$  (range 22,600 to  $444,300 \text{ mU/L}$ ). Defects of the following axes were seen at diagnosis LH/FSH-Testosterone/Oestrogen ( $n = 10$ , 63%), TSH-T4 ( $n = 6$ , 38%), ACTH-Cortisol ( $n = 4$ , 25%), (50% of patients did not have full adrenal axis assessment prior to treatment). 3 patients underwent surgical decompression prior to starting medical treatment (2 of whom had surgery before prolactin measured). All patients had primary or post-surgical treatment with either cabergoline (63%) or bromocriptine (38%). Median period of follow up was 3 years (range 1 month to 19 years) with latest median prolactin level of 215 (30 to  $11,500 \text{ mU/L}$ ; (excluding  $27,570 \text{ mU/L}$  in one recent, non-compliant patient). In 75% of patients the latest prolactin was  $< 650 \text{ mU/L}$ . Recovery of visual fields was seen in 90% of patients and in the LH/FSH-Testosterone/Oestrogen axis ( $n = 2$ , 20%), TSH-T4 ( $n = 1$ , 17%), ACTH-Cortisol ( $n = 1$ , 25%).

#### Conclusion

75% of percent of patients with prolactin  $> 20,000 \text{ mU/L}$  presented with mass rather than biochemical effects. Not all patients had pituitary insufficiency and some surprisingly, even had normal FSH/LH/Testosterone/Oestradiol levels. This study highlights the importance in measuring serum prolactin in all patients with skull-base tumours prior to considering surgery, and for early endocrine review of hyperprolactinaemic patients, as there is generally a good response visually and biochemically to medical treatment.

## P195

### Long-term outcome in men with microprolactinoma

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#### Introduction

It is accepted that hyperprolactinaemia associated with microprolactinoma may prove self-limiting in 25–30% women, and there is some suggestion from series of mixed gender that the same may be true in men.

#### Methods

We have therefore reviewed the outcome in all men with microprolactinomas managed at the Department of Diabetes and Endocrinology, City Hospital, Nottingham, between 1994 and 2002. All had sustained and previously untreated hyperprolactinaemia ( $> 600 \text{ mU/L}$ ). Macroprolactinoma and drug-induced disease were excluded.

#### Results

There were 10 men with a median age of 55.5 years (range 40–70). Pituitary imaging prior to treatment (MRI/CT) revealed microadenomas in 2 but was normal in 8. Other pituitary function was normal in all cases. Initial prolactin (confirmed elevated in at least one other specimen) was  $1305 \text{ mU/L}$  (624–26000). Median serum testosterone was  $7 \text{ nmol/L}$  (3.7–17.6) (reference range 8–27 nmol/L). Other pituitary function was normal. Two (baseline PRL 624, 784) opted not to be treated, and serum prolactin fell to, and remained, within the reference range in both during 75 and 30 months of follow up. The remaining 8 received dopamine agonist therapy (bromocriptine, quinagolide or cabergoline) in conventional modest doses. Therapy was discontinued irregularly and serum prolactin monitored each 6 to 12 months. Treatment was permanently discontinued in three after 47, 48 and 50 months and most recent serum prolactin (measured after an interval of 27, 29 and 35 months, respectively, was 817, 558 and  $893 \text{ mU/L}$ . Pre-treatment prolactin concentrations in these three were 1300, 4600 and 1310. Treatment was discontinued in a fourth man after 78 months and serum prolactin was  $300 \text{ mU/L}$  when measured one month later.

#### Conclusion

These data suggest that idiopathic hyperprolactinaemia (with or without demonstrable microadenoma) may prove self-limiting in men, but the possibility needs to be substantiated in a larger cohort.

## P196

### Ophthalmic findings in turner syndrome

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Turner syndrome (TS) is associated with more frequent ophthalmic morbidity. The goal

To investigate the prevalence and severity of ophthalmic problems in patients with TS diagnosis established on a detailed karyotype analysis.

#### Patients and method

73 girls with TS aged 2–30 (mean  $14.34 \pm 6.39$ ) were involved in the study. 45,X monosomy was found in 57.8% of them, different mosaic pattern in 33.8% and structural aberration in 8.4%. Full ophthalmic examination was performed (visual acuity, refraction, ocular tension, colour vision and eye fundus examination with a lens of Volk).

#### Results

An organ of sight was changed in 37 (50.7%) of the patients. 31 (42.5%) of the patients suffered from an inappropriate visual acuity: 10 from myopia (2 with astigmatism), 21 from hypermetropia (12 with astigmatism). Amblyopia was present in 11 girls. Convergent squint was found in 15 (20.55%). In 2 of them an epicanthus coexisted, in 1 a nystagmus. Another patient suffered from congenital bilateral ectropion, which led to chronic inflammation and persistent lacrimation. A divergent squint was present in one girl. 4 patients suffered from a defective colour vision. An ocular hypertension and increased corneal thickness on pachymetry was also found in one of them. In 2 patient anterior segment defect occurred. It was PEX in 1 and malformation of the iris in 1. Eye fundus abnormalities were present in 5 girls. Tortuous vessels on the ocular fundi were found in 1 patient, a choroidal nevus in 2 and drusen of the optic nerve disc in another one. On ophthalmoscopy optic nerve discs oedema was present in 1 patient.

There was no correlation between the ocular findings and the pattern of karyotype.

#### Conclusion

Our study confirms that ocular problems occur in TS frequently. A routine ophthalmologic examination is recommended early in TS to diagnose and treat confirmed abnormalities.

**P197**

**Early detection of diabetic autonomic neuropathy with a new device**

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**Introduction**

Diabetic patients are at high risk of developing diabetic autonomic neuropathy (DAN). The aim of this study was to compare autonomic scoring and measurements from a new device the ANSiscope™ (Dyansys, Inc) of diabetic patients who do not suffer from DAN complications.

**Method**

After approval of the Ethics committee, 18 diabetic patients (mean age 48 ± 7.5) without any complication due to DAN were included in the study. They all underwent 3 standard autonomic tests: Valsalva Manoeuvre, Respiratory sinus arrhythmia, ratio 30:15 and blood pressure fall after tilt test. Classification between healthy (H)/early(E) and advanced(A) DAN was deduced from scoring as described by Bellavere et al. The ANSiscope™ computes a percentage of dysautonomia from a recording of 571 RR intervals recordings for patients at rest in supine position. They are then classified as having healthy(H)/early(E)/late(L)/advanced(A) or most advanced(MA) DAN. Resting heart rate was used to confirm parasympathetic dysfunction in patients with advanced DAN.

**Table 1**

	Patient classification (number of patient – group)			
ANSiscope™	2 H	3 E	4 L	9 A (incl.2 MA)
Aut. scoring	2 H	3H	3 H + 1 E	5 H + 4 E

**Results**

2 MA patients had a resting heart rate > 100beats/min

**Discussion**

Autonomic scoring was only able to detect 2 groups of patients: 13 H and 5 E whereas the ANSiscope™ detected 5 groups: 2H, 3E, 4L and 9A (including 2 MA cases). The results obtained by the ANSiscope™ were stable and reproducible. The 2 MA cases had a resting heart rate > 100, which confirms parasympathetic dysfunction. These cases were classified as healthy by autonomic scoring.

**Conclusion**

When no complication indicates a dysfunction of autonomic nervous system and autonomic scoring detects only early stage of DAN, the ANSiscope™ was able to classify the patients in a reproducible manner. This device may be a useful tool to assess DAN in patients without clinical complication and help stratify patients at high risk to develop these symptoms.

**P198**

**Adult growth hormone replacement therapy after the institution of NICE guidelines**

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Guidelines for the use of Growth Hormone (GH) replacement in adults with GH deficiency (GHD) were published by the National Institute for Clinical Excellence (NICE) in 2003. We undertook an audit to ensure that patients attending our adult endocrinology clinic were being prescribed GH in accordance with NICE guidelines.

Patients commenced on GH replacement between June 2004 and June 2005 were included in the audit. Data was collected from medical notes, and was compared to the 6 NICE standards.

25 patients had a biochemical diagnosis of GHD, however 5 of these had an AGDHA score of <11. 19 patients fulfilled NICE criteria for GH replacement, 2 of whom had childhood onset GHD. The average time taken to receive written approval from GP's was 110 ± 24 days. Patients then waited a further 48 ± 9 (mean ± SEM) days before starting GH.

Results are shown as the number fulfilling NICE criteria/total number of patients within each standard. S1- biochemical diagnosis of GHD, AGDHA score >11 and other pituitary hormone deficiencies (15/17); S2-improvement of 7 points in AGDHA score after a 9 month trial period (13/13); S3-reassessment of patients with childhood onset of GH (once achieved linear growth/peak bone mass)(1/2); S4-as for S3 but applying to patients in early adulthood (1/1); S5-Clinical care is undertaken by a

consultant endocrinologist (19/19); S6- Primary care involvement via agreed shared care protocol (10/10).

Our practice shows good adherence to NICE guidelines. The unexpected delay in the time taken to start GH incorporates time taken for patient education. No GP's refused to prescribe GH, however this does not reflect more recent experience. One GP prescribed GH outside NICE guidelines. These data demonstrate the reliance on AGDHA score which may penalise patients who benefit from GH in other ways such as protection against cardiovascular risk, and maintenance of bone density.

**P199**

**Clinical evaluation of Sheehan syndrome**

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The term Sheehan syndrome refers to the development of pituitary necrosis due to ischemia within a few hours of delivery. Usually, antecedent hypotension and shock are present, resulting most commonly from obstetric hemorrhage. In such instances evidence of adrenal and thyroid insufficiency should be sought. A classical symptom of Sheehan's syndrome is the absence of lactation after delivery related to prolactin deficiency. We retrospectively analysed 64 patients who were diagnosed as Sheehan syndrome in Istanbul Faculty of Medicine, Endocrinology and Metabolism Clinic, in 1980–2005.

Mean ages at delivery and diagnosis were 29.14 ± 5.66 (16–40) and 39.69 ± 8.21 (24–60), respectively. Unfortunately, diagnosis was delayed 9.92 ± 7.78 years. All patients had a history of massive and prolonged hemorrhage period at delivery. Six of them were twin pregnancies, and eight of them lasted by fetal death. Fourty-nine (% 76.6) patients neither menstruate nor lactate any more. The clinical presentations were as acute form of Sheehan' syndrome in 14 patients (% 21.9). One patient was diagnosed after generalized tonic-clonic convulsion induced by hypoglycemia. Twenty-three patients had low serum glucose concentrations (minimum 16 mg/dl). Eight patients had hyponatremia (minimum sodium concentration was 118 mEq/L). All patients (100%) had hypogonadism. Sixty patients (93.75%) had primary, four patients (6.25%) had secondary hypothyroidism. Fifty six (87.5%) patients were hypocortisolemic. We performed corticotropin stimulation test with 250 µg ACTH for patients who were diagnosed ≥ 5 years after delivery. Ten patients had adrenal insufficiency, too. None of the patients had diabetes insipidus. Total cholesterol, LDL-cholesterol, VLDL-cholesterol and triglyceride levels had positive correlation with years passed for diagnosis (P < 0.001).

**P200**

**Is a repeat or resting prolactin necessary in the investigation of hyperprolactinaemia?**

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**Background**

Prolactin levels are affected by stress, and in patients with moderate hyperprolactinaemia, a repeat test and/or a resting prolactin has been recommended, but there are very few data addressing the utility of these additional measurements.

**Aim**

To study the value of: A) Repeat measurement and B) Resting measurement of serum prolactin in mild to moderate prolactin excess (510–7500 IU/L).

**Methods and subjects**

Case note review of 75 patients referred for evaluation of high prolactin ('referral prolactin', P0). All patients had a repeat sample done at our investigation unit immediately following insertion of a forearm cannula ('repeat prolactin', P1) and a second sample drawn after 120 min bed rest ('resting prolactin', P2). P1 and P2 values were corrected for macroprolactin to achieve an estimate of monomeric prolactin. 14/75 patients who were on dopamine receptor antagonists were excluded from the analysis.

**Results**

P0 median was 1229, range 561 to 5949 IU/L. P1 was normal in 17/61 patients (27%), one due to macroprolactin; amongst these 17, P0 median

was 892 IU/L (range 616–1800). Of the remaining 44, eight showed normalisation of resting prolactin, P2; amongst these the P0 median was 1445 IU/L (range 561–2412). However, the highest P1 in those in whom hyperprolactinaemia was excluded was 1062 IU/L.

	P0 P1 P2	No. (%)	P0 median (range) IU/L
Repeat normal	Hi Lo Lo	17 (28%)	892 (616–1800)
Resting normal	Hi Hi Lo	8 (13%)	1445 (561–2412)
Confirmed	Hi Hi Hi	36 (59%)	1484 (577–5949)
Total		61	1229 (561–5949)

#### Conclusions

41% of patients with elevated prolactin normalised on further testing. Most of these false positive results would have been corrected by a simple repeat sample. A resting prolactin is probably unnecessary if a simple repeat value is twice the upper limit of normal.

#### P201

##### Knowledge of testosterone replacement therapy is significantly correlated with patient satisfaction suggesting greater need for education

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The purpose of this study was to explore the use of testosterone treatment and patients' knowledge in male hypogonadism. A questionnaire was sent to all 213 patients on testosterone replacement therapy recorded in our clinic database, with a response rate of 35.7% ( $n = 76$ ). Respondents' age ranged from 19 to 87 years (mean = 44.5; SD = 16.2). Causes of hypogonadism for this group are presented in Table 1.

Duration of testosterone treatment ranged from 0.4 to 35 years (mean = 10.4; SD = 8.9). The most widely used form of treatment at the time of the study was Sustanon 250 mg (39.5%) followed by Testogel (35.5%). For 62 patients (82%), this treatment was chosen by the hospital endocrinologist. Of the 76 respondents, only 70% had received adequate information about their treatment. Patients' level of knowledge was correlated with satisfaction with their form of treatment ( $r = 0.436$ ;  $P < 0.001$ ). Only one patient could list all the available forms of testosterone treatment whilst 39 (51.3%) patients could list only up to two. Similarly, 55 (72.3%) patients had used up to two testosterone products; 67 (88.2%) had at some point been on Sustanon. The number of products used was not correlated to the years of treatment but was significantly correlated to the number of treatment forms patients knew of ( $r = 0.611$ ;  $P < 0.001$ ). Respondents were also asked to list any positive and negative features of each testosterone product they had used; comments were analysed using content analysis.

This survey concluded that patients have inadequate knowledge regarding testosterone replacement therapy and almost half of them (47.2%) were

**Table 1** Respondents' cause of hypogonadism ( $n = 76$ ).

<b>A</b>	Primary hypogonadism $n = 14$ (18.4%)	Klinefelter's Syndrome ( $n = 9$ ), Primary testicular failure ( $n = 5$ ), Chemotherapy (3), Mumps ( $n = 1$ ), Haemochromatosis ( $n = 1$ ), Testicular cancer ( $n = 1$ )
<b>B</b>	Secondary hypogonadism $n = 47$ (61.8%)	Post inguinal hernia repair ( $n = 1$ ) Pituitary surgery ( $n = 23$ ), Craniopharyngioma ( $n = 6$ ) Idiopathic hypopituitarism ( $n = 10$ ), Hypogonadotrophic Hypogonadism ( $n = 7$ ) Kallman's syndrome ( $n = 1$ ), HIV ( $n = 1$ )
<b>C</b>	Did not respond to this question $n = 7$ (9.2%)	

unsatisfied with their treatment. The need for patient education is imperative in order to provide individualised care tailored to each patient's needs.

#### P202

##### Cushing's disease – an audit of outcomes following pituitary surgery from a single centre

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We evaluate the short and long-term outcomes following diagnosis and treatment of pituitary dependent Cushing's disease from a single centre. From 1971, there were 47 patients with proven endogenous cortisol excess, of whom, 43 (92%) had pituitary dependent Cushing's disease. The median age of this cohort was  $38 \pm 11$  years and the majority (77%) were females. 17 patients had primary treatment either with radiotherapy ( $n = 9$ ), bilateral adrenalectomy ( $n = 7$ ) or long term medical therapy ( $n = 1$ ) and this group was excluded from further analysis. Of the remaining 26 individuals who had pituitary dependent Cushing's and had pituitary surgery, the pre-operative imaging findings were, 19 (73%) microadenoma, 4 (15%) macroadenoma and 3 (12%) normal pituitary tissue. Pituitary surgery performed were, selective adenomectomy ( $n = 15$ , 58%), hemi or subtotal hypophysectomy ( $n = 8$ , 31%), total hypophysectomy ( $n = 1$ , 4%) and surgery abandoned following tumour bleed ( $n = 2$ , 7%). At the 6 week post pituitary surgery evaluation, 7 (27%) had normal adrenal function, 13 (50%) had adrenal deficiency requiring steroids and 6 (23%) remained biochemically Cushingoid. All 6 of the early relapsers were subsequently subjected to bilateral adrenalectomy and cured off their Cushing's. Over a median follow up of 8 years (range 1–15 years), a further 7 of the initially cured group ( $n = 20$ ) relapsed later (median time to relapse 18 months post original pituitary surgery). Further management in this group included bilateral adrenalectomy ( $n = 2$ ), further pituitary surgery and subsequent radiotherapy ( $n = 1$ ), further pituitary surgery and subsequent adrenalectomy ( $n = 3$ ) and radiotherapy and subsequent adrenalectomy ( $n = 1$ ). At the time of final evaluation ( $n = 26$ ), 6 (23%) had normal adrenal function, 19 (73%) had adrenal deficiency requiring steroid support and 1 (4%) remained biochemically Cushingoid currently on long term medical treatment. Overall, the cure rate following 1 pituitary surgery in individuals with pituitary dependent Cushing's were 77% in the short term and 50% in the longer term.

#### P203

##### Clinical characterization of patients with hyperprolactinaemia due to macroprolactin

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Prolactin (PRL) exists in different forms in human serum. The predominant form is little PRL (23 kDa) with smaller amounts of big PRL (50–60 kDa), that may be also associated with big big PRL (bbPRL) or macroprolactin (150–170 kDa). A lower biological activity *in vivo* of bbPRL was reported. Aim of the study was to evaluate the clinical impact of macroprolactin and the association between macroprolactinaemia and polycystic ovary syndrome (PCOS). A group of 115 female patients with diagnosis of hyperprolactinaemia were included in our study. All samples collected from these patients were subjected to a PEG precipitation test to detect the presence of macroprolactin according to Vieira method. Recovery  $\leq 40\%$  was considered indicative of the predominance of macroprolactin form. Forty-four patients presented bbPRL as prevalent form of prolactin, 39 of whom were in reproductive age. By converse, in the remaining 71 patients PEG precipitation test demonstrated a true hyperprolactinaemia. Subsequent MRI studies demonstrated the presence of a pituitary lesion in 58 out of these 71 patients, while the remaining 13 subjects had idiopathic hyperprolactinaemia. As far as the clinical picture was concerned, oligo-amenorrhoea and galactorrhoea were more frequent in macroprolactinaemic in respect to non-macroprolactinaemic patients (46 vs 59%,  $p < 0.019$ , and 23 vs 39%,  $P < 0.019$ , respectively). Twenty-five macroprolactinaemic patients were further investigated for the presence of PCOS according to diagnostic criteria of 2003 Rotterdam Consensus Conference. Interestingly,



40% of these patients were found to be affected by PCOS. In conclusion, our data confirm a lower biological activity *in vivo* of bbPRL as demonstrated by the lower prevalence of oligo-amenorrhoea and galactorrhoea observed in macroprolactinaemic as compared to non-macroprolactinaemic patients. Finally, further studies are needed in order to confirm the possible association between macroprolactinaemia and PCOS.

## P204

### Total cortisol and calculated free cortisol during the 250 µg ACTH test

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#### Objective

Evaluate the influence of altered cortisol binding globulin (CBG) concentration on total cortisol and calculated free cortisol (CFC) during the 250 µg ACTH test.

#### Methods

We included 100 unmedicated healthy individuals (HI)(50M:50F), 13 women taking oral contraception(OC), and 5 men with nephrotic syndrome(NS, prior to glucocorticoid treatment; hypoalbuminaemia < 9 g/l; proteinuria > 1.6 g/24 h). An ACTH-test was performed between 0800 and 1000, after an overnight fast, administering 250 µg ACTH intravenously. Blood was collected at baseline and 30 min. and analysed for cortisol and CBG. CFC was calculated from Coolens formula.<sup>1</sup> Approved by local Ethical Committee.

#### Results

Baseline and stimulated total cortisol concentrations were 2-3 fold higher in OC compared to HI and NS ( $P < 0.0001$ ), HI and NS not differing significantly. Accordingly, OC showed 2-3 times elevated CBG compared to HI and NS. CBG concentrations remained unchanged in all groups during stimulation.

Baseline CFC was significantly elevated in NS compared to HI and OC (75.6 (31.5–99.4)(median (range)) vs. 23.3 nmol/l (12.8–32.2) and 24.1 nmol/l (6.7–60.0), respectively; $P < 0.005$ ), HI and OC not differing significantly. After stimulation, CFC remained elevated in NS compared to the other groups ( $P < 0.01$ ), whereas post-stimulatory CFC was lower in OC compared to HI and NS (31.6 (24.9–40.5) vs. 64.5 (31.8–185.0) and 87.5 (66.4–163.7), respectively; $P < 0.0001$ ).

#### Conclusion

Altered CBG concentrations highly affect total cortisol. Baseline CFC has been shown to be within the normal range in patients with altered CBG concentrations and otherwise normal adrenocortical function. CFC has therefore been advocated as an alternative to total cortisol in the evaluation of the adrenocortical function in those cases. Focusing on the stimulated CFC we conclude that in OC women it is difficult to perpetuate the total as well as indices of free cortisol in the evaluation of the adrenocortical function. Elevated CFC in NS is possibly explained by active disease.

1. JL Coolens. J Steroid Biochem 1987; 26: 197–202.

## P205

### Comparison of the effects of transdermal and oral oestrogen treatments on serum and salivary cortisol concentrations

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#### Objective

To determine whether transdermal oestrogen (E<sub>2</sub>) preparations alter total cortisol and cortisol binding globulin (CBG) concentration similarly to oral E<sub>2</sub> treatment.

#### Methods

This cross-sectional, observational study compared levels of total serum cortisol, CBG, the free cortisol index (FCI) and salivary cortisol levels (as a measure of free cortisol) in oestrogen naïve women ( $n = 15$ ), women taking oral oestrogen ( $n = 14$ ) and females using transdermal oestrogen treatment ( $n = 8$ ). 37 women attending the endocrine unit at our Hospitals were studied. Single blood and salivary samples were collected from each patient, between day 10–18 of their menstrual cycle (where applicable) at 0830-0930 am. The study was approved by the local Research Ethics Committee.

#### Results

Total circulating cortisol levels were higher in those receiving oral E<sub>2</sub> than either the transdermal group ( $P < 0.01$ ) or controls ( $P < 0.01$ , oral group  $645 \pm 139$  nmol/l, mean  $\pm$  SD vs transdermal  $368 \pm 111$  vs control  $399 \pm 92$ ). CBG was similarly higher in those on oral E<sub>2</sub> (with no difference observed between control and transdermal subjects (oral group  $112 \pm 39$  mg/l vs transdermal  $51 \pm 12$  ( $P < 0.05$ ) vs control  $50 \pm 21$  ( $P < 0.01$ )). The FCI and salivary concentrations were similar between the groups (Salivary cortisol: oral group  $5.5 \pm 1.8$  nmol/ml vs transdermal  $5.5 \pm 2.0$  vs control  $5.2 \pm 2.1$ ).

#### Conclusions

Oral E<sub>2</sub> increased total cortisol concentrations, by increasing levels of CBG. Transdermal E<sub>2</sub> did not result in increased CBG and thus did not alter the level of total cortisol. Free cortisol was appropriately similar between control subjects and those on both oral and transdermal E<sub>2</sub>. This pilot study indicates that transdermal E<sub>2</sub>, unlike oral E<sub>2</sub> has no significant effect on cortisol levels. It is probably not necessary to discontinue transdermal E<sub>2</sub> in patients undergoing assessment of the HPA axis or hydrocortisone replacement. Transdermal E<sub>2</sub> preparations may be the most suitable form of E<sub>2</sub> replacement for many hypopituitary women.

## P206

### A survey of gender dysphoria (transsexual) patients attending an endocrine clinic

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Management of patients with gender dysphoria (transsexualism) is often difficult, and most patients are routinely managed outwith specialist gender identity clinics. We carried out a retrospective case-note survey of 21 patients attending a routine adult endocrine clinic (ages 24–64y; 13 male-to-female (MF) and 8 female-to-male (FM)).

All MF and 6 of 8 FM patients were on hormonal treatment. 6 of 13 MF patients took oestrogen alone, 6 used oestrogen with antiandrogen and 1 also took progestagen. First reported experience of gender dysphoria was <14y in all patients, but FM patients started living in the preferred gender role younger than MF. Mean age for FM to live full-time in the preferred role was 22y, for taking cross hormones, 27y, and for first surgical intervention 29y, compared to 37, 40 and 43y respectively in MF.

Social disruption was commoner in MF patients. 9 of 13 were separated/single or otherwise socially isolated. 6 of 8 FM patients lacked any evidence of social disruption post-diagnosis and only one was described as socially isolated. MF patients had more evidence of past or current depression than FM but both groups had high rates of attempted suicide (MF 5 of 13, FM 2 of 8). 8 of 11 MF were attracted to females and 3 of 11 to males. 5 of 6 FM had relationships with females and one was bisexual. Divorce rates were higher in MF (5 of 13 compared with 1 of 8 FM). More FM went on to higher education than MF but unemployment rates in both groups were high.

In this small population, MF and FM transsexual patients appeared distinct. Both groups had significant social difficulties, but MF patients were worse affected. Social isolation and depression are frequently encountered but with more accepting general and medical attitudes this may improve.

## P207

### Osteoporosis, osteopaenia and osteoarthritis in autoimmune hypoadrenalism

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Bone loss in treated Addison's disease (autoimmune hypoadrenalism) is often attributed to supraphysiological doses of glucocorticoid. The largest international survey to date ( $N = 613$ ) suggests that other factors are also likely to be associated with bone loss in these patients and that an intrinsic risk of bone loss in autoimmune hypoadrenalism cannot be ruled out. This survey also found significant rates of osteoarthritis among autoimmune hypoadrenalism patients. 13% of patients with autoimmune adrenal failure reported osteoporosis or osteopaenia ( $N = 82$ ). A further 12% reported osteoarthritis ( $N = 73$ ). A well-matched control group ( $N = 612$ ) reported less than 2% osteoporosis/osteopaenia and 3% osteoarthritis.<sup>1</sup> Analysis of



the reported glucocorticoid dosage and years since diagnosis for those with osteoporosis/osteopaenia identified no significant correlation (correlation = 0.06). Only 20% were on doses > 30 mg hydrocortisone per day; whereas 34% were on doses of 20 mg or less. This is well below the mean dose (26 mg hydrocortisone) for the patient sample as a whole, 9% had both been diagnosed less than five years ago and took 20 mg hydrocortisone per day or less. Other researchers have identified an intrinsic risk of bone loss for diabetes (Selby PL, 1988), rheumatoid arthritis (Mueller MN 1976) and asthma (Reid DM et al, 1986). These factors do not appear to be correlated for the patients in this survey; premature ovarian failure, gender and age appear to be only weakly associated. Loss of adrenal androgens has been implicated in glucocorticoid-induced bone loss (Hampson G et al, 2002). Our data, drawn from the largest international survey of autoimmune hypoadrenalism to date, suggests that loss of adrenal androgens deserves analysis as a factor potentially contributing to an intrinsic risk of bone loss in autoimmune hypoadrenalism and that there is merit to screening patients for bone loss early in the course of their disease.

<sup>1</sup>The control group were matched by age, gender. DEXA scans would undoubtedly reveal higher rates of osteoporosis/osteopaenia among both groups. Hypoadrenalism patients are not routinely offered a bone scan in any of the countries participating in this survey.

## P208

### Autoimmune hypoadrenalism: symptoms at diagnosis

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Addison's disease is notoriously difficult to diagnose and has been labelled "the master of unforgiving disguise". In the largest international survey of autoimmune Addison's disease to date ( $N = 613$ ), we asked patients to recall their symptoms at diagnosis. The results confirm the challenges of diagnosis, in that no patients recalled all the standard symptoms.

Hyperpigmentation with dizziness on standing/blackouts and weight loss, which in an extremely ill patient are often the most revealing indicators of autoimmune hypoadrenalism, were only recalled by 79% – 86% of respondents. Only three-quarters recalled a loss of appetite or nausea, while just over half recalled salt cravings, vomiting or difficulty in concentrating. Only one patient recalled blue gums, suggesting that most patients are diagnosed in less advanced stages of the disease than was the case historically. Only three patients (0.5%) recalled that they were in or near a coma, suggesting that fewer patients die from undiagnosed hypoadrenalism than would have been the case historically.

62% said their symptoms had been attributed to other conditions prior to diagnosis and 33% said they had been told by a medical professional that their symptoms were "all psychological". 31% said they had been ill for more than 12 months prior to diagnosis. Around half said it took more than 3 months to recover their health after diagnosis. Indicative of the non-specific but severe nature of the illness, 40% said they had received hospital treatment for their Addison's symptoms prior to diagnosis; 9% had been hospitalised three times or more prior to diagnosis.

These findings, drawn from the largest international survey of Addison's disease to date, suggest that hypoadrenalism cannot be excluded from a differential diagnosis without laboratory tests and that medical professionals must remain open to the possibility of Addison's disease even where hyperpigmentation is absent.

## P209

### Does HT still have a place? The impact on menopausal-related symptoms

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Hormonal treatment (HT) represents the only complete therapy of the consequences of the postmenopausal estrogenic deficiency. Quality of life in postmenopausal women is often compromised. We evaluated the quality of life (appreciated with IQL questionnaire) in 80 postmenopausal women – 40 with intact uterus, treated with natural estrogens associated with progestin, and 40 non-treated women. Group selection (treated / not treated) was made function of the informed consent of the patients. No significant differences in

age, age at menopause, and chronic diseases and socio-economic status were present in the two groups. The follow-up period was 3 months. The questionnaire was completed at the base line and 3 months after. A significant amelioration of the symptoms (diminution of the global score from -2.8 to 1.8,  $P < 0.01$ ) was found in the treated group (T), but practically no modification was seen in the non-treated (NT) one (from 2.5 to 2.2,  $p > 0.05$ ): Hot flushes score was more important at the base-line in the T group and decreased from 2.9 to 1.8 in the T group while in the NT they had only a slight, non-significant diminution (from 2.6 to 2.2). Perspirations have also improved in the T group (2.97 vs 1.78) but not in the NT (2.4 vs 2.17). In the T group palpitations and insomnia diminished from 2.61 to 1.93, respectively from 2.19 to 1.59, while in the NT group they did not change (2.35 vs 2.05, 2.6 vs 2.2). Asthenia has improved in both groups. HT remains the election treatment of the menopausal symptoms. In the light of the new studies, the estrogen type and dosage, the period and administration route must be established together with the well-informed patient.

## P210

### High mortality rate in hospital inpatients with hyponatraemia

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Hyponatraemia is the commonest electrolyte abnormality in hospitalised patients. It is often seen in patients with complex medical problems and in the critically ill. We determined the outcome for patients identified to have hyponatraemia over a one month period.

#### Methods

We reviewed all in-patients with severe hyponatraemia, defined as serum sodium < 125 mmol/l (135-146) at Hope Hospital during April 2005. Patients were identified retrospectively from laboratory computer data and case notes retrieved.

#### Results

We identified 336 patients with serum sodium < 135 mmol/l of whom 40 had serum sodium < 125 mmol/l. 36 were available for study, 4 from general surgical, 2 from neurosurgical and 30 from general medical wards. The median age was 66.5 years, range 43–90 years and median serum sodium was 113 mmol/l, range 102–124 mmol/l. Heart rate, blood pressure and chest examination were recorded in 100% but jugular venous pressure was assessed in only 17/36 (47.2%). 5/36 (13.9%) were stated to be "dry/dehydrated" but there was no evidence that the patient's tissue hydration had been assessed in any reliable way. Fluid balance was charted in 18/36 (50%). 8/36 (22.2%) were clinically hypovolaemic, 6/36 (16.7%) were hypervolaemic, and the remainder 22/36 (61.1%) were assumed to be euvoalaemic. There was written evidence the patient's medication had been reviewed in 28/36 (77.8%); potentially contributing medication was identified in 14 patients but only stopped in 8 patients. Mortality rate was surprisingly high at 31% (11/36) and of those who died 91% (10/11) patients were still hyponatraemic at death.

#### Conclusion

Many patients had not been assessed in enough detail to guide the management of this disease, which has a high mortality. Assessment of the tissue signs of hypovolaemia and jugular venous pressure was particularly poor. Hyponatraemia persisted in many patients and had a very high mortality rate.

## Comparative endocrinology

### P211

#### The patients with HIV lipodystrophy syndrome have lower total fat mass but similar free fat mass

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#### Introduction

The highly active antiretroviral therapy (HAART) completely changed the natural history and body composition of the infected patients. The

lipodystrophy syndrome of HIV-infected patients (IP) is characterized by fat mass redistribution in the limbs, face lipoatrophy and abdominal lipohypertrophy. Dual-energy x-ray absorptiometry scan (DEXA) and bioelectrical impedance (BIA) are techniques to determine body composition (BC).

**Aims**

Evaluate the BC in HIV-IP treated with HAART with (CL) and without clinical lipodystrophy (WCL).

**Patients and methods**

We studied 117 HIV-IP, 25 women and 92 men, by anthropometry, BIA (Tanita) and DEXA.

**Table 1**

	CL (n = 82)	WCL (n = 35)	P
Age (years)	48.8 ± 10.9	40.4 ± 10.1	<0.001
Duration of disease (years)	8.6 ± 4.1	6.5 ± 3.3	<0.05
Duration of treatment HAART (years)	7.7 ± 4.0	4.8 ± 3.2	<0.005
BMI (Kg/m <sup>2</sup> )	24.4 ± 3.8	25.9 ± 4.9	<0.05
Waist circumference (WC) (cm)	90.2 ± 10.2	92.6 ± 13.2	ns
Hip circumference (HC) (cm)	91.9 ± 7.1	99.0 ± 8.4	<0.001
Thigh circumference (TC) (cm)	46.3 ± 4.8	50.4 ± 5.8	<0.001
Total Fat Mass (TFM) (%) (BIA) (n = 117)	18.8 ± 7.6	24.3 ± 10.7	<0.05
Total Fat Mass (TFM) (%) (DEXA) (n = 83)	15.2 ± 8.9	27.2 ± 10.8	<0.001
Fat Free Mass (FFM) (Kg) (BIA) (n = 117)	53.3 ± 7.4	53.4 ± 8.6	ns
Fat Free Mass (FFM) (Kg) (DEXA) (n = 83)	51.2 ± 7.2	50.6 ± 9.3	ns
Total Body Water (Kg) (BIA) (n = 117)	39.0 ± 5.4	39.1 ± 6.3	ns

To compare the variables we used Mann-Whitney test.

**Results**

The patients with CL had similar WC to WCL but lower HC and TC. In patients with CL we observed a % TFM significantly lower (BIA and DEXA) but similar FFM and TBW.

**Conclusions**

The patients with HIV-Lipodystrophy Syndrome had WC, FFM and TBW similar to the patients WCL. The TFM of the same patients is significantly reduced.

**P212**

**Dynamic macroprolactin and adjusted prolactin responses in hyperprolactinaemic patients**

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**Background**

Prolactin is found in serum in different molecular forms, differing in molecular size. Macroprolactin is a complex of prolactin with immunoglobulin and has limited biological activity due to failure to cross capillary wall because of its high molecular weight complex.

**Objective**

To assess the response of the inactive macroprolactin and the biologically active monomeric prolactin, in patients with hyperprolactinaemia, during TRH and metoclopramide tests.

**Design**

Eleven patients (8 females) with hyperprolactinaemia had a TRH test and eight (6 females) had a metoclopramide test. The macroprolactin and monomeric prolactin levels were assessed at baseline and at 20 minutes. Adjusted prolactin and macroprolactin levels were measured following standard PEG precipitation technique.

**Results**

The mean response, from baseline, of macroprolactin to TRH stimulation was 17.7% (Range: -40% → +92.5%) and the mean response of monomeric

prolactin was 160% (Range: -0.5% → +485%), *P* < 0.01. Following the metoclopramide challenge the mean rise, from baseline, of macroprolactin was 69.2% (Range: -1.2% → +199%) and the rise of monomeric prolactin was 297% (Range: +28.3% → +1164.6%), *P* = 0.07.

**Conclusion**

These data demonstrate that there is rise of the biologically inactive macroprolactin, which is less than that observed in the bioactive monomeric prolactin, following both the TRH and metoclopramide challenge. This would support the hypothesis that monomeric prolactin is the principal secretory product from lactotrophs and that macroprolactin is a post-secretory complex with immunoglobulin.

**P213**

**Retrospective study of usefulness of Sestamibi scan in primary hyperparathyroidism**

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**Background and aims**

Primary Hyperparathyroidism is fairly common and the diagnosis is established by demonstrating raised blood parathormone levels in the presence of hypercalcaemia. Sestamibi scan is an investigation used primarily to identify the offending gland which would help in surgery. The aim of this study was to assess the usefulness of sestamibi scan in Primary Hyperparathyroidism.

**Method**

Case notes for patients with Primary Hyperparathyroidism from 2000 to 2004 were reviewed. In total 71 patients were diagnosed with the condition. 53 patients had undergone sestamibi scan while only 49 of them underwent parathyroid operation. All the histology specimens were reviewed.

**Results**

Sestamibi scans for the 49 patients who underwent parathyroidectomy showed 33 were positive with 31 single and 2 multiple. 13 single, the 2 multiple and 7 scan negative were hyperplasias, while the rest were adenomas (18 scan positive and 9 scan negative). 17 patients with hyperplasia had 2 or more glands removed. 8 patients with adenomas and 3 patients with hyperplasia re-presented with hyperparathyroidism. On reviewing the histology of 48 patients, over 50% with the diagnosis of adenomas had to be corrected according to the recent classification. Out of the 27 adenomas, 14 were reclassified as hyperplasia (5 scan negative) and 1 had early features of malignancy (scan negative). 1 other patient with a diagnosis of carcinoma histologically had a negative scan.

**Conclusion**

This retrospective study shows the sestamibi scan is useful in identifying an offending gland. Both adenoma and hyperplasia had fairly equal positive and negative scans. Therefore the scan was unable to delineate a particular pathological cause except that multiple positive spots suggested hyperplasia. Patients with the diagnosis of carcinoma had a negative scan.

**P214**

**Assessment of oxidative stress in patients with polycystic ovarian syndrome treated with either Orlistat or Metformin**

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Orlistat and Metformin have both been shown to be beneficial in the treatment of Polycystic Ovarian Syndrome (PCOS), however, because Orlistat works by decreasing the absorption of dietary lipids, there is the potential to decrease absorption of lipophilic antioxidant substances with consequent decreased protection from oxidative stress. The objective of this study was to evaluate and compare the effect of treatment with orlistat vs. metformin on oxidative stress in patients with polycystic ovarian syndrome.

Twenty Caucasian women with PCOS [mean (± SEM) age 27 ± 0.9 yr and body mass index 36.7 ± 3.3 kg/m<sup>2</sup>] participated in this prospective, randomized, open-labeled study. All subjects had an 8-wk run-in period of dietary modification and then randomized to receive either metformin *n* = 10 (500 mg three times daily) or **orlistat** *n* = 10 (120 mg three times daily) for 3 months. Fasting blood samples were taken at randomisation and on completion (1). Malondialdehyde, (MDA) used as a marker of oxidative stress, was measured in fasting plasma samples taken at randomisation and on completion.

There was a significant reduction in weight with orlistat and a reduction in androgen levels in both groups and a trend to a reduction in insulin resistance (1). When compared with baseline, treatment with both **orlistat** (mean  $\pm$  SEM) [ $0.61 \pm 0.06$  umol/L vs.  $0.61$  umol/L  $\pm 0.04$ ,  $P = 0.99$ ] and metformin [ $0.711 \pm 0.067$  umol/L vs.  $0.74 \pm 0.085$  umol/L,  $P = 0.69$ ] produced no significant change in oxidative stress as assessed using MDA.

The reduction in weight and androgen levels by Orlistat and Metformin are not associated with changes in oxidative stress in patients with PCOS.

1. Jayagopal V *et al.* J Clin Endocrinol Metab 2005; 90: 729–733.

## P215

### Prognostication in canine parvoviral diarrhoea using basal serum cortisol concentrations

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In human critical care, patients with the highest cortisol concentrations generally have the highest mortality rates, with resultant prognostic classifications based on basal cortisol and/or delta cortisol concentrations. In contrast, comparative data on canine critical illness does not exist. Parvoviral diarrhoea is a severe infectious disease inducing a sepsis-like state predominantly in paediatric canine patients. The objective of this study was to evaluate the prognostic value of basal serum cortisol concentrations in canine parvoviral diarrhoea. A prospective, *in vivo* study was conducted on clinical cases of parvoviral diarrhoea meeting the criteria for admission to a high care ward in a veterinary academic hospital. Forty-five patients were enrolled and had their basal serum cortisol concentrations measured at admission and daily until death or discharge. Mortality was assessed as a function of basal cortisol concentrations obtained at day 2 in the whole cohort. Specificity and sensitivity data were obtained using empirical ROC curves.

Serum cortisol	Dead	Survived	Total
< 224 nmol/l	2	34	36
> 224 nmol/l	6	0	6
	8	34	42

Day 2 cortisol concentrations of >224 nmol/l had a specificity of 100%, a sensitivity of 75% and a 1.00 positive predictive value for mortality in paediatric canine patients with parvoviral diarrhoea. Basal cortisol concentrations in canine patients are considerably lower than in human patients, reflecting the lower reference range (10–160 nmol/l) of this species. The results are in accordance with human critical care data, allowing for prognostication based on basal cortisol concentrations. This data contributes to the affirmation of the dog as a model for conducting studies in the human critical care field. Our data suggest that basal cortisol concentrations have a good prognostic value and could be helpful in identifying patients with parvoviral diarrhoea at high risk of death. Further studies will have to be conducted to determine whether the same is true for the short Synacthen test, as is the case in humans.

## P216

### Short-term effects of propionate on the leptin system and on G protein-coupled receptor GPR41 mRNA levels in adipose tissue of goats

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Propionate upregulates leptin synthesis through the G protein-coupled receptor 41 (GPR41) in mice. In contrast to monogastric mammals, ruminants almost entirely depend on short chain fatty acids as energy source, but the reports about the physiological relevance of propionate for leptin secretion in these species are controversial and the importance of the GPR41 for its regulation is not yet clarified. Here we characterise the effects of intravenously infused propionate on the leptin system and on

GPR41 mRNA in goats. Castrated male goats (Deutsche-Edelziege, 10 to 12 months old) were allocated to infusions through jugular catheters after an over-night fast. They received propionate infusion ( $96 \mu\text{mol/kg}\cdot\text{min}$ ;  $n = 8$ ) or NaCl-solution of the equivalent Na-concentration ( $n = 5$ ). After recording leptin baseline concentrations for 1 h, infusions were carried out for 260 minutes. Blood samples were collected in 10 to 15 min intervals and analyzed for leptin by ELISA. The mRNA of leptin, leptin receptor (long form) and GPR41 from subcutaneous adipose tissue (SAT) and perirenal adipose tissue (PAT) were quantified by real-time PCR after euthanasia (propionate group:  $n = 4$ ; NaCl group:  $n = 5$ ). Repeated measurement analysis of serum leptin values showed an increase in the propionate versus the control group ( $P < 0.05$ ). Estimation of the mRNA expression data indicated a tendency for an increase of leptin mRNA in PAT ( $P = 0.081$ ) but not in SAT ( $P = 0.106$ ). The leptin receptor mRNA decreased in PAT ( $P = 0.042$ ) and in SAT, the latter decrease, however, did not reach the level of significance ( $P = 0.286$ ). GPR41 mRNA was elevated in SAT ( $P = 0.029$ ) but not in PAT ( $P = 0.756$ ). The differential response of GPR41 mRNA in SAT versus PAT towards propionate might be involved in the regulation of the leptin system whereby the decrease of leptin receptor mRNA might be attributable to the increasing serum concentrations of leptin.

## P217

### Ontogeny of galanin and vasotocin immunoreactivity in the supraoptic nucleus (SON) and the bed nucleus of the stria terminalis (BSTM) in chickens

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Vasotocin (AVT), the avian homolog of vasopressin, is synthesized in the BSTM of chickens in addition to the hypothalamo hypophysial axis. In previous reports, sex specific AVT-immunoreactivity(ir) in male BSTM (Jurkevich *et al.* 1999) and its ontogeny were demonstrated (Grossmann *et al.* 2002). In addition, galanin (Gal) was found in male BSTM colocalized to AVT. In the present study, the ontogeny of AVT- and Gal-ir was investigated in the SON as part of the magnocellular AVT system and the limbic BSTM. Chickens at the age of days D2, D14, D35, D70, and D112 and adults were processed for double immunohistochemistry and confocal microscopy. In SON, only AVT was detected in chicks of D2 to D70 in either sex. At D112, galanin was found in both sexes with up to 75% colocalization to AVT. In adult chickens, Gal was not visible in male and female SON. In BSTM, Gal as well as AVT were expressed in both sexes at D2. Significantly reduced cell numbers and ir-intensities of both peptides were recorded at D14. At D35, neither of the peptides were detected. Gal and AVT ir neurons were clearly detected again in BSTM of D70 males only. At D112, the pattern of AVT and Gal ir were not different compared to adult males. In contrast to mammals, female chickens synthesize no Gal in BSTM after D35.

This study demonstrates for the first time close relationship between AVT and Gal peptidergic systems in BSTM and provides first evidence for a sex, age and region dependent Gal-expression in chickens.

## Cytokines and growth factors

### P218

#### The role of IL-4 and IFN-gamma as prognostic factors of major adverse cardiac events in patients with ST-elevation myocardial infarction assigned primary percutaneous coronary intervention

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Mediators of inflammatory reaction are present at every stage of forming of atheromatous plaque, its proliferation and destabilization. Cytokines play a key role in regulation of intensity of chronic inflammatory reaction. We aimed at investigation if IL-4 and IFN-gamma are predictors of MACE in a group of patients with acute myocardial infarction (AMI).

A total of 50 patients: 40 males and 10 females, aged  $56 \pm 10$  years (mean  $\pm$  SD), with diagnosis of AMI, who underwent primary percutaneous coronary intervention (PCI) were analysed. All patients were followed for an average period of  $17 \pm 5$  months. The event rate during follow-up for

combined end point (MACE) of death, myocardial infarction and repeated revascularization was assessed. Plasma levels of IFN-gamma and IL-4 measured in venous blood samples, collected before and immediately after successful PCI as well as at the 2nd and 72nd hour after intervention. Patients with MACE (group I) were compared with the remaining ones (group II). In a long-term observation MACE were observed in 6 pts (12%). In the group I plasma levels of IL-4, before PCI procedure were significantly lower ( $13.4 \pm 1.27$  pg/ml vs  $17.98 \pm 3.21$  pg/ml,  $P < 0.0001$ ). In this group, plasma levels of IFN-gamma obtained immediately after PCI were significantly higher ( $12.6$  (range 1.8-34.0) pg/ml vs  $0.89$  (range 0-2.1) pg/ml,  $P = 0.0001$ ) comparing to the IFN-gamma plasma level in the group II. Initial plasma level of IL-4 proved to be a predictive factor of adverse cardiac events occurrence in patients with acute myocardial infarction undergoing percutaneous coronary intervention. Elevated IFN-gamma plasma level measured directly after percutaneous coronary intervention was an unfavorable predictive factor of adverse cardiac events in patients with acute myocardial infarction.

## P219

### Adiponectin and resistin concentrations during oral glucose tolerance test (OGTT) in insulin-resistant subjects

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#### Background and aims

Mechanism underlying the IR (Insulin-resistance) remains yet to be identified. Resistin has been shown to antagonize insulin action, and thus might be implicated in the pathogenesis of IR. In contrast, adiponectin has been shown to increase insulin sensitivity and improve glucose tolerance. We aimed to test the hypothesis that resistin levels might correlate positively with the indices of insulin resistance, while the opposite might be true for adiponectin.

#### Materials and methods

The study included 18 patients with established insulin-resistance: (W-16, M-2), age- $35.8 \pm 11.6$  years, BMI- $30.23 \pm 10.1$  kg/m<sup>2</sup>. IR was assessed both in a fasting state: HOMA-R, QUICKI and during the oral glucose tolerance test (OGTT); this allows the assessment of compensatory hyperinsulinaemia, sometimes observed only in response to meals. Insulin Resistance Index (IRI) was calculated through the formula:  $2/[1/(INSp \times GLYp)] + 1$ , where INSp and GLYp are the measured insulin and glycaemic areas. The concentrations of: adiponectin, resistin, insulin, glucose were assessed using kits dedicated to clinical purposes.

#### Results

The concentrations of: (G)lucose (mmol/l), (I)nsulin (mU/l), (A)diponectin ( $\mu$ g/ml), (R)esistin (ng/ml) in 0, 60, 120 minutes during the OGTT are presented below: (G)-  $4.88 \pm 0.88$ ,  $8.69 \pm 3.72$ ,  $6.99 \pm 2.60$ , (I)-  $14.33 \pm 10.12$ ,  $122.33 \pm 64.84$ ,  $87.94 \pm 65.03$ , (A)-  $11.49 \pm 4.71$ ,  $12.61 \pm 5.73$ ,  $14.92 \pm 7.58$ , (R)-  $7.29 \pm 1.59$ ,  $7.36 \pm 1.14$ ,  $7.18 \pm 1.26$ , (A/R)- 1.58, 1.71, 2.08, (IRD):  $1.34 \pm 0.29$ , HOMA-R:  $2.99 \pm 1.74$ , QUICKI:  $0.58 \pm 0.09$ .

There was a significant correlation ( $P < 0.05$ ) among those 3 methods of insulin-resistance assessment. A negative correlation between fasting serum adiponectin and resistin was observed ( $-0.56$ ;  $P < 0.05$ ). There was no correlation between indices of IR and the levels of adiponectin and resistin.

#### Conclusions

We raise the hypothesis that fasting serum adiponectin/resistin ratio may be useful in prediction of future cardiovascular risk among people with established IR.

## P220

### Effects of IGF-2 on glucose metabolism

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The effects of IGF-2 on glucose homeostasis have been more understood from individuals with fasting hypoglycaemia associated with non-islet cell tumours. Endogenous IGFs which circulate in adults fail to exert their

immense potential hypoglycaemic activity because they are largely trapped within the vascular space due to their sequestration in a high molecular weight protein complex. The tumours produce excessive amounts of 'big IGF-2' which is less readily bound by circulating IGFBP-3 and more available for interaction with cell surface receptors. However the exact mechanisms or effects on glucose metabolism are not well defined.

We had the opportunity to do a euglycaemic clamp study [with stable isotope infusion] on a 55 year old man who had an IGF-2 producing retroperitoneal haemangiopericytoma. He presented with recurrent hypoglycaemia [ $2.5$  mmol/L] and was shown to have significantly raised IGF-2 levels. His C peptide and serum Insulin levels were normal. During the clamp study we measured the amount of glucose infusion needed to maintain euglycaemia, hepatic glucose production and peripheral glucose utilisation. We also looked at the effects of IGF-2 on IGFBP's, free fatty acids and counter regulatory hormones. We compared the patient's results with those of normal sedentary males who also went on to have low dose [ $0.3$  mU/kg] insulin and high dose insulin [ $1.5$  mU/kg].

We conclude that IGF-2 leads to an increased peripheral glucose uptake in different tissues as well as inhibition of hepatic gluconeogenesis and lipolysis. We also discuss the IGF-2 effects compared to that of low dose and high dose insulin.

## P221

### Interleukin 6 and transforming growth factor beta1 concentration in serum in patients with coronary vessels stenosis

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Cytokines play a role in cardiovascular diseases by inducing inflammation, cell proliferation and apoptosis. It is well known that activated macrophages are one of the main sources of inflammatory cytokines. Previous studies have shown that the cells found in the vascular wall produce various kind of cytokines which are involved in cardiovascular diseases. It has been reported that some cytokines (IL-6, IL-8) are increased in acute coronary syndrome. The results of some other studies have suggested that PTCA induces inflammatory response in the circulation.

In the present study we investigated changes in concentration of several cytokines that are suspected to be involved in cascade mechanism after vascular injury; IL-6, a multifunctional cytokine playing a central role in inflammation and tissue injury and TGF beta which may influence atherogenesis and has a dominant protective role on coronary arteries.

We studied 36 patients with myocardial infarction who underwent stent implantation following PTCA. They underwent control coronarography after 12 months and they have been examined for lumen loss. During this follow-up the IL-6 and TGF-beta1 plasma concentration was measured in peripheral blood sample. Patients with decompensated heart failure, renal failure, hepatic failure. All patients gave informed consent. IL-6 and TGF beta concentration were measured in duplicate by ELISA using commercially available kits (R&D System). The lower limits of detection of IL-6 and TGF beta1 were  $0.7$  pg/ml and  $7.0$  pg/ml respectively. Data are expressed as mean (SEM). Comparison between groups were analysed using unpaired Student's *t* test. Additionally U Mann-Whitney test and Spearman rang correlation test were used.  $P < 0.05$  were considered significant. Correlation between IL-6 and lumen loss and artery stenosis were established after one year follow-up. There were significant correlation between IL-6 concentration and lumen loss and there was no significant correlation between TGF beta 1 plasma concentration and artery stenosis.

## P222

### Immunosuppressive effect of recombinant human erythropoietin

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Erythropoietin (EPO) is a glycoprotein hormone produced in kidneys in response to tissue hypoxia and is the main regulator of red blood cell (RBC) production in the body as well. New studies show the effects of EPO on



immune system. In this study the antihuman leukocyte (anti-HLA) antibody titer were determined in five groups of rats, which were sensitized with human lymphocyte. Also, the effects of stimulation's frequency and dose of recombinant human erythropoietin (rHuEPO) on anti-HLA antibody titer were studied. Two groups of rats received 20 and 100 IU/Kg rHuEPO respectively, after twice sensitization with human lymphocyte. The other two groups were given 20 and 100 IU/Kg rHuEPO, but after three times sensitization with human lymphocyte. Control group did not receive rHuEPO. Microlymphocytotoxicity method was used to detect anti-HLA antibodies. The results show that the anti-HLA antibody titer has been decreased significantly compared to control group. This statistically significant decrease was seen in groups which received 100 IU/Kg rHuEPO and also in those which received 20 IU/Kg after 2 antigenic stimulations. This could be due to the effects of rHuEPO on the number or the activity of B cells and T cells. Moreover, the dose of rHuEPO, length of treatment and the level of sensitization with human lymphocyte might affect anti-HLA antibody titer.

## P223

### Low dose dexamethasone does not alter serum adiponectin or resistin concentrations

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#### Background

Adiponectin and resistin belong to the group of adipocytokines, that are thought to be involved in regulation of insulin sensitivity, and that also exert the opposite effects on inflammatory processes related to atherosclerosis and cardiovascular risk. Glucocorticoids have anti-inflammatory properties, but also increase insulin resistance. There are, however, conflicting data on the effects of glucocorticoids on serum concentrations of adiponectin and resistin.

#### Material and methods

We assessed serum adiponectin, resistin, fasting glucose and insulin in 17 subjects (14 female), age:  $42.53 \pm 16.41$  years (mean  $\pm$  SD), BMI:  $37.88 \pm 8.53$  kg/m<sup>2</sup>, before (day 0), after 24 hours (day 1), and after 48 hours (day 2) of oral administration of dexamethasone (0.5 mg every 6 hours for 48 hours). This dose of dexamethasone is used during the low dose suppression test intended to rule out Cushing's syndrome.

#### Results

There was no significant change of adiponectin ( $12992.5 \pm 8172.3$  ng/ml,  $14607 \pm 10464.4$  ng/ml,  $13420.7 \pm 8344.2$  ng/ml on day 0, 1 and 2, respectively,  $P = ns$ ), or resistin ( $30.56 \pm 10.99$  ng/ml,  $35.12 \pm 16.43$  ng/ml,  $30.18 \pm 11.99$  ng/ml on day 0, 1 and 2, respectively,  $P = ns$ ). Insulin resistance assessed by HOMA and QUICKI models increased from day 0 to day 2 ( $P < 0.05$ ). Both HOMA and QUICKI models were equally effective in detecting the changes in insulin sensitivity ( $r = 0.99$ ,  $P < 0.001$ ).

#### Conclusions

Administration of this dose of dexamethasone (2 grams per day for 48 hours) does not significantly change serum adiponectin and resistin concentrations in overweight and obese individuals despite an increase in insulin resistance assessed by HOMA and QUICKI models. It remains to be established, whether higher doses of glucocorticoids may affect serum concentrations of these adipocytokines.

## P224

### Interleukin-6 and TNF-alpha in hemodialysis patients: association with obesity and metabolic syndrome

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Recent studies revealed that the elevated tumor necrosis factor-alpha (TNF-alpha) and interleukin-6 (IL-6) serum levels were associated with body fat, especially visceral adiposity, in obese healthy subjects. We investigated the relationship between circulating TNF-alpha and IL-6, parameters of body fatness, and the metabolic syndrome in chronic renal failure maintenance hemodialysis patients.

#### Methods

We explored the relationships between fatness and visceral obesity parameters [by anthropometry and bioelectrical impedance analysis] and adipocytokines TNF-alpha and IL-6 by enzyme-linked immunosorbent assay, and examined their associations with components of metabolic syndrome, in 56 [37 female (66%) and 19 male (34%)] patients on chronic maintenance hemodialysis, with median 48 months (IQR 24.5-82.0) dialysis duration.

#### Results

According to tertiles of total body fat percents we selected 28 well-nourished or middle-malnourished ( $12.5 < \text{FAT\%} \leq 25.6$ ) and 28 overweight or obese ( $\text{FAT\%} > 25.6$ ) HD patients for our study. The prevalence of metabolic syndrome with higher value of IR-HOMA was present in 16 (57%) overweight and in 12 (43%) nonobese HD patients. Levels of circulating TNF-alpha [ $221.66(110.50-332.16)$  pg/ml vs.  $181.87(90.30-272.70)$  pg/ml,  $P = 0.04$ ;  $247.32(123.48-481.14)$  pg/ml vs.  $212.46(58.90-338.58)$  pg/ml,  $P = 0.75$ ] were higher in patients with metabolic syndrome than without it, but only in obese group. In multivariate regression analyses  $\text{FAT\%}$  and leukocyte number were independent predictors of the circulating TNF-alpha levels (Adjusted  $R^2 = 0.48$ ,  $P = 0.02$ ).

#### Conclusion

Our results showed that circulating levels of TNF-alpha was related with metabolic syndrome in overweight HD patients. General adiposity, as a strong inflammatory stimulus and total white blood cell count, as participant of low-grade inflammation, explained 48% of variation in circulating TNF-alpha levels in overweight hemodialysis patients.

## P225

### Vegf and sVCAM in patients with adrenal gland tumors

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The study presents the outcome of determination of serum VEGF and sVCAM in 58 patients (43 women and 15 men aged 20–76 years) with adrenal tumors: adrenal cortical adenoma (34), adrenal cortical carcinoma (12), pheochromocytoma (9), myelolipoma (2) and lymphangioma (1). Control group comprised 25 practically healthy volunteers (13 women and 12 men aged 17–67 years). Serum VEGF was measured by enzyme immunoassay using reactants from "R&D" (USA), sVCAM – "BenderMedSystems" (Austria). In patients with adrenal tumors mean VEGF was significantly higher ( $304.3 \pm 31.0$  pg/ml) than in the control ( $150.8 \pm 22.7$  pg/ml) ( $P = 0.002$ ). No differences in serum sVCAM in patients ( $635.7 \pm 37.8$  ng/ml) and in the control ( $615.7 \pm 54.3$  ng/ml) were found. Direct correlation between VEGF level and patient's age was revealed ( $r = 0.42$ ,  $P = 0.001$ ). The highest serum VEGF ( $474.4 \pm 87.9$  pg/ml) and sVCAM ( $688.2 \pm 87.7$  ng/ml) were found in adrenal cortical carcinoma patients. VEGF were markedly elevated in patients with advanced stage of the adrenal cortical carcinoma as compared to patients with early stage. In patients with nonfunctional adrenal cortical carcinoma serum VEGF ( $594.5 \pm 147.0$  pg/ml) was higher than patients with Cushing's syndrome ( $410.5 \pm 72.4$  pg/ml). No association between serum sVCAM and tumor stage was revealed in adrenal cortical carcinoma patients. In patients with adrenal cortical adenoma mean VEGF and sVCAM comprised  $244.4 \pm 26.3$  pg/ml and  $611.8 \pm 64.4$  ng/ml, accordingly. Direct correlation between VEGF level and tumor size was found in patients with aldosterone-producing adenoma ( $r = 0.44$ ,  $P = 0.015$ ). Mean VEGF and sVCAM in pheochromocytoma's patients comprised  $319.8 \pm 105.4$  pg/ml and  $542.1 \pm 66.4$  ng/ml, accordingly. No correlations between serum VEGF, sVCAM and catecholamines were found in these patients. VEGF and sVCAM can play the role in the pathogenesis of adrenal tumors.

## P226

### Prolactin deficiency is independently associated with reduced IGF-I status in severely GHD adults

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#### Background

Considerable overlap of IGF-I levels exists between normal subjects and those with severe GHD determined by conventional testing of GH status.

Recognised factors influencing IGF-I status in GHD patients include age, gender, timing of onset of GHD and exogenous oestrogen therapy, but these do not fully explain the GH/IGF-I discordance in severe GHD. The primary structures of prolactin and GH are similar. Effects of hypoprolactinaemia are not well described in humans but laboratory studies have suggested a role for prolactin in hepatic IGF-I release, possibly through the STAT5B pathway. A potential impact of prolactin status on IGF-I status in severely GHD adults is investigated here.

#### Patients and methods

The study was approved by the hospital and local research ethics committees. Using multiple regression analysis techniques the contributions of the following variables to age-adjusted IGF-I SDS were evaluated in 162 (86 female, 76 male) GHD adults; Gender, timing of onset of GHD (childhood: CO or adult: AO), presence or absence of prolactin deficiency, BMI, number of additional pituitary deficits and underlying pathology.

#### Results

Timing of onset of GHD ( $P < 0.0001$ ) and presence of prolactin deficiency ( $P < 0.0001$ ) were independently associated with reduced IGF-I status. The contributions of CO-GHD and prolactin deficiency to IGF-I SDS were  $-2.64$  and  $-2.36$  respectively. Gender ( $P = 0.07$ ), BMI ( $P = 0.99$ ), number of additional pituitary deficits ( $P = 0.65$ ) and underlying pathology ( $P = 0.06$ ) did not significantly influence IGF-I status.

#### Conclusions

Prolactin deficiency is independently associated with reduced IGF-I status in hypopituitary adults with GHD. The prolactin deficiency may simply reflect the severity of the GHD, implying a GHD paradigm undetected by conventional GH provocative tests, or alternatively that pituitary prolactin contributes to IGF-I release in GHD, possibly through the STAT5B pathway.

## P227

### GH/IGF-I axis and haematological alterations in anorexia nervosa

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Anorexia nervosa (AN) is a psychiatric disorder featuring multiple endocrine abnormalities, comprising a decrease in IGF-I production and an increase in GH concentrations. This resistance to GH action may contribute to impaired haematopoiesis in AN as GH/IGF-I modulate blood cell proliferation and GH therapy exerts beneficial effects on hematocrit values in GH deficient patients.

Aim of the present study is to evaluate the link between GH/IGF-I status and haematologic parameters in anorexia nervosa.

#### Patients and methods

We studied 10 patients (9 women, 1 man, age range 15–38 years, BMI range 11.5–16.4 kg/m<sup>2</sup>) affected by active restrictive eating disorder. Haematologic parameters including serum iron, transferrin, erythropoietin, folic acid, vitamin B12, reticulocytes were measured in all patients and correlated with baseline GH and IGF-I concentrations. Results. As expected, IGF-I values were low ( $66.3 \pm 12.84\%$  of age range) and GH concentrations increased ( $5.2 \pm 1.57$  ng/ml, normal  $<4$ ). Haemoglobin and leukocyte counts were on average low-normal ( $12.1 \pm 0.34$  g/dl and  $3.400 \pm 0.22$ /mm<sup>3</sup>, respectively) with 4 patients presenting frank anemia (Hb  $< 11.0$  g/dl) and 7 leukopenia ( $< 4.100$ /mm<sup>3</sup>). However, erythropoiesis was not activated (normal erythropoietin and reticulocytes) and iron, folic acid or vitamin B12 stores were preserved. BMI was a significant determinant of IGF-I levels ( $r = 0.63$ ,  $P < 0.005$ ) whereas no correlation was detected between BMI and GH or between GH, IGF-I, BMI and parameters of erythropoiesis. Interestingly, leukocyte counts were significantly correlated with both BMI and IGF-I at covariance analysis ( $r = 0.7$ ,  $P < 0.05$ ).

#### Conclusions

No clear correlation between GH/IGF-I axis and erythropoiesis was detected in our series, suggesting that IGF-I is not a primary determinant of red blood cell hypoplasia in AN. Conversely, IGF-I was positively correlated with leukocyte counts and BMI. This is an additional, important overlap between endocrine and nonendocrine alterations in AN which warrants further investigations on a larger series of patients.

## P228

### Selected hormones serum levels in dependence on BMI and sex

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#### Objectives

To investigate the influence of body weight and sex on serum levels of insulin, leptin, ghrelin, IGF1, adiponectin and TSH.

#### Methods

Serum levels of insulin, IGF1, TSH and ghrelin were measured by RIA, leptin by IRMA and adiponectin by ELISA in 90 individuals (42 women, average age  $49.8 \pm 8.9$  years (48 men, average age  $49.1 \pm 6.6$ ). The sample was divided into three subgroups: normal weight (BMI = 15.5–24.9), excess weight (BMI = 25.0–29.9) and obesity (BMI  $> 30$ ). Results were correlated to BMI, to each other and compared with the endothelium function. The Ethical Committee approval was obtained for the research in 2002.

#### Results

Serum levels of measured hormones were influenced by body weight in both sexes. The influence was stronger in the case of insulin, leptin and adiponectin in women and in the case of IGF1 in men. Serum levels of ghrelin were not influenced by sex. The highest serum levels of insulin and leptin and the lowest levels of adiponectin, ghrelin and IGF1 were found in individuals with BMI over 30. Positive correlation was confirmed between leptin and insulin ( $r = 0.504$ ,  $P = 0.001$ ). Negative correlation was observed between adiponectin and insulin ( $r = -0.377$ ,  $P = 0.02$ ) and between adiponectin and leptin ( $r = -0.265$ ,  $P = 0.02$ ). Subjects with endothelium dysfunction had higher BMI, insulin and leptin and lower ghrelin, adiponectin and IGF1. No correlation was found between TSH and BMI, sex, other examined hormones and the endothelium function.

#### Conclusions

Higher levels of leptin and lower levels of adiponectin may increase the level of insulin. Higher levels of leptin lead to the decrease of ghrelin, which may enhance oxidative stress. Insulin resistance and obesity are main risk factors of the development of metabolic syndrome and process of atherosclerosis. Leptin, ghrelin, adiponectin and IGF may be considered as markers of metabolic syndrome and connected complications.

## P229

### Abnormalities of humoral immunity with reduced immunogenicity to pneumococcal vaccine in panhypopituitarism

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Although prolactin is well recognised as a cytokine, effects of hypoprolactinaemia on biological endpoints are not well described in humans. In-vitro and clinical studies suggest an influence on humoral and cell mediated immunity and autoimmunity. We studied basal and stimulated immune function in panhypopituitary adults with and without prolactin deficiency and age and gender matched healthy controls. The study was approved by hospital and local research ethics committees. Nine hypopituitary patients with prolactin deficiency (5 female, age  $52.0 \pm 15.2$  yrs; group 1), 12 matched hypopituitary subjects of normal prolactin status (4 female, age  $46.5 \pm 13$  yrs; group 2) and 12 matched volunteers (5 female, age  $47.5 \pm 17.6$  yrs; group 3) were studied. Baseline humoral immunity, pneumococcal titres (9 strains) 1 month after vaccination, T cell numbers and function using in-vitro response to gamma interferon and cross-sectional immunity to T cell dependent antigen tetanus toxoid were studied.

Absolute CD19+ cell numbers were reduced in group 1 ( $236.5 \pm 124$ ) and elevated in group 2 ( $432.8 \pm 209.3$ ), compared with group 3 ( $275 \pm 115$ ),  $P = 0.02$ . CD19+ correlated with prolactin level in the whole cohort ( $R = 0.44$ ,  $P = 0.01$ ). T cell numbers, immunoglobulins and T cell function were comparable between groups. Susceptibility for invasive pneumococcal infection one month after vaccination remained in 5 of 9 patients (55.6%) in groups 1 and 4 of 12 (33.3%) in group 2, compared with 100% immunogenicity in group 3, ( $P = 0.01$ ; comparison between groups 1&2 versus 3). Pattern of response (post versus pre-vaccination contrast in pneumococcal antibodies) was similar between groups with a proportionately attenuated response in groups 1 and 2. Immunity to tetanus toxoid was protective in all subjects ( $> 0.01$  IU/mL). Abnormalities of humoral immune response are present in hypopituitary adults. CD19 expression appears to correlate positively with prolactin level, in keeping with previous data linking prolactin and autoimmunity. Panhypopituitarism is also associated with reduced response to pneumococcal vaccine.

**P230****Lack of influence of an IGF-I gene polymorphism on circulating IGF-I levels in severely GHD adults**

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Considerable overlap in IGF-I levels exists between normal and severely GHD subjects; the mechanisms underlying this observation remain poorly understood. Under various clinical circumstances therefore, IGF-I estimation is an unreliable marker of GH status. A polymorphism in the IGF-I gene influences IGF-I levels in normal populations; a higher IGF-I is associated with the presence of 2 wild type alleles of the IGF-I gene (WTG). The possibility that an IGF-I gene polymorphism could contribute to overlap of unstimulated IGF-I levels between normal and severely GHD adults is investigated.

The study was approved by the local research ethics committee. IGF-I genotype was determined in 128 (72 female, age range 18–82 years) severely GHD adults. Using multiple regression analysis, contributions of IGF-I genotype and the following variables capable of affecting age-adjusted IGF-I in severe GHD were evaluated; gender, timing of onset of GHD, presence or absence of prolactin deficiency, BMI, number of additional pituitary deficits and underlying pathology.

Ninety-eight (76.6%) and 30 (23.4%) subjects possessed WTG and variant IGF-I genotype (VG) respectively. Eighty-seven (68%) had adult-onset GHD. Median IGF-I level was 114.0 µg/l and 121.5 µg/l in WTG and VG respectively ( $P = 0.45$ ). There was no significant difference in weight and height between subjects with WTG and VG. Mean BMI was  $29.4 \pm 7.22$  and  $25.7 \pm 5.75$  for WTG and VG groups respectively ( $P = 0.01$ ). No independent contribution of IGF-I genotype ( $P = 0.78$ ), gender ( $P = 0.38$ ) BMI ( $P = 0.48$ ) and number of additional pituitary deficits ( $P = 0.9$ ) to IGF-I status were detected.

The higher IGF-I level associated with the presence of IGF-I WTG appears lost in severe GHD, consistent with the disturbed GH/IGF-I secretory physiology associated with this condition. Although the confidence intervals are wide for this observation, the presence of factors other than IGF-I genotype, appear to have a greater influence on IGF-I status in severely GHD adults.

**P231****Serum TGF-β-1 levels in non-alcoholic steatohepatitis**

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**Aim**

Non-alcoholic steatohepatitis is one of the most common causes of chronic liver disease and the incidence in the population varies from % 10–24. TGF-β – 1 is the most important TGF- β isoform responsible for fibrosis and its effect on hepatic fibrosis was demonstrated in-vitro and in chronic viral hepatitis. Besides TGF- β is an immunosuppressive cytokine and it is known that low TGF- β activity can trigger autoimmune diseases and inflammation. However, studies about serum TGF- β -1 levels in patients with NASH failed to show a direct relationship between NASH and TGF- β -1. The aim of this study is to demonstrate serum TGF- β -1 levels in NASH and to determine the possible correlations between serum TGF-β-1, and other laboratory and anthropometric parameters.

**Materials and methods**

31 patients with NASH (diagnosed with liver biopsy) and 31 healthy subjects were included. The mean age of the patient group (17 men, 14 women) and healthy subjects (17 men, 14 women) was 51.7 and 50.6 respectively. The metabolic, anthropometric and laboratory parameters were recorded.

**Results**

The mean body mass index of the patient group was significantly higher than the healthy subjects ( $P = 0.009$ ). The mean serum TGF- β -1 level of patients was significantly lower than the control group (8.17 ng/ml and 11.07 ng/ml respectively,  $P = 0.018$ ). In the patient group serum TGF- β 1 levels were found to be correlated with fasting insulin ( $r = 0.531$

$P < 0.05$ ). In the multiple regression analysis fasting insulin was found to be the most important parameter effecting serum TGF- β -1 levels in NASH. Conclusion

In the literature there is a small number of human studies present determining the serum TGF- β -1 levels in NASH and the results are contradictory. In our study in spite of their high BMI the patient group had low serum TGF- β -1 levels. It is known that TGF- β has immunosuppressive properties and low TGF-beta activity can trigger autoimmune diseases. It was demonstrated that TGF- β knock-out mice developed multi-organ inflammatory changes. That's why the low serum TGF- β -1 levels in NASH may be responsible as a trigger for the inflammatory process.

**P232****Subclinical hypothyroidism could be regarded as benign condition in oxidative stress but not in atherosclerosis**

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**Introduction**

Whether subclinical hypothyroidism (SCH) is related to risk for cardiovascular disease is controversial. Inflammation and oxidation of lipoproteins play an important role in the progression and complications of atherosclerosis.

Therefore we aimed to evaluate the plasma levels of thiobarbituric acid reactive substances (TBARS) as a marker of oxidative stress, high sensitive CRP (hsCRP) and lipid profile as a risk factor for atherosclerosis in SCH before and after L-thyroxine replacement therapy, and compare to control group.

**Methods**

The study was performed in age- and sex-matched 26 subclinical hypothyroid patients and 27 healthy controls. Exclusion criteria were known atherosclerotic disease, diabetes, morbid obesity, familial hyperlipidemias, coagulation disorders and severe systemic diseases. The patients, who were treated with lipid lowering drugs, estrogen and acetylsalicylic acid, were not enrolled in the study. Blood samples were obtained from patients with SCH before levothyroxine replacement, and one month after achieving a euthyroid state with levothyroxine.

**Results**

SCH patients had higher hsCRP levels than control group at the beginning (4.28 mg/L vs 1.88 mg/L;  $P = 0.018$ ). High levels of hsCRP were negatively correlated with free thyroid hormone levels ( $P = 0.026$  for FT<sub>3</sub> and  $P = 0.021$  for FT<sub>4</sub>). After achieving euthyroid state with levothyroxine replacement, hsCRP levels decreased significantly in patients with SCH (4.28 vs 2.32;  $P = 0.006$ ). In our study, the L-thyroxine treatment in doses which normalize TSH secretion was not associated with significant changes in serum lipid profile ( $P > 0.05$ ). TBARS levels of SCH patients before and after restoration of euthyroidism were similar to control group ( $P > 0.05$ ).

**Discussion**

Our findings suggest that hsCRP values increase in patients with subclinical hypothyroidism, and may count as an additional risk factor for the development of atherosclerosis and cardiovascular disease. Normalization of thyroid state by levothyroxine replacement seems to effectively reduce serum hsCRP levels in subclinical hypothyroidism without any correlation with TBARS activity and with similar lipid levels in SCH.

**P233****Low TGF beta-1 serum levels in idiopathic male osteoporosis**

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**Introduction**

Although the etiology of osteoporosis is different between men and women, the underlying pathophysiologic mechanism is similar, namely an absolute or relative increase in bone resorption, leading to progressive bone loss. Transforming growth factor (TGF) beta-1 is a growth factor in



human bone, which is produced by osteoblasts and inhibits osteoclast proliferation and activity and stimulates proliferation and differentiation of preosteoblasts.

The aim of our study was to determine serum TGF beta-1 levels in male patients with idiopathic osteoporosis.

#### Methods

Twenty five male with idiopathic osteoporosis and 25 age-matched controls were studied. Osteoporosis was defined by a T score  $< -2.5$  in the lumbar spine or at the femoral neck. Exclusion criteria were known atherosclerotic disease, diabetes, morbid obesity, familial hyperlipidemias and severe systemic diseases. The patients, who had any secondary cause of osteoporosis or other metabolic bone diseases, were not enrolled in the study. We measured levels of TGF beta-1, estradiol and bioactive testosterone. Various markers of bone remodeling were also measured.

#### Results

TGF beta-1 was significantly lower in the osteoporotic patients than in controls ( $4.6 \pm 3.08$  vs  $8.97 \pm 4.4$ ;  $p = 0.000$ ). Moreover, TGF beta levels were negatively correlated with bone mineral density (BMD) at the lumbar spine ( $r = -0.41$ ,  $P = 0.042$ ) and at the femoral neck ( $r = -0.44$ ,  $P = 0.028$ ). No correlation was found between serum estradiol, bioactive testosterone and TGF beta-1 levels.

#### Discussion

Our study suggests that TGF beta may play a key role in male patients with idiopathic osteoporosis. Serum TGF beta-1 levels are decreased in osteoporotic men and negatively correlated with hip and spine BMD. Serum TGF beta-1 levels appears to have potential as a marker for osteoporosis.

### P234

#### Possible predictive value of IGF-II mRNA expression in breast cancer (BC)

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Insuline like growth factor II (IGF II) is an important regulator of neoplastic growth and it is stromal in origin. Estrogen and Progesterone receptor (ER, PR) are considered a good prognostic predictors in BC. The aim of the study was to evaluate the impact of IGF II mRNA expression in the clinical outcome in breast malignancy. The study group included 75 women (mean age  $\pm$  SD =  $53.3 \pm 15.6$  yr) submitted to radical mastectomy for ductal infiltrating breast carcinoma. The BC specimens were assessed for IGF II mRNA using *in situ* hybridization method and ER and PR by immunohistochemistry. Five years clinical follow-up was available in 65/79 BC (82.3%) and 46/65 (70.8%) were still alive and relapse free. ER + was found in 39/65 (60%), PR + in 30/65 (46.2%) and stromal IGF II mRNA expression (IGF II +) in 33/65 (50.8%). 22/65 (33.8%) BC were IGF II + ER + and 19/65 (29.2%) IGF II + PR +. No relationship was found between ER, PR, IGF II separately examined and clinical outcome. The better 5yr survival was found in ER + IGF II + (16/22: 72.7%) and IGF II + PR + BC (14/19: 73.7%) and in contrast, the worse survival was found in IGF II + ER - (6/11: 54.5%) and IGF II + PR - (57.1%) groups, ( $P = 0.006$ ,  $P = 0.02$ , respectively). These data indicate that stromal IGF II may be considered a new important predictive factor in BC.

In particular IGF II have a good prognostic value in ER + /PR + and in contrast the poor predictive significance in ER - /PR - BC. These findings indicate that IGF II may have an important role in the differentiation or proliferation of BC cells.

### P235

#### IGF II protein expression in breast cancer. Relationship with the most important predictive parameters in breast malignancy

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Recent *in situ* hybridization data showed high content of IGF-II mRNA in the stroma of breast cancer. The aim of this study was to examine the expression of IGF-II protein and its relationship with several prognostic parameters in 75 ductal infiltrating carcinoma (DIC) of the breast.

The tissue sections were evaluated for IGF II protein, proliferating activity, ER, PR, p53, P21 oncogene expression using immunohistochemical procedures. The amount of the stromal proliferation was assessed in all cases. The menopausal status, the axillary nodes involvement and the nuclear degree were known.

Thirty-five patients (35/75: 44.3%) were pre-menopausal and 47 (62.6%) had node metastases. Marked stromal proliferation was found in 34 (45.3%) specimens and high nuclear grade in 20 (26.5%). Eighteen tumors (18/75: 24%) showed no IGF-II immunostaining. In the positive cases, IGF-II was detected in the stroma as well as in the cytoplasm of epithelial cancer cells: marked IGF-II content was found in 12 specimens (12/75: 16.0%), slight in 14 (18.7%) and moderate in 31(41.3%). Twenty-four tumors (24/75: 32.0%) showed high proliferating activity. Both ER and PR were expressed in the nucleus of cancer cells: 49/75 DIC (65.3%) were ER positive (ER +) and 34 (45.3%) PR positive (PR +). p21 protein was detected in 37 tumors (37/75: 49.6%) and p53 in 12 (16%).

IGF-II protein was not related with menopausal status, lymph-node metastases, nuclear grade, proliferating activity, ER and p53 oncogene. In contrast, IGF-II was strongly correlated with stromal proliferation ( $P < 0.001$ ), with PR ( $P:0.04$ ) and with p21 oncogene ( $P:0.01$ ).

The results of this study demonstrate that in DIC of the breast IGF-II protein is expressed in the epithelium as well as in stroma of the majority of tumors and that it is correlated with stromal amount, with PR and p21 oncogene expression. These findings indicate that in breast cancer, IGF-II expression is connected with the stromal proliferation and with two important regulators of breast cancer growth and differentiation.

### P236

#### The effect of growth hormone replacement on the diurnal and ultradian dynamics of ghrelin and leptin in adult growth hormone deficiency

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Rhythmic, timed secretion of ghrelin and leptin is integral to the intricate temporal interplay of hormonal signals that maintains energy balance, feeding and body weight. Growth hormone replacement (GHR) in adult growth hormone deficient (AGHD) patients has been shown to alter body composition, energy balance and insulin sensitivity with changes in leptin secretory pattern and concentration. With GHR, single time point ghrelin concentrations have been shown to either increase, decrease or not change. Therefore, we studied the secretory pattern of ghrelin prior to and following GHR in AGHD and its relationship with leptin.

#### Methods

Blood samples were collected for ghrelin and leptin at half-hourly intervals over 24 h in 4 AGHD patients (2 men) prior to, then 1, 6 and 12 months following GHR. Circadian rhythm analysis (chronolab), pulse analysis (ULTRA) and cross correlation analyses were performed.

#### Results

Circulating ghrelin and leptin exhibited significant circadian rhythms ( $P < 0.001$ ) and ultradian pulsatility prior to and following GHR. Ghrelin concentrations were higher during the day (acrophase range - 16:30 to 17:40) and were out of phase with leptin which demonstrated higher nocturnal concentrations. Maximum negative cross-correlation coefficient was observed when changes in leptin concentration preceded changes in ghrelin by half an hour ( $r = -0.6$ ). GHR had significant effects on the day-time and nocturnal concentration changes of both leptin and ghrelin as well as an increase in leptin pulsatility. Ghrelin secretion also showed an ultradian pulsatility with a mean of  $7.3 \pm 1.3$  pulses/24h in untreated AGHD but with no significant change following GHR.

#### Discussion

GHR has significant effects on the secretory rhythm of both leptin and ghrelin which may contribute to the restoration of their organised, coordinated and timed secretion and inter-communication needed to favourably change and maintain energy balance, body composition and insulin sensitivity on a daily basis in treated AGHD patients.



**P237**

**Growth hormone receptor extracellular domain linked to glycerophosphatidyl inositol (GHR-GPI); a potential growth hormone receptor antagonist**

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**Introduction**

The growth hormone receptor (GHR) consists of 620 residues and belongs to the class I cytokine receptor family. It is a single membrane spanning protein that binds its ligand, GH, via the extracellular domain. GH binding to GHR induces a conformational change in the preformed receptor dimer, which leads to intracellular signalling. Correct functional dimerisation of two GHR molecules is essential for GH signalling. We have previously shown that membrane bound truncated extracellular GHR can act as a dominant negative inhibitor of GHR signalling. We have now generated a GHR extracellular molecule linked to a GPI lipid moiety, to insert into membranes, in order to produce a potential cytokine receptor antagonist.

**Methods**

The GHR-GPI was cloned and expressed in *Dictyostelium discoideum* strain AX-2, generating  $5 \times 10^6$  amoebae ml<sup>-1</sup> in the late exponential phase of growth. The harvested cells were used to produce cell membranes containing GHR-GPI. The membranes were then solubilised using n-octylglucoside and the GHR-GPI purified using affinity chromatography involving GH covalently linked to activated sepharose. Elutions were analysed by western blotting to determine the presence of GHR-GPI and assayed using the Bradford protein assay. Efficiency of insertion of GHR-GPI into cell membranes was measured using flow cytometry techniques.

**Results**

Western blotting results showed that the GHR-GPI containing membranes could be extracted, solubilised and the GHR-GPI protein purified. Flow cytometry analysis showed that the GHR-GPI protein inserted into the cell membrane to high levels.

**Conclusions**

The GHR-GPI protein can insert into cell membranes, where it may potentially disrupt the preformed GHR dimer, and theoretically block GH signalling.

**P238**

**The depot specific expression of NPY in human adipose tissue and its upregulation under hyperinsulinaemic conditions in isolated human adipocytes**

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Neuropeptide Y (NPY) is a centrally expressed peptide with potent orexigenic properties, released post-prandially to stimulate appetite, regulated by insulin and altered by obesity. NPY is expressed in adipose tissue which suggests it may have a potential effect on altering appetite control in obesity. To date no study has investigated NPY and its regulation in human adipose tissue. Therefore, the aims of this study were to 1) determine the depot-specific expression in abdominal and gluteo-femoral fat 2) identify the adipocyte as a source of NPY in adipose tissue and 3) examine whether *in vitro* NPY secretion is stimulated by hyperinsulinaemic conditions. For this study, human adipose tissue was isolated from patients undergoing elective surgery (age  $\pm$  SD:  $42.7 \pm 1.5$  yr; BMI  $\pm$  SD:  $26.2 \text{ Kg/m}^2 \pm 0.7$ ; n = 38), with local ethics approval. Western blot analysis was then used to determine depot specific NPY expression. Furthermore, isolated human abdominal subcutaneous (AbdSc) adipocytes were treated with insulin (1-1000 nM) for 48 hours and NPY levels in the conditioned media measured via ELISA. The *ex vivo* studies demonstrated that NPY protein expression in AbdSc ((AbdSc) n = 20) was approximately 2 fold higher than both omental ((Om) n = 8) and thigh (n = 7); (AbdSc:  $1.87 \pm 0.23$ , Om:  $1.03 \pm 0.15$ , thigh:  $1.0 \pm 0.29$ , P = 0.029 and P = 0.035, respectively). Western blotting also determined the presence of NPY protein within isolated AbdSc adipocytes, which was confirmed by immunohistochemical analysis. Analysis of NPY secretion (n = 14) identified that insulin significantly increased NPY secretion (P < 0.05). In summary, our present findings show that NPY is differentially expressed in human adipose tissue, with AbdSc demonstrating the highest levels. Further we have shown that NPY is secreted by isolated adipocytes and stimulated by insulin. In conclusion, NPY is expressed and secreted in human adipose

tissue. Therefore increased NPY secretion from adipocytes, due to elevated insulin concentration, may contribute to the link between hyperinsulinaemia and continuing weight gain.

**P239**

**The role of BMPs in the establishment of zona glomerulosa in the adrenal gland**

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The adrenal gland is composed of the medulla (neurodermal origin) and the cortex (mesodermal origin). The cortex is further subdivided into three zones: zona glomerulosa (zG), zona fasciculata (zF) and zona reticularis (zR). The zones of the cortex are functionally characterised by their ability to synthesise different steroids and consequently they express different steroidogenic enzymes. These and other markers of the zones have been described but so far no good candidate for a determining factor of zonal establishment has been discovered. Bone morphogenetic proteins (BMPs) are multifunctional cytokines belonging to the transforming growth factor- $\beta$  (TGF- $\beta$ ) superfamily. In a microarray analysis of transcripts from the zG and zF, we have discovered that some BMPs are potentially zG specific. Various members of the BMP family and their receptors were further analysed by Real-Time PCR, using rat adrenal zG and zF tissues, and some showed preferential or exclusive expression in zG. These results were also seen in H295R cells (human adrenocorticocarcinoma cell line) after the cells had been differentiated into a zG (by Angiotensin II) and zF (by Forskolin) phenotype. These observations suggest a possible involvement of BMP signalling in the establishment of the adrenal zG. In order to investigate this further, we have influenced H295R differentiation by the addition of exogenous BMPs and blocked endogenous BMPs by siRNA. We have also confirmed the Real-Time PCR results by immunohistochemistry and Western blotting.

**P240**

**Anosmin-1: a novel cofactor for fibroblast growth factor receptor 1**

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**Introduction**

Autosomal dominant Kallmann's syndrome (AKS) results from mutations within the fibroblast growth factor receptor 1 (FGFR1: KAL-2). The FGFR family of tyrosine kinases are involved in a multitude of biological processes from embryogenesis to adult homeostasis. The crystal structure of FGF-FGFR-heparan sulphate (HS) ternary complex has provided a basis for understanding the way in which FGF, FGFR and HS cooperate to assist FGFR dimerization. Anosmin-1, KAL-1 gene product is mutated in X-linked KS. We have previously demonstrated that anosmin-1 can modulate neuronal differentiation of human embryonic olfactory neuroblast through the interaction with FGFR1/FGF2/HS complex to stimulate p42/44 and p38 MAP kinase and cdc42/Rac, acting as a novel modulator for FGFR1 signalling.

**Aim**

To understand the role of anosmin-1 in FGFR1/FGF2/HS ternary complex assembly.

**Methods**

ELISA was performed using immobilised murine FGFR1IIIc and decasaccharide derived from bovine lung heparin. The 6His-tagged wild type anosmin-1 and its three KS-associated missense mutations were used as soluble ligands. Bound anosmin-1 was detected by using anti-His HRP conjugated antibody. The solution structure of anosmin-1 was studied by analytical ultracentrifugation, X-ray scattering and homology modelling.

**Results**

Anosmin-1 binds to FGFR1IIIc in a dose-dependent manner, while BSA and three missense mutants do not. The presence of FGF2 and heparin does not change the binding of anosmin-1 to FGFR1IIIc. Anosmin-1 binds to oligosaccharide as short as the 10mer. The six domains of anosmin-1 are in an extended arrangement with flexible inter-domain linker.

**Conclusion**

Anosmin-1 can directly bind to FGFR1 with the requirement of its first and third FnIII domains and to oligosaccharide with only ten saccharide residues. The extended domain arrangement of anosmin-1 may provide the binding platform for multi-molecules of FGFR1/FGF2/HS complex; this

assembly presumably amplifies and synergises downstream FGFR1 signalling, thereby eliciting a receptor-specific cellular response within olfactory system ontogenesis.

## P241

### Effect of growth hormone deficiency in adults on insulin action and gene expression of selected adipokines in subcutaneous adipose tissue

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Growth hormone deficiency (GHD) in adults seems to predispose to insulin resistance (IR). Development of GHD related IR could be associated with adipose tissue (AT) derived signals. To investigate the nature of this pathological regulation we 1) measured gene expressions for leptin, adiponectin, TNFalpha, IL-6, 11beta HSD1, SREBP, PPARgamma in the AT of untreated GHD adults, and 2) assessed effect of euglycemic hyperinsulinemia (HI) at the gene level.

#### Methods

17 patients (9M/8F) aged 21-41 yrs with GH deficiency were studied. Controls (C) were matched with patients for BMI, age and sex. Subcutaneous (s.c.) AT biopsies were performed once after overnight fast before an oGTT, and 2nd time in the 3rd hr of an euglycemic HI clamp. mRNA levels were assessed using real-time RT-PCR.

#### Results

Compared to the matched controls, the GHD subjects did not differ in the *in vivo* insulin action and triglyceride levels. In the basal state GHD patients had raised RNA levels for leptin ( $P < 0.001$ ), TNFalpha ( $P < 0.05$ ) and SREBP ( $P < 0.05$ ) as well as for IL-6 ( $P < 0.05$ ) and 11beta HSD-1 ( $P < 0.05$ ). In both groups, euglycemic HI led to elevation of mRNA for SREBP ( $P < 0.001$ ) and PPAR gamma ( $P < 0.05$ ). Gene expression for leptin increased further in the GHD patients only.

#### Conclusions

(1) the whole body insulin-induced glucose utilization of GHD patients was not altered when compared to BMI, age and sex matched controls; (2) nevertheless, GHD patients had already raised s.c. AT gene expression for lipostatic /leptin/, proinflammatory /TNFalpha, IL-6/ and proadipogenic /11beta HSD-1, SREBP/ pathways in basal state, and (3) euglycemic HI increased gene expression for leptin only. Thus, changes in s.c. AT at the gene level are present in GHD subjects with features of the metabolic syndrome who seem not to be IR due to a GH deficit. Supported by STAA No. 51-0406/02.

## P242

### Soluble interleukin 2 receptor is elevated in sera of women with Graves' disease and has relation to serum free thyroxin and thyroid volume

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#### Introduction

Although some studies have shown elevated soluble interleukin 2 receptor (sIL-2R) in sera of patients with hematological malignancies and some autoimmune diseases, its role in serum is not fully understood.

#### Patients and methods

The aim was to compare serum levels of sIL-2R in four groups of women (11 with Graves' disease, 10 with Hashimoto's thyroiditis, 11 with breast cancer and 10 healthy controls) and to test their possible relationship to parameters of thyroid function (TSH, FT4), thyroid antibodies (against thyroid peroxidase-TPOAb, thyroglobulin-TgAb and TSH receptor-TRAK) and thyroid volume. Serum levels of sIL-2R were measured using a commercially available ELISA kit, the other parameters using routine laboratory methods. All subjects underwent thyroid ultrasonography (Phillips Envisor) with measurement of thyroid volume.

#### Results

Serum levels of sIL-2R were significantly higher in women with Graves' disease compared to Hashimoto's thyroiditis ( $1.46 \pm 0.50$  vs.

$0.833 \pm 0.184$  ng/ml,  $P < 0.001$ ), breast cancer ( $1.46 \pm 0.50$  vs.  $0.91 \pm 0.51$ ,  $P = 0.021$ ) and controls ( $1.46 \pm 0.50$  vs.  $0.81 \pm 0.21$ ,  $P = 0.016$ ). In the whole group, strong positive correlations between sIL-2R and FT4 ( $r = 0.688$ ,  $P = 0.0000357$ ,  $n = 42$ ) and sIL-2R and thyroid volume ( $r = 0.636$ ,  $P = 0.000363$ ,  $n = 42$ ) were found. No significant correlations were found between sIL-2R and TPOAb, TgAb and TRAK.

#### Conclusions

The close relation of sIL-2R to FT4 suggests that serum sIL-2R is rather the consequence of changes of thyroid function than thyroid autoimmunity. In contrast to hematological malignancies, sIL-2R was not elevated in sera of women with breast cancer.

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## P243

### Molecular mechanisms of idiopathic muscle hypertrophy in humans

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Understanding the molecular factors that regulate human muscle mass is acquiring increasing scientific interest. Hitherto, known (+ve) endocrine regulators of lean body mass included the GH-IGF-1 system, anabolic steroids, and (-ve) myostatin. In order to elucidate this process further, we have investigated a clinical case at a genetic and phenotypic level. A 46 year old man with idiopathic muscle hypertrophy requiring multiple therapeutic fasciotomies underwent clinical and biochemical (endocrine) assessment followed by molecular diagnostics of his muscle tissue including Affymetrix<sup>TM</sup> microarray expression profiling, using statistical modelling to identify high probability candidate genes.

#### Method

Using 2 different vastus lateralis biopsies, total RNA was extracted from flash frozen muscle tissue using standard techniques. Following one round amplification, labelled cRNA was hybridised to U133A array chips run in duplicate; having passed all the quality control steps according to MIAME (minimum information about a microarray experiment) guidelines. The expression profiling data ( $n = 4$ ) was further analysed using an unsupervised *in silico* model comparing the results with a database of >120 human skeletal muscle samples processed in an identical manner. Diagnostic categorisation was performed using a tree and nodal system.

#### Results

Clinical examination revealed a hypermuscular individual, of Ht 1.87 m Wt 141.8 kg and BMI of 41. Baseline biochemistry, CK, 09.00 testosterone, LH/FSH, multiple GH sampling, and IGF-1 estimation were normal, as was the GH response to an oral glucose challenge. Electromyography, immunohistochemistry and western blot analysis for dystrophic conditions were also normal. Statistical modelling revealed a unique gene expression signature, in a distinct category. Our data failed to demonstrate over-expression of anabolic factors e.g. IGF-1 or underexpression of myostatin. However, the microarray data suggested significant downregulation of SOCS-2 ( $P < 0.00001$ ), suggesting a novel, physiologically plausible, candidate gene for detailed onward investigation.

## P244

### CD4 T-cell count improves during sustained IGF-I response following low dose growth hormone therapy in HIV-infected patients on stable antiretroviral regimens. A pilot study

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**Objective**

High dose growth hormone (rhGH) regimens (2 to 4 mg/day) have been shown to increase circulating insulin-like growth factor (IGF)-I to supra-physiological levels in human immunodeficiency virus (HIV)-infected patients on combined antiretroviral therapy (CART). This setting may improve immunologic output. However, a high plasma IGF-I concentration has detrimental effects on glucose metabolism, which hampers the use of high dose rhGH regimens.

**Methods**

We have reported previously on a group of 6 HIV-infected patients on stable CART (>28 months), in whom 16 weeks on rhGH 0.7 mg/day increased total (+117%,  $P < 0.01$ ) and free (+155%,  $P < 0.01$ ) IGF-I to high in physiological range. This study was extended to examine whether continued use of low-dose rhGH (0.7 mg/day until week 60; 0.4 mg/day from week 60 to week 140) would keep IGF-I expediently high in the normal range and improve CD4 T-cell response.

**Results**

Total and free IGF-I remained expediently increased at week 36 (+97%,  $P < 0.01$  and +125%,  $P < 0.01$ ) and week 60 (+77%,  $P = 0.01$  and +125%,  $P < 0.01$ ) compared to baseline ( $161 \pm 15 \mu\text{g/L}$  and  $0.75 \pm 0.11 \mu\text{g/L}$ ). CD4 T-cell count, which was unchanged after 16 weeks ( $P > 0.7$ ), was increased at week 36 (+15%,  $P < 0.05$ ) and week 60 (+31%,  $P = 0.01$ ), compared to baseline ( $CD4 456 \pm 55 \text{ cells}/\mu\text{L}$ ). After rhGH dose-reduction, total IGF-I remained increased at week 88 (+44%,  $P = 0.01$ ) and week 140 (+46%,  $P = 0.07$ ) compared to baseline, whereas free IGF-I returned to baseline ( $P > 0.3$ ). CD4 cell count remained increased at week 88 (+33%,  $P < 0.01$ ) and week 140 (+36%,  $P = 0.02$ ) compared to baseline.

**Conclusions**

These data suggest that a low-dose rhGH regimen through a substantial increment in circulating IGF-I may improve immunologic response in terms of increased CD4 T-cell output in HIV-infected patients on stable CART. A well-powered, randomized, double-blind, placebo-controlled clinical trial is ongoing aiming to validate these preliminary data.

**P245****The differential influence of specific plant lectins on insulin and IGFs binding to solubilised placental membrane IGF and insulin receptors**

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The influence of the plant lectins on binding of IGF-I, IGF-II and insulin to the cognate receptors from solubilised human placental cell membranes was examined. The lectins (wheat germ agglutinin, WGA; concanavalin A, Con A; phytohaemagglutinin, PHA and *Sambucus nigra* agglutinin, SNA) were chosen according to their sugar specificity and were expected to bind to the most abundant saccharides present as terminal residues on N-glycan moieties of insulin receptor (IR) and IGF receptors (IGFRs): GlcNAc, Man, Gal and Sia. The lectin effects were tested using competitive ligand binding assays (CLBA) in which the binding of  $^{125}\text{I}$ -labelled insulin or IGFs (tracers) to solubilised receptors, as competed by unlabelled insulin and IGFs, was carried out in the absence or presence of the lectins. WGA, Con A and PHA exerted different effects on the binding of the different tracers to the receptors, ranging from an inhibition to a significant enhancement of binding. These effects were also lectin specific and they could be blocked by specific sugars. The effect of a particular lectin depended on the order of addition of the receptors, ligands and lectins. The competition curves had different shapes for different lectins indicating the different mechanisms involved in the change of receptor affinity. The results of CLBA were in accordance with the results obtained from the lectin affinity chromatography and electrophoresis, where multiple glycoforms of the IR and IGFRs were shown to exist. The data from this work strongly suggested the change in affinity and specificity of placental IR and IGFRs for the homologous and heterologous ligands, under the influence of specific plant lectins. The fact that some plant lectins can modulate the interaction of placental IR and IGFRs with their ligands may imply the existence of placental lectins of the similar sugar specificity with the same function.

**P246****GH and GH antagonist B2036 effect on the immune system**

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**Introduction**

Growth Hormone (GH) receptors are expressed on, and GH is secreted by, human peripheral blood mononuclear cells (PBMCs). However, the role of GH in immunity has remained elusive and studies have given conflicting results. Experimental data strongly implicates a role for pituitary GH in the immune response of rodents. The data from knockout mice suggests that GH acts not as an obligate immunoregulator but as an anabolic and stress-modulating hormone. Despite the clear evidence for GH immunomodulatory actions in animal studies, the evidence from humans has been contradictory.

**Aim**

To clarify the role of GH in the human immunophenotype and human PBMCs proliferation.

**Methods**

Blood from GH deficient patients and patients with acromegaly ( $n = 10$ ), prior to treatment, was collected and PBMCs were immunophenotyped by FACS analysis (CD3, CD4, CD8, B, NK, NKT, CD4CD45RA, CD4CD45RO, CD4CD25, CD8CD28, gamma/delta, CD8CD45RA, CD8CD45RO). To analyse the effects on immune cell proliferation, PBMCs of normal individuals were isolated by Ficoll and the effects of GH and the specific GH antagonist B2036 were analysed after proliferation activation with anti-CD3 antibody (OKT3).

**Results**

Surface markers on T lymphocytes (CD3, CD4, CD8), B lymphocytes (CD19) and NK cells (CD16/56) were normal in all acromegalic and all but one GH deficient patient. This single GH deficient patient showed only elevated NK cells percentage. Neither GH at low (25 ng/ml) to high (500 ng/ml) concentrations, nor the GH antagonist B2036 nor the combination had any effect on the OKT3-induced proliferation of PBMCs whether with maximal or submaximal doses of OKT3.

**Conclusions**

GH status in patients did not seem to significantly influence the immune phenotype. Neither exogenous nor endogenous GH production by PBMCs influenced their proliferation. The results suggest that in contrast to rodents, GH in humans does not act as a strong immunoregulator.

**P247****IGF-I modulates HIF-1 $\alpha$  and HIF-2 $\alpha$  in Kaposi Sarcoma**

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Neoangiogenesis is essential for tumor development. Hypoxia inducible factor (HIF), a transcriptional factor composed of two subunits ( $\alpha$  and  $\beta$ ) plays a key role in this process, activating proangiogenic factors, such as VEGF. The HIF  $\alpha$  subunits are critically regulated by oxygen but also modulated by growth factors. Kaposi Sarcoma (KS) is a highly vascular tumor which releases large amounts of VEGF and for which we have recently described an essential role for Insulin-like Growth Factor (IGF) system. We therefore investigated the expression of HIF  $\alpha$  subunits in biopsies from KS tumors and their modulation by IGF-I in KSIMM, a KS cell line.

Both HIF-1 $\alpha$  and HIF-2 $\alpha$  are expressed in KS biopsies in all tumoral stages. HIF-1 $\alpha$  immunopositivity increases through the tumor development with highest expression in late nodular stages.

In KSIMM cells, IGF-I induces accumulation of both HIF  $\alpha$  subunits (western blot). The induction suggests a translation mechanism as demonstrated by cycloheximide (CHX) chase experiment coupled with constant RNA levels as evaluated by qRT-PCR. IGF-I induced HIF  $\alpha$  accumulation is followed by increase of HIF function as documented by reporter gene assay and by induction of endogenous target genes as evaluated by qRT-PCR (VEGF-A and GLUT-1) and ELISA (VEGF-A). Blocking the IGF-IR with picropodophyllin (PPP), a specific IGF-IR tyrosine kinase inhibitor, diminishes the basal and IGF-I-dependent



induction of both HIF  $\alpha$  congeners and VEGF. These novel findings shed lights on the coupling between the IGF system and HIF pathway in KS and suggest their contribution in the tumor characteristically vascular phenotype.

## P248

### Proinflammatory cytokine gene expression shows intrinsic circadian oscillation in peripheral cells

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An extraordinary array of behavioural and physiological systems is regulated by endogenous circadian timekeepers within mammals. This circadian machinery is comprised of intricate autoregulatory transcription feedback loops, which interact to generate a time delay of approximately 24 hours in the cyclical transcription of components. In addition to the master clock in the suprachiasmatic nucleus it is clear that peripheral tissues also express clock components. The central clock entrains these in part through the HPA axis and changes in serum glucocorticoid levels.

Several inflammatory diseases show distinct diurnal profiles of activity eg asthma and rheumatoid arthritis. Serum concentrations of pro-inflammatory cytokines, IL-6 and TNF $\alpha$ , and also glucocorticoids oscillate in circadian manner suggesting a direct causative effect. It is uncertain if the IL-6 and TNF $\alpha$  cycles are regulated by changes in the HPA axis or whether their expression is driven by peripheral clock mechanisms.

We now show that IL-6 promoter activity is directly regulated by expression of core clock gene *clock* and *BMAL1*, but with a phase delay compared to the index clock controlled gene *RevErb $\alpha$* . We then generated stable cell lines expressing IL-6-luc and TNF $\alpha$ -luc. The cells were synchronised with serum shock and continuous, broad-field, integrated luciferase activity was measured at 60 s intervals for up to 10 days. The data showed unexpectedly strong, robust oscillations in TNF promoter activity with a period of approximately 24 h. Less marked oscillations were seen in IL-6-luc, but again with a 24 h period. A key upstream mediator of cytokine expression is C/EBP $\beta$ , or NF-IL6. Strikingly, there was also a robust circadian oscillation in C/EBP $\beta$  activity, measured with reporter gene. This is the first demonstration of endogenous clock control of proinflammatory cytokine gene expression. This finding implies that circadian variation in disease severity has a significant, and previously unexpected peripheral clock component.

## P249

### Plasma interleukin 18 in patients with polycystic ovary syndrome – relation to insulin resistance

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#### Aim of the study

Polycystic ovary syndrome (PCOS) is common disease affecting women in reproductive age which leads to anovulation. In pathogenesis of PCOS insulin resistance plays an important role. The aim of the present study was to look for possible relationship between interleukin 18 (IL-18) and insulin resistance in patients with PCOS.

#### Material and methods

The study was done in 76 patients with PCOS and 40 women without menstrual disturbances. The oral glucose tolerance test with glucose and insulin estimations was performed in all the women. In the fasting plasma concentration of IL-18, sTNFR1, sTNFR2, adiponectin, IL-6, sIL6R, SHBG and hormonal parameters were estimated. Hyperinsulinemic, euglycemic clamp was done to determine insulin sensitivity. The study was approved by the local Ethics Committee.

#### Results

In the PCOS group, lower insulin sensitivity index ( $P = 0.0063$ ), higher fasting glucose ( $P = 0.024$ ), insulin ( $P = 0.0014$ ) as well as higher post-load glucose ( $P = 0.014$ ) and insulin ( $P = 0.046$ ) were observed. PCOS group had significantly higher testosterone ( $P = 0.000$ ) and LH concentration ( $P = 0.000$ ), whereas SHBG concentration was markedly decreased ( $P = 0.001$ ). IL-18, IL-6, and sIL6R concentrations were not

statistically different between studied groups. In the patients with PCOS increase of sTNFR2 was observed ( $P = 0.039$ ). Correlation analysis revealed significant inverse correlation between IL-18 and insulin sensitivity index ( $r = -0.39$ ;  $P = 0.0008$ ), adiponectin ( $r = -0.46$ ;  $P = 0.033$ ), HDL-cholesterol ( $r = -0.29$ ;  $P = 0.015$ ). IL-18 was related to BMI ( $r = 0.27$ ;  $P = 0.017$ ), waist girth ( $r = 0.32$ ;  $P = 0.003$ ), WHR ( $r = 0.38$ ;  $P = 0.0006$ ), sTNFR1 ( $r = 0.41$ ;  $P = 0.00029$ ), sTNFR2 ( $r = 0.22$ ;  $P = 0.058$ ), IL-6 ( $r = 0.31$ ;  $P = 0.006$ ).

#### Conclusion

The obtained results suggest that IL-18 could play a role in pathogenesis of insulin resistance in patients with PCOS.

## P250

### Expression of functional toll-like receptors in adipocytes

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Various proinflammatory cytokines have been demonstrated to be involved in insulin resistance and other obesity related diseases. Pre-adipocytes and adipocytes are likely to modulate inflammatory and immune processes by intrinsic mechanisms. Toll-like receptors (TLRs) which are - together with CD14 - involved in the innate immunity and are able to recognize microbial components. Microbial components and immunosuppressive drugs modulate the cytokine expression. We address here the influence of immunosuppressive drugs and microbial components (lipopolysaccharide [LPS], lipoteichoic Acid [LTA] and Pam-3-Cys) of TLR2-, TLR4-, IL-6- and TNF $\alpha$  expression. The regulation of TLRs and cytokine mRNA levels was investigated by using the TaqMan quantitative PCR method. To detect the possible pro- or anti-inflammatory effects of microbial components and immunosuppressive drugs we measured the IL-6- and TNF $\alpha$  expression in 3T3-L1 adipocytes by TaqMan quantitative PCR and its secretion by ELISA, respectively. 3T3-L1 adipocytes expressed TLR2, TLR4, IL-6- and TNF $\alpha$  on mRNA and protein levels, as detected by RT-PCR and immunocytochemistry. LPS enhanced the TLR4 and cytokine expression, while LTA barely regulated the expression of TLRs and cytokines. Pam-3-Cys induced TLR2 and cytokine expression. Dexamethasone inhibited TLR and cytokine expression. We provide evidence that TLRs can activate the pathway of innate immunity in adipocytes which result in the secretion of immunomodulatory molecules. The pharmacological regulation of TLR and cytokine expression may provide a novel mechanism in the treatment of insulin resistance and other obesity related diseases.

## Diabetes, metabolism and cardiovascular

### P251

#### The involvement of GH-IGF-I axis in a mouse model of type II diabetic nephropathy

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Type II diabetic nephropathy (DN) has become the single most common cause of end-stage renal disease in the Western world. In previous studies we have shown a significant involvement of the GH-IGF-I axis in a mouse model of type I DM. The purpose of this study was to examine the endocrine and renal changes in GH-IGF-I axis key molecules in db/db mice, a model of type II DM lacking a functional leptin receptor. Obese (D) and lean (heterozygote) (C) animals were followed, beginning at 6 weeks of age (at hyperglycemia onset), for a total of 4 weeks. Mean serum glucose levels in D animals during the study were  $445 \pm 48$  mg % vs  $116 \pm 10$  mg % in C. Serum GH levels at sacrifice were decreased in D ( $16 \pm 2$  compared to  $26 \pm 5$  ng/ml in C;  $P < 0.05$ ). A similar decrease was seen for circulating IGF-I ( $237 \pm 7$  vs  $433 \pm 28$  ng/ml in D vs C,  $P < 0.05$ ). Kidney weight was increased in D ( $169 \pm 3$  vs  $134 \pm 3$  mg in C;  $P < 0.05$ ). In addition, hyperfiltration ( $214 \pm 18\%$  of C), albuminuria ( $160 \pm 20\%$  of C), glomerular hypertrophy ( $205 \pm 20\%$  of C) and increased glomerular PAS deposition were observed in D animals. Renal IGFBP-1 protein was significantly increased ( $241 \pm 39\%$  of C) and renal IGF-I mRNA was decreased ( $70 \pm 7\%$  of C) in D animals.

In conclusion, renal and glomerular hypertrophy, hyperfiltration, albuminuria and renal IGFBP-1 accumulation are seen in diabetic db/db mice already after 4 weeks of hyperglycemia, similar to finding in type I DM



models. However, these renal changes are associated with low circulating IGF-I and GH levels, contrary to type I DM. This suggests that the renal complication in type II DM may be more affected by local diabetic environment.

## P252

### Sodium bicarbonate reacts with polysaccharides starch to enhance exercise

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#### Objectives

To study the mechanism by which sodium bicarbonate act to enhance exercise.

#### Methods

Fifteen healthy volunteers, different age groups varies between 20 to 32 years, body weight between 65 to 70 kg, male subjects, were put for exercise test under three conditions: following ingestion of 300mg sodium bicarbonate per kg of body mass (i); following ingestion of a placebo (100mg sodium chloride isotonic solution per kg of body mass) (ii); and following ingestion of neither (iii). A double-blind protocol was used between the (i) and (ii) trials. All volunteers had a starchy meal before going to bed the night before exercise. Each condition was repeated so that the volunteers underwent treadmill exercise for six times, 100 min before commencing treadmill exercise was allowed after ingesting substances in (i), (ii) and (iii). The volunteers exercised until fatigue. Fourteen of the volunteers completed all the tests.

#### Results

The volunteer's average times for trials (i), (ii) and (iii) were 4.01, 4.34 and 4.36.0s, respectively. The data were analysed using a two-way ANOVA with replicates and Tukey tests. This revealed a difference between trial (i) and trials (ii) and (iii) ( $P < 0.05$ ), but no difference between trials (ii) and (iii).

#### Conclusion

The findings therefore, indicated that sodium bicarbonate has an ergogenic effect upon exercise by increasing glycolysis and this is due to its reaction with polysaccharides and starch. Sodium Bicarbonate disintegrate and disrupt polysaccharides starch compounds and free sugar molecules in blood and tissues.

## P253

### New function for the enzyme amylase discovered: amylase works as a catalyst/hydrolyzing agent to break down, disaccharides (sucrose, lactose, maltose... etc) and polysaccharides (starch)

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Amylase is a digestive enzyme made primarily by the pancreas and salivary glands.

#### Objectives

To determine other functions for the enzyme amylase.

#### Design

*In vitro* tests combined with prospective study on one hundred and fifty healthy volunteers.

#### Methods

Twenty five mls of Human Saliva alpha amylase were added to 5 mls of sucrose (table sugar) and 5 mls of flour (starch) in different samples. Twenty five mls of normal saline, bread yeast solution, sodium bicarbonate solution, distilled water and pure plant amylase (50mg) were added to 5 mls of sucrose solution and 5 mls of flour (starch) solution. **Hexokinase and glucoseoxidase** were used to read sucrose and flour (starch) in each sample. Readings were obtained immediately, after five minutes, then half hourly for eight hours.

#### Results

Traces of sugar were detected immediately on the plant amylase (sucrose and flour) samples. Traces of sugars were detected in 148 samples of saliva-sucrose within 30 to 90 minutes (in two samples traces of sugar were detected within 90 to 120 minutes). One cross of sugar detected after two hours in 148 samples of saliva-sucrose. Two to three crosses of sugar were obtained after eight hours in all amylase-sucrose-samples. Traces of sugar were detected in all saliva-flour samples after 8 hours, and one to two crosses of sugar detected after 9 to 10 hours or more (up to 24 hours) in all samples.

#### Conclusion

This study demonstrated that the function of the enzyme amylase is to break down (hydrolyzes) polysaccharides starches and disaccharides.

## P254

### Liver GH receptor signaling in type I diabetic mice

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High levels of GH and low levels of IGF-I are found in the serum of type I DM. GH binds to its receptor (GHR) and leads to a series of signal transduction reactions, involving phosphorylation of intracellular proteins, including IGF-I and angiotensin II receptor type I (AT1R). It has been previously shown that in type I DM mice there is a decrease in liver GHR levels and circulating IGF-I in spite of increased circulating GH, suggesting a state of GH resistance, although its exact pathway is not clear yet. The aim of this study is to check the expression of proteins mediating GHR signalling in liver tissue of type I DM mice. DM was induced in adult male mice by the injection of streptozotocin (STZ). Diabetic mice (D), nondiabetic control mice (C), and control and diabetic mice injected with bovine GH, (DGH and CGH, respectively), were used. Liver tissue was examined for the following proteins (by WB): GHR, pJAK2, pSTAT5, pERK, IGF1R and AT1R. GHR and IGF-1 mRNA levels were also determined.

#### Results

Diabetic mice treated with or without GH, showed a significant decrease in liver GHR mRNA levels. Liver IGF-I mRNA levels were unchanged. While total STAT5 and JAK2 levels remained unchanged, the levels of phosphorylated STAT5 were increased in both CGH and DGH. Levels of IGF1R and IRS were decreased in D and DGH groups. AT1R and pERK were increased in both D and DGH groups.

#### In summary

GH resistance in DM is exemplified here by the decrease in liver GHR expression. The known decrease in serum IGF-I in type I DM is not supported by its unchanged liver mRNA levels. GHR signalling machinery was intact using this experimental model. The associated signalling systems with potential relevance for diabetic complications (IGF-IR and AT1R) were independently changed, irrespective of GH stimulation. The regulatory pathways involved in this depression of GHR expression in type I DM remain to be determined.

## P255

### The effects of changes in insulin sensitivity on androgen supply values in diabetic men at different age

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It is well known that there exists a correlative relationship between diabetes mellitus (DM) and sex hormone levels. However, it has not yet been found if it is caused either by hyperinsulinemia, or hyperglycemia or insulin resistance (IR). Eighty-one men with DM and 35 healthy men with normal glucose levels were examined. In each group the persons were divided into four age subgroups: 36–45 years old, 46–55, 56–65 and 66–75 years old. IR was estimated using HOMA-IR, F1R1, QUICKI and Raynaud index. As a result, it has been established that T and FTI level tend to decrease with the increasing age. As compared to men with normal insulin sensitivity the most considerable decrease was shown in healthy men aged 46–55 with IR ( $12.4 \pm 2.37$  and  $7.28 \pm 1.2$  nmol/l, respectively,  $P < 0.05$ ) and diabetic patients with IR aged 66–75 ( $14.82 \pm 0.71$  and  $5.91 \pm 1.11$  nmol/l, respectively,  $P < 0.05$ ). SHBG level increased with age in diabetic patients and men without diabetes mellitus. However, in diabetic patients SHBG level was shown to be lower than in men with normal blood glucose content irrespective of insulin sensitivity ( $P < 0.05$ ). The lowest values were registered in diabetic patients with IR aged 36–45 ( $P < 0.05$ ). A pronounced increase in free testosterone index (FTI) as compared to men without diabetes mellitus and diabetic patients with IR in all age groups ( $P < 0.05$ ) was observed in diabetic patients with normal insulin sensitivity. In other age groups low SHBG values were not accompanied by FTI increase. In the senior age group (66–75 years) SHBG levels in diabetic patients were similar to those in normal persons. A significant decrease in SHBG level was also found in men without diabetes mellitus with IR as compared to those with normal insulin sensitivity ( $P < 0.05$ ). Conclusion. The most pronounced decrease in T, FTI and SHBG values is observed in

diabetic patients with IR in older age groups. Low SHBG levels in diabetic patients aged 36–45 were accompanied by higher FTI values in contrast to healthy men with normal blood glucose values. The disorders found did not depend upon glycemia level, duration of the disease, the treatment given and existent complications but they were caused by insulin sensitivity.

## P256

### The effect, in clinical practice, of long-acting fluvastatin (LAF) on low density lipoprotein-cholesterol (LDL-Ch) in patients with type 2 diabetes mellitus (T2DM) in which other statins have failed to achieve the goals

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There is an association between high LDL-Ch levels and cardiovascular disease in T2DM patients. Clinical trials have shown that reduction of LDL-Ch with statins has a beneficial effect on macrovascular complications in patients with T2DM, but is this a relevant effect in clinical practice? LAF has a slower absorption, longer action time and less side effects than other statins.

#### Objective

To assess in clinical practice, the effect of LAF on LDL-Ch and their action on other serum lipids (SL), according to the European Diabetes Policy Group (EDPG) goals, in T2DM patients in which maximum dose of other statins have failed to achieve the recommended goals.

#### Material and methods

Four hundred fifty two of 788 patients with T2DM and dyslipidaemia attending our Endocrine Clinic, who were on maximum dose of simvastatin, lovastatin, pravastatin or atorvastatin were selected. Eighty four of them had LDL-Ch levels > 115 mg/dl, according the EDPG guidelines, and were included in this study. In these patients we measured total cholesterol (Ch), high density lipoprotein-Ch (HDL-Ch) and triglycerides (TGs), and calculated LDL-Ch, before and after 9 months shift from the previous statin treatment to LAF 80 mg/day. The percent of patients who reached the LDL-Ch target and the mean ( $\pm$ SD) values for SL, before and after 9 months LAF treatment were compared. For continuous variables a paired samples "t" test, and for categorical variables a related samples MacNemar test were used. All analyses were performed using the SPSS statistical program (versión 6.0). A level of  $P < 0.05$  was considered significant.

#### Results

After 9 months LAF treatment, the 57% of patients achieved the LDL-Ch target according EDPG guidelines ( $P = 0.0000$ ). There was also a significant decrease in mean Ch and LDL-Ch ( $P = 0.000$ , for two); and an increase in HDL-Ch ( $P = 0.000$ ).

#### Conclusions

In clinical practice, LAF treatment got the LDL-Ch target, according EDPG, in more than 50% of patients with T2DM in which maximum dose of other statins have failed to achieve the recommended goals. In addition to this effect, the increase in HDL-Ch observed after LAF treatment show the importance of this statin in the treatment of T2DM patients with dyslipidaemia.

## P257

### Nutritional outcome in end-stage renal disease diabetic patients: short term follow-up of patients initiating/escaping dialysis

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#### Introduction

Undernutrition is common in haemodialysed diabetic patients. To estimate the nutritional status before and after dialysis, we followed up prospectively initially undialysed uremic diabetic patients.

#### Methods

All 20 patients had initial body composition (Lean Body Mass, LBM, using DEXA) and serum albumin (SA) measurements. Ten patients underwent hemodialysis (HD) after 15 m (mean). They had a similar LBM & SA evaluation 6.5 m after starting dialysis. The 10 other patients with end stage renal disease (ESRD, glomerular filtration rate, GFR < 30 mL/min/1.73 m<sup>2</sup> using 51Cr-EDTA) but not HD had a second evaluation after 24 m. The results (means  $\pm$  sem) were compared within and between groups (paired and unpaired Student's *t* test, respectively).

Results Initially, no difference was found between HD and ESRD patients. Clinically, the initial characteristics of patients were: age  $61.6 \pm 10.3$  vs  $65.6 \pm 9.6$  yr, sex 7 vs 5 men, 80% vs 80% type 2 diabetes. Biologically, we found GFR  $16.2 \pm 5.3$  vs  $19.7 \pm 6.2$  mL/min/1.73 m<sup>2</sup>, weight  $73.9 \pm 16.8$  vs  $73.7 \pm 12.5$  kg, LBM  $49.9 \pm 10.4$  vs  $51.2 \pm 11.1$  kg and SA  $34.4 \pm 3.2$  vs  $36.6 \pm 2.4$  g/l in HD and ESRD respectively.

At the second time point, for HD patients, total body weight tended to decrease  $-3.4 \pm 6.2$  kg ( $p > 0.05$  within group & compared to ESRD) due to a significant loss of LBM  $-4.2 \pm 5.7$  kg ( $P = 0.046$  within group;  $p > 0.05$  compared to ESRD) associated to a maintained SA level  $+0.3 \pm 1.1$  g/l ( $p > 0.05$  within group;  $P = 0.039$  compared to ESRD). Conversely for ESRD patients (GFR  $+1.7 \pm 8.9$  mL/min/1.73 m<sup>2</sup>), total body weight  $+0.3 \pm 6.0$  kg, LBM  $+1.6 \pm 2.8$  kg were unchanged and SA levels significantly improved  $+3.2 \pm 1.4$  g/l ( $P = 0.01$ ).

#### Conclusion

The nutritional status of HD patients altered compared to the status of ESRD patients. Whether this is due to initiating haemodialysis therapy remains to be investigated. Alternatively, it may be due to the disease's evolution *per se*. In any case, adapted nutritional advice should be provided at this period.

## P258

### Improvement of nutritional status in non-dialysed uremic diabetic patients

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#### Introduction

End-Stage Renal Disease is a common cause of undernutrition, with a high risk of morbidity and mortality in dialysis. To improve nutritional status before dialysis, we followed up 35 non-dialysed uremic diabetic patients in a prospective and cooperative study.

#### Methods

We analysed glomerular filtration rate (GFR, using 51Cr-EDTA), glycemic control (HbA1c), and nutritional status: body composition (lean body mass, LBM, by DEXA), serum albumin (SA) initially and after 2 yr. The results (means  $\pm$  sem) were compared by paired (initial time vs 2 yr) or unpaired Student's *t* tests (groups with rapid or slow GFR evolution).

#### Results

The 35 patients (67% men, 67% type 2 diabetes) were followed up for 2 yr without dialysis. Initially their characteristics were: age  $66 \pm 10$  yr, BMI  $26.5 \pm 4.2$  kg/m<sup>2</sup>, HbA1c  $8.0 \pm 1.2\%$ , serum creatinine  $162 \pm 64$   $\mu$ mol/L, GFR  $42.5 \pm 20.5$  mL/min/1.73 m<sup>2</sup>, SA  $36.3 \pm 3.2$  g/L.

After 2 yr, GFR decreased  $-6.5 \pm 16.7$  mL/min/1.73 m<sup>2</sup> ( $P = 0.028$ ). However, HbA1c  $-0.7 \pm 1.1$  ( $P = 0.001$ ) and nutritional status were improved: weight  $+2.3 \pm 12.9$  kg ( $P = 0.026$ ), BMI  $+0.7 \pm 4.4$  kg/m<sup>2</sup> ( $P = 0.003$ ), LBM  $+1.5 \pm 8.3$  kg ( $P = 0.003$ ) and SA  $+3.1 \pm 3.4$  g/L ( $P = 0.048$ ).

Initial BMI were higher in the 15 slowly progressing than in the 20 rapidly progressing patients ( $28.0 \pm 4.1$  vs  $24.9 \pm 3.7$  kg,  $P = 0.025$ ). The former had stable GFR  $+4.7 \pm 10.6$  mL/min/1.73 m<sup>2</sup> (NS) the latter had decreasing GFR  $-21.5 \pm 10.4$  mL/min/1.73 m<sup>2</sup> ( $P = 0.001$ ). Overall, the decrease of GFR was negatively correlated with the initial BMI ( $r = -0.37$ ,  $P = 0.031$ ).

#### Conclusion

Despite an alteration of GFR, patients improved their nutritional status with this cooperative follow-up. This is important because the higher the initial BMI the better evolution of GFR ("slowly progressing"). Moreover a high BMI is known as associated with an improved prognosis in haemodialysed patients (Johansen, *Am J Clin Nutr* 2004).

## P259

### Subthalamic nucleus stimulation in parkinsonian patients does not increase serum ghrelin levels

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**Introduction**

Patients with Parkinson's disease on pharmacological treatment frequently lose weight but regain weight after subthalamic nucleus deep brain stimulation (SNBS). This is, at least in part, due to an increase in resting energy expenditure (Perlemaire *et al.*, Br J Nutrition 2005; 93). As SNBS electrodes are located close to the hypothalamic centre regulating food intake, we investigated whether ghrelin levels would vary with SNBS and/or L-DOPA treatment.

**Methods**

Two types of patients suffering from Parkinson's disease were investigated: patients with chronic L-DOPA treatment ( $n = 12$ ) and patients with SNBS associated to L-DOPA ( $n = 12$ ). They were investigated before & after receiving L-DOPA, and depending on the group with and without neurostimulation. Total fasting ghrelin was assayed in duplicate with an RIA kit. Paired *t*-tests were used to compare subjects within groups.

**Results**

When the patients were sorted according to their chronic treatment, L-DOPA had a significant acute effect on ghrelin levels neither in non-SNBS patients ( $936 \pm 393$  vs  $919 \pm 317$  pg/ml) nor in SNBS patients off neurostimulation ( $880 \pm 155$  vs  $883 \pm 201$  pg/ml) ( $P > 0.05$ ). L-DOPA had no significant acute effect on ghrelin levels when all patients were considered together ( $908 \pm 231$  vs  $901 \pm 260$  pg/ml;  $P > 0.05$ ).

Neurostimulation itself did not elicit any modification of ghrelin levels in patients off L-DOPA ( $932 \pm 177$  vs  $880 \pm 155$  pg/ml;  $P > 0.05$ ).

In SNBS patients on neurostimulation L-DOPA nearly achieved a significant effect ( $932 \pm 177$  vs  $879 \pm 178$  pg/ml;  $P = 0.05$ ).

**Conclusion**

Total circulating ghrelin does not play an important role in the modification of weight homeostasis in patients treated for Parkinson's disease. This is in agreement with recent findings that although patients with hypothalamic damage (tumour) show impaired satiety, there is no change in circulating ghrelin concentrations in response to a test meal (Daousi *et al.* JCEM 2005 90:5025).

**P260****Hyperandrogenism as a risk factor of coronary artery disease in young women**

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The aim of the study: an attempt to assess relative roles of common risk factors of coronary artery disease (CAD) and sex hormones in the pathogenesis of CAD in young menstruating women (W).

**Materials**

CAD-W group: 52 W in the age of 38–48 years with angiographically proven critical changes in coronary arteries. 38 of them suffered from myocardial infarction. H-W group: 15 healthy W in the age of 35–45 years.

**Methods**

In all W occurrence of common risk factors of CAD was defined. Data on menstruation, fertility and physical signs of hyperandrogenism were collected. In 4–7 day of sexual cycle at 8.00 a.m. blood from cubital vein was taken to measure by immunological methods concentrations of hormones: estradiol (E2), testosterone (T), dehydroepiandrosterone sulphate (DHEAS), follicle-stimulating hormone (FSH), luteinizing hormone (LH), prolactin (PRL), thyrotropin (TSH), progesterone (P), cortisol (Cort) and sex-hormone binding globulin (SHBG).

**Results**

In CAD-W, comparing to H-W, higher frequency of arterial hypertension (60% vs 7%), smoking (92% vs 47%), dyslipidaemia and occurrence of hirsutism (84% vs 30%) was found. No significant differences in pattern of sex circle between W of both groups was revealed. Conc of T was significantly higher in CAD-W than in healthy ones ( $3.5 \pm 1.7$  nmol/l- vs  $2.4 \pm 1.0$ ,  $P < 0.02$ ). Conc of E2, DHEAS, FSH, LH, PRL, TSH, P, Cort and SHBG were similar between CAD-W and H-W. In logistic regression analysis was proven that a cluster of 3 parameters, visceral obesity, dyslipidaemia and hirsutism, was of the best goodness of fit with occurrence of CAD.

**Conclusions**

In women in the reproductive age hyperandrogenism may be involved in pathogenesis of CAD apart from known common risk factors as visceral obesity and unfavourable lipid profile.

**P261****Comparison of urinary N-Acetyl  $\beta$ -D glucoseaminidase activity in diabetic patients and controls**

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Urinary N-Acetyl  $\beta$ -D glucoseaminidase EC: 3.2.1.31 is a lysosomal enzyme which is classified in hexosaminidases group. This enzyme is found in different tissues such as, kidney, liver and spleen, but the kidney has maximum level of enzyme.

The first step was the standardization of colourimetry and flourimetry of enzyme assay. In colourimetry method para-nitrophenyl N-acetyl  $\beta$ -D-glucosamine is used as substrate and in flourimetry method the substrate is 4-methyl umbelliferyl N-acetyl  $\beta$ -D-glucosamine.

In this study the effect of incubation time, dilution of urine and substrate concentration was analysed. Between and within CV for both methods were also calculated. The enzyme activity was measured with the two methods in fifty diabetic patients type 1 (3-30 years) and eighty diabetic patients type 2 (30-60 years) and thirty controls for any group, and its correlation with proteinuria was examined.

Mean enzyme activity of  $\beta$  NAG in diabetic patients type 1 and 2 (even in patients with normal proteinuria) was higher than in controls. In diabetic patients type 1, the correlation rate between urine protein and enzyme activity ( $\beta$  NAG) was,  $r = 0.72$   $P < 0.001$  and in diabetic patients type 2 was  $r = 0.70$   $P < 0.001$ . We did not find any correlation between age of patients and duration of diabetes with urine enzyme activity.

**P262****Association of hypogonadism and type 2 diabetes in men attending an outpatient erectile dysfunction clinic**

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**Introduction and objectives**

Aim of this study is to evaluate the psycho-biological correlates of DM associated hypogonadism (DMAH).

**Methods**

The Structured Interview SIEDY was employed along with several biochemical, psychological and instrumental investigations. We defined hypogonadal subjects (HY) those with a circulating total testosterone (T) below 10.4 nmol.

**Results**

We studied 1246 patients. More than 15% resulted affected by type 2 DM and 1/3 of them were also HY. DMAH was associated with a significant reduction of the androgen-dependent marker PSA, as indicating the presence of a biological, more than biochemical, hypogonadism. In addition, DMAH was associated with hypogonadism-related symptoms, such as reduction in libido, leading to a decrease number of sexual attempts. In DMAH, testis size and LH concentrations were significantly reduced, suggesting a central origin of the disease. DMAH was associated with higher prevalence of depression symptoms than the rest of type 2 DM sample. DMAH patients showed a lower duration of disease and lower DM-associated complications, as nephropathy when compared to the rest of type 2 DM sample. However, they were showed higher BMI and higher triglyceride and lower HDL cholesterol than the rest of type 2 DM patients. A negative association was found between BMI and T plasma levels ( $-0.353$ ;  $P < 0.0001$ ). At penile Duplex ultrasound (PDU), DMAH was associated with a decrease in acceleration rate and basal peak systolic velocity. PDU parameters were inversely related to DMAH, even after adjusting for age and BMI.

**Conclusion**

Hypogonadism frequently complicates type 2 DM. DMAH might exacerbate sexual dysfunction by reducing libido and mood and further compromising penile vascular reactivity and lipid metabolism. Hence, testing circulating T is strongly recommended in ED subjects with type 2 DM.



## P263

### Estimation of the lipid metabolism in women with gestational diabetes mellitus (part 2). Differences resulting from the method of treatment

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#### Aim

The aim of the study was a comparison of the selected parameters of the lipid metabolism (total cholesterol – TC, LDL cholesterol – LDL, HDL cholesterol – HDL and triglycerides – TG) in pregnant women with gestational diabetes mellitus (GDM) depending on the method of diabetes treatment. Material and methods. A group of 121 women with GDM was analyzed. There were 85 women treated with diet alone (G<sub>1</sub>) and 36 women treated with diet and insulin (G<sub>2</sub>). The parameters were analyzed after making a diabetes mellitus diagnosis in GDM women and at the end of the third trimester (T3).

#### Results

Improvement of diabetes control presented by a statistically significant decrease of fasting blood glucose (FBG), of mean blood glucose (MBG) and of fructosamine level (F) was observed in both groups (for G<sub>1</sub>: 4.5 ± 0.8 vs 4.1 ± 0.4;  $P < 0.001$ ; 5.5 ± 0.9 vs 5.1 ± 0.4,  $P < 0.001$ ; 1.9 ± 0.2 vs 1.8 ± 0.2, NS mmol/l and for G<sub>2</sub>: 5.5 ± 1.3 vs 4.6 ± 0.4,  $P < 0.001$ ; 6.9 ± 1.4 vs 5.6 ± 0.4,  $P < 0.001$ ; 2.1 ± 0.3 vs 1.9 ± 0.1,  $P < 0.001$  mmol/l, respectively). While making GDM diagnosis in G<sub>1</sub> a significantly higher values of TC, LDL and HDL, as compared to G<sub>2</sub>, were observed (6.4 ± 1.2 vs 5.7 ± 1.1,  $P < 0.01$ ; 3.6 ± 1.0 vs 3.1 ± 1.0,  $P < 0.05$ ; 1.7 ± 0.4 vs 1.5 ± 0.4,  $P < 0.05$  mmol/l, respectively). In T3, as compared to T1 (GDM diagnosed), TG increase was significant in both groups (in G<sub>1</sub>: 2.8 ± 1.0 vs 3.3 ± 0.9  $P < 0.01$  mmol/l, in G<sub>2</sub>: 2.7 ± 1.0 vs 3.3 ± 1.1  $P < 0.05$  mmol/l). HDL concentration increased statistically in G<sub>2</sub> in T3 (1.5 ± 0.4 vs 1.7 ± 0.4 mmol/l,  $P < 0.05$ ).

#### Conclusion

Intensive hypoglycemic treatment of GDM improves significantly glycemic values. A better short-term diabetes control, expressed by the fructosamine concentration, was observed in G<sub>2</sub>. The intensive insulin therapy in G<sub>2</sub> women favorably increased the HDL concentration as compared to G<sub>1</sub> women.

## P264

### Estimation of some parameters of lipid metabolism in pregnant women with gestational diabetes mellitus

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The aim of the study was to estimate the parameters of lipid metabolism (total cholesterol – TC, LDL and HDL cholesterol and triglycerides – TG) in women with GDM. Material and method. The study was carried out in 121 GDM women being at the age of 30.5 ± 5.7. The control group (CG) were 36 healthy women at the age of 25.3 ± 4.4.

#### Results

The parameters were analyzed while making diabetes mellitus diagnosis in GDM women at the end of the second gestational trimester in CG and at the end of the third trimester in both groups. During pregnancy period the improvement of glycaemic control was observed, which was demonstrated by a statistically significant decrease of fasting blood glucose (FBG), of mean blood glucose and a fructosamin level in blood. No significant of an improvement of HbA<sub>1c</sub> value was found. At the end of gestation period a significant increase of TG level was observed in the patients with GDM while a considerable increase of TG and TC was found in the control group. In the third trimester of pregnancy the TC level was significantly higher in CG than in GDM patients. In the GDM group a considerably lower HDL level was observed in the first trimester in women suffering from obesity before pregnancy, and significantly lower TC and LDL levels were found in the third pregnancy trimester in the same group of women. There were no significant TG value differences between the groups of pregnant women being in the first and in the third trimester. However, the TG values in overweight women before pregnancy at the moment of making a GDM diagnosis were significantly higher than in those non-obese women.

#### Conclusion

It was proved that in the GDM group the changes in the lipid levels were lower than the changes in the control group. It is worth noticing that the GDM obese patients before pregnancy showed lower lipoprotein values than

healthy, obese or non obese patients. This can result from the influence of more rigorous diet treatment and from more frequent insulin therapy in the GDM group of patients.

## P265

### The role of a lifestyle modification in preventing type II diabetes mellitus in subjects with high-risk factors

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The aim of our study is to assess the efficacy of a lifestyle modification including altered diet composition and physical activity in preventing diabetes mellitus type 2 (DM 2) in individuals with impaired glucose tolerance and impaired fasting glucose (IGT/IFG)

#### Materials and methods

The study included 327 patients (68 m 258 f) 25-65 years. Patients were divided into 2 groups matched by sex, age, weight, body mass index (BMI), waist-to-hip ratio (WHR). Research group included 183 patients (32 m, 150 f) who received and carried out individual recommendations of a balanced diet and physical activity. Control group included 144 patients (36 m 108 f) who did not lifestyle modification. The study was 48 weeks. We measured fasting plasma glucose (FPG), 2-hour plasma glucose concentrations (2-h PG) following a 75-g oral glucose tolerance test.

#### Results

Patients of the research group demonstrated reduction of body weight (-5.3 kg), BMI (-2.6 kg/m<sup>2</sup>) and WHR (-0.2) ( $P < 0.01$ ). They had positive dynamics of FPG and 2-h PG concentrations also (-0.4 ± 0.6 and -0.9 ± 0.7 mmol/l respectively ( $P < 0.001$ )). Persons of the control group had significant increase in weight, BMI and WHR and FPG and 2-h PG concentrations elevated ( $P < 0.05$ ). Among subjects with IGT at baseline, glucose levels normalized in 56% of patients from the research group and 4.5% in control group ( $P < 0.001$ ). By the end of the study 12% of non-diabetic subjects with obese of control group have developed DM 2, 28% subjects - IFG and 48% - IGT, while in the research group there were no changes in glucose concentrations among subjects with NGT ( $P < 0.001$ ). Among patients of the research group was a reduction of DM 2 by 34.9% and an increase in the control group by 17.4%

#### Conclusion

Thereby, lifestyle modifications are effective in preventing or delaying type 2 diabetes development in individuals with high-risk factors.

## P266

### The effect of Greek mediterranean diet on trace elements and blood coagulation factors in type 2 diabetic patients

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#### Background and aims

DM2 has been associated with altered levels of trace elements and a prothrombotic state. We investigated the effect of a 28-day Greek mediterranean diet (rich in fiber, mono- and polyunsaturated fatty acids and complex carbohydrates) on serum concentration of trace elements and blood factors participating in coagulation and fibrinolysis in 58 patients with DM2.

#### Materials and methods

Blood trace elements and blood coagulation factors were evaluated in 35 men aged (Means ± SEM) 61.6 ± 1.57 years and 23 women aged 58.8 ± 1.97 years with DM2, on treatment with diet or/and oral hypoglycaemic agents as well as in 22 healthy controls matched for sex and age. The food, rich in olive oil, vegetable and fruit, was prepared and provided daily by two commercial firms. The diet was isocaloric to the current so that patients could not reduce their weight during the test period. The BMI and HbA<sub>1c</sub> of patients were less than 28 and 7%. The study subjects were assessed before and after the end of the 28 day-period on the diet. The Wilcoxon matched paired test was applied for the statistical analysis. All values are expressed as means ± SEM.

#### Results

BMI did not change significantly following the diet but waist perimeter was reduced in diabetic men and in the control group. HbA<sub>1c</sub> fell in the patients (6.45 ± 0.21% versus 7.00 ± 0.18%,  $P < 0.001$ ). Serum ferritin was drastically reduced in the patients (95.24 ± 11.7 versus 109.83 ± 11.08 ng/ml,  $P = 0.0006$ ), whereas plasma levels of Mg and P increased following the diet in patients and controls ( $P < 0.02$ ). Analysis of



parameters involved in haemostasis revealed a significant reduction of fibrinogen in patients ( $374.76 \pm 9.78$  versus  $393.96 \pm 10.05$ ,  $P = 0.016$ ) but a decrease in the activity of the anticoagulant proteins C and S both in patients ( $P < 0.001$ ) and controls ( $P < 0.04$ ).

#### Conclusions

It is concluded that adherence to mediterranean diet even for a short period improves glucose control, induces increases in plasma levels of Mg and P and reduces serum ferritin which is positively correlated to insulin resistance. Additionally it lowers blood fibrinogen levels but also reduces the activity of proteins C and S, an effect the significance of which remains to be elucidated.

## P267

### 12 month audit of insulin glargine use in the diabetic clinic

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#### Introduction

Glargine is an analogue insulin with a flat pharmacokinetic profile and duration of action of 24 h providing stable 'background' insulin on which fast acting insulin can be added during meal times. Clinical trials have indicated that this leads to more satisfactory and stable glycaemic control with reduced frequency of hypoglycaemia. We carried out a prospective audit to see if these reported benefits are confirmed in routine clinical practice.

#### Methods

123 adults with Diabetes Mellitus on an insulin regimen were changed over to a regimen including Glargine between September 2002 and September 2003 and were monitored over a period of 12 months. They included 96 patients with Type 1 Diabetes and 27 patients with Type 2 Diabetes. Indications for transferring to Glargine were: poor control (34%), hypoglycaemia (40%), nocturnal hypoglycaemia (25%), patient's choice (1%).

#### Results

Median HbA1c improved significantly from 9% to 8.5% ( $P = 0.001$ ). The frequency of hypoglycaemia was significantly reduced following the conversion to Glargine (from 49 to 16,  $P < 0.001$ ) and the frequency of nocturnal hypoglycaemia was reduced in 20 of 30 patients. However, there was a small but significant increase in body weight with the median Body Mass Index increasing from 25.5 to 26.5 ( $P < 0.001$ ).

#### Conclusion

Introducing Glargine led to a significant improvement in glycaemic control and a reduction in the frequency of hypoglycaemia. Patients, particularly those working shifts, also reported an improvement of quality of life. There was however a rise in Body Mass Index.

## P268

### Increased expression of the adipokines resistin and fiaf in hypoxic/ischemic mouse brain

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Obesity and adipokines are associated with a state of chronic low grade inflammation (Trayhurn & Wood 2004). In view of our findings that the adipokines *resistin* and *fiaf* (*fasting-induced adipose factor*) are also expressed in mouse brain and pituitary gland (Wiesner et al., 2004), we tested the hypothesis that these factors would be induced in brain tissue following cerebral hypoxia/ischemia (H/I). Neonatal H/I (postnatal day 8; unilateral common carotid artery occlusion under isoflurane anaesthesia, plus exposure to 8% oxygen; 60 mins) increased *fiaf* expression (Real Time RT-PCR), but not *resistin* mRNA, in ipsilateral mouse cerebral cortex and hippocampus at 2 days (1.4 to 1.9-fold;  $P < 0.01$ ) and 7 days (1.8-fold;  $P < 0.001$ ) post-H/I. In contrast *resistin* mRNA was significantly increased (1.8-fold;  $P < 0.001$ ) in cortex only after 21 days, when *fiaf* mRNA levels had returned to baseline. Since *fiaf* is a PPAR $\alpha$  target gene, we also tested the effects of the PPAR $\alpha$  agonist WY-14643 (Tocris; 50 microgm per gm b.wt; s.c.; male mice; 28 days old). This drug acutely (3 hr) increased *fiaf* (2.2-fold;  $P < 0.01$ ), but not *resistin*, expression in cerebral cortex, but induced down-regulation of *fiaf*

mRNA ( $P < 0.01$ ) in cortex, pituitary and fat after 3 daily injections. Our data suggest that brain-derived adipokines, such as FIAF and resistin, are involved in cerebral inflammation following ischemic injury. Funded by NSHRF, CIHR and NHF (Australia).

## P269

### Prevalence of chronic cardiovascular complications in newly diagnosed type 2 diabetic patients

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The prevalence of cardiovascular diseases (CVD) has increased sharply in the developing countries and because Type 2 diabetic patients are at increased risk for CVD.

#### Objective

To determine the prevalence of patients with past history of a myocardial infarction or cerebral stroke in newly diagnosed type 2 diabetic patients in Georgia.

#### Design

Cross-sectional study. PATIENTS: Newly diagnosed type 2 diabetic patients ( $n = 648$ , age (M  $\pm$  SD)  $53.9 \pm 10.11$ ; female 58%). MEASUREMENTS: Blood pressure, body mass index, past history of CVD.

#### Results

There was a linear relationship between BMI and age at diagnosis of type 2 diabetes ( $P < 0.01$ ). Adults with type 2 diabetes were more obese (BMI  $30.0 \pm 5.34$  kg/m<sup>2</sup>) versus control subjects (BMI  $27.6 \pm 4.51$  kg/m<sup>2</sup>;  $P < 0.001$ ). Patients, with newly diagnosed type 2 diabetes without an arterial hypertension had the greater prevalence of a past history of myocardium infarction (13/299), than persons of control group (0/194,  $\chi^2 = 8.66$ ,  $P = 0.003$ ). According to present data, we can make conclusion, that atherosclerotic vascular complications starts to develop long before the onset of clinical diabetes, due to metabolic disorders during the prediabetic state. In newly diagnosed type 2 diabetes patients, the risk of coronary heart disease (CHD) is more prevalent, than the risk of cerebrovascular atherosclerotic disease. At the stage of newly diagnosed diabetes the risk of cardiovascular disease is more frequently present in male patients, than in female. The coexistence of hypertension and type 2 diabetes significantly rises the risk of cerebrovascular disease, and not significantly changes the high risk of CHD disease. In newly diagnosed type 2 diabetes patients with hypertension the risk of cerebro-vascular disease is 4 fold prevalent in the case of male patients.

## P270

### Hormonal profile associated with the insulin sensitive obese subjects

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Recently, several groups have described a subset of obese women, who despite having excessive total body fat, are insulin sensitive (ISO for Insulin Sensitive Obese), normotensive and present a favorable lipid profile. Adiponectin, ghrelin and leptin play important roles in the regulation of energy metabolism and food intake. Moreover, all three hormones have been reported to be closely linked to insulin resistance and regulated by insulin. Therefore, we propose to investigate whether ISO and "Insulin Resistant Obese" (IRO) individuals display differing adiponectin, ghrelin and leptin profiles during a hyperinsulinemic state.

#### Materials and methods

Eighty-nine non-diabetic obese post-menopausal women underwent a series of tests to evaluate their insulin sensitivity (euglycemic/hyperinsulinemic clamp; EHC), body composition, blood lipid profile, blood pressure and an inflammatory marker (hsCRP). Ghrelin (acylated and non-acylated), adiponectin, and leptin profile were assessed in the fasting state and during an EHC (0, 60, 160, 170 and 180 min). Subjects within the highest tertile of

insulin sensitivity were described as ISO, while those within the lowest tertile of insulin sensitivity were considered as IRO.

#### Results

Total adiponectin profile and AUC along with maximal insulin-regulated inhibition of ghrelin, were significantly greater ( $P < 0.05$ ) in ISO than in IRO. The AUC for leptin, active ghrelin and % active ghrelin along with end of EHC circulating active ghrelin were significantly decreased ( $P < 0.05$ ) in ISO individuals. Adiponectin AUC ( $r = 0.39$ ,  $P < 0.05$ ) as well as total and active ghrelin maximal inhibition during the EHC ( $r = -0.31$ ,  $P < 0.05$  and  $r = -0.29$ ,  $P < 0.05$  respectively) were significantly correlated with insulin sensitivity.

#### Conclusion

In a hyperinsulinemic state, total adiponectin concentrations, maximal inhibition of total ghrelin, AUC and end of EHC % active ghrelin are different between ISO and IRO subjects. These findings suggest a potential regulatory involvement of the different forms of leptin, adiponectin and the different forms of ghrelin in the ISO profile.

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### P271

#### AT<sub>1</sub> receptor activation mediated oxidative stress and uncoupling protein-2 driven beta-cell dysfunction in obesity-induced Type 2 diabetes mellitus

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#### Background/Objectives

We have previously shown the existence of a pancreatic islet renin-angiotensin system and its key component AT<sub>1</sub> receptor (AT<sub>1</sub>R) was upregulated in an obese Type 2 diabetes mellitus (T2DM) mouse model. Blockade of the AT<sub>1</sub>R was found to improve beta-cell function and glucose-stimulated insulin secretion in this T2DM model. However, the mechanism(s) remain equivocal. In this context, oxidative stress was suggested to be a critical regulator for the beta-cell dysfunction and apoptosis. On the other hand, uncoupling protein-2 (UCP-2) was demonstrated to be activated by oxidative stress and down regulating insulin secretion. The present study is, therefore, aimed at investigating the role of AT<sub>1</sub>R mediated oxidative stress and UCP-2 induced beta-cell dysfunction using an obesity-induced T2DM model.

#### Methods

10 mg/kg/day losartan was given to 4-week-old obese db/db mice for 8 weeks so as to block the AT<sub>1</sub>R activation chronically. Water-fed db/db and m + /db mice were employed as the positive and negative controls, respectively. After 8-week treatment, the islets were isolated from the mice for analyses. Levels of oxidative stress were determined by the mRNA and protein expression of NADPH oxidase subunits (p22 phox and gp91 phox) and nitrotyrosine. Apoptosis of beta cells was examined by the staining of the fragmented DNA using the TUNEL method. The mRNA and protein expression of UCP-2 were assessed by real-time PCR and Western blot, respectively. The secretory function of the beta cells was monitored by measuring islet insulin release.

#### Results

Results showed that blockade of AT<sub>1</sub>R inhibited oxidative stress production via the down regulation of NADPH oxidase, which in turn suppressed UCP-2 expression. In addition, the levels of apoptosis were significantly lowered. On the other hand, beta-cell function was consequently improved as evidenced by an increase of insulin secretion.

#### Conclusions

The data suggest that AT<sub>1</sub>R blockade improves beta-cell function and inhibits apoptosis in an obesity-induced mouse model of T2DM, probably via a reduction of oxidative stress and down regulation of UCP-2 expression.

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### P272

#### Effects of hyperglycemia on the AT<sub>1</sub> receptor expression and secretory function in an INS-1E beta-cell line

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#### Objectives

We have recently identified an islet renin-angiotensin system in the pancreas which was subjected to upregulation in an obesity-induced mouse model of Type 2 diabetes mellitus (T2DM). Blockade of the AT<sub>1</sub> receptor (AT<sub>1</sub>R) activation in this diabetic model improves islet function. It is well known

that hyperglycemia plays a pivotal role in beta-cell dysfunction and T2DM. Nevertheless, glucotoxicity-induced AT<sub>1</sub>R activation and its consequence in oxidative stress-mediated beta-cell dysfunction are largely undefined. Accordingly, the present study was designed to investigate the in-vitro effects of chronic hyperglycemia on the expression changes in AT<sub>1</sub>R and insulin release using an INS-1E beta-cell line.

#### Methods

An INS-1E beta-cell line was cultured and incubated in different concentrations of glucose with varying time course. Immunocytochemistry was employed for precise localization of AT<sub>1</sub>R in INS-1E cells. The effects of hyperglycemia-induced AT<sub>1</sub>R expression changes in gene and protein levels were examined by real-time PCR and Western blot analysis, respectively. Glucotoxicity-induced AT<sub>1</sub>R activation-mediated secretory dysfunction was assessed by insulin release from INS-1E cells. AT<sub>1</sub>R activation mediated oxidative stress was also assessed by changes in NADPH oxidase expression.

#### Results

Immunoreactivity for AT<sub>1</sub>R was specifically localized to the cell membrane of INS-1E cells. AT<sub>1</sub>R expression at the gene and protein levels was dose dependently upregulated by chronic exposure to hyperglycemia, as demonstrated by real-time PCR and Western blot analyses, respectively. Chronic exposure of INS-1E cells to hyperglycemia impairs glucose-stimulated insulin release, which was specifically mediated by AT<sub>1</sub>R activation. AT<sub>1</sub>R-mediated NADPH oxidase activity/expression was also upregulated by hyperglycemia.

#### Conclusions

These data indicate that chronic hyperglycemia upregulates AT<sub>1</sub>R located on beta cells, thus impairing insulin secretion. The regulatory pathway may be mediated by an AT<sub>1</sub>R-mediated NADPH oxidase-dependent generation of reactive oxygen species.

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### P273

#### Evaluation of Garlic extract effect on serum angiotensin converting enzyme (ACE) level in streptozotocin-diabetic rats

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The renin-angiotensin system (RAS) has been implicated in the development of diabetic vascular complications and angiotensin converting enzyme (ACE) has a major role in this system. This study sought to examine the *in vivo* effect of aqueous extract of garlic (*Allium sativum*) on the level of ACE activity of serum from streptozotocin (STZ)-diabetic and non-diabetic rats. Serum ACE activity measured by Cushman and Cheung colorimetric assay in the beginning of diabetes induction, after 4 weeks and after 8 weeks. Although treatment with garlic extract had no significant effect on serum glucose but it significantly decreased the level of serum ACE activity. Results showed that ACE activity in diabetic rats was higher than non diabetic animals but in diabetic animals treated by garlic extract the elevation of ACE activity did not find. These findings was more remarkable after 8-week in compare with 4-week period. Results suggest that garlic extract can be introduced as a useful ACE inhibitor to prevent some vascular complications of diabetes mellitus.

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### P274

#### Determinants of metabolic syndrome and insulin resistance in hemodialysis patients

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Insulin resistance is an integral part of the metabolic syndrome. Insulin resistance and metabolic syndrome predisposes to chronic kidney disease. However, the determinants of insulin resistance (measured by the homeostasis model, HOMA-IR) and metabolic syndrome are not known in hemodialysis (HD) patients. Thus, we studied 157 HD patients.

#### Results

The prevalence of metabolic syndrome was 46.5%. Patients with metabolic syndrome had higher HOMA-IR ( $5.6 \pm 1$  vs.  $1.8 \pm 0.3$ ,  $P < 0.001$ ),

interdialytic weight gain ( $3.3 \pm 0.2$  vs.  $3 \pm 0.1$  kg,  $P < 0.05$ ) and intradialytic DBP drop ( $12 \pm 1$  vs.  $7 \pm 1$  mmHg,  $P < 0.05$ ) and lower Kt/V ( $1.5 \pm 0.03$  vs.  $1.54 \pm 0.02$ ,  $P < 0.05$ ), serum creatinine ( $9.9 \pm 0.3$  vs.  $10.4 \pm 0.2$  mg/dl,  $P < 0.05$ ). The determinants of metabolic syndrome were: female, diabetes, HOMA-IR, cholesterol, interdialytic weight gain, magnesium and low Kt/V. The risk of metabolic syndrome reached a plateau when HOMA-IR  $\geq 1.0$ . The risk of metabolic syndrome was U-shaped with the nadir of magnesium at 2.4 mg/dl. The risk of metabolic syndrome was also U-shaped with the nadir of Kt/V at 1.7. The determinants of HOMA-IR were: age, short duration of HD, HBsAg, triglyceride, low HDL-cholesterol, uric acid, ferritin, intradialytic drop in SBP and low PTH.

#### Conclusion

The prevalence of metabolic syndrome was much higher in HD patients than the general population (46.5% vs. 11.6%). The determinants of metabolic syndrome and HOMA-IR were different. Therefore, metabolic syndrome and insulin resistance are similar but distinct entities.

## P275

### Hyperlactatemia caused by acute myocardial infarction in patients with type 2 diabetes mellitus

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#### Background

Metabolic disturbances could contribute to poor outcome after acute myocardial infarction (AMI) in type 2 diabetic patients.

#### Objective

We examined the acid base disturbances that could contribute to poor prognosis after MI among type 2 diabetics suffering AMI.

#### Methods

Parameters of acid base status and blood lactate level were compared (using one and two – way ANOVA) in 160 age-matched individuals. Forty of them were type 2 diabetics in acute phase of MI; 40 were patients with AMI and normal blood glucose, and another 40 were type 2 diabetics with no evidence of CAD; control group consisted of 40 healthy persons. Different clinical metabolic and hormonal influences were examined in multiple correlation analysis.

#### Results

Blood lactate levels were significantly higher ( $P < 0.01$ ) in type 2 diabetics suffering AIM (DM + AIM) than in other examined groups. Blood lactate levels in DM + AIM group correlated significantly with degree of hearth insufficiency ( $R = 0.41$ ), hearth rhythm and conduction disturbances ( $R = 0.54$ ), CK level ( $R = 0.68$ ), blood glucose ( $R = 0.46$ ) SaO<sub>2</sub> ( $R = 0.68$ ) and serum noradrenalin level ( $R = 0.44$ ).

#### Conclusion

Incidence of hyperlactatemia in acute of myocardial infarction in type 2 diabetics is much higher than in non-diabetics with acute myocardial infarction. Stress-related diabetic ketoacidosis occurs less frequently than lactic acidosis in type 2 diabetics suffering AIM.

## P276

### The safety and efficacy of chinese herbal medicine PHS-e2 (Glucoherb) in streptozotocin – induced diabetic wistar rats

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Traditional herbal medicine has been used for centuries to treat various chronic diseases – There have been some anecdotal evidence to suggest that the use of some of these herbal medicine may be both safe & efficacious in patients and laboratory animals with diabetes. Many patients with diabetes worldwide use some of these medicines without informing their caring physicians. PHS-E2 (Glucoherb) is a Chinese traditional herbal medicine, which is extracted from two medicinal plants named Dahuang and Heshouwa. Which are originally used as vegetables. We tested the safety and efficacy of their agent after earlier studies in China suggested significant hypoglycemia properties. Diabetes was induced in 26 out of 30 Wister rats following streptozotocin i.p as a dose rate of 60 mg/kg. Acute, sub acute and chronic toxicity were tested in these animals. Also the efficacy of this agent improving hypoglycemia was compared with control and with the oral hypoglycemic agent glibenclamide. In acute, subacute and chronic toxicity studies, no difference in weight of the experimental

animals occur, nor there was any significant change in level, of aspartate aminotrasferase (AST), alanine aminotrasferase (ALT), and bilirubin between actively treated rats and control group. There was significant improvement in level of blood glucose at various dosage of glucoherb (7 mg/kg, 15 mg/kg and 25 mg/kg) comparable to the effect of glibenclamide (dose 0.6 mg/kg).

These results suggest that PHS-E2 (Glucoherb) is both safe and efficacious in STZ diabetic Wistar rats. This outcome is awaited to be reproduced from human studies which is currently is underway.

## P277

### Education for diabetic patients for fasting of Ramadan: a questionnaire study

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Fasting of Holy month of Ramadan may be beneficial for patients with Type-2 diabetes but patient education and advice is of paramount importance to offset any effect from changing of dietary habits and treatment schedule. We conducted a questionnaire study few weeks before, during Ramadan and few weeks after Ramadan 1424 H/ 2004. A cross sectional random sample of patients with diabetes attending our services was analyzed. 50 patients mean age 65 yeas (20–83), (median, range) diabetes duration 15 years (1–35), 24 females & 26 males accepted to join the study. 22 patients were insulin treated, and 28 patients were taking different oral hypoglycemia agents including sulphonylurase, metformin or a glitazone, either single or in different combinations. 40% of participants never received any specific advice for fasting and 30% received education and advice in recent visits, while only 18% received some form of advice in the past. Specific dietary advice was only given to 18% of patients. However, only one patient who is taking oral hypoglyraemic agent (a SU, metformin and a glitazone), suffered an episode of severe hypoglycemia necessitating admission to hospital, and on the other hand only one patient who is inculin Rx, developed ketoacidosis. This survey points to major deficit in the way patients with diabetes are advised regarding fasting of Ramadan. Specific educational programmes, including patient's counseling and educational leaflets should be widely available for diabetic patients who are keen to fast Ramadan.

## P278

### Alcohol intake deteriorates insulin receptors and adipokines expression in rat adipose tissue

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The effect of short-term (10 days) and long-term (28 days) intake of alcohol (6% ethanol in tap water) on insulin receptor properties in adipose tissue plasma membranes and adipokines expression in adipose tissue of *ad libitum* fed male Wistar rats was studied. Control animals (C) had free access to standard laboratory diet. After 10 days of alcohol intake lower body mass gain with no changes in size of both epididymal fat pads and adipocytes was observed. However, long-term ethanol consumption resulted in notably lower body mass gain, reduced adipose tissue weight and smaller fat cell size as compared with controls. In both ethanol groups increased glycemia was positively correlated with plasma insulin levels (contrary to physiological negative relation between insulinemia and glycemia in C group), indicating impaired insulin control of glucose level. Alcohol intake abolished down regulation of insulin receptor (IR) protein by insulinemia in adipose tissue plasma membranes and positive correlation between insulin level and the expression of IR-alpha protein subunit was present. Concomitantly with lower protein expression of IR-alpha subunit in both experimental groups attenuated expression of adipose tissue IR mRNA was detected. The presence of decreased IRS-1 mRNA level in fat tissue was observed. Rats of both alcohol groups displayed diminished expression of leptin mRNA in adipose tissue, these values were positively correlated with insulinemia. Gene expression of adiponectin in adipose tissue was not affected by ethanol consumption, without any relation to tested variables.



These results demonstrate the disturbed insulin tolerance on the peripheral tissues as well as impaired IR intracellular signalling and hormonal function of rat white adipose tissue. This study was supported by the grant VEGA 2/4030/4.

## P279

### Des-acyl ghrelin regulates glucose and lipid metabolism in HL-1 cardiomyocytes

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#### Background

Des-acyl ghrelin (Des-G) is an isoform of ghrelin without the n-octanoyl modification at Ser3 present in this peptide. It is mainly produced in endocrine cells located in the stomach, in the mucosal layer of the fundus, and it is secreted into bloodstream where it circulates in higher amounts than ghrelin (2:1).

Although Des-G was previously thought of as a non-functional peptide, since it does not activate GHS-R1a, recent studies have shown that it is able to share with ghrelin antiproliferative effects on breast and prostate cancer cell lines, and can stimulate adipogenesis and regulate glucose secretion in hepatocytes.

The aim of our study was to investigate if Des-G could also regulate cardiomyocytes viability and metabolism.

#### Materials and methods

Using MTT assay we demonstrate that Des-G did not change HL-1 cardiomyocytes metabolic activity. However, pre-treatment with Des-G (0.1  $\mu$ M) for 12 hours prevented the apoptosis induced by treatment with cytosine arabinoside (AraC, 0.1  $\mu$ M) in HL-1 cells. Des-G (1 and 3  $\mu$ M), increased significantly the fatty acid uptake (assayed by Bodipy) in HL-1 cardiomyocytes while ghrelin inhibited this stimulatory effect of Des-G. Ghrelin (30 min, 3  $\mu$ M) also inhibited 2-deoxy-D-[<sup>3</sup>H]glucose uptake induced by insulin (1 hour, 100 nM) in HL-1 cardiomyocytes while Des-G lacked this effect. Furthermore Des-G (in equimolar doses) was able to prevent the inhibitory effect of ghrelin on insulin-induced glucose uptake.

#### Conclusions

Des-G, as ghrelin does, has a protective effect against the apoptosis induced by AraC in HL-1 cells. In terms of cardiomyocytes glucose and lipid metabolism Des-G counteracts the negative effect of ghrelin in insulin-induced glucose uptake, while it stimulates long chain fatty acid uptake in this cell type.

## P280

### Regulation of brain-derived resistin and FIAF in a novel hypothalamic neuronal cell line

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Adipokines such as resistin and FIAF (fasting-induced adipose factor) are predominantly, but not exclusively, expressed in adipose tissue and may modulate insulin sensitivity and lipid metabolism. We reported that *resistin* and *fiarf* mRNA, and resistin protein, are detectable in the mouse hypothalamus and pituitary gland (see eg. Wiesner et al., 2004). We are investigating the regulation of these genes in a novel hypothalamic neuronal cell line (N-1; Belsham et al., 2004). Over-expressing the transcription factor CEBP $\alpha$  increased *resistin* mRNA (8-fold,  $P < 0.01$ ), but reduced *fiarf* by 45% ( $P < 0.01$ ) and SOCS-3, an inhibitor of leptin signalling, by 35% ( $P < 0.01$ ; Real time RT-PCR). Conversely, attenuating resistin gene expression using a *resistin*-specific small interfering RNA (siRNA) reduced *resistin* expression by 55% (100 nM;  $P < 0.001$ ), but increased *fiarf* and SOCS-3 by 30% ( $P < 0.01$ ) and 22% ( $P < 0.01$ ) respectively. In contrast, a *fiarf*-specific siRNA reduced *fiarf* expression by 57% (100 nM;  $P < 0.001$ ), but SOCS-3 and *resistin* mRNA remained unchanged. A *fiarf*-encoding plasmid significantly increased *fiarf* mRNA 230-fold ( $P < 0.01$ ) in N-1 cells, but once again no changes in *resistin* or SOCS-3 expression were detected. These data suggest that

brain-derived resistin can attenuate *fiarf* and SOCS-3 expression, and may contribute to the regulation of hypothalamic energy homeostasis. Funded by NSHRF, IWK, UIMRF/Capital Health and CIHR.

## P281

### Myocardial ischemia in type 1 diabetic patients – re-examination after four years

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The silent myocardial ischemia is a serious diagnostic problem especially in patients with long-standing diabetes of type 1 (DM). Resting as well as exercise myocardial perfusion scintigraphy tests allow to recognize the silent myocardial ischemia in these groups of patients and to start the relevant treatment. The aim of the study was to establish the prevalence of myocardial ischemia in patients with type 1 DM, without clinical symptoms of ischemic heart disease (IHD) and with normal rest electrocardiography (ECG) record. The first examinations have been carried out in 2000 and the following re-examination took place at the end of 2004 year. The study group consisted of 20 diabetic patients (14-male, 6-female) aged 24 – 51 years (on average 35.4) treated from 4 to 37 years (on average 13.6). The patients were divided into two groups – 10 persons each. The first group with the duration of DM shorter than 10 years (4-9 year) and the second group – with DM lasting longer than 10 years (10-37 years). The functional insulinotherapy was administered to all of patients. ECG as well as resting and exercise myocardial perfusion scintigraphy (SPECT) tests have been performed in all patients. The symptoms of IHD in ECG were not observed whilst 16 persons (80%) during the first examination and 15 (75%) during re-examination abnormal results of SPECT have been found. Specifically, 70% of the 1st group patients and 90% of the second group showed abnormal results of SPECT at the beginning of the study. Alternatively, four-years later the abnormal results were observed in 90% of the first group and in 60% of the second group, respectively. It can be assumed that the duration of DM type 1 effects on emerging of myocardial ischemia. Otherwise, the proper compensation of DM will be a good protection against the increase of ischemic changes.

## P282

### The metabolic syndrome presence in peripheral arterial occlusive disease of diabetic patients

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#### Background and aims

The aim of our investigation was to evaluate the relationship between parameters of the metabolic syndrome (MSDR; NCEP/ATPIII Panel) in randomly selected diabetic patients (DM) with peripheral arterial occlusive disease (PAOD).

#### Materials and methods

1366 diabetics, male 680 (49.7%), age (mean + SD) 52.6 + 14.4yrs. (16–84), were prospectively examined using doppler examination and neuropathy disability scoring (NDS). PAOD was confirmed in 67 T2DM pts. (4.8%; G<sub>1</sub> group) while mediosclerosis in 10 (0.73%; G<sub>2</sub> group).

#### Results

Age difference (t test) was found between G<sub>1</sub> and G<sub>3</sub> (control) groups (62.5 + 8.3 yrs. vs. 64.2 + 5.9 yrs,  $P < 0.05$ ). Age of G<sub>2</sub> group was 56.5 + 13.7 yrs. VPT was significantly lower in both G<sub>1</sub> and G<sub>2</sub> groups in relation to G<sub>3</sub> (5.1 + 2.6 vs. 3.2 + 2.9 vs. 6.7 + 1.7;  $P < 0.001$ ). Significantly higher ankle reflexes score was present in G<sub>1</sub> comparing with G<sub>3</sub> group (3.3 + 1.3 vs. 2.4 + 1.6;  $P < 0.001$ ). MSDR was present in 51 pts. (76.1%; contingency tables, Chi sq.,  $P < 0.001$ ) with 3 components in 38.8%, 4 in 23.9% and 5 in 13.4%pts. In the 16 (23.9%) nonMSDR pts., 8 (11.9%) had cholesterol > 6.5 mmol/l, and 7 (10.4%) were smoker. In G<sub>1</sub> group high BP was in 48 pts. (71.6%;  $P < 0.001$ ), hyperTG in 52 pts. (77.7%;  $P < 0.001$ ). Multiple regression analysis with Winsor's index as dependent variable showed following significant constelations: TG, HDL and W ( $P < 0.06$ ); TG, HDL, W and cigarette number ( $P < 0.02$ ); cholesterolemia and cigarette number ( $P < 0.001$ ).



#### Conclusions

T2DM patients with PAOD have a significant presence of MSRD. The fact that MSRD, hyperCHOL and current smoking covers almost all PAOD cases should increase awareness of a PAOD as the part of cardiovascular health.

#### P283

##### A TCF1 mutation may cause transient congenital hyperinsulinism followed by MODY3

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In congenital hyperinsulinism (CHI), mutations have been found in 5 different genes, ABCC8, KCNJ11, GCK, GLUD1 and SCHAD. In approximately 50% of all cases, however, no genetic explanation can be found.

A mature newborn girl presented macrosomic, birth weight 4378 g, and a blood glucose down to 1.3 mmol/L at day 1. Hyperinsulinism was documented. The child responded to diazoxide treatment, initially in combination with prednisolone, chlorothiazide and octreotide.

The parents were non-consanguineous, the mother had no diabetes. The father and several members of his family had MODY 3 segregating with the previously described R159Q mutation in the TCF1 gene encoding HNF-1 $\alpha$ . The mutation was found in the girl, too. The girl had no mutations in the ABCC8, KCNJ11 and GCK genes (DHPLC and sequencing). Her p-ammonia and urine  $\beta$ -hydroxy glutaric acid were normal, excluding the possibility of a GLUD1 mutation and a SCHAD mutation. Sequencing of the MODY1 gene (HNF-4 $\alpha$ ) showed no mutations. Diazoxide was discontinued at the age of 3 y, no diet restrictions. Subsequent fasting blood glucose values, OGTT, p-proinsulin and p-C-peptide values were normal (p-insulin failed).

In the other members of the MODY3 family, no signs of hypoglycaemia in the neonatal or infancy period were recorded by questioning. Our patient had neonatal onset CHI, but no mutations in the candidate genes for CHI; GLUD1 and SCHAD mutations were ruled out by biochemical data. The hyperinsulinism was transient with normalisation after 3 years.

MODY3 has until now never been associated with transient CHI. MODY3 is characterised by a progressive insulin secretion defect. It is hypothesised, that the TCF1 mutation R159Q, perhaps influenced by unknown other genetic factors, may cause a transient hypersecretion of insulin in the neonatal period and infancy followed by euglycaemia and later MODY3. Hyperinsulinaemic hypoglycaemia may occur in other young children of MODY3 families.

#### P284

##### Waist circumference cut-offs for categorisation among Tunisian adult population

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#### Aims

Waist circumference is a convenient measure of abdominal adipose tissue, it correlates closely with BMI and is strongly linked to obesity-related risk factors. The sex specific waist circumference currently proposed for European male is 94 centimetres and for females is 80 cm. Thus, there is a need to develop sex specific waist circumference cut-offs points appropriate for different population (W.H.O).

The aims are to determine the appropriate waist cut-offs for identifying people with body mass index ( $\geq 25$  kg/m<sup>2</sup>) and those with B.M.I. ( $\geq 30$  kg/m<sup>2</sup>) among Tunisian adult population (north Africa country).

#### Materials and methods

We used a sample of the Tunisian National Nutrition Survey a cross-sectional health survey, conducted in 1996 on a large nationally representative sample which included 2927 adults over 20 years old who had measurements of height, body weight, waist circumference, blood pressure, fasting plasma glucose, total cholesterol and triglycerides. Receiver operating characteristic (ROC) analysis was used to identify

waist circumference values corresponding to BMI cut-offs for over weight ( $\geq 25$  kg/m<sup>2</sup>) and obesity ( $\geq 30$  kg/m<sup>2</sup>) in both men and women.

#### Results

Waist circumference exceeding 85 cm in men and 80 cm in women correctly identified subjects with a body mass index of  $\geq 25$  kg/m<sup>2</sup> with sensitivity of  $>90\%$  and specificity of  $>83\%$ . Whereas waist circumference exceeding 90 cm in men and 85 cm in women identified subjects with a body mass index of  $\geq 30$  kg/m<sup>2</sup> with a sensitivity of  $>95\%$  and specificity of  $>78\%$  compared with those with waist circumference below the first cut-offs points.

#### Conclusion

Waist circumference of 85 cm was most sensitive and specific in men whereas in women it was 80 cm to identify most subjects with a body mass index  $\geq 25$  kg/m<sup>2</sup>. These cut-offs are derived by identifying waist circumference values corresponding to BMI cut-offs for over weight ( $\geq 25$  kg/m<sup>2</sup>) in Tunisian adult population.

#### P285

##### Associations between bmi and smoking with reference to other life style factors, the 4th and the 5th Tromsø studies

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#### Objective

To explore the relationship between body mass index (BMI) and smoking in a cross-sectional and longitudinal study.

#### Methods

In the 4th and 5th Tromsø studies in 1994 and 2001, 27158 and 8130 subjects participated. Height and weight were measured and a questionnaire on life style factors filled in.

#### Results

Complete datasets were available for 10920 men and 12092 women in the 4th Tromsø study. Among these subjects, 2364 men and 2738 women also participated in the 5th Tromsø study. In the 4th Tromsø study, smokers as a group had lower BMI (mean  $\pm$  SD) compared to non-smokers ( $25.0 \pm 3.4$  vs.  $25.9 \pm 3.3$  kg/m<sup>2</sup>,  $P < 0.001$ ). However, among smokers there was an increase in BMI with increasing number of cigarettes smoked, particularly in males, where those smoking 1-5 cigarettes daily had BMI  $24.9 \pm 3.3$  versus  $25.9 \pm 3.3$  kg/m<sup>2</sup> in those smoking  $\geq 21$  cigarettes daily. The increase in BMI with number of cigarettes was paralleled by an increase in coffee and alcohol consumption. In the longitudinal study, all smoking subgroups increased in BMI from 1994 to 2001. The smallest increase was among those who started smoking during this period, whereas the highest increase was among those who quit smoking. For those that stopped smoking between 1994 and 2001, the increase in BMI was positively related to the number of cigarettes smoked in 1994. Changes in coffee consumption occurred in parallel with changes in smoking habits.

#### Conclusion

There is a U-shaped relationship between smoking and BMI. Heavy smoking is associated with an unhealthy lifestyle, which appears to override the weight reducing effect of cigarette smoking.

#### P286

##### Gene variants at calpain-5 locus are associated with obesity and other features of metabolic syndrome

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#### Introduction

Genes involved in common complex disorders such as obesity, type 2 diabetes mellitus (T2DM), dyslipidemia or hypertension are not disease specific, since clinically related disorders also share genetic components. These diseases cluster together in the Metabolic Syndrome, a generalized pathology with increased cardiovascular risk in which insulin resistance is considered a key feature. Cysteine protease Calpain-10 (CAPN10) has been associated with T2DM, polycystic ovary syndrome (PCOS), hypertension, hypercholesterolemia and increased body mass index (BMI). Calpain-5 gene is a CAPN10 homologue that lies in 11q13, a chromosomal region that has been linked to T2DM. We have analyzed the CAPN5 gene in several

anthropometric and biochemical determinations related to metabolic syndrome.

**Patients and methods**

We have performed a QTL association analysis of four polymorphic variants of the CAPN5 gene in 606 individuals randomly chosen from a cross-sectional population-based epidemiological survey in the province of Segovia in Central Spain (Castille), recruited to investigate the prevalence of anthropometric and physiological parameters related to obesity and other components of the metabolic syndrome. Phenotype measures analyzed include BMI, waist circumference, blood pressure, fasting and 2h-glucose levels, fasting insulin, insulin resistance estimated as HOMA, total cholesterol, LDL-c, HDL-c and triglyceride levels. The study protocol was approved by the Ethics Committee of the reference Hospital.

**Results**

Genotype analysis was significant for BMI ( $P \leq 0.041$ ), diastolic blood pressure ( $P = 0.015$ ) and HDL-cholesterol levels ( $P = 0.025$ ). Different haplotypes were also associated with BMI ( $0.022 \leq P \leq 0.043$ ), diastolic blood pressure ( $0.0005 \leq P \leq 0.010$ ) and total cholesterol levels ( $0.001 \leq P \leq 0.048$ ). In accordance with the quantitative haplotype analysis, the AACA haplotype is more prevalent in individuals with obesity ( $P = 0.031$ ), obesity with hypertension ( $P = 0.023$ ) and metabolic syndrome ( $P = 0.029$ ).

**Conclusions**

Our results suggest that Calpain-5 gene variants could contribute to the development of obesity and related cardiovascular risk factors such as hypertension and dyslipidemia in the context of metabolic syndrome.

**P287**

**Plasma concentrations of asymmetric dimethyl arginine and total homocysteine in siblings of type 2 diabetes mellitus**

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Diabetes mellitus (DM) includes a group of carbohydrate metabolism disorders that is characterized by hyperglycemia and leads long-term macro- and microvascular complications. Also siblings of type 2 diabetic patients have increased relative risk of micro and macrovascular outcome of DM even though their plasma glucose levels in normal range. Genetically determined hyperinsulinemia, insulin resistance and associated dyslipidemia have crucial role in the processes of vascular alterations in siblings of DM. In addition to classical cardiovascular risk factors, recently introduced risk factors of endothelial dysfunction such as asymmetric dimethyl arginine (ADMA) and total homocysteine (tHcy) may exert their deleterious effect on the endothelial cells. The aim of this study was to determine the plasma ADMA and tHcy concentrations in siblings of DM.

Study population consists of healthy subjects. All subjects were divided into two groups according to existing DM in their first degree relatives. Concentrations of plasma ADMA were measured by high-performance liquid chromatography (HPLC). tHcy levels were measured by ELISA. Plasma ADMA and tHcy levels were comparable between the siblings of DM and the controls ( $0.406 \pm 0.077$  vs  $0.400 \pm 0.080$   $\mu\text{mol/L}$ ;  $20.02 \pm 30.09$  vs  $21.73 \pm 25.95$   $\mu\text{mol/L}$ ) respectively. There is no correlation between ADMA and tHcy levels. In conclusion, results of this study suggested that plasma ADMA and tHcy concentrations were not increased in siblings of type 2 diabetic patients.

**P288**

**Mortality risks of diabetic patients who reach end stage renal disease (ESRD) in the united states: age and gender differences**

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Prior studies have demonstrated that diabetes as a cause of or contributing factor to end stage renal disease (ESRD) has a detrimental impact on survival. What is unclear is whether this effect varies by age and gender.

**Methods**

The objective of this study was to evaluate mortality differences by age and gender among diabetic and non-diabetic ESRD patients in a national cohort.

Data on all new ESRD patients ( $N = 451,296$ ) who were initiated on dialysis in the U.S. between 5/1995-12/2000 and followed until 12/2001 were obtained from the U.S. Renal Data System. Mortality risks were compared for diabetic and non-diabetic patients using Cox regression adjusting for sociodemographic characteristics and 18 comorbid indicators recorded at ESRD onset. The cohort was stratified by age group ( $< 50$ ,  $50-70$  and  $> 70$  yrs) and gender.

**Results**

The adjusted relative hazard ratios (RR) for death for Diabetic (DM) vs non-Diabetic (Non-DM) patients are shown.

Group	Adjusted RR of death (Diabetic vs Non Diabetic)					
	<50 yrs		50-70 yrs		>70 yrs	
	Non-DM	DM	Non-DM	DM	Non-DM	DM
All Patients	1.00	1.33**	1.00	1.10**	1.00	1.05*
Male	1.00	1.21**	1.00	1.05*	1.00	1.11**
Female	1.00	1.51**	1.00	1.14**	1.00	0.93

\*  $P < 0.01$ ; \*\*  $P < 0.001$

**Conclusions**

Mortality risks were significantly greater in diabetic than non-diabetic ESRD patients. These risks were greatest for patients  $< 50$  yrs and females. Whether these differences represent inequalities in delivered diabetic care or greater disease severity deserves further attention.

**P289**

**Hormonal profile in men suffering from coronary artery disease and metabolic syndrome**

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**Aim**

To assess possible changes in concentrations (conc) of hormones in men (M) suffering from metabolic syndrome (MS) which may be involved in pathogenesis of coronary artery disease (CAD).

**Material**

CAD-M group: 33 M with MS and CAD in the age  $51.5 \pm 6.4$  years with angiographically defined CAD. 14 of them were normoglycemic, 19 with impaired glucose tolerance or diabetes mellitus t. II. H-M group: 13 healthy M in the same age.

**Methods**

In M of both groups the occurrence of common risk factors of CAD was determined. By immunological methods conc of luteinising hormone (LH), follicle-stimulating hormone (FSH), estradiol (E2), progesterone (P), 17-hydroxy-progesterone (17-OH-P), testosterone (T), dehydroepiandrosterone sulfate (DHEAS), insulin (Ins), somatomedin-1 (IGF-1), cortisol (Cort) and leptin (Lept) in blood was measured.

**Results**

In CAD-M significantly higher conc of Lept ( $23.9 \pm 14.1$  vs  $14.2 \pm 13$   $\mu\text{g/ml}$ ,  $P < 0.02$ ), Ins ( $26.3 \pm 37.4$  vs  $9.7 \pm 4.9$   $\mu\text{U/ml}$ ,  $P < 0.00005$ ) and Cort ( $12.6 \pm 3.8$  vs  $9.0 \pm 3.8$   $\mu\text{g/dl}$ ,  $P < 0.04$ ) than in H-M was revealed. Conc of LH was lower ( $3.7 \pm 2.3$  vs  $6.0 \pm 4.8$  IU/l,  $P < 0.01$ ) and conc of P was higher ( $0.034 \pm 0.029$  vs  $0.013 \pm 0.01$  nmol/l,  $P < 0.001$ ) and there was a trend towards lower conc of FSH and T and higher conc of E2 in CAD- M. Conc of IGF-1, 17-OH-P and DHEAS were similar in M of both groups.

**Conclusions**

Higher conc of Ins influence atherogenesis in M. Elevated conc of Lept may constitute a risk factor of CAD in M. Higher conc of P and lower conc of LH indicates changes in function of pituitary-gonadal axis in men with CAD and MS.

Elevated conc of Cort in CAD-M may suggest proatherogenic changes in adrenal steroidogenesis.

**P290****The impact of vitamins and/or minerals supplementation on blood pressure in type 2 diabetes**Maryam Farvid<sup>1</sup>, M Jalali<sup>2</sup> & F Siassi<sup>2</sup><sup>1</sup>School of Nutrition and Food Technology, Shaheed Beheshti University of Medical Sciences, Tehran, Iran; <sup>2</sup>School of Public Health, Tehran University of Medical Sciences, Tehran, Iran.**Objective**

The present study designed to assess the effect of Mg + Zn, vitamin C + E, and combination of these micronutrients on blood pressure in type 2 diabetic patients.

**Materials and methods**

In a randomized, double-blind, placebo controlled clinical trial, 69 type 2 diabetic patients were randomly divided into four groups, each group receiving one of the following daily supplement for 3 months; group M: 200 mg Mg and 30 mg Zn ( $n = 16$ ), group V: 200 mg vitamin C and 150 mg vitamin E ( $n = 18$ ), group MV: minerals plus vitamins ( $n = 17$ ), group P: placebo ( $n = 18$ ). Blood pressure was measured at the beginning and at the end of the trial. Treatment effects were analyzed by general linear modelling.

**Results**

Results indicate that after 3 months of supplementation levels of systolic, diastolic and mean blood pressure decreased significantly in the MV group by 8 mmHg ( $122 \pm 16$  vs.  $130 \pm 19$  mmHg), 6 mmHg ( $77 \pm 9$  vs.  $83 \pm 11$  mmHg), and 7 mmHg ( $92 \pm 9$  vs.  $99 \pm 13$  mmHg), respectively ( $P < 0.05$ ). Also combination of vitamin and mineral supplementation had significantly effects in increasing serum potassium and decreasing serum sodium to potassium ratio ( $P < 0.05$ ) and in decreasing serum malondialdehyde ( $P < 0.05$ ). There was no significant change in the levels of these parameters in the other 3 groups.

**Conclusion**

In conclusion, the results of the present study indicated that in type 2 diabetic patients, combination of vitamins and minerals rather than vitamin C and E, or Mg and Zn, might decrease blood pressure.

**P291****Pro12Ala PPAR gamma2 gene polymorphism in PCOS women – the role of the satiety and insulin activity regulating compounds**B Bidzinska-Speichert<sup>1</sup>, U Tworowska<sup>1</sup>, M Demissie<sup>1</sup>, G Bednarek-Tupikowska<sup>1</sup> & R Slezak<sup>2</sup><sup>1</sup>Department of Endocrinology and Diabetology, Medical University of Wrocław, Wrocław, Poland; <sup>2</sup>Department of Genetics, Medical University of Wrocław, Wrocław, Poland.

Peroxisome proliferator-activated receptors (PPARs) regulate adipocyte differentiation and gene expression by an interplay with other transcription factors. They are the nuclear receptor that are mostly expressed in adipocytes and some nonadipose tissues like intestinal wall, muscles, liver and macrophages. The aim of the study was to assess the possible association between Pro12Ala PPAR-gamma2 receptor gene polymorphism and satiety factors, insulin resistance and androgen excess in PCOS women.

**Material and methods**

Two groups of women were studied: a control group (51) and PCOS women (54). Women were divided into a group of non-obese with BMI  $< 30$  kg/m<sup>2</sup> and obese women with BMI  $\geq 30$  kg/m<sup>2</sup>. Examinations: BMI and WHR, measurements: insulin and glucose during OGTT, leptin, NPY, galanin and CCK. Pro12Ala polymorphism in the gene of PPAR gamma2. The study protocol was approved by local Ethical Committee.

**Results**

Frequency of Ala allele in the control group was estimated at 26.47% and in the PCOS patients at 23.15%. PCOS was connected with occurrence of hyperleptinemia and higher NPY levels. PCOS, obese and non-obese subjects had significantly lower levels of galanin. Significantly lower levels of CCK in obese women with PCOS were observed.

**Conclusions**

Frequency of Pro12Ala polymorphism evaluated in investigated patients is significantly higher than shown in other European populations. Pro12Ala polymorphism in women with PCOS carrying a single Ala allele has protective role as far as hyperleptinemia, hyperandrogenemia and insulin resistance is concerned. PCOS patients may reveal a disrupted loop of central leptin/NPY feedback. The constellation of high leptin/NPY and low galanin/CCK levels in women with PCOS indicates some shifts in food intake.

**P292****Glucose metabolism, insulin secretion and insulin sensitivity in juvenile hemochromatosis**N Angelopoulos<sup>1</sup>, M Noutsou<sup>2</sup>, A Goula<sup>1</sup> & G Tolis<sup>1</sup><sup>1</sup>Hippocrateio Hospital, Department of Endocrinology, Athens, Greece; <sup>2</sup>Hippocrateio Hospital, Department of Diabetes, Athens, Greece.

Among specific diabetes subtypes secondary to pancreatopathies, hereditary hemochromatosis is an inherited disorder of iron metabolism, resulting to excessive iron overload and tissue damage in various organs. We report here the case of a man with the young-onset form of the disease and we describe his glycaemic status, before and during venesection therapy. A 25-year-old man attended our clinic in Athens, Greece, with hypogonadotropic hypogonadism due to hereditary hemochromatosis. Genetic analysis revealed that he was suffered from juvenile aggressive form, and treatment initiated with frequent phlebotomies, among with androgen substitution. Within 18 months of therapy ferritin level was normalized and hypogonadism was fully restored. Despite severe iron overload glucose tolerance remained normal during the various stages of the disease, although alterations in both insulin secretion and sensitivity were detected. In conclusion, glucose metabolism in juvenile hemochromatosis is a unique clinical entity and mainly depends on the efficacy of chelation therapy and the duration that patient remains under the iron-overload stage.

**P293****Glucocorticoids are metabolised within perivascular adipose: the link between metabolic diseases and their vascular consequences?**

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The metabolism of glucocorticoids within adipose tissue, by the enzyme 11beta-hydroxysteroid dehydrogenase type 1 (11HSD1), is known to be altered in obesity and following high fat feeding. Until recently, little attention has been paid to the fat depots found directly adjacent to blood vessels. Emerging evidence suggests that perivascular adipose may play a key role in modulating vascular function. The presence of steroidogenic enzymes within perivascular adipose, and the effect of obesity and high fat feeding on enzyme activity have not been previously explored. We hypothesized that 11HSD1 mRNA and activity is present in rodent peri-aortic adipose, and that this enzyme is enhanced by obesity, and reduced by high fat feeding. Peri-aortic adipose tissue was obtained from male C57B6J mice following 8 weeks of high fat or chow diet, and lean and obese Zucker rats. 11HSD1 enzyme activity was determined in homogenized tissue *in vitro* by conversion of tritiated corticosterone to 11-dehydrocorticosterone. mRNA for 11HSD1, 5alpha-reductase and glucocorticoid receptor (GR) in peri-aortic adipose from lean and obese Zucker rats was quantified by real time PCR and corrected for cyclophilin and 18S. Data are mean  $\pm$  SEM. 11HSD1 activity was detected in murine peri-aortic adipose ( $56 \pm 7\%$  conversion after 2 hrs) and was significantly reduced following high fat feeding ( $25 \pm 2\%$  conversion,  $n = 6$ ,  $P < 0.001$ ). There was an increase in 11HSD1 activity ( $20 \pm 2\%$  conversion after 4 hrs vs  $14 \pm 1\%$  conversion in controls,  $n = 8$ ,  $P = 0.05$ ) and expression ( $1.1 \pm 0.2$  vs  $0.6 \pm 0.1$ ,  $n = 8$ ,  $P < 0.005$ ) in peri-aortic adipose from obese Zucker rats. In addition, obesity was associated with a reduction in 5alpha-reductase ( $0.7 \pm 0.1$  vs  $1.0 \pm 0.1$  in controls,  $n = 8$ ,  $P < 0.05$ ) and an elevation in GR ( $1.0 \pm 0.1$  cf  $0.7 \pm 0.1$ ,  $n = 8$ ,  $P < 0.01$ ) mRNA. These data demonstrate that perivascular adipose is a novel site of glucocorticoid metabolism. Altered glucocorticoid metabolism within adipose at this location may contribute to vascular dysfunction in obesity.

**P294****Absence of sexual dimorphism in the symptomatic responses to hypoglycaemia in adults with and without type 1 diabetes**J Geddes<sup>1</sup>, RE Warren<sup>1</sup>, AJ Sommerfield<sup>1</sup>, V McAulay<sup>1</sup>, MWJ Strachan<sup>1</sup>, KV Allen<sup>1</sup>, IJ Deary<sup>2</sup> & BM Frier<sup>1</sup><sup>1</sup>The Royal Infirmary of Edinburgh, Edinburgh, United Kingdom; <sup>2</sup>The University of Edinburgh, Edinburgh, United Kingdom.



#### Objective

In humans insulin-induced hypoglycaemia activates the autonomic nervous system and provokes counterregulatory hormonal responses, the magnitude of which is lower in healthy non-diabetic females and in women with type 1 diabetes compared to male counterparts, although the glycaemic thresholds at which these responses are triggered are similar in both sexes. The Edinburgh Hypoglycaemia Score (a validated method of symptom assessment) was used to examine symptoms to ascertain whether hypoglycaemia symptomatic responses differ between the sexes.

#### Research design and methods

Symptom score data from 8 hypoglycaemia studies (induced using the hyperinsulinaemic glucose clamp technique) were analysed in 160 subjects (age range 18-45 years). The subjects with type 1 diabetes ( $n = 72$ , 31 female) all had diabetes for at least one year (median 6.9 years, range 1.1-30.9), with reasonable glycaemic control (mean HbA1c 7.9% SD 2.0) and normal awareness of hypoglycaemia. The results were compared with symptoms in healthy non-diabetic subjects ( $n = 88$ , 45 females).

#### Results

Scores for the autonomic, neuroglycopenic and general malaise symptoms were all significantly higher during hypoglycaemia, compared to euglycaemia ( $P < 0.0005$ ). The non-diabetic subjects had higher autonomic symptom scores than those with type 1 diabetes, this difference being statistically significant ( $P = 0.011$ ,  $F = 0.176$ ). No differences were observed in the autonomic symptom scores between gender in either cohort ( $P = 0.196$ ,  $F = 1.683$ ). No statistical difference in neuroglycopenic symptom scores was found between the non-diabetic subjects and those with type 1 diabetes, nor was any effect of gender evident.

#### Conclusion

The sexual dimorphism that is recognised to affect counterregulatory hormonal responses to hypoglycaemia and is manifested by physiological differences, was not apparent with respect to symptomatic responses, suggesting that the symptoms of hypoglycaemia do not differ between men and women.

### P295

#### Metabolic syndrome and insulin sensitivity in early glycoregulation disorders

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#### Background and aims

Early glycoregulation disorders, impaired fasting glucose (IFG) and impaired glucose tolerance (IGT) are associated with metabolic syndrome (MS). The aim of this study is: (a) to analyze parameters of insulin sensitivity, glycoregulation, lipid status, microalbuminuria (MI) and homocysteine (HCY) in patients with obesity (I), IFG (II), IGT (III) and newly diagnosed DM type 2 (IV) patients; (b) to determine the percentage of metabolic syndrome at these patients.

#### Materials and methods

The study included 148 obese individuals (BMI > 25 kg/m<sup>2</sup>) over 45 years. The oral glucose tolerance test (OGTT)- WHO, WG, 1998, revealed obesity in 23.6%, IFG in 12.2%, IGT in 35.8%, diabetes mellitus type 2 (DM2) in 28.4% of patients. Insulin sensitivity was determined by HOMA IR; lipid status was determined by spectrophotometry (total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides) and immunochemical method Lp(a)<sub>1</sub>.

#### Results

The metabolic syndrome accounted for 38.2% in obese patients, while it was almost double in other groups (IFG - 72.2%, IGT - 76.0%, DM2 - 70.7%). BMI indicated extreme obesity in all groups. HOMA IR was elevated in IGT and DM2 groups. LDL/HDL ratio was at the upper maximum level and did not differ statistically between groups. Lp(a) was slightly increased in IGT patients and considerably increased in IFG ones. Blood pressure was moderately increased in all groups of patients, with

no statistical difference between groups. Homocystein was at upper maximum level ( $11.7 \pm 3.0$ ,  $12.3 \pm 4.6$ ,  $12.3 \pm 4.6 \mu\text{mol/l}$ ) and there was no important statistical difference between groups. MI showed a statistical importance ( $P < 0.05$ ) at obese patients with MS compared to those without MS.

#### Conclusions

Results obtained indicate that a metabolic syndrome exists at two thirds of patients with early glycoregulation disorders (IFG, IGT) and newly diagnosed diabetes mellitus type 2, and that it is associated with decreased insulin sensitivity, hypertension and hyperlipoproteinemia.

### P296

#### Biochemical predictors of the cardiovascular complications in patients with obesity and type 2 diabetes

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#### Background

The impact of hyperglycemia on atherogenesis is regulated through the increase of the oxidative stress, increase of the rest of the glycosylated products of metabolism and development of the endothelium dysfunction.

#### Aim

To compare the biochemical indexes and investigate their relationship with the development of cardiovascular disease in obese patients with and without type 2 diabetes.

#### Materials and methods

21 patients (mean age 22-65 years) divided in 2 groups were investigated. I group comprised the obese patients with type 2 diabetes documented with clinical data, II group - obese patients without diabetes. In I group 8 patients had extension of heart chambers, 7- diastolic dysfunction of the left ventricle, 3 - hypertrophy of IVS, 7 - ischemic alterations, 6 - arterial hypertension. In II group - 8 patients had extension of heart chambers, 5- diastolic dysfunction of the left ventricle, 7 - hypertrophy of IVS, 3 - ischemic alterations, 2 - arterial hypertension.

#### Results

Patients with type 2 diabetes had increased BMI. Elevation of Cholesterol, Tryglicerids, LDL and the lowering of HDL was practically equal in both groups. The level of glycosylated hemoglobin in patients with diabetes was elevated what is risk for the development of the cardiovascular diseases.

#### Conclusions

In both of the groups was the anatomic and functional alteration of the heart observed, although the cardiovascular complications significantly prevail over in patients with type 2 diabetes. In obese patients with diabetes the main damage is disturbance of energetic metabolism in cardiomyocytes, what reduces the ability of the myocard for the functional adaptation and more early clinical manifestation of the cardiovascular diseases.

### P297

#### Decreased cardiac and arterial noradrenaline and dopamine concentrations occur concomitantly with the development of type II diabetes in rats treated neonatally with monosodium glutamate (MSG): a possible basis for autonomic neuropathy?

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Neonatal rats were treated with MSG (4G/Kg daily for 7 days). Glucose tolerance tests (GTT) were performed at 32, 65 and 70 weeks. After one year there was evidence of Type II diabetes, and the blood glucose levels were significantly greater than the controls for several points in the GTT; islet beta-cells were unchanged, cataracts were often present and there was central obesity.

The amines in the heart, proximal tail artery, kidneys and adrenals were extracted after decapitation and analysed using HPLC. At 32 weeks of age



there were no significant differences between MSG-treated and control rats in the GTT and in amine concentrations, except that the renal level of adrenaline (ADR) and serotonin (SER) increased by around 50% in the experimental group.

At 65 weeks of age, 3 out of 5 points in the GTT were significantly different from controls. There were significant reductions in cardiac noradrenaline (NA), ADR, 5-hydroxyindoleacetic acid (HIAA) and dopamine (DOP), and in arterial NA, ADR, DOP and SER. Adrenal NA, ADR and SER increased significantly. Renal SER remained significantly increased, but adrenaline fell.

At 70 weeks of age, 5 out of 5 points in the GTT were significantly greater than controls. Cardiac NA fell, but not significantly, and DOP and HIAA were significantly less than controls; arterial NA, SER and DOP also fell significantly. Adrenal NA and ADR were significantly increased compared with the controls. Renal SER remained high, and DOP fell significantly.

There is evidence of a decline of [NA] in the heart and artery, no change in the kidney and an increase in the adrenal after Type II diabetes become established. At the same time, [DOP] in heart and artery also fell. These findings may be of relevance to autonomic neuropathy and cardiovascular control in Type II diabetes.

## P298

### Gender distribution in Ukrainian adult insulin-treated diabetics depending of age at diagnosis

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#### Background

The male preponderance among 15–30 yrs old patients with type 1 diabetes mellitus (T1DM) was repeatedly described. All-age gender distribution assessment was not done yet because of the difficulties in the type of DM recognition in insulin-treated (IT) patients diagnosed after 30 yrs of age. The possibility of such assessment has arisen from the use of population-based DM registers by patients stratification depending of their disease history.

#### Methods

A cross-sectional analysis of Ukrainian DM nation-wide register (105 364 IT patients  $\geq 30$  yrs of age) was conducted. Patients were stratified into subgroups according to the age, when DM was diagnosed: "T1DM"  $\leq 30$  yrs, intermediate group ("IG") 31–39 yrs, and "T2DM"  $\geq 40$  yrs at diagnosis. Gender distribution was assessed in each group and adjusted according to the 2001 Ukraine national census. The data sets were analyzed using a 2-way chi-square analysis. Percentage data were arcsine transformed.

#### Results

The percentage of patients who had insulin-free period  $< 2$  yrs was decreased and proportion of females as well as female/male RR were increased with increasing of age at the time of DM diagnosis (see Table).

Group (age at diagnosis, yrs)	N	Percentage of patients with insulin-free period $< 2$ yrs (%; 95% CI)	Proportion of females (%; 95% CI)	Female/male RR (95% CI)
T1DM ( $\leq 30$ )	16821	88.2 (87.7–88.7)	47.5 (46.7–48.2)	0.69 (0.67–0.71)
IG (31–39)	16754	63.3 (62.5–64.0)	49.4 (48.6–50.1)	0.74 (0.72–0.76)
T2DM ( $\geq 40$ )	71789	40.6 (40.2–40.9)	64.0 (63.7–64.4)	1.27 (1.25–1.29)
p		$< 0.001$ (between groups)	$< 0.001$ (between groups)	$< 0.0001$ (for each group)

## Conclusions

The male preponderance in adult IT patients which are rather T1DM and female ones which are rather T2DM was revealed in Ukrainian diabetic population. Gender distribution is related to patient age at DM diagnosis. Further investigation could clarify if it depends upon the sex hormones dynamics in ontogenesis.

## P299

### Effects of arm exercise on metabolic parameters in type 2 diabetic patients

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#### Background

Arm swing is a Chinese style of exercise. During the exercise, participants stand and simultaneously move their both arms forward and then backward for about 1 round per second.

#### Abstract

The present study aims to investigate effects of arm exercise training on metabolic responses in type 2 diabetic Thai patients.

#### Methods

Fourteen male and 47 female diabetic patients (aged  $57.5 \pm 1.4$  yrs) without cardiovascular disease were recruited in the present study. All subjects were informed verbally and in writing before they signed the consent form approved by the Ethical Committee of the University of Nottingham Medical School. Subjects did not take part in regular physical training. The experiment consisted of 2 eight-week periods. During the first period, subjects maintained normal daily life without regular exercise. During the next consecutive period, they performed 30-min arm swing per day, 3 days per week. Fasting blood glucose, HbA<sub>1c</sub> concentrations, insulin sensitivity, lipid profiles and anthropometry were measured before and after each period. Insulin sensitivity was determined by the Quantitative Insulin Sensitivity Check Index (QUICKI).

#### Results

There were no significant differences on fasting blood glucose and HbA<sub>1c</sub> concentrations, insulin sensitivity, lipid profiles, and anthropometric parameters between both periods.

#### Conclusions

These results suggest that thirty-min arm swing for 8 weeks may have no effects on glucose and lipid metabolism in Diabetes Mellitus type 2. Either the exercise intensity or frequency of arm swing may be too low to have a significant effect on metabolism in type 2 diabetes patients.

## P300

### The ethanolic extract of *Combretum decandrum* Roxb. improved glucose tolerance and increased glucose uptake of hyperinsulinemic rats

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Abstract withdrawn.

### P301

#### In-patients food intake and reasons of non-consumption

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#### Background

In-patients inadequate food intake can worsen the prevalence and degree of malnutrition.

#### Patients and methods

A "one day" questionnaire was used in medicine (M-Dpts) geriatric (G-Dpts) and surgery (S-Dpts) departments of a university hospital to: (i) evaluate nutritional status with the Detky score, (ii) quantify food intake, (iii) understand the reasons of non-consumption of in-patients (n 286, 46 & 104 in M-, G- & S-Dpts, respectively).

#### Results

29, 33 and 30% patients in M-Dpts, G-Dpts and S-Dpts respectively had a potential malnutrition according to the Detsky score.

When breakfast, lunch and dinner were considered separately, patients who had eaten: the whole breakfast were 77, 67 and 74%, the whole lunch 73, 69 and 69% and the whole dinner 80, 73 and 72% in M-, G- and S-Dpts respectively. 46, 24 and 33% patients from M-Dpts, G-Dpts and S-Dpts have eaten the total menu of the day.

Patients were more likely to eat the totality of fruits & vegetables than all the meat any (fruits & vegetables vs meat: 72 vs 40%, 74 vs 51%, 81 vs 69% in M-, S- and G-Dpts respectively,  $P < 0.05$ ).

Patients who had lost more than 3 kg during the last 3 m, who had not eaten the entire menu or who had not eaten the totality of the meat were more likely to have a potential malnutrition. Too many dishes on the tray and the illness itself are the 2 main reasons mentioned by the patients for not consuming the food served.

#### Conclusion

Food intake is insufficient in in-patients particularly in terms of protein and for patients who already have potential malnutrition. Given energy enriched food could be one solution to improve in-patients food intake.

### P302

#### Renal expression of alpha-ENaC is increased in testosterone treated rats

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#### Introduction

There is no difference in blood pressure between boys and girls, but following puberty, blood pressure increases more in men than in women. The higher blood pressure and stronger progression of hypertension in men is associated with a higher risk and mortality for cardiovascular diseases than in women. Androgens are known to play an important role in renal tubular epithelial cell growth, hypertrophy and erythropoietin production and may be important determinants of sex-specific differences in blood pressure. Modulation of epithelial sodium reabsorption through the epithelial sodium channel (ENaC) is an important component in the control of sodium balance. The promoter of the gene encoding for alpha-ENaC harbours an androgen receptor (AR) binding site. We investigated the *in vivo* effect of testosterone treatment on ENaC expression in rat kidneys.

#### Methods

Male Wistar rats aged 8–10 weeks were orchietomized and treated either with a long-lasting testosterone undecanoate (100 mg/kg or 500 mg/kg), or with a 5alpha-dihydrotestosterone preparation (75 mg/pellet per 21-d release) or with placebo (each group  $n = 4$ ). After 14 days the kidneys were removed. RNA was extracted and semi-quantitative PCR was performed using a Typhoon 8600 (molecular dynamics). Relative expressions were normalized by calculating the target gene/18S ratio.

#### Results

AR was expressed in male rat kidneys and its expression was not influenced by androgen treatment. Renal alpha-ENaC expression was significantly ( $P < 0.01$ ) higher in testosterone (500 mg/kg) than in placebo treated animals. beta- and gamma-ENaC showed a trend towards higher expression in testosterone treated animals. Surprisingly, 5alpha-dihydrotestosterone did not significantly alter the expressions of ENaC subunits or AR.

#### Conclusions

These data show that alpha-ENaC expression in the rat kidney is regulated by testosterone *in vivo*. It highlights a potential mechanism explaining the reported gender differences in blood pressure.

### P303

#### Insulin resistance syndrome (IRS) and non-alcoholic fatty liver disease (NAFLD) in obese children: influence of ethnic background, sex, age and family history of type 2 diabetes (T2DM)

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#### Background

IRS consists of  $\geq 3$  of the following components: obesity (BMI  $> 98$ th centile), abnormal insulin glucose homeostasis, hypertension, dyslipidaemia. Elevated serum alanine aminotransferase (ALT) is a marker of NAFLD.

#### Objective

To define the prevalence of IRS and NAFLD in obese children referred to our centre.

#### Methods

109 subjects: mean BMI-SDS  $+6$  ( $+0.6$  to  $+14$ ), median age 13.4 years (3–19), female 66%, British Asian 24%, underwent an oral glucose tolerance test with results categorised by WHO criteria: impaired fasting glucose (IFG), impaired glucose tolerance (IGT), T2DM. Fasting hyperinsulinaemia (FH) and 120 minute hyperinsulinaemia (HI) were defined  $>26$  and  $>95$  mIU/L respectively. HOMA-IR was calculated (normal  $<2.5$ ). Fasting lipids were measured in 42, ALT in 29 and blood pressure in 24. Abnormalities were defined: cholesterol  $> 95$ th centile for age and sex, triglycerides  $\geq 1.75$  mmol/l, ALT  $> 45$  IU/l, systolic BP  $> 95$ th centile for age and sex.

#### Results

Abnormalities of glucose and insulin homeostasis were observed in: IFG 4%, IGT 13%, T2DM 6% (only one child with IGT was identified with IFG), HOMA-IR  $>2.5$  78%, FH 42%, HI 38% and acanthosis nigricans 40%. British Asians were more likely than British White to have acanthosis nigricans (OR 4.1, 95%CI 1.3–12.7). Elevated lipids, ALT and BP were identified in 33.5% (cholesterol 9.5%, triglycerides 24%), 42% and 54% respectively. BP was correlated with BMI ( $r^2 = 0.17$ ,  $P = 0.05$ ) and HOMA-IR contributed 16% to the variance of ALT ( $P = 0.04$ ). Controlling for age and puberty, HOMA-IR was determined by female sex ( $P = 0.002$ ), acanthosis nigricans ( $P = 0.002$ ) and BMI-SDS ( $P = 0.04$ ). 36% had obesity alone, 43% had 2 components IRS and 21% had  $\geq 3$  components IRS. The number of components of IRS was determined by increasing age ( $P = 0.03$ ) and family history of T2DM ( $P = 0.02$ ).

#### Conclusion

In obese children, female sex, British Asian background, increasing age and family history of T2DM appear to be risk factors for IRS. Increased insulin resistance was observed in children with NAFLD.

### P304

#### Serum ghrelin levels during oral glucose challenge – new insights from gastrectomized patients

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Ghrelin is gastric peptide with appetite stimulating and growth hormone (GH) releasing properties. Circulating ghrelin levels decrease after nutrient ingestion, oral and iv glucose challenge. It has been hypothesized that vagal system may have a major role in initiating this decrease.

The aim of this study was to investigate circulating ghrelin levels during oral glucose challenge in gastrectomized (GASTRX)-vagotomized patients. The study was approved by the local ethics committee.

In six GASTRX- vagotomized patients and eleven healthy age and sex matched subjects standard (75 g) oral glucose tolerance test (OGTT) was performed. Glycemia, insulin, GH and ghrelin levels were determined at baseline and every 30 min for two hours.

Serum ghrelin levels at baseline were reduced by 55% in GASTRX- vagotomized patients compared to the control group,  $P < 0.01$ . OGTT induced significant reduction in serum ghrelin levels in healthy but not in

GASTRX-vagotomized subjects ( $\Delta_{\text{ghrelin}}$  214.2  $\pm$  44.0 vs 19.5  $\pm$  11.8 pg/ml). Significantly higher increase in blood glucose ( $\Delta_{\text{glycemia}}$  9.2  $\pm$  1.3 mmol/l vs. 2.8  $\pm$  0.8 mmol/l,  $P < 0.01$ ) and serum insulin levels ( $\Delta_{\text{insulin}}$  75.0  $\pm$  15.6 mU/l vs. 30.6  $\pm$  5.7 mU/l,  $P < 0.01$ ) were observed in GASTRX-vagotomized patients compared to healthy controls during OGTT. GH response to OGTT in GASTRX-vagotomized patients was not different from the control group.

In conclusion, circulating ghrelin levels in GASTRX-vagotomized patients do not decline after oral glucose administration supporting the hypothesis that in human subjects post glucose fall in ghrelin levels may be vagally mediated.

### P305

#### A gene gene interaction of single nucleotide polymorphisms within two oxidative stress genes affects arterial compliance in patients with coronary artery disease

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We hypothesised that SNPs within oxidative stress genes influence vascular compliance in patients with coronary artery disease.

A SNP within the gene encoding the eNOS (*NOS3*) which encodes a Glu298  $\rightarrow$  Asp amino acid substitution has been associated with cardiovascular disorders in which NO bioactivity is impaired. The gene coding for p22phox, a critical component of the NADH/NAD(P)H oxidase enzyme system, a major source of vascular SO, is *CYBA*. An allelic polymorphisms of *CYBA*, C242T, is associated with progression of coronary atherosclerosis. Consistent characteristic changes in the pulse pressure wave shape are associated with ageing, risk factors for cardiovascular disease and impaired NO bioactivity. Radial applanation tonometry with pulse waveform analysis can be utilized to quantify arterial compliance and dynamic changes in NO bioactivity.

Local ethical committee approval was obtained for the study. 103 patients undergoing coronary bypass graft surgery were recruited. Genotypes were determined with polymerase chain reaction and restriction digestion. Radial artery pressure waveforms were recorded using a calibrated tonometer. Windkessel based diastolic pressure decay analysis was used to generate large (C1) and small (C2) artery compliance values.

The distribution of the genotypes in either gene was in Hardy-Weinberg equilibrium. Homozygosity for the *NOS3* polymorphism (894 G  $\rightarrow$  T) was associated with decreased small artery compliance ( $P = 0.01$ ). In contrast the *CYBA* 242T allele was associated with decreased large artery compliance ( $P = 0.0001$ ). A gene-gene interaction was evident with patients homozygous for the *NOS3*T allele and possessing the *CYBA* 242T allele having lower large ( $P = 0.01$ ) and small ( $P = 0.01$ ) artery compliance than patients homozygous for the *NOS3* G allele and homozygous for the *CYBA* C allele.

We confirmed our hypothesis that SNPs in the *NOS3* and *CYBA* genes contribute to vascular compliance.

### P306

#### The variable number of tandem repeat polymorphism in the *NOS3* gene is associated with arterial compliance and insulin resistance in coronary artery disease

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A common *NOS3* polymorphism (the gene which encodes endothelial nitric oxide synthase), a variable number of tandem repeats within Intron 4 has been associated with cardiovascular disorders in which nitric oxide bioactivity is impaired including myocardial infarction. Consistent characteristic changes in the pressure pulse wave shape have been associated with ageing, risk factors for cardiovascular disease and impaired NO bioactivity. Therefore pulse waveform analysis can be utilized to quantify arterial compliance as well as dynamic changes in NO bioactivity.

Parameters associated with Insulin resistance have also been associated with both arterial stiffness and ischaemic heart disease. We hypothesise that this polymorphism within *NOS3* would influence vascular compliances and parameters of insulin resistance in patients with coronary artery disease.

Ethical approval was obtained from the local committee. We recruited 101 patients with angiographically documented coronary artery disease. Genotypes were determined with polymerase chain reaction and restriction digestion. Radial artery pressure waveforms were recorded using a calibrated tonometer at the radial artery to derive a measurement of systemic arterial compliance (augmentation index). Moreover we measured biochemical parameters associated with insulin resistance: ICAM, IL-6 and sensitive CRP. Differences between genotype groups were analysed using unequal variance unpaired Student's *t* tests.

The distribution of the genotypes in did not differ significantly from that expected under Hardy Weinberg equilibrium. We found that the AA subtype was associated with increased systemic arterial stiffness ( $P = 0.03$ ), and higher levels of biochemical parameters associated with biochemical parameters of insulin resistance including CRP ( $P = 0.01$ ), IL 6 ( $P = 0.04$ ) and ICAM ( $P = 0.05$ ).

We confirmed our hypothesis that this polymorphism of the *NOS3* gene affects arterial compliance and biochemical parameters of insulin resistance.

### P307

#### Altered cholesterol and bile acid homeostasis in the hexose-6-phosphate dehydrogenase (H6PDH) knockout mouse

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In humans, glucocorticoids (GC) are implicated in the pathogenesis of obesity and insulin resistance. GCs are regulated at the prereceptor level by 11beta-hydroxysteroid dehydrogenases (11 $\beta$ -HSD). 11 $\beta$ -HSD type 1 (11 $\beta$ -HSD1) predominantly displays oxo-reductase activity, converting cortisone in man, 11-dehydrocorticosterone in rodents, to cortisol and corticosterone respectively – a reaction requiring the cofactor NADPH. The generation of a hexose-6-phosphate dehydrogenase (H6PDH) null mouse has shown that H6PDH is required for generating NADPH and thereby allowing oxo-reductase activity of 11 $\beta$ -HSD1. In this study, we used the H6PDH KO mice to assess the effects of altered GCs on lipid and carbohydrate metabolism in the liver. Histological examination of liver sections revealed no obvious difference between the KO and WT livers, although hepatic cholesterol levels were found to be lower in the KO mice compared with WT ( $n = 4$ ). Pooled liver RNA from 3 WT and 3 KO male mice were used to assess changes in expression of genes involved in a variety of metabolic pathways, using Affymetrix arrays. Peroxisomal acyl-CoA thioesterase 2A (PTE-2A), an enzyme involved in peroxisomal  $\beta$ -oxidation and proposed to regulate bile acid formation and excretion, was up regulated 7.4 fold in the H6PDH KO mice compared with WT controls ( $P < 0.001$ ). This increase in expression was confirmed by real time PCR, where there was an 18.4 fold increase in the KO compared to WT ( $P < 0.001$ ). Cyp7a1, which encodes cholesterol-7a hydroxylase, the rate limiting step in bile acid formation, was increased 1.6 fold in the H6PDH KO male mice compared to WT mice ( $P < 0.001$ ), and the very low density lipoprotein receptor (VLDLR) was increased 2 fold in the KO mice compared with WT ( $P < 0.001$ ). These data suggest a potentially novel role for H6PDH and possibly GCs in the maintenance of cholesterol and/or bile acid homeostasis.

### P308

#### Insulin secretion in HBV and HCV infected patients with type 2 diabetes mellitus

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Background and aims

Earlier we reported, that GADA and ICA revealed more often in HBV and HCV infected type 2 diabetic patients, than in noninfected. The goal was to determine the role of HBV- and HCV-infections in insulin insufficiency mechanisms in type 2 diabetic patients.

Materials and methods

173 patients with type 2 DM (41 male, 132 female, middle age 57.5  $\pm$  0.8 year, average diabetes duration 8.55  $\pm$  0.53 years) were surveyed. Markers of a viral hepatitis B and C, glutamic acid decarboxylase antibodies (GADA), islet cells antibodies (ICA) and basal C-peptide level were studied by immune-enzyme assay.

**Results**

In HBV and HCV infected, GADA and/or ICA-positive diabetic patients ( $n = 18$ , patients with 0 pmol/l C-peptide level were excluded) the C-peptide median was essentially lower, than in GADA and/or ICA-negative diabetic patients ( $n = 10$ ) (0.84 vs 1.49 pmol/l respectively;  $P = 0.027$ , Mann-Whitney U test). The survival analysis (Kaplan–Meier product limit method with use of Cox F-test) has been lead before an outcome (decrease C-peptide) depending on duration DM. It is revealed, that in HBV- and HCV-infected type 2 diabetic group a share of persons, which are not having C-peptide level decrease, was less, than in noninfected; the difference made from 8 up to 13% ( $F(158.66) = 1.59, P = 0.016$ ).

**Conclusion**

In HBV and HCV infected patients with type 2 DM, with increase in duration of disease, decrease of insulin secretion faster progress than in noninfected. One of the pathogenetic mechanisms of insulin secretion decrease can be autoimmune aggression directed to pancreatic  $\beta$ -cells, initiated by HBV- and HCV-infection.

**P309**

**The pancreatic beta-cells autoantibodies in HBV and HCV infested patients with type 2 diabetes mellitus**

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**Background and aims**

High prevalence of diabetes mellitus (DM) in patients with viral hepatitis is observed. Trigger role of HBV and HCV infection in development of autoimmune reactions in diabetic patients is discussed. The goal was to study influence of hepatotropic viruses (HBV and HCV) on development of autoimmune processes to pancreatic  $\beta$ -cells.

**Materials and methods**

173 patients with type 2 DM (41 male, 132 female, middle age  $57.5 \pm 0.8$  year, average diabetes duration  $8.55 \pm 0.53$  years) were surveyed. Patients were distributed into five groups: first group ( $n = 7$ ) – diabetic patients with HBV infection in replicative phase; second ( $n = 68$ ) – HBV infected in nonreplicative phase; third ( $n = 23$ ) – HCV infected in replicative phase; fourth ( $n = 13$ ) – HCV infected in nonreplicative phase; fifth ( $n = 62$ ) – noninfected type 2 diabetic patients. Markers of a viral hepatitis B and C, glutamic acid decarboxylase antibodies (GADA) and islet cells antibodies (ICA) were studied by immune-enzyme assay, viral DNA and RNA – by polymerase chain reaction.

**Results**

GADA was revealed more often in HBV and HCV infected type 2 DM patients in comparison with noninfected: in group 1–57.1%,  $P = 0.007$ ; in 2–36.7%,  $P = 0.003$ ; in 3–34.8%,  $P = 0.003$ ; in 4–53.9%,  $P = 0.01$  vs. group 5–9.7% (chi-square test). ICA in infected patients also met more often, than in noninfected: in group 1–28.6%,  $P = 0.019$ ; in 2–29.4%,  $P < 0.001$ ; in 3–17.4%,  $P = 0.026$ , in 4–15.4%,  $P = 0.15$  vs group 5–1.6% (chi-square test). GADA and ICA revealing frequency did not depend on a virus replication. In 13.5% HBV- and HCV-infected patients both kinds of antibodies were determined. In noninfected patients simultaneously ICA and GADA didn't revealed.

**Conclusion**

In type 2 diabetic HBV and HCV infected patients antibodies to pancreatic  $\beta$ -cells are found out statistically significantly more often, than in noninfected DM patients.

**P310**

**Adrenomedullin increases the expression of CLR and RAMP2 in microvascular endothelial cells**

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Adrenomedullin (AM) is a multifunctional peptide hormone which plays a significant role in vasodilation and angiogenesis, implicating it in hypertension as well as in carcinogenesis. AM exerts its effects via the Calcitonin Receptor-Like Receptor (CLR) complexed with either Receptor Activity Modifying Protein (RAMP) 2 or 3.

The aim of the present study is to investigate the biological actions, functions and possible target genes of AM in immortalized Human Microvascular Endothelial Cells (HMECs). We showed in this cell line the expression of AM receptor message and protein using PCR and western blotting respectively. Treatment of cells with AM elevated cAMP in a time (5–45 min) and dose ( $10^{-6}$ – $10^{-14}$  M) dependent manner, as well as increasing phosphorylation of ERK 1/2 after 5 min of treatment. Even though the phosphorylated ERK 1/2 signal is translocated into the nucleus, this did not result in increased cell proliferation. However, using real-time PCR we were able to show for the first time that AM up-regulates mRNA expression of VEGF after 2h of treatment as well as increasing mRNA levels of its own receptor (CLR/RAMP2) after 2 and 24 h of treatment. The biological function of this new finding is currently under investigation.

Additionally we established the correct transcription start site of RAMP2 using Rapid Amplification of cDNA Ends (RACE) PCR. Analysis of 1000 bp promoter region revealed a TATA-less promoter with several zinc fingers as well as an AP1 transcription site, suggesting possible utilisation of other signalling pathways. Taken together with the already published characterisation of the CLR promoter the comparison of potential transcriptional elements in both promoter regions may help to elucidate a common regulatory mechanism.

**P311**

**Sympathetic function in carriers of MC4R mutations**

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Activation of the melanocortin-4 receptor (MC4R) leads to a decrease of appetite and an elevation of sympathetic nerve activity (SNA). The sympathoexcitatory property of leptin is mediated by the melanocortin system and a functional MC4R is a prerequisite for the development of hypertension associated with obesity in animal model. To test the hypothesis that functional mutations of the MC4R lead to a diminished SNA in humans, ten carriers of MC4R mutations and a control group of 17 subjects who were matched for gender, body mass index (BMI) and age were investigated. Muscle sympathetic nerve activity (MSNA) recordings from the superficial peroneal nerve were obtained by microneurography. Frequency domain analysis of heart rate variability (HRV) was also performed for low frequency (LF, 0.045–0.15 Hz) and high-frequency (HF, 0.15–1.0 Hz) components. Carriers with MC4R mutations showed a significantly lower heart rate. However, neither HRV parameters of cardiac sympathetic activity nor MSNA differed between both groups. Results are shown in the following table:

	MSNA (bursts/min)	LF (nu)	HF (nu)	HR (bpm)
MC4R-Mut.	22.7 ± 4.6	51.8 ± 6.5	41 ± 5.7	64.7* ± 2.9
Control	22.1 ± 2.1	50.0 ± 4.6	42.3 ± 4.1	73.3 ± 2.7

± SEM; \* $P < 0.05$ .

Blood pressure was similar in both groups. In both groups MSNA was positively correlated with age (MC4R-mut.  $R = 0.61$ ;  $P = 0.059$ ; contr.  $R = 0.67$ ,  $P = 0.004$ ). In carriers of MC4R mutations but not in the control group heart rate was positively correlated with HOMA-IR as an marker of insulin sensitivity (MC4R-mut.  $R = -0.27$ ;  $P = 0.45$ ; contr.  $R = 0.65$ ,  $P = 0.005$ ). In conclusion, both sympathetic nerve activity to the muscle vascular bed and to the heart is not altered in carriers of MC4R mutations. Although the lower heart rate could indicate altered autonomic function in MC4R mutation carriers our study does not indicate to a depressed sympathetic nerve activity to the heart or the muscle vascular bed.



**P312****A case of myocardial infarction induced by factitious hypoglycaemia in type 2 diabetes patient**R Srinivasan<sup>1</sup>, E Sanders<sup>2</sup> & M Flynn<sup>3</sup><sup>1</sup>Guys and St Thomas Hospital, London, United Kingdom; <sup>2</sup>North Durham University Hospital, Durham, United Kingdom; <sup>3</sup>Kent and Canterbury Hospital, Canterbury, United Kingdom.**Introduction**

Insulin is widely used in Type 2 patients to control the blood sugar when the oral hypoglycaemic agents fail. This is a case report of a type 2 diabetes patient who developed Myocardial Infarction after factitious hypoglycaemia with insulin.

**Case report**

A 64-year-old gentleman with known Ischaemic Heart Disease was treated with insulin (Mixtard 30) and metformin for his type 2 diabetes. He presented to diabetes Specialist nurse with persistent hypoglycaemia. His insulin dose was initially reduced and eventually stopped. As he continued to have hypoglycaemia even after the insulin was stopped he was investigated as inpatient. Blood tests done as inpatient were as follows, blood sugar- 1.9mmols/L, Insulin - 158 Iunits/L and C-peptide - <9 Units/litre. This confirms that he has factitious hypoglycaemia. On confrontation he confessed that he has given himself excess of insulin. He was managed with oral hypoglycaemic agents and counselling was offered. As his blood sugars were high in the subsequent weeks, he was restarted on Insulin again. He presented 4 weeks later with chest pain and symptoms of hypoglycaemia. ECG and Troponin showed that he had Acute Myocardial infarction. His insulin, c-peptide and blood sugar repeated again, confirmed that he has taken excess of insulin again. The insulin was stopped completely and was started on sulphonylurea with referral to psychiatrist for counselling.

**Conclusion**

Hypoglycaemia can increase myocardial workload by sympathetic drive, it has also been hypothesised that hypoglycaemia can lead to coronary artery spasm. These can lead to acute myocardial infarction. So prolonged severe hypoglycaemia should be avoided in high-risk patients.

**P313****Orlistat improves postprandial triglyceride metabolism in obese patients with or without diabetes**I Sahin<sup>1</sup>, R Bentli<sup>1</sup>, L Keskin<sup>1</sup>, C Taskapan<sup>2</sup>, S Senel<sup>1</sup> & S Yologlu<sup>3</sup><sup>1</sup>Inonu University, Department of Endocrinology and Metabolism, Malatya, Turkey; <sup>2</sup>Inonu University, Department of Biochemistry, Malatya, Turkey; <sup>3</sup>Inonu University, Department of Biostatistics, Malatya, Turkey.**Objective**

Accumulating data suggests that post-prandial hypertriglyceridemia is important in the pathogenesis and progression of atherosclerosis, and thus cardiovascular diseases. We aimed to assess the acute effect of the orlistat vs. placebo on post-prandial triglyceride (TG) level in diabetic and non-diabetic obese patients.

**Research design and methods**

Study population consisted of 49 patients with obesity (25 non-diabetics, 24 diabetic). Patients were randomized to take either single dose of orlistat 120 mg (26 patients) or placebo (23 patients) before a standard mixed meal containing 50 g of fat. Plasma TG, glucose and insulin levels were measured at baseline and post-prandially at 2-h interval for 6-h. Post-prandial curves were calculated as the total area under curve (AUC) for TG. The incremental area under the curves (d AUC) for TG was also calculated.

**Results**

There were no statistically significant difference between the orlistat and placebo groups with regard to age, sex, BMI, A1c, basal triglyceride and basal total cholesterol. Post-prandial glucose and insulin responses to test meal were not significant between the orlistat and the placebo groups. Significant post-prandial TG reduction in TG 4-h ( $202.2 \pm 103.1$  vs.  $297.5 \pm 121$  mg/dl,  $p = 0.005$ ) and in TG 6-h ( $151.9 \pm 78.7$  vs.  $233.9 \pm 93.1$  mg/dl,  $p = 0.002$ ) were achieved with orlistat, which resulted in a significant lower TG AUC ( $1163.3 \pm 566.2$  vs.  $1475.6 \pm 561.7$ ,  $p = 0.042$ ) and TG dAUC ( $269.7 \pm 250.7$  vs.  $571.8 \pm 296.2$   $P < 0.001$ ) compared to the placebo group.

The effect of orlistat on time course of the change in postprandial TG concentrations (except TG at 6-h), TG AUC, TG dAUC during 6-h postprandial period were not different between diabetic and non-diabetic obese.

**Conclusion**

Orlistat improves postprandial TG concentrations in both diabetic and non-diabetic obese patients.

**P314****Challenge in achieving target Hba1c in a relatively rare diabetes complication**

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We present a case of 55 years old obese man with type 2 diabetes of 13 years duration, seen in diabetes clinic. His HbA1c had been 8–10% for about 7 years despite being on maximum doses of sulphonylurea and metformin.

It was decided to start him on 30/70 mixed insulin and Metformin was continued. His HbA1c did not improve despite aggressive insulin titration. His injections became painful as insulin doses increased and resistance was noted on injecting. Hard lumps formed at injection sites and insulin could not be injected at all at some sites. There was no improvement on using different size needles.

Examination revealed "pebble" like palpable lumps at injection sites. Skin was noted to be tight over the abdominal wall, upper back and thighs.

**Investigations**

Positive anti-Scl 70 antibody but other connective tissue screening was normal. CT scan-abundant intra-abdominal fat but scanty subcutaneous fat. A skin biopsy showed a thick lesion and confirmed a diagnosis of 'Scleredema Diabeticorum'.

A further attempt with oral hypoglycaemics including rosiglitazone, was unsuccessful (HbA1c 11.1%). CSSI was not tolerated due to pain.

After failure of above attempts to improve HbA1c, it was decided to try needle free injection device. This delivers insulin via high-pressure stream into subcutaneous tissue. This system revolutionised insulin administration for this patient. Seventeen months on and he was injecting 30/70 insulin bd along with metformin 1 g bd. Target HbA1c has been achieved (6.9%) and no problems have been reported.

We have highlighted the need to consider needle free device early in Scleredema diabeticorum to improve glycaemic control and to consider this condition in patients who are having difficulty with injecting insulin.

**P315****Depot specific activation of inflammatory signalling molecules JNK and NFκB in human abdominal subcutaneous and omental adipose tissue**

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Central obesity is strongly associated with sub-clinical inflammation, insulin resistance (IR) and type 2 diabetes mellitus (T2DM), but the intracellular mechanisms involved in its pathogenesis remain unclear. Recent Murine studies have implicated C-jun N terminal kinase (JNK) as an important molecule linking insulin action and inflammation, therefore we investigated JNK in human abdominal adipose tissue (AT). JNK expression/activity was assessed in abdominal subcutaneous (AbdSc  $n = 20$ ), omental (Om  $n = 10$ ) and thigh ( $n = 12$ ) depots (age:  $47.8 \pm (\text{mean} \pm \text{SD})5.0$  yrs; BMI:  $25.0 \pm 0.6$  kg/m<sup>2</sup>), as well as the effect of adiposity and T2DM. The effect of differential JNK activity on the intracellular signalling pathway involving I-kappaB kinase (IKK)β and IKKγ, IκBα and NFκB was also investigated. Finally the effect of TNF-α and its antagonist as a JNK modulator in human AbdSc adipocytes was examined. Our *ex vivo* studies demonstrated total JNK protein expression was increased in the Om versus AbdSc (Om:  $5.79 \pm (\text{mean} \pm \text{SEM})0.87$  ng/mL; AbdSc:  $2.98 \pm 0.43$  ng/mL;  $P < 0.05$ ), independent of macrophage content. Phosphospecific JNK1 was increased 5.8 fold in the AbdSc depot versus Om AT (AbdSc JNK1/2,  $P < 0.01$  vs OM), whilst JNK1/2 expression was unaltered with adiposity or diabetes. From this, we determined that Om AT exhibited the lowest JNK activity compared with AbdSc ( $P < 0.01$ ). We also showed that other intracellular pathways may operate in omental fat to stimulate an inflammatory response by increased expression of the IKKβ ( $P < 0.001$ ), IKKγ and both phosphorylated IκBα (IκBα-P) and IκBα in Om compared with AbdSc AT ( $P < 0.01$ ). Finally JNK activity was unaffected in AbdSc adipocytes treated with TNF-α. In conclusion, this is the first study to demonstrate depot specific JNK expression and functional activity in human abdominal AT. Furthermore, JNK may be a potential mediator of inflammation and IR in AbdSc AT, whilst other intracellular signalling pathways may play a more central role in activation of inflammation and IR in omental AT.

### P316

#### Endothelial, inflammatory and endocrine markers in women with PCOS before and after metformin treatment

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Polycystic ovary syndrome (PCOS) affects more than 5% of women in reproductive age and is characterized by a heterogeneous spectrum of oligoanovulation and biochemical or clinical evidence of hyperandrogenism. Insulin resistance and compensatory hyperinsulinaemia play a significant role in pathogenesis of PCOS.

Chronic low level inflammation and an increased risk for cardiovascular disease and type2-diabetes are reported in women with PCOS.

Women with PCOS ( $n = 68$ ) and age- and body mass index-matched healthy women ( $n = 30$ ) with regular menses and no signs of hyperandrogenism were investigated.

PCOS patients ( $n = 27$ ) accepting participation in our study were treated with metformin (850 mg twice daily). Metabolic, endothelial and endocrine markers including OGTT were investigated in all women and in the treatment group before and after 6 months on metformin. In addition intima media thickness (IMT) as surrogate marker of cardiovascular disease was measured. Among the endothelial markers we were particular interested in the concentration of asymmetric dimethylarginine (ADMA), an endogenous inhibitor of NO synthase and independent risk factor for cardiovascular morbidity and mortality.

Levels of asymmetric dimethylarginine (ADMA), androgens, hs-CRP and proinflammatory cytokines (IL-6, IL-18) were significantly increased in women with PCOS and were found to be positively correlated with parameters of insulin sensitivity (HOMA-IR and AUCinsulin<sub>0-120min</sub>).

Treatment with metformin positively affected metabolic and endocrine profile as well as menstrual function and led to spontaneous pregnancies.

In conclusion, our data show that women with PCOS have elevated levels of inflammatory and endothelial markers, that was partly reversed after a 6 month metformin treatment.

### P317

#### Expression and regulation of adiponectin receptors (AdR-1 and AdR-2) in pancreatic beta-cells

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Obesity is a risk factor for the metabolic syndrome and type 2 diabetes. Adiponectin, a 30 kDa peptide secreted from adipocytes has been shown to be anti-atherogenic, and anti-inflammatory, as well as affecting insulin resistance and pancreatic beta-cell function. This study investigated the levels of expression of the adiponectin receptor subtypes 1 and 2 (AdR-1 and AdR-2) and regulation of their expression in the pancreatic BRIN-BD11 beta-cells line. Cells were cultured in RPMI-1640 and treated with rosiglitazone (10 micromolar), leptin (10 ng/ml and 50 ng/ml), oleic acid (50 micromolar), palmitic acid (50 micromolar), elaidic acid (50 micromolar) and the PPAR alpha agonist WY14643 (10 micromolar) for 24 hours. Total cellular RNA was extracted and mRNA was assessed by real-time PCR. Expression of AdR-1 was 30 fold higher than that of AdR-2 ( $P < 0.001$ ) at both low and high glucose concentrations. Rosiglitazone treatment resulted in a 32% decrease in AdR-2 mRNA expression ( $P = 0.029$ ) but had no effect on AdR-1. Oleic acid treatment conversely resulted in a decrease in AdR-1 expression of 28% ( $P = 0.031$ ). Leptin had no effect on expression of either receptor type. These observations confirm the differential expression levels of AdR-1 and -2 in pancreatic beta-cells, and suggest a role for adiponectin in beta-cell function which may be altered in obesity and insulin resistant states.

### P318

#### Cardiovascular risk factors and macrovascular complications in type 2 diabetes

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The frequency of type 2 diabetes increases in all of the world. The risk for atherosclerosis vascular disease is greatest in type 2 diabetic patients who have other known risk factors such obesity, smoking, hypertension and dyslipidemia.

The aim of our study is to determine the frequency of cardiovascular risk factors associated to diabetes and the frequency of macrovascular complications in type 2 diabetic patients.

It is a retrospective multicentric study of 708 patients (375 (53%) women and 333 (47%) men) in 7 departments of diabetes in Tunisia.

#### Results

The mean age of patients is  $58.8 \pm 11.6$  years.

#### Cardiovascular risk factors

Obesity is showed in 32.8% of patients, fat android distribution is noted in 92.5% and smoking in 68.5% of patients. Hypertension is confirmed in 59.1% of patients 31.9% of patients have hyper triglyceridemia and 26.8% have hypercholesterolemia

#### Macrovascular complications

Coronary insufficiency showed in 17.6% % and myocardial infarction in 3.7% of patient. Arteritis of lower lumbs is noted in 16.6% of patients and amputation in 4.3%.

Our study confirmed that type 2 diabetic have frequently several cardiovascular risk factors associated to the diabetes. All this factors should be considered in the treatment of type 2 diabetic patients to reduce complications.

Early insulintherapy is recommended to improve glycemic control. Management of type 2 diabetes must be global including treatment of all cardiovascular risk factors.

### P319

#### Effects of Ezetimibe on serum lipoproteins in patients with homozygous familial hypercholesterolemia undergoing LDL cholesterol apheresis and high dose atorvastatin therapy

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Homozygous familial hypercholesterolemia (HoFH) represents a potentially fatal condition that is caused by low density lipoprotein (LDL) cholesterol receptor deficiency. Statins alone are not effective in HoFH. In recent years, semiselective or selective LDL cholesterol apheresis systems have been shown to prolong life. However, applying LDLC apheresis therapy, recent evidence also suggests that more intense LDL cholesterol reduction is associated with more clinical benefit. Ezetimibe is the first of a new class of cholesterol absorption inhibitors that potently inhibits dietary and biliary cholesterol absorption.

The aim of this study was tested whether ezetimibe can induce a further reduction of LDL cholesterol levels in patients with high dose atorvastatin therapy or LDL cholesterol apheresis.

Four patients were recruited from our apheresis unit. All patients were treated by atorvastatin 80 mg/day and regular LDL cholesterol apheresis treatment every two weeks. LDL cholesterol apheresis was carried out using the cascade filtration (Dideco, Italy). Ezetimibe was given at a dose of 10 mg/day after a meal once every day for 6 weeks.

At the end of treatment period, changes in LDL cholesterol -10.6%, in total cholesterol -9.53% in VLDL cholesterol -9.83% were detected significantly ( $P < 0.01$ ) ( $P < 0.05$ ). Changes in HDL cholesterol and triglyceride were not significantly detected. No adverse events were observed with ezetimibe treatment.

Our results show that LDL cholesterol reduction by ezetimibe is a safe and effective therapy in patient with combined therapy with high dose atorvastatin and LDL cholesterol apheresis for HoFH.

### P320

#### Effect of micronised fenofibrate on plasma levels of c-reactive protein in combined hyperlipidemia

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C-reactive protein (CRP) is a non-specific but sensitive marker of underlying systemic inflammation. High CRP levels correlate with risk for future cardiovascular events. Statins can lower the circulating levels of CRP. In contrast, the evidence that fibrates lower CRP levels is very limited. This retrospective study evaluates the effect of micronised fenofibrate on plasma levels of CRP in patients with combined hyperlipidemia.

Sixty-four patients with combined hyperlipidemia were treated with micronised fenofibrate for a median of 22 months (range 11–38 months). Plasma lipids, serum liver function tests, alkaline phosphatase (AP), creatinine kinase (CK) and hs-CRP levels were measured before and sixth months of 200 mg per day micronised fenofibrate treatment.

At the end of 6 months of treatment with micronised fenofibrate total serum cholesterol decreased from  $264 \pm 27$  to  $205 \pm 19$  mg/dl (a 22% decrease,  $P < 0.001$ ), low density lipoprotein cholesterol decreased from  $152 \pm 30$  to  $102 \pm 21$  mg/dl (a 33% decrease,  $P < 0.001$ ), high density lipoprotein cholesterol increased from  $47 \pm 8$  to  $59 \pm 11$  mg/dl (a 25% increase,  $P < 0.001$ ) and triglyceride decreased from  $317 \pm 112$  to  $161 \pm 53$  mg/dl (a 51% decrease,  $P < 0.001$ ). CRP levels decreased from  $4.8 \pm 4.1$  to  $2.6 \pm 2.3$  mg/l (a 50% decrease,  $P < 0.001$ ).

In the present study, it might be concluded that micronised fenofibrate significantly decreased of serum levels of hs-CRP in addition to lipid modifying effect.

### P321

#### Long term safety of Atorvastatin and Fenofibrate therapy in patients with combined hyperlipidemia

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Combined hyperlipidemia is characterized by elevated levels of total cholesterol, low density lipoprotein (LDL), triglyceride and mild decrease in high density lipoprotein (HDL) cholesterol. It is difficult to achieve desirable decreases of LDL cholesterol, triglycerides and increasing of HDL cholesterol with monotherapy in patients with mixed hyperlipoproteinemia. This retrospective study was carried out to assess the safety atorvastatin-fenofibrate combination therapy.

One hundred-nine consecutive patients received atorvastatin 20 mg/day and micronised fenofibrate 200 mg/day combination for resistant hyperlipidemia to either agent therapy. Treatment continued for 2 years in all patients. To evaluate side effects, participant's hepatic transaminase, creatine kinase (CK), blood urea nitrogen (BUN), and creatinine levels have been measured every three months. In our experience participants have been suffering insomnia and muscle weakness but there have not been encountered laboratory adverse effects in laboratory parameters.

In conclusion; our results suggested that combining atorvastatin and micronised fenofibrates in patient with combined hyperlipidemia could be used safely in Turkish hyperlipidemic patients.

### P322

#### Use of insulin glargine during pregnancy in women with type 1 diabetes mellitus

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Glargine is an analogue insulin which has a peakless action profile over approximately 24 hours. As a result it has been shown to reduce the frequency of hypoglycaemia thereby enabling achievement of tight glycaemic control. This is important during pregnancy in order to lower the risk of foetal malformations.

#### Method

17 women, mean age 28 years, with type 1 Diabetes Mellitus on a basal bolus regimen including Glargine became pregnant between January 2004 and January 2005. Two women were switched back to conventional insulins when pregnancy was confirmed due to concerns about safety with Glargine but the remainder continued with Glargine throughout gestation.

#### Results

There were 3 early miscarriages in women who had poor glycaemic control at conception with a mean HbA1c of 9.6%. In the remaining pregnancies the

mean HbA1c at conception was 8%. Glycaemic control improved significantly during gestation and the mean HbA1c dropped to 7.3% (6–8%). One woman developed hypertension but the rest of the pregnancies were uncomplicated. All women were delivered at term, 2 vaginally and 12 by Caesarean section. There was 1 baby born with an absent forearm but no other congenital abnormalities.

#### Conclusion

By improving glycaemic control Glargine may help to reduce the risk of diabetes-related complications in pregnancy. However, further data are necessary regarding its safety before it is used routinely in pregnancy.

### P323

#### Serum ferritin levels and obesity

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#### Introduction

Serum ferritin concentration was found to be related with dyslipidemia, hypertension and abdominal adiposity. The aim of this study is to evaluate the correlations between serum ferritin concentrations and metabolic risk markers in obesity.

#### Methods

Obese 534 women were enrolled into the study. The patients were divided into three tertiles according to the serum ferritin concentrations. The mean serum ferritin concentration was  $9.96 \pm 4.16$  ng/mL in Group I ( $n = 175$ ),  $28.03 \pm 6.85$  ng/mL in Group II ( $n = 179$ ), and  $81.62 \pm 42.10$  ng/mL in Group III ( $n = 180$ ). The groups were compared for metabolic risk markers regards of cardiovascular disease.

#### Results

The mean serum ferritin concentration of the whole group was 40.16 ng/mL. Body mass index (BMI), waist circumference, waist-to-hip ratio (WHR), cholesterol, triglyceride, blood pressure, fasting glucose and insulin, HOMA and uric acid were significantly high in Group III compared to Group I ( $P < 0.05$ ). Except for uric acid all these parameters were significantly higher in Group III compared to Group II ( $P < 0.05$ ) as well.

#### Conclusion

The correlation between whole body iron storage and the components of metabolic syndrome was reported in several studies previously. Ferritin is also known to be a marker of inflammation and increases in cardiovascular disease. Here in this study we defined the correlation between increment of serum ferritin concentrations and metabolic risk markers regards of cardiovascular risk. So serum ferritin levels can be suggested as a risk marker for atherosclerotic disease

### P324

#### Utility of continuous glucose monitoring (CGMS) to explore glycaemic patterns in patients with diabetes mellitus in the UAE

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#### Introduction

Prevalence of diabetes mellitus (DM) in the UAE is around 20% amongst adults. Lifestyle including meal timings are different from the West and may necessitate different treatment schedules. We have used continuous glucose monitoring (CGMS) to look at glycaemic patterns in patients with type 1 and type 2 diabetes in the UAE.

#### Methods

Available CGMS data (using methods previously described) on forty-four patients (thirty-one with type 2 DM and thirteen with type 1 DM) and seven non-diabetic subjects was pooled and summarized. Interstitial Glucose Values (IGV) at 288 time points for 3 consecutive days were available for each subject. Mean (IGV) for each time point for 3 groups of subjects was calculated. HbA1c was also noted in all subjects.

#### Results

Mean  $\pm$  SD CGMS readings in type 1 and type 2 DM were  $182 \pm 39.8$  and  $153 \pm 19.8$  mg/dl respectively. Corresponding HbA1c's were  $8.6 \pm 1.8\%$  and  $8.4 \pm 1.4\%$  Mean IGV in non-diabetic subjects was  $101 \pm 6.1$  mg/dl. In patients with type 2 DM, IGV was always less than 200 mg/dl. Four peaks in IGV per day were noted. In type 1 DM, glycaemic control was less



satisfactory with two peaks in IGV at 9 AM and 3 AM respectively. In non-diabetic subjects glucose level was stable and in euglycemic range throughout 72 hours.

#### Conclusion

In the group studied there was relatively good glycemic control among patients with type 2 DM. Patients with type 1 DM had poor control, especially for the afternoon and the evening. The pattern of control suggests poor compliance with diet and also need for rethinking the conventional insulin regimes to suit the culture in the UAE.

### P325

#### Dyslipidemia in premenopausal and postmenopausal obese women

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#### Introduction

The body fat distribution changes with menopause in women. The gluteo-femoral fat dominance alters with central adiposity via menopause. The incidence of cardiovascular atherosclerosis increases with this period. Altered lipid profile and the increment in abdominal adipose tissue are suggested to be the influencing factors of this situation. The aim of this study was to evaluate the metabolic and anthropometric features of premenopausal and postmenopausal obese women and the influencing factors of dyslipidemia.

#### Methods

Obese 382 and overweight 1960 women, between 40–65 years old, were included into the study. The patients with surgical menopause and under the treatment of hormone replacement therapy were excluded. Group I ( $n = 1080$ ) was consisted of premenopausal and Group II ( $n = 1262$ ) was consisted of postmenopausal women.

#### Results

The women were significantly older in Group 2 ( $52.70 \pm 5.70$  vs  $44.87 \pm 4.28$  yrs. Triglyceride, cholesterol, HDL-C, LDL-C, VLDL-C and cholesterol /HDL were significantly higher in Group 2 ( $P < 0.05$ ). HOMA-IR, HOMA-B and QUICKI were significantly different in Group 2 as well ( $P = 0.03$ ,  $p = 0.01$ ,  $p = 0.02$  respectively). Despite there was no difference for weight, BMI and body fat mass-regards of whole body adiposity- between the groups, waist circumference, waist-to-hip ratio, sagittal waist height and calculated abdominal fat mass-regards of intraabdominal adiposity- were higher in Group II ( $P < 0.05$ ).

#### Conclusion

In this study, women in postmenopausal period were found to have higher lipid parameters and significant abdominal adiposity. Although the advanced age and hypoeostrogenemia simplify dyslipidemia, the effect of abdominal adiposity has to be taken into consideration. In postmenopausal period body fat distribution alters from gluteofemoral region to intraabdominal region where the tissue is more sensitive to lipolysis. The increased insulin resistance parameters with accelerated dyslipidemia can be suggested as a result of this alteration as well.

### P326

#### The effect of moxonidine on endothelial dysfunction in metabolic syndrome

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#### Introduction

Metabolic syndrome (MS) is a clustering of risk factors. Endothelial dysfunction has been reported in patients with MS and even in healthy obese individuals with a normal metabolic profile. Sympathetic activity commonly is increased in obese hypertensives, and moxonidine, is found to be effective in lowering blood pressure and improving insulin sensitivity. The aim of this study is to evaluate the efficacy of moxonidine on endothelial dysfunction in insulin-resistant obese patients.

#### Method

The study group was consisted of 52 patients, 26 (Group 1; 19 women, 7 men, mean age  $40.6 \pm 5.0$  yrs) were found to have mild hypertension and besides hypocaloric diet they were treated with moxonidine for three months. The remainders (Group 2; 16 women, 10 men, mean age  $39.6 \pm 5.9$  yrs) were followed up with calorie restriction solely. Anthropometric and metabolic features of the groups and flow mediated dilatation (FMD) were evaluated. Insulin resistance was calculated by homeostasis model assessment formula (HOMA) and quantitative insulin sensitivity check index (QUICKI).

#### Results

Systolic and diastolic blood pressures ( $P = 0.000$ ) and waist circumference ( $P = 0.29$ ) were high, and QUICKI ( $P = 0.04$ ) and FMD ( $P = 0.01$ ) were significantly low in Group 1. On the third month of the follow-up nearly all the study parameters were found to be improved within each group. Not only the decrement in blood pressure, but improvement in metabolic and anthropometric chances with increment of FMD was significant in moxonidine treated group.

#### Conclusion

Despite most of the risk markers of MS was found to be improved with diet, moxonidine had an additive effect not only on body fat mass and insulin resistance but on improvement of FMD as well. Moxonidine can be proposed as a valuable molecule for treating mild/moderate hypertension in obese and insulin resistant patients with metabolic syndrome especially for the improvement of endothelial dysfunction.

### P327

#### The BsmI and FokI polymorphisms in the vitamin D receptor gene in relation to anthropometric and biochemical parameters describing metabolic syndrome

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#### Introduction

It was found that vitamin D could have a direct effect on adipocyte differentiation and metabolism and might be involved in glucose regulation of insulin secretion, which may be speculated from finding of a nuclear localization of  $1,25\text{-(OH)}_2\text{D}_3$  in the pancreatic islets. In recent years several polymorphisms in VDR gene, which are potent to alter the activity of VDR protein, have been described. BsmI and FokI polymorphisms were described in relation to obesity and type 2 diabetes. AIM of the study was to find out whether there is an association between BsmI and FokI polymorphisms and anthropometric (BMI, WHR, BP) and biochemical parameters describing metabolic syndrome.

#### Materials and methods

We have studied 176 randomly selected men, aged 25–65 years (mean  $54.16$ ) with mean BMI of  $26.16 \text{ kg/m}^2$ . Two polymorphisms of the VDR gene (FokI and BsmI) were explored using PCR-RFLP method. Serum glucose, insulin, total cholesterol, LDL, HDL and TG were measured using commercially available kits. The study protocol was approved by the local Ethical Committee.

#### Results

We found that BB carriers tend to have higher BMI comparing to bB and bb genotypes ( $29.0 \pm 3.7$  vs  $28.6 \pm 4.6$  vs  $26.6 \pm 3.6$  respectively,  $P = 0.0297$ ). Similarly, FF carriers had higher fasting insulin levels comparing to fF and ff genotypes ( $12.4 \pm 11.5$  vs  $9.3 \pm 4.7$  vs  $5.8 \pm 2.8$  respectively,  $P = 0.0187$ ). Beside these no significant difference were found.

#### Conclusions

The BsmI VDR polymorphism seems to influence BMI, while the FokI VDR polymorphism appears to affect insulin sensitivity.

### P328

#### Comparison between NPH insulin and insulin glargine in intensive insulin therapy in type 1 diabetes mellitus

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#### Background and aims

Insulin glargine is a long acting insulin analog that mimics normal basal insulin secretion without pronounced peaks. The aim of this study was to



compare insulin glargine with NPH insulin for basal insulin supply in patients with type 1 diabetes.

#### Materials and methods

A total of 48 type 1 diabetics (mean age 27 yr, BMI 23.1 kg/m<sup>2</sup>) on long term intensive insulin therapy (IIT) were randomized into 3 different regimens of basal insulin substitution: 1. continuation of NPH insulin once daily at bedtime with more intensive self monitoring ( $n = 15$ ); 2. NPH insulin twice daily ( $n = 15$ ); 3. insulin glargine once daily ( $n = 18$ ). Meal time insulin aspart was continued in all groups.

#### Results

Fasting blood glucose (FBG) was lower in glargine group ( $7.3 \pm 1.2$  mmol/l) than in twice daily NPH group ( $7.5 \pm 1.9$  mmol/l) but without significant difference. FBG was significant higher in once daily NPH group ( $8.4 \pm 2.1$  mmol/l;  $P < 0.05$ ). HbA1c after 3 months did not change in one daily NPH group, but decreased in glargine group (from  $7.7 \pm 1.2$  to  $6.9 \pm 0.5\%$ ) as well as with NPH insulin twice daily (from  $7.8 \pm 1.0$  to  $7.0 \pm 1.2\%$ ). Total daily insulin doses were similar in all groups but only in glargine group there was no an increase of basal and decrease of meal related insulin doses. Frequency of mild hypoglycaemia was significantly lower in glargine group ( $6.7 \pm 1.0$ ) than in both NPH groups ( $9.5 \pm 1.7$  in twice daily NPH group and  $8.2 \pm 2.4$  in other NPH group) (episodes/patients-month,  $P < 0.05$ ).

#### Conclusion

Basal insulin supplementation in type 1 diabetes mellitus with either twice daily NPH insulin or glargine can result in similar glycaemic control when combined with meal time insulin aspart. However, with glargine regimen FBG, HbA1c and frequency of hypoglycaemic events are lower. These facts contribute to better patients' satisfaction with insulin glargine versus NPH insulin in IIT in type 1 diabetics.

### P329

#### Association of c-reactive protein with insulin resistance in first degree relatives of diabetic patients

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#### Objective

C-reactive protein (CRP) is a nontraditional risk factor that predicts cardiovascular disease but its relationship between diabetes and insulin resistance is not well documented. We evaluated the association of CRP levels and insulin resistance in a group of patients at high risk for diabetes.

#### Materials and methods

In this study we evaluated 22 first degree relatives of type 2 diabetic patients with impaired glucose tolerance test (Group 1) and 24 first degree relatives of type 2 diabetic patients with normal glucose tolerance test (Group 2) and 22 control subjects (Group 3) matched for age, sex and BMI. Anthropometric measurements, biochemical analyses and insulin and CRP levels were performed in each subject. Insulin resistance was assessed using the homeostasis model assessment of insulin resistance (HOMA).

#### Results

CRP levels were significantly higher in Group 1 than in Group 2 and Group 3 ( $4.12 \pm 1.7$  vs.  $3.7 \pm 2.1$  mg/L,  $P < 0.05$  and  $4.12 \pm 1.7$  vs.  $2.1 \pm 0.8$  mg/L,  $P < 0.05$ , respectively), and also in Group 2 compared with Group 3 ( $3.7 \pm 2.1$  vs.  $2.1 \pm 0.8$  mg/L,  $P < 0.05$ ). HOMA levels were significantly higher in Group 1 than in Group 2 ( $3.2 \pm 1.7$  vs.  $2.4 \pm 0.9$  mg/l,  $P < 0.05$  and  $3.2 \pm 1.7$  vs.  $1.1 \pm 0.4$  mg/L,  $P < 0.05$ , respectively) and Group 3, and also in Group 2 compared with Group 3 ( $2.4 \pm 0.9$  mg/l vs.  $1.1 \pm 0.4$  mg/L,  $P < 0.05$ ). CRP levels were correlated with HOMA ( $r = 0.326$ ,  $P < 0.05$ ) in the whole group of patients. CRP was found to be related to HOMA independently of fat mass ( $r^2 = +0.22$ ,  $P < 0.05$ ).

#### Conclusion

These findings support that subclinical inflammation is associated with insulin resistance. CRP can provide additional prognostic information in first degree relatives of type 2 diabetic patients.

### P330

#### How does glucose insulin potassium improve haemodynamic performance? Evidence for beta-adrenoreceptor and sarcoplasmic reticulum calcium ATPase up-regulation

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#### Objectives

Glucose insulin potassium (GIK) improves haemodynamic performance following coronary artery bypass graft surgery (CABG). We postulated that this might be secondary to beta-1 adrenergic receptor (ADRB1) up-regulation and changes in myocyte calcium handling.

#### Methods

We performed a randomised double-blind placebo-controlled trial on patients undergoing first time elective/urgent on-pump CABG (LREC approval obtained). A cohort of 48 patients randomised to receive either pre-ischaemic placebo (5% dextrose) ( $n = 24$ ) or GIK (40% dextrose,  $K^+100$  mmol.L<sup>-1</sup>, Insulin 70u.L<sup>-1</sup>) ( $0.75$  ml.kg<sup>-1</sup>.h<sup>-1</sup>) underwent left ventricular biopsy immediately prior to aortic cross clamp (AXC), before release of AXC and 10 minutes post-reperfusion. GIK therapy was infused for a mean of  $79 \pm 21$  minutes pre ischaemia. GIK/placebo therapy was terminated 6 hours after removal of AXC. Serial haemodynamic measurements were performed at baseline until 12 hours post removal of AXC. Biopsies were snap frozen and stored at  $-80^\circ\text{C}$ . mRNA was extracted and reverse transcribed. Taqman real time PCR was performed to investigate expression of ADRB1 and sarcoplasmic reticulum Ca-ATPase (SERCA2a).

#### Results

Repeated measures analysis demonstrated a statistically significant increase in cardiac index (CI) for the GIK group in the first 6 hours ( $P = 0.037$ ). Taqman RT-PCR showed significantly greater ADRB1 mRNA expression at all time points (4.9-, 7.4- and 15.6-fold increase respectively,  $P < 0.001$ ) and significantly greater SERCA2a mRNA expression after reperfusion (13.2-fold,  $P < 0.001$ ) in the GIK group.

#### Conclusions

The increased haemodynamic performance seen with GIK therapy is associated with increased ADRB1 and SERCA2a mRNA expression. We postulate that ADRB1 and myocyte calcium handling modulation contribute to the beneficial effects of GIK.

### P331

#### Effects of weight loss on the coronary risk profile in obese patients two years after bariatric surgery

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#### Objective

To analyse the effects of bariatric surgery on the global coronary risk profile two years after the surgical procedure.

#### Methods

A total of 32 class III obese patients, 6 men and 26 women, mean age  $34.3 \pm 8.5$  years, were included. Total Cholesterol (TC), HDL, triglycerides, Blood Pressure (BP), presence of diabetes and smoking were evaluated before and two years after bariatric surgery. The 10 year Framingham coronary heart disease risk score was used to assess the global risk profile. Laparoscopic gastric banding was performed in nineteen (59.4%) patients and gastric bypass in eleven (34.4%); the other surgeries were a duodenal switch and a vertical gastropasty.

#### Results

Mean weight loss was  $35.0 \pm 26.4$  Kg and mean percentage weight loss was  $23.8 \pm 14.4\%$ . The initial prevalence of diabetes, dyslipidemia, hypertension and smoking were 28.1%, 46.9%, 43.8% and 26.5%, respectively. Normalization of the metabolic alteration was observed in 55.6% of patients with diabetes, 26.7% of patients with dyslipidemia and 28.6% of patients with hypertension. None of the patients quit smoking. The changes in BP (systolic  $136.3 \pm 14.1$  vs  $127.9 \pm 15.8$  mmHg,  $P < 0.005$ ; diastolic  $84.2 \pm 10.4$  vs  $80.5 \pm 8.2$  mmHg,  $P < 0.05$ ), TC ( $201.4 \pm 55.7$  vs  $180.8 \pm 44.7$  mg/dL,  $P < 0.05$ ), HDL ( $43.1 \pm 9.5$  vs  $47.6 \pm 12.7$  mg/dL,  $P < 0.05$ ), triglycerides ( $170.7 \pm 86.9$  vs  $125.9 \pm 83.2$  mg/dL,  $P < 0.001$ ) and fasting glucose levels ( $107.7 \pm 29.4$  vs  $91.3 \pm 18.1$  mg/dL,  $P < 0.001$ ) were statistically significant. The 10 year Framingham risk score was significantly reduced ( $3.7 \pm 3.9\%$  vs  $2.6 \pm 3.1\%$ ,  $P < 0.001$ ). Percentage weight loss was only significantly related to the reduction of triglyceride values ( $r_s = 0.37$ ;  $P < 0.05$ ) and was not related to 10 year Framingham risk score.

#### Conclusions

Weight loss observed in the first two years after bariatric surgery was associated with a significant improvement of single cardiovascular risk factors and global risk. However, the extent of weight loss was poorly

related to the magnitude of improvement in cardiovascular risk. The continuous follow-up of these patients may elucidate the true meaning of these findings.

### P332

#### The severity of cardiovascular disease in women is modified by estrogen receptor alpha polymorphic variants

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Impaired estrogen (E2) action is important for cardiovascular disease (CAD) in both men and women. Associations of CAD with estrogen receptor polymorphisms, which may influence sensitivity to E2 have been reported for men but have not been confirmed for women. The aim of the present study was to investigate the association of estrogen receptor common polymorphisms with the severity of coronary disease in women undergoing coronary angiography.

Estrogen receptor alpha polymorphisms at positions c.454-397 T > C (PVU2) and c.454-351 A > G (Xba1) were studied in 129 postmenopausal women (age 37–88 yrs). The severity of CAD was assessed by the number of arteries (0, 1, 2 or 3) with >50% stenosis in the angiography. The protocol was approved by the Institution's ethical committee. Patients gave their informed consent. Biochemical parameters were assessed.

60 women had 0 vessel disease, 27 had 1, 27 had 2 and 15 had 3 vessel disease. Several classical risk factors for CAD were significantly associated with the severity of CAD, such as smoking, hyperlipidemia, positive family history, waist perimeter and the presence of diabetes mellitus. There was a significant association between the TT, TC and CC genotypes (PVU2) and the severity of CAD ( $P < 0.01$ , Mantel Haenzel test for linear association); similar results were obtained with the Xba1 polymorphism.

We conclude that common estrogen receptor alpha polymorphisms probably affecting sensitivity to estrogen may influence the severity of CAD in women undergoing coronary angiography, as they probably reflect the life time exposure to estrogen. Similar associations have been reported for men with coronary artery disease. These polymorphisms should probably be taken into account when associations with estrogen action are examined.

### P333

#### Adult pancreatic islet progenitor cells exhibit plasticity *in vitro*

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Adult progenitor cells exist in most adult tissues, their differentiation being primed by insult, repopulating damaged or dysfunctional tissue. Utilisation of resident progenitor cells has the potential to cure many diseases, including neurodegenerative disorders or diabetes which presently can only be managed. This potential is limited in the brain by constraints on endogenous progenitor cell mobilisation and by difficulties in progenitor cell harvesting for *ex vivo* expansion and differentiation. The regenerative capacity of progenitors is dependent on both their phenotype and microenvironment, and their plasticity may allow cross-tissue transplantation. Since adult pancreatic endocrine and neural progenitors share similarities in lineage gene expression and developmental pathways, we hypothesise that, given neural environmental conditions, cultured pancreatic progenitors will expand and differentiate along neuronal lineages. We have shown that adult mouse pancreatic islets contain resident progenitor cell populations that can be isolated, cultured and characterised by immunolocalisation of the stem cell markers c-kit and CD34. Mouse pancreatic islets were isolated by collagenase digestion and dextran gradient separation. The cell preparation consisted of 90% or better dithizone-positive islets. The prepared islets were embedded in rat tail collagen and cultured in DMEM/F12, cholera toxin and EGF to develop expanded epithelial monolayers enriched with c-kit, nestin, c-Met and CD34 positive cells after 4 weeks in culture. When prepared pancreatic islets were cultured on poly-L-ornithine/laminin coated plates in DMEM/F12, N2 supplement, glucose and FGF-2 (a neural environment), they also formed a monolayer

and within three weeks the cells had differentiated into multiple phenotypes including neuron-like cells, as defined by neurofilament immunoreactivity. We conclude that pancreatic islets have the capacity to differentiate into multiple cell types that are dependent on the environment, not the tissue of origin. Given the correct stimuli, pancreatic islet progenitor cells *in vitro* exhibit plasticity and the ability to differentiate along neuronal lineages.

### P334

#### Regulation of hexose-6-phosphate dehydrogenase (H6PDH) in human fetal liver WRL-68 cells

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Excessive glucocorticoid exposure has been implicated in the pathogenesis of obesity and the metabolic syndrome. The *in vivo* conversion of inactive to active glucocorticoids is catalysed by 11 $\beta$ -hydroxysteroid dehydrogenase type 1 (11 $\beta$ -HSD1), requiring NADPH as a cofactor. Hexose-6-phosphate dehydrogenase (H6PDH) is co-localised with 11 $\beta$ -HSD1 in the lumen of the endoplasmic reticulum and controls local NADPH availability. Thus H6PDH plays an important role in the directionality of 11 $\beta$ -HSD1 and the activation of glucocorticoids. While various regulators of 11 $\beta$ -HSD1 have been identified, little is currently known about the regulation of H6PDH. This study aimed to assess changes in H6PDH gene expression after the administration of a variety of treatments, using a human fetal liver cell line (WRL-68). Following 24 h serum starvation, confluent cells were treated with a range of concentrations of dexamethasone (1–1000 nM), testosterone (0.1–10  $\mu$ g/ml) or estradiol (0.1–10  $\mu$ g/ml) for 8 or 24 h. RNA was extracted from the cells to measure H6PDH mRNA expression by real-time PCR, and Western Blots and H6PDH activity assays were utilised to assess protein expression levels. Increasing concentrations of dexamethasone (>250 nM) resulted in a dose-dependent increase in H6PDH mRNA (fold change  $36.9 \pm 14.1$ ,  $P < 0.006$ ,  $n = 4$ ). This effect was seen at 8 and 24 hours of culture (fold change:  $59.5 \pm 3.2$  (8 h) vs  $11.0 \pm 0.2$  (24 h);  $P < 0.004$ ). H6PDH protein expression also increased in a dose-dependent manner. Upregulation of H6PDH mRNA also occurred following treatment with testosterone (fold change: control  $1.0 \pm 0.0$ , 0.1  $\mu$ g/ml  $1.1 \pm 0.03$ , 1.0  $\mu$ g/ml  $2.3 \pm 0.12$ , 10  $\mu$ g/ml  $3.5 \pm 0.03$ ;  $P < 0.001$ ,  $n = 2$ ) and estradiol (fold change: control  $1.0 \pm 0.0$ , 0.1  $\mu$ g/ml  $0.6 \pm 0.08$ , 1.0  $\mu$ g/ml  $1.2 \pm 0.05$ , 10  $\mu$ g/ml  $5.1 \pm 0.49$ ;  $P < 0.05$ ,  $n = 2$ ). Glucocorticoids and sex hormones appear to play a role in the regulation of H6PDH within liver. This regulation of H6PDH could have implications for the pathology of the metabolic syndrome.

### P335

#### Relationships between plasma adiponectin and testosterone concentrations in middle-aged men and women

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Abstract withdrawn.

### P336

#### Relationship between testosterone and *in vivo* expression of adipor1 in skeletal muscle in middle aged Caucasian men

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Abstract withdrawn.

**P337****Testosterone secretion and melatonin rhythm in men with the metabolic syndrome**R Robeva, G Kirilov & Ph Kumanov  
Sbaleng, Sofia, Bulgaria.**Introduction**

Low endogenous testosterone levels are related to the metabolic syndrome /MS/ and diabetes type 2 in middle-aged and elderly men, but little is known about interrelations testosterone-insulin in younger hyperinsulinemic men. Recent studies have shown that the night melatonin in males with hypogonadotropic hypogonadism is significantly higher in comparison with the healthy men, while it is significantly reduced in patients with hypergonadotropic hypogonadism. However, it's still not clear whether the melatonin rhythm is changed in cases of low normal testosterone levels or mild hypogonadism.

**Objectives**

To compare the testosterone levels in young (within the reproductive age) men with MS to healthy controls and to investigate the possible changes in their melatonin rhythm.

**Subjects and methods**

BMI, HOMA-IR, serum lipids, fasting insulin and testosterone were investigated in 22 age-matched men (mean age  $31.00 \pm 2.42$  years) (at 8 h): 11 healthy controls (HC) and 11 men with metabolic syndrome (MSP). Melatonin and LH were measured at 19 h, 03 h and 11 h.

**Results**

A significant difference was found between the testosterone levels in HC ( $21.1 \pm 2.17$ ) and MSP ( $11.88 \pm 1.09$ ),  $P = 0.001$ . Testosterone levels correlated significantly with fasting insulin ( $r = -0.521$ ;  $P = 0.015$ ), HOMA-IR ( $r = -0.516$ ;  $P = 0.017$ ) and BMI ( $r = -0.549$ ;  $P = 0.008$ ). No changes in the melatonin rhythm of the two groups were found. LH levels in both groups were similar, however a tendency to higher night LH levels in MS patients was observed (LH /HC) =  $3.51 \pm 0.46$ ; (LH /MS) =  $5.17 \pm 0.68$ ;  $P = 0.057$ .

**Conclusion**

Endogenous testosterone levels are significantly lower in young men with metabolic syndrome compared to healthy age-matched controls. The mild hypogonadism in hyperinsulinemic patients is not related with changes in melatonin rhythm, suggesting that factors different from the testosterone are responsible for the melatonin fluctuations by hypo- and hypergonadotropic hypogonadism.

**P338****Mortality and morbidity are objective epidemiological indices that reflect state of diabetic aid in region**IV Misnikova, YA Kovaleva & AV Dreval  
Moscow Regional Research Clinical Institute, Moscow, Russia.**Material and methods**

Data for 124 554 DM patients is contained in the DM register of Moscow region (MR) for 2004. Mortality is evaluated yearly.

**Results**

Mortality as result of T1D was 1.92, and of T2D - 41.82 people for 100 thousand population. Mortality among men surpasses mortality among women for T1D (2.11 men/1.76 women), whereas the opposite was observed for T2D (58.79 women/21.81 men). Life expectancy after a diagnosis of sugar diabetes is made is 16 years for T1D, and 10.72 years for T2D.

Leading cause of death for both types of DM is cardiovascular disease: 36.88% for T1D, 68.5% for T2D. 18% of patients with T1D and 1.9% for T2D died as result of cardiovascular diseases (CVD). However, absolute number of patients who died as result of CVD is much larger for T2D. Quite large percentage of deaths when there is T1D are deaths from comas (4%). During the analysis of morbidity, higher indices were noted for T2D (3.1%) than for T1D (2.6%). Morbidity is bit higher among men than among women for both types of DM. The maximum morbidity for T2D is in older age group of 80 and over (7.64%), and lowest (under 0.59%) in 40-44 y.o. age group. Bit of increase in morbidity is noted in younger age group: 0.72% in 25-29 y.o. age group, 1.28% in 35-39 y.o. age group, and 2.35% in 30-34 y.o. age group.

**Conclusion**

Leading cause of death among diabetes patients is CVD. Maximum mortality figures for T1D come at the age of 55-59 y.o., and mortality figures for T2D grow in proportion to age of patients.

**P339****Prevalence of diabetic foot syndrome among diabetes patients**IV Bakharev, IV Misnikova & AV Dreval  
Moscow Regional Research Clinical Institute, Moscow, Russia.**Background and aims**

Diabetic foot ulcers are a common problem and result in lower extremity amputations. Research on the prevalence of diabetic foot syndrome (DFS), food ulcers, and high amputations reflects the state of diabetic aid.

**Materials and methods**

The data from the Moscow Region DM register on the detectability of DFS, the number of high amputations, the presence of late complications that enable the development of ulcer defects - diabetic neuropathy (DN) and diabetic macro-angiopathy (DMA) for 2004 is presented. The DM register is made up of 124 281 patients.

**Results**

DFS is registered among 11.22% of T1D patients and 5.58% of T2D patients. Conditions with an increased risk for DFS developing: DN is discovered among 37.1% of T1D patients, 19.14% of T2D patients, whereas DM is discovered among 15.66% of T1D patients and 11.54% of T2D patients. The frequency of high amputation in 2004 made up 0.85% of T1D patients and 0.61% of T2D patients. The total percent of patients who previously had high amputations with T1D was 2.38%, and 2.87% for T2D. Small amputations were conducted among 1.76% of T1D patients, and 1.2% of T2D patients in 2004. Morbidity as the result of sepsis from gangrene among patients with DFS made up 3.15% among T1D patients and 2.74% among T2D patients.

**Conclusion**

The analysis of attained data showed that the prevalence of diabetic foot corresponds to the average European figures. However, the frequency with which diabetic macro-angiopathy is encountered requires intensification of the amount medical aid for this category of patients.

**P340****Decreased cutaneous vasomotor responses in patients with insulin resistance**P Tretjakovs<sup>1</sup>, A Jurka<sup>1</sup>, K Geldenere<sup>1</sup>, A Stifts<sup>2</sup>, JI Aivars<sup>1</sup> & V Pirags<sup>1</sup>  
<sup>1</sup> University of Latvia, Riga, Latvia; <sup>2</sup> Pauls Stradins Clinical University Hospital, Riga, Latvia.**Aim**

To evaluate the relationship between insulin resistance and cutaneous vasomotor responses (endothelial-dependent vasodilatation and peripheral sympathetic failure: noradrenergic control of smooth muscle cells - vasoconstriction and neuropeptides induced vasodilatation) in patients with metabolic syndrome (MS).

**Methods and subjects**

Patients with insulin resistance and MS (defined according to "The 2005 IDF definition of the metabolic syndrome"), but without hypertension were divided into two groups: 18 patients with type-2 diabetes mellitus (without insulin therapy and without pronounced diabetic complications) (DM) and 18 patients without DM. 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. We recorded changes in laser Doppler flux (LDF; PeriFlux 4001, Perimed) on the big toe. Basal LDF (b-LDF), postocclusive hyperemia (m<sub>1</sub>-LDF), vasoconstrictor response (v-LDF) to deep inspiration on the pulp (apical skin); and heat (44 °C; PeriTemp 4005) induced hyperemia (m<sub>2</sub>-LDF) on the dorsum (non-apical skin) of the big toe were estimated using a PeriSoft for Windows program.

**Results**

B-LDF and local skin temperature were without differences among the study groups ( $P > 0.05$ ). v-LDF was significantly less pronounced only in diabetics compared to healthy subjects (DM  $31.8 \pm 13.7$  vs. C  $52.6 \pm 8.5\%$ ,  $P < 0.05$ ). m<sub>1</sub>-LDF was decreased in both patient groups in comparison with the controls ( $P < 0.05$ ), but the decrease of m<sub>2</sub>-LDF was pronounced only in diabetics (DM  $134 \pm 61$  vs. C  $192 \pm 78$  PU,  $P < 0.05$ ).

**Conclusion**

Our findings show that patients with insulin resistance have significant cutaneous vasomotor dysfunction despite the absence of pronounced diabetic or macrovascular complications.



### P341

#### Lipid profile in normal weight and obese women with polycystic ovary syndrome

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#### Background

Women with polycystic ovary syndrome (PCOS) appear at increased cardiovascular risk due in part to a dyslipidemia characterized by increased plasma triglyceride and reduced high density lipoprotein (HDL) cholesterol levels. Obesity, insulin resistance and hyperandrogenemia are features of PCOS and potentially affect lipid metabolism. On the other hand, obesity has an important influence on the lipid metabolism.

#### Objectives

The aim of this study was to compare lipid profile in lean and obese women with PCOS with normal weight and obese controls.

#### Methods

The study group consisted of 106 women divided in four subgroups (1. lean PCOS,  $n = 48$ ; age  $25.7 \pm 6.2$ ; 2. obese PCOS,  $n = 38$ ; age  $26.6 \pm 7.3$ ; 3. lean controls,  $n = 10$ , age  $27.7 \pm 7.6$ ; 4. obese controls,  $n = 10$ ,  $26.8 \pm 7.57$ ). Data were analyzed by ANOVA and Games-Howell post-hoc test. Obesity was defined by  $BMI > 29 \text{ kg/m}^2$ .

#### Results

Cholesterol, LDL cholesterol and triglyceride levels were significantly higher in obese PCOS group than in lean PCOS group (mean cholesterol was  $5.22 \pm 1.03$  vs.  $4.56 \pm 0.89$ ;  $P = 0.015$ ; mean LDL cholesterol was  $3.41 \pm 0.94$  vs.  $2.81 \pm 0.77$ ;  $P = 0.025$ ; mean triglyceride level was  $1.79 \pm 1.05$  vs.  $1.05 \pm 0.44$ ;  $P = 0.001$ ). On the contrary, HDL cholesterol levels were significantly lower in obese PCOS group than in lean PCOS group ( $1.08 \pm 0.29$  vs.  $1.32 \pm 0.35$ ;  $P = 0.009$ ). There was no statistically significant difference in lipid profile between obese PCOS group and obese control. Comparing lean PCOS and lean controls, only triglyceride levels reached statistically significant difference ( $1.05 \pm 0.44$  vs.  $0.73 \pm 0.22$ ;  $P = 0.009$ ).

#### Conclusions

These data suggest that obesity affects lipid metabolism in PCOS subject, especially by reducing HDL cholesterol levels, suggesting a reduced capacity for cholesterol removal from tissues with diminished antiatherogenic potential. PCOS per se, affects only triglyceride levels.

### P342

#### Is ghrelin a potential signal of decreased fat-free mass in elderly subjects?

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Aging is associated with weight loss, appetite decline, reduced fat-free mass (FFM) and increased fat mass (FM). Dysregulation of the ghrelin and leptin systems could reduce hunger and promote early satiety in elderly subjects, leading to impairment of the nutritional status. To better clarify this issue, we evaluated the response of plasma ghrelin and leptin to a standardised oral mixed nutrient load (SOMNL) in elderly subjects with different body composition. The study population, including 36 elderly subjects (14/12 F/M,  $68.6 \pm 4$  yrs; 16 overweight/obese and 10 normal-weight) and 10 young healthy controls, was subjected to air plethysmography (BOD POD) for FM and FFM and DEXA for appendicular skeletal muscle mass (ASMM) to assess body composition. Plasma ghrelin, leptin, growth hormone (GH), glucose and insulin concentrations were measured before and 1 h after SOMNL (19% protein, 35% lipids, 46% carbohydrates; 478 Kcal).

In obese elderly subjects, basal ghrelin was lower and leptin was higher than in normal-weight elderly subjects, as expected. Leptin was higher in elderly subjects than in young controls, whereas ghrelin was similar in both age groups. After SOMNL, ghrelin was more suppressed in normal-weight than in obese elderly subjects ( $-23\%$  vs.  $-12.4\%$ ,  $P < 0.05$ ) and in controls, whereas leptin and GH variations were similar. Basal and post-SOMNL ghrelin concentrations were inversely related to FFM, ASMM and to insulin sensitivity (QUICKI), whereas basal and post-SOMNL leptin levels were directly related to FM.

The present data suggest that, in elderly subjects, ghrelin concentrations, both basal and after SOMNL, are sensitive to the depletion of FFM (and specifically to the amount of skeletal muscle), similar to the sensitivity

of leptin concentrations to relative increments of FM. The greater ghrelin suppression in normal-weight elderly subjects could represent a signal of early satiety, leading to a reduction of food intake.

### P343

#### Obesity and expression of serotonin transporter (SERT) in human platelets

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Serotonergic transmission is involved in eating behaviour and body weight control. Comparison of SERT properties in blood platelets and serotonergic synaptosomes suggests that the human platelet can serve as an appropriate model for the transport, metabolism and release of serotonin by serotonergic neurons. Aim of this study was to evaluate SERT expression in platelets obtained from overweight or obese subjects (OB) as compared with normal weight healthy volunteers (C), and to look for possible correlations between SERT expression and various clinical or biochemical parameters. 34 OB (BMI range: 25–55) were compared with a group of C (BMI range: 18.5–24.9). SERT expression was evaluated in platelet membranes by the specific binding of [<sup>3</sup>H]paroxetine, and both maximal binding capacity (Bmax, fmol/mg protein) and dissociation constant (Kd, nM) were estimated. Results: Kd values in the two groups did not show significant difference. The mean Bmax of [<sup>3</sup>H]paroxetine was significantly lower in OB than in C (Bmax:  $1083.23 \pm 276.9$  vs.  $1443.8 \pm 242.55$  fmol/mg protein, respectively,  $P < 0.005$ ). An inverse correlation was observed between BMI and Bmax values, as assessed by simple linear regression ( $R = -0.563$ ;  $P < 0.0001$ ). A significant negative correlation was also found between SERT Bmax and waist circumference ( $P = 0.0046$ ;  $R = -0.424$ ), hip circumference ( $P = 0.0009$ ;  $R = -0.49$ ), systolic blood pressure ( $P = 0.0237$ ;  $R = -0.341$ ), fasting serum glucose ( $P = 0.001$ ;  $R = -0.479$ ), insulin ( $P < 0.0001$ ;  $R = -0.626$ ), tryglycerides ( $P = 0.0071$ ;  $R = -0.4$ ) C reactive protein ( $P = 0.01$ ;  $R = -0.39$ ), VES ( $P = 0.021$ ;  $R = -0.359$ ), haptoglobin ( $P = 0.0135$ ;  $R = -0.37$ ), leptin ( $P = 0.0002$ ;  $R = -0.535$ ) and PAI-1 ( $P < 0.0001$ ;  $R = -0.559$ ), while a positive correlation was found between SERT Bmax and HDL cholesterol ( $P = 0.026$ ;  $R = 0.338$ ). After multivariate analysis only insulin and PAI-1 were correlated with SERT Bmax independently of BMI.

In conclusion, our results suggest that adipose tissue may regulate SERT density on platelets (and possibly on serotonergic neurons). This effect could be mediated by circulating molecules related to adiposity.

### P344

#### Acute and chronic effect of teriparatide on glucose metabolism in women with established osteoporosis

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#### Introduction

There is indirect evidence of unfavorable effects of parathyroid hormone (PTH) on glucose metabolism. Teriparatide (recombinant human PTH 1-34 - TPTD) has been recently available for the treatment of osteoporosis.

#### Aim

To evaluate the acute and chronic effect of TPTD on blood glucose and insulin levels in women with established osteoporosis.

#### Patients and methods

Twenty-three postmenopausal women with established osteoporosis (mean age  $65.6 \pm 1.8$  years) received daily injections of  $20 \mu\text{g}$  TPTD for six months. Three oral glucose tolerance tests (OGTT) were performed: one day before the first injection (OGTT-basal), one hour after (OGTT-acute) and six months after initiation of therapy (OGTT-chronic).

#### Results

There were significant differences between the OGTT-basal and OGTT-acute values in glucose at 90 min ( $168.3 \pm 9.8$  vs.  $180.6 \pm 9.2$ ,  $P < 0.05$ ) and 120 min ( $152.0 \pm 8.7$  vs.  $170.5 \pm 7.8$ ,  $P < 0.01$ ), between the OGTT-basal and OGTT-chronic values for glucose at 90 min ( $168.3 \pm 9.8$  vs.  $184.5 \pm 13.3$ ,  $P < 0.05$ ) and between the OGTT-basal and OGTT-acute for insulin at 90 min ( $56.7 \pm 7.4$  vs.  $68.7 \pm 8.2$ ,  $P < 0.01$ ). These differences



remained significant for the subgroup of patients with normal ( $n = 8$ ) but not impaired glucose tolerance or diabetes mellitus ( $n = 15$ ).

#### Conclusions

TPTD seems to have an acute, subclinical adverse impact on stimulated glucose levels possibly due to insulin resistance. This impact is not seen at six months. On clinical grounds, it seems that TPTD administration has no significant impact on glucose metabolism in osteoporotic patients.

### P345

#### Adipose glucocorticoid synthesis: transcription of the 11 $\beta$ -hydroxylase gene in omental but not subcutaneous adipose tissue

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The major glucocorticoids (cortisol in man and corticosterone in the rat) play an important role in obesity and its attendant hypertension. However, there is no consistent evidence of glucocorticoid excess in simple obesity, suggesting that its role in the regulation of adipose tissue mass results from a more complex mechanism than adrenal hypersecretion of glucocorticoid. Glucocorticoid biosynthesis is not limited to the adrenal cortex; mRNA and enzymatic activity of 11 $\beta$ -hydroxylase, the enzyme responsible for the terminal stage of corticosterone production, have been demonstrated in the rat brain. In this study we sought evidence for 11 $\beta$ -hydroxylase gene transcription in adipose tissue. We used a fully quantitative RT-PCR system utilising homologous RNA standards to detect 11 $\beta$ -hydroxylase mRNA in adipose tissue taken from the Zucker rat model of obesity.

We analysed omental and subcutaneous adipose tissue from the Zucker obese rat and its lean control ( $n = 5$  for each group). 11 $\beta$ -Hydroxylase transcripts were easily and reproducibly detected in omental tissue ( $4.7 \times 10^5 \pm 9.4 \times 10^4$  mRNA copies/ $\mu$ g total RNA) at levels approximately 300-fold lower than the adrenal; adipose mRNA levels did not differ significantly between lean and obese animals. No transcripts could be detected in subcutaneous adipose RNA. Further experiments showed all adipose RNA samples to contain transcripts for the Steroidogenic Acute Regulatory (StAR) protein and the side-chain cleavage enzyme (P450scc) which are vital to *de novo* synthesis of glucocorticoid from cholesterol.

The local activation of glucocorticoid by the type 1 11 $\beta$ -hydroxysteroid dehydrogenase enzyme (11 $\beta$ -HSD1) in omental adipose tissue has attracted a great deal of interest and may play a role in the development of obesity by regulating adipocyte differentiation. Here we demonstrate omentum-specific 11 $\beta$ -hydroxylase expression for the first time, thus providing an additional mechanism for the local generation of glucocorticoid in adipose tissue.

### P346

#### Metabolic syndrome among patients with primary aldosteronism: a common feature?

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In the light of recent data reporting a high rate of cardiovascular events in Primary Aldosteronism (PA) we investigated whether the Metabolic Syndrome (MS) represents a common feature in patients with PA. A cohort of 86 patients, mean age  $51 \pm 12$  yr was analysed: 59 patients (26 females and 39 males) with idiopathic hyperaldosteronism (IHA) and 27 patients (13 females and 14 males) with aldosterone producing adenoma (APA). Anthropometric parameters (height, weight, waist circumference), lipid profile (total cholesterol HDL cholesterol, triglycerides) and glucose profile (fasting plasma glucose and insulin) were evaluated in all subjects. The MS was defined according to ATP III criteria, requiring the presence of at least three of the following factors: hypertension (Ht), obesity (O), high triglycerides levels (T), low HDL cholesterol levels (C), high fasting plasma glucose (G). Thirty seven patients with PA fulfilled ATP III criteria with an overall prevalence of the MS of 43%: 8 patients with APA (30%) and 29 patients with IHA (49%). Considering the distribution within gender, 24 male patients (51%) had MS compared to 13 female patients (33%). No differences were observed between male/female distribution in APA and IHA (5/3 and 19/10, respectively). Considering the distribution of three or more factors:

	HtOT	HtOC	HtOG	HtTC	HtGC	>3	No SM
APA % (n)	0	0	0	11%(3)	4%(1)	15%(4)	70%(19)
IHA % (n)	3%(2)	10%(6)	3%(2)	7%(4)	5%(3)	21%(12)	51%(30)

Gender analysis showed that while female patients display more frequently obesity, male patients have more often alterations of the lipid and glucose profiles. Our data show that the prevalence of the MS in PA patients is about double than in the age-matched Italian population. Moreover the MS is more frequent in IHA patients than in APA, underlying that this variant of PA is more similar to essential hypertension. Finally, the presence of more than 3 ATP III factors in many PA patients underline that this secondary form of hypertension is not so benign and is strongly associated with metabolic alterations that may be involved in the development of cardiovascular events.

### P347

#### Metabolic parameters in patients with primary aldosteronism: relation to snps of the adiponectin gene

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Adiponectin, a recently discovered protein which is secreted by the adipose tissue, exerts anti-inflammatory and anti atherogenic properties, but also promotes glucose uptake by skeletal muscle and fatty acids oxidation. Patients with hypertension and obesity have reduced plasma levels of adiponectin so that they lack its beneficial metabolic effects. However no data are available in patients with primary aldosteronism (PA). In order to investigate the role of the adiponectin gene variants on glucose and lipids metabolism in patients with PA, we analysed data from 89 patients: 23 with aldosterone producing adenoma (APA) and 66 with idiopathic hyperaldosteronism (IHA). Two single nucleotide polymorphisms were studied, the T45G in exon 2 and the T276G in intron 2, using enzymatic restriction analysis. The same SNPs were also evaluated in 45 patients with essential hypertension (EH). No differences in genotype distribution were observed between PA and EH patients. Allele and genotype frequency distributions were in Hardy-Weinberg equilibrium for both SNPs in both PA and EH. Genotype distribution for T45G was: TT = 63, GT = 25, GG = 1. No significant correlations were observed between genotype and allele distribution and blood pressure values nor glucose and lipid profiles. The G allele was associated with lower values of HOMA-IR ( $1.7 \pm 1.2$  vs  $3.9 \pm 4.7$   $P < 0.05$ ). Although not significantly different, the G allele was also associated to lower waist circumference values, lower serum aldosterone levels, plasma glucose and insulin after OGTT. Genotype distribution for T276G was: GG = 51, GT = 33, TT = 5. The genotype 276 T/T was associated with higher levels of both systolic and diastolic blood pressure levels ( $P < 0.005$ ), triglycerides ( $P < 0.05$ ), and fasting plasma glucose ( $P < 0.005$ ) and insulin levels ( $P < 0.05$ ). Moreover, the T allele was associated with higher values of HOMA-IR index ( $3.9 \pm 5.5$  vs  $2.6 \pm 1.8$ ,  $P < 0.05$ ).

In conclusion, our data show that the genotype 45G/G-G/T has a protective role against the development of metabolic complications in PA patients, while the genotype 276 T/T defines PA patients with a worse glucose and lipids profiles.

### P348

#### Coronary artery disease and heart failure in diabetic patients in the north of portugal

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#### Aims

The aim of our study was to evaluate the incidence of coronary artery disease (CAD) and heart failure (HF) in diabetic and nondiabetic patients admitted to a central hospital in the North of Portugal between 1989 and 2004.

#### Materials and methods

Data from the Hospital Coding Centre were retrospectively analysed according to International Classification of Diseases (9th version) criteria. We have studied the distribution by age, sex, procedures, causes and duration of admissions. Statistical analysis was performed with Student's

t-test,  $\chi^2$  test or Fisher exact test. A two-tailed  $P$  value  $<0.05$  was considered significant

#### Results

A total of 698847 admissions were registered during the studied period. There were 14715 admissions with a diagnosis of CAD or HF without diabetes and 4733 admissions with a diagnosis of CAD or HF and diabetes. There were no significant differences in the age of diabetic patients [42.5% men (M) and 57.5% women (W)] and nondiabetic patients (48.4% M and 51.6% W) ( $66.1 \pm 10.5$  vs  $66.0 \pm 4.7$  years,  $P = \text{NS}$ ). There were no significant differences in the duration of hospitalization in diabetic patients and in nondiabetic patients ( $12.8 \pm 6.6$  vs  $12.9 \pm 12.3$  days,  $P = \text{NS}$ ). The incidence of angina pectoris was significantly higher in diabetic than in nondiabetic patients (2.2% vs 1.6%,  $P = 0.007$ ). There was an excess of myocardial infarction among the diabetic patients (5.6% vs 4.6%,  $P = 0.03$ ). The incidence of HF was significantly higher in diabetic than in nondiabetic patients (6.6% vs 5.0%,  $P < 0.001$ ).

#### Conclusions

The high incidence of CAD and HF in diabetic patients implies that the screening and the aggressive treatment of these pathologies are critical components of the health care in our population.

### P349

#### Physiological concentrations of testosterone inhibit extracellular calcium entry via voltage-gated calcium channels in the A7r5 vascular smooth muscle cell line - a non-genomic effect

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Testosterone (T) acts as a coronary vasodilator and reduces myocardial ischemia in men with coronary heart disease. We have previously demonstrated that T inhibits the pore forming  $\alpha_1c$  subunit of the cardiovascular L-type calcium channel in HEK293 cells. In this study we investigated the effects of T and  $5\beta$ -dihydrotestosterone ( $5\beta$ -DHT) on extracellular calcium entry in A7r5 vascular smooth muscle cells (VSMCs). A7r5 cells grown on coverslips in 12-well plates in DMEM + 10% foetal bovine serum were incubated in medium containing the calcium fluorescence probe Fura2-AM 4  $\mu\text{M}$  for 40-min, at 37°C. Coverslip fragments were placed in a perfusion chamber and changes in calcium were indicated by fluorescence emitted at 510 nm after excitation at 340 and 380 nm, in the presence of test substances. High potassium ( $\text{K}^+$ ) induced a change in cellular fluorescence of  $0.12 \pm 0.02$  ratio units. Subsequent recordings are expressed as a percentage of this response. Compared to ethanol (0.1%), 2-min incubation with T (1, 3, 10, 100 nM) caused a concentration-dependent inhibition of this response;  $94.2 \pm 7.4\% \text{K}^+$ ,  $98.4 \pm 5.1\% \text{K}^+$ ,  $67.6 \pm 4.0\% \text{K}^+$  ( $P < 0.01$ ),  $51.5 \pm 5.8\% \text{K}^+$ ,  $40.9 \pm 2.6\% \text{K}^+$  all  $P < 0.001$  respectively.  $\text{IC}_{50} = 3.1$  nM. 2-min incubation with  $5\beta$ -DHT (1, 10, 100 nM) also caused an inhibition of this response;  $58.3 \pm 9.8\% \text{K}^+$  ( $P < 0.01$ ),  $44.9 \pm 2.5\% \text{K}^+$ ,  $39.3 \pm 6.1\% \text{K}^+$  (both  $P < 0.001$ ). Incubation with nifedipine (L-type calcium-channel blocker) (5  $\mu\text{M}$ ) also caused similar inhibition of this response;  $46.7 \pm 4.1\% \text{K}^+$  ( $P < 0.001$ ), as did pimoizide (T-Type calcium channel blocker) (1  $\mu\text{M}$ )  $31.3 \pm 4.4\% \text{K}^+$  ( $P < 0.001$ ) and calcium-free buffer almost abolished the response  $6.5 \pm 1.3\% \text{K}^+$  ( $P < 0.001$ ), as did co-incubation with nifedipine (5  $\mu\text{M}$ ) + pimoizide (1  $\mu\text{M}$ )  $12.3 \pm 1.3\% \text{K}^+$  ( $P < 0.001$ ). Co-incubation with T (10 nM) + nifedipine (5  $\mu\text{M}$ ) showed no extra inhibition;  $44.1 \pm 6.8\% \text{K}^+$  ( $P < 0.001$ ) compared to nifedipine and T only. We conclude that physiological concentrations of T and  $5\beta$ -DHT inhibit extracellular calcium entry via L-type voltage-gated calcium channels in VSMC's via a non-genomic manner, with an effect similar to that of nifedipine.

### P350

#### Comparison between premixed insulin aspart 30 vs premixed human insulin 30/70 in combination with metformin in obese type 2 diabetic patients

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#### Background and aims

Many obese patients with type 2 diabetes mellitus (T2DM) are not adequately controlled with oral antidiabetic therapy (OAD), even with combination between metformin and sulfonylurea (SU). When OADs no longer maintain good glycaemic control in obese T2DM, it is necessary to add insulin therapy. The aim of this study is to compare the effect of adding premixed insulin aspart 30(BIAsp30) vs. premixed human insulin 30/70 (BHI30) to support metformin therapy in obese T2DM patients.

#### Materials and methods

50 obese type T2DM patients, BMI  $34 \pm 2$  kg/m<sup>2</sup>, poorly controlled by OADs (metformin + SU) in maximal doses, HbA1c  $9.4 \pm 1.3\%$ . 30 of them were treated with adding BHI30 in combination with metformin. 20 of them were treated with BIAsp30 in combination with metformin. Duration of the study was 3 months. Efficacy was assessed by analysis of HbA1c, FPG, postprandial glycaemia, blood glucose profile, and hypoglycaemic episodes.

#### Results

HbA1c decreased in both groups, but significantly lower in BIAsp30 + metformin group vs. BHI30 + metformin group. FPG decreased during the study in both groups, but postprandial glycaemia was significantly lower in BIAsp30 + metformin group. Hypoglycaemic episodes didn't differ significantly in both groups.

#### Conclusion

In obese patients with T2DM, poorly controlled by OADs, insulinisation with BIAsp30 and BHI30 in combination with metformin provides significant improvement in HbA1c and FPG. BIAsp30 reduce postprandial glucose and HbA1c significantly greater than BHI30. For that reason BIAsp30 added to metformin could be optimal therapeutic option for achieving good glycaemic control in obese T2DM patients.

### P351

#### Effect of repaglinide vs glimepirid on glycaemic control in overweight and obese patients with type 2 diabetes mellitus uncontrolled by metformin

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#### Background and aims

Metformin is the drug of first choice in obese patients with type 2 diabetes (T2DM), but in some patients it is not possible to achieve good glycaemic control by using metformin only. Starting from a fact that T2DM is a heterogeneous disorder due to the interaction of impaired early insulin secretion and peripheral insulin resistance, the aim of the present study was to evaluate and compare the efficacy of repaglinide vs. glimepirid on glycaemic control of overweight and obese patients with T2DM, previously treated by metformin.

#### Materials and methods

Overweight and obese T2DM patients, BMI  $29 \pm 3$  kg/m<sup>2</sup>, diabetes duration  $5 \pm 2.3$  years, HbA1c  $8.5 \pm 1.1\%$ . All patients were at metformin therapy, metformin dose 2000 mg/day. Half of them were treated with metformin (2000 mg/day) + repaglinide (2 mg/meal) and the others were treated with metformin (2000 mg/day) + glimepirid (3 mg/day). Duration of the study was 3 months. Parameters assessed were HbA1c, FPG, blood glucose profile, change in body weight and hypoglycaemic episodes.

#### Results

HbA1c values during the study decreased in each group, but significantly lower in Rep + Met group 7.3 (0.1%), vs. 7.9 (0.1%),  $P = 0.03$ . FPG, preprandial and postprandial glycaemia decreased during the study in both groups, but postprandial glycaemia was significantly lower during Rep + Met treatment. There was not significant variation in BMI. The hypoglycaemic episodes were more frequent in Glim + Met group.

#### Conclusion

These results indicate that combination between repaglinide and metformin has superior effect on glycaemic control, specially in controlling postprandial hyperglycaemia, compared with combination between glimepirid and metformin.

### P352

#### Association of an insertion/deletion polymorphism in the alpha 2B adrenergic receptor with essential hypertension in diabetic patients

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**Background**

The alpha2B adrenoceptor gene, a candidate gene for essential hypertension, harbours a functional insertion/deletion (I/D) polymorphism of three glutamate residues.

**Objectives**

The aim of this study was to investigate whether the presence of the D allele is associated with hypertension in Greek patients with type 2 diabetes mellitus (T2DM).

**Patients and methods**

One hundred and twenty-nine hypertensive (64 male, mean age:  $66.5 \pm 9.3$ , mean diabetes duration:  $11.9 \pm 8$ ) and 70 age-matched normotensive (32 male, mean age:  $65.8 \pm 9.6$ , mean diabetes duration:  $10.7 \pm 9.2$ ) T2DM subjects were included in the study. Hypertension was defined as known or newly diagnosed hypertension according to current guidelines (JNC7). All patients included were normoalbuminuric with normal renal function. The I/D polymorphism was determined by polymerase chain reaction (PCR). Differences in the genotype and allele frequencies in the study and control groups were analyzed by use of Yates-corrected chi square-test.

**Results**

Alpha2B adrenoceptor I/D genotypes were distributed in T2DM hypertensive patients as follows: 81 (62.8%) II, 40 (31%) ID, 8 (6.2%) DD. The distribution was similar in T2DM normotensives: II in 62.9% (44), ID in 31.4% (22), and DD in 5.7% (4). There was no statistically significant difference in genotypic and allelic frequencies of the I/D polymorphism between the hypertensive and normotensive diabetic patients (Yates-corrected chi-square = 0.814,  $P = 0.36$  for the allelic frequencies). In T2DM hypertensive patients, those carrying the D allele showed significantly higher diastolic blood pressure ( $P = 0.002$ ). Total cholesterol and atheromatous index were also higher in this group of patients ( $P = 0.033$  and  $P = 0.004$  respectively).

**Discussion**

The D variant of the alpha2B-adrenoceptor gene was not associated with hypertension in T2DM patients. However, diabetic hypertensive patients carrying the D allele are probably prone to diastolic hypertension.

**P353****The metabolic syndrome and insulin resistance in polycystic ovary syndrome – study over 40 patients**

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Insulin resistance is possibly playing an underlying pathogenic role in the polycystic ovary syndrome (PCOS) and although is not a part of its definition, it appears in 50–90% of PCOS women. Polycystic ovary syndrome is also frequently associated with obesity; women suffering of PCOS seem to be at a great risk of developing a metabolic syndrome.

**Objectives**

The aim of our study was to determine the prevalence of the metabolic syndrome in a population of 40 women with PCOS and to establish a relationship between this syndrome and insulin resistance.

**Material and method**

A retrospective study was carried out with 40 women diagnosed with PCOS in our clinic. The patients underwent complete clinical and biochemical measures, including fasting glycemia and insulinemia, lipid profile and total plasmatic testosterone. We appreciated insulin resistance by calculating HOMA index and the metabolic syndrome was appreciated using the updated ATP III (2005) criteria.

**Results**

Insulin resistance appeared in 55% cases. Prevalence of the MS in our group study was 40% (16 patients out of 40), near 2-fold higher than that of the control group. Except for one, the patients with PCOS and MS were also insulin resistant. Central obesity and low levels of HDL-cholesterol were present in 100%, respectively 87.5% of cases diagnosed with metabolic syndrome. High blood pressure appeared in 68.75% of these patients; high levels of triglycerides and fasting glycemia  $>100$ mg/dl were present in 37.5% of cases.

**Conclusions**

We conclude that the MS is more common in women with PCOS than in the control group. Central obesity has a good correlation both with insulin resistance and the metabolic syndrome appearance. Abdominal obesity and low levels of HDL cholesterol were the most prevalent individual components of the metabolic syndrome.

We found no other correlation between the independent criteria for MS and insulin resistance.

There was no correlation between the metabolic parameters and the biochemical androgenism expressed by total plasmatic testosterone.

**P354****Prevalence of registered diabetic nephropathy and its terminal stages**

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**Background**

Diabetic nephropathy is leading cause of end-stage renal disease (ESRD), which requires dialysis or a transplant. Progression of diabetic nephropathy (DN) leads to development of chronic renal failure (CRF).

**Material and methods**

Data for 124 554 DM patients who live in Moscow region (MR) is in the MR DM register for 2004. The number of T1D- 8747, T2D patients is 115, 534. These registers only contain information about registered cases of DN.

**Results**

Registered prevalence of DN when there is T1D is 21.7%, and 6.9% when there is T2D. The prevalence of DN when there is T1D is bit higher among men, and among women when there is T2D. When children have T1D it makes up 4.3%, 13.1% among adolescents, and 23.9% among adults. Percent of DN reaches its maximum when there has been long-term diabetes for over 15 years: 40.8% when there is T1D, and 15.4% for T2D, and also when there is an increase in the severity of disease, making up 21.1% given a severe form.

Prevalence of CRF among T1D patients is 0.65%, and 0.2% for T2D. Dialysis gets 60 patients, which makes up 0.6% of DM patients with DN. Among them 45.0% are hemodialysis, 53.3% are peritoneal dialysis. 1 patient gets renal transplantation. The duration of peritoneal dialysis among DM patients varies from 2 to 78 months. DM patients make up 13.5% of all patients who receive dialysis (8.9% are on hemodialysis, 22.5% are on peritoneal dialysis). DM patients on peritoneal dialysis make up 21.2% of overall number of CRF patients.

**Conclusion**

Diagnosis of diabetic nephropathy in irreversible stage and large number of severe forms requires prophylactic measures.

**P356****Noninsulinoma pancreatogenous hypoglycaemia syndrome (NIPHS) caused by an activating glucokinase mutation**

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NIPHS is a rare cause of adult onset hyperinsulinaemic hypoglycaemia with islet hypertrophy/nesidioblastosis, but without mutations in the ABCC8 and KCNJ11 genes coding for the beta cell  $K_{ATP}$ -channel subunits SUR1 and Kir6.2. NIPHS patients with GCK mutations have never been described.

We report of a 42-y-old woman with asymptomatic hypoglycaemia down to 2.9 mmol/l with simultaneous p-insulin 208 pmol/l (12–77 pmol/l), p-C-peptide 1574 pmol/l (130–760 pmol/l), p-proinsulin 88 pmol/l (2–23 pmol/l), (normal ranges at euglycaemia in brackets by Delphia method). Her son had congenital hyperinsulinism with remission at the age of 8 months, but relaps at age 14 y treated with diazoxide, thiazide and octreotide up to the actual age of 20.

No insulinoma was detected. ABCC8 and KCNJ11 genetic analysis was normal. In the glucokinase gene, however, a novel activating mutation, A456V, was found in the mother and child. Functional mutation analysis showed a left shift of the glucose dependency curve (in contrast to MODY 2). After 5 years of asymptomatic hyperinsulinaemic hypoglycaemia without treatment, the mother had an attack of convulsions and unconsciousness during a slimming diet. Blood glucose was 3.2 mmol/l on admission. Treatment with diazoxide and thiazide was commenced.

During a slimming diet, the 24 y-old daughter of the mother's brother had an accident and was found hypoglycaemic, 2.7 mmol/l, with a simultaneous increased p-insulin of 88 pmol/l and p-C-peptide, 1014 pmol/l. After a) 6 h fasting; and b) 3½ h after an OGTT, she had hypoglycaemic symptoms and a blood glucose of a) 1.6 mmol/l; and b) 1.9 mmol/l. She also had the GCK mutation A456V and responded to octreotide treatment.

**Conclusion**

NIPHS due to an activating GCK mutation was demonstrated in 3 family members with an onset ranging from the neonatal period to 47 years of age. NIPHS-GCK has so far been medical responsive.



**P357**

**Relationship between plasma visfatin levels and the metabolic variables in metabolic syndrome**

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**Objective**

Visfatin, a novel adipocytokine, is predominantly expressed in visceral fat and correlated with obesity. The pathophysiologic role of visfatin in metabolic syndrome (MS) is not clear. We aimed to investigate the relationship between plasma visfatin levels and components of MS, and also the effect of therapeutic lifestyle change (TLC) on these parameters.

**Design and methods**

Nineteen patients (5 male, 14 female; mean age 51.0 ± 9.3 years; body mass index (BMI) 31.0 ± 5.1 kg/m<sup>2</sup>) and 20 healthy controls (6 male, 14 female; mean age 45.4 ± 9.31 years; BMI 30.1 ± 4.0 kg/m<sup>2</sup>) were enrolled. Plasma visfatin levels were measured along with the BMI, waist circumference (WC), blood pressure, lipids, glucose, immunoreactive insulin, adiponectin and hsCRP levels both before and six weeks after the TLC. The insulin sensitivity index was quantified using homeostasis model assessment index (HOMA). The local ethic committee of university approved the study protocol and informed consent was obtained from all subjects.

**Results**

Both groups had similar age, sex and BMI. Lipids, insulin and HOMA levels of the patients were significantly higher and adiponectin levels were significantly lower than the controls. The plasma visfatin levels were not significantly different in both groups. After six weeks of TLC; the BMI, WC, triglyceride, insulin, HOMA and visfatin levels of the patients decreased while the adiponectin levels increased significantly. No correlation was established between the plasma visfatin levels and the other parameters.

**Conclusions**

Our results indicate that TLC in MS significantly reduces plasma visfatin levels, despite the lack of any significant correlation with the components of the MS. Further studies are needed to investigate the role of plasma visfatin in the pathogenesis of MS.

significant difference in the magnitude of the response to oestrogen between the sexes.

**Conclusion**

Oestrogen acts as an efficacious vasodilator in the human pulmonary circulation, with no marked differences observed in the response dependant on sex. Oestrogen may therefore be a potential novel agent in the treatment of pulmonary vascular disease, namely pulmonary hypertension.

**P359**

**The effect of fluvastatin on adiponectin and insulin sensitivity**

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**Objective**

The pleiotropic functions of statins are crucial in reducing cardiovascular events. There is controversy about their effects on insulin resistance. The aim of this study was to investigate the effect of Fluvastatin treatment on plasma adiponectin and insulin levels in a group of dyslipidemic population who had no confounding factors for insulin resistance such as diabetes or hypertension.

**Design and methods**

Forty nine (27 male, 22 female; mean age 47.2 ± 10.3 years; BMI 29.64 ± 3.2 kg/m<sup>2</sup>) consecutive patients and 20 control subjects (6 male, 14 female; mean age 45.3 ± 9.31 years; BMI 30.07 ± 4.04 kg/m<sup>2</sup>) were enrolled. Patients were treated with therapeutic lifestyle changes (TLC) for six weeks. Then, the follow up was maintained for additional 12 weeks in the remaining 43 patients. The remaining patients were allocated to Fluvastatin 80mg daily plus TLC (24 patients; 14 male and 10 female) or to TLC alone (19 patients; 9 male and 10 female). The insulin sensitivity index was quantified using homeostasis model assessment index (HOMA). The local ethic committee of university approved the study protocol and informed consent was obtained from all subjects.

**Results**

TLC caused significant improvement in plasma insulin levels and HOMA indexes ( $P = 0.02$  and  $P = 0.02$  respectively) along with a significant elevation of plasma adiponectin levels ( $P = 0.002$ ). Fluvastatin treatment made significant contributions on the decrement of total cholesterol, LDL cholesterol ( $P = 0.001$ ,  $P = 0.003$  respectively) and the elevation of plasma adiponectin levels ( $P = 0.001$ ). However, no significant effect of Fluvastatin treatment was established on plasma insulin or HOMA level.

**Conclusions**

These results indicate that Fluvastatin treatment has no effect on insulin sensitivity while it causes significant elevation in plasma adiponectin levels.

**P358**

**The vasodilatory action of oestrogen in isolated human pulmonary arteries**

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**Background**

This study was carried out to assess the vasodilatory effect of oestrogen in the human pulmonary circulation. The influence of gender upon the response to oestrogen was also assessed.

**Method**

Isolated human pulmonary arteries were studied by wire myography. Vessels were obtained from male ( $n = 6$ , age 70 ± 9 years) and female ( $n = 6$ , age 59 ± 9 years) patients. The study was approved by the local ethics committee. Vessels were precontracted with U46619 (1 µM) and endothelial integrity was tested with acetylcholine (1 µM). Vessels were then washed before the addition of increasing concentrations of U46619 (1 nM-100 µM) prior to exposing them to either oestrogen (1 nM-100 µM) or ethanol vehicle.

**Results**

Results are shown in Table 1 as: Change in mean active tension (%) ± SEM. A statistically significant relaxation to oestrogen was seen, with male vessels dilating at significantly lower doses of oestrogen. There was however no

**Table 1**

Gender	0.1µM	1.0µM	3µM	10µM	30µM	100µM
Male	-2.3 ± 0.9*	-3.3 ± 1.0*	-3.3 ± 1.2*	-5.1 ± 1.6*	-10.8 ± 3.2*	-26.1 ± 3.6*
Female	-0.1 ± 1.8	-0.5 ± 1.6	-0.8 ± 1.5	-2.1 ± 1.6	-3.8 ± 1.7	-25.2 ± 7.37*

\* Represents significant dilation compared to ethanol vehicle ( $P < 0.05$  via students t test for independent variables).

**P360**

**Leptin as a regulator of serum acylated ghrelin in normal-weight and obese premenopausal women**

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**Introduction**

Besides of the neuroendocrine actions including the stimulatory effects on growth hormone, prolactin and adrenocorticotropin release, acylated ghrelin (AG) plays a role in the regulation of metabolic processes, resulting in the decrease of insulin secretion and increase of hepatic glucose release. Despite of many earlier studies, the mechanisms involved in AG secretion have not been established. The aim of study was to assess the influence of leptin on AG.

**Material and methods**

The study was performed on 32 normal-weight (BMI 18.9–24.2 kg/m<sup>2</sup>) healthy women aged 22–47 yr, and 80 obese women (BMI 30.1–51.4 kg/m<sup>2</sup>) aged 22–46 yr without a diagnosis of metabolic syndrome according to ATP III criteria. Basal serum AG, leptin, insulin and glucose were measured. Insulin sensitivity was assessed by homeostatic model of assessment (HOMA).

**Results**

In normal-weight women a positive correlation between leptin and AG was found ( $R = 0.375$ ;  $P = 0.034$ ), and in multiply regression analysis leptin



positively influenced AG serum level ( $P = 0.001$ ). In obese women with HOMA  $< 2.5$  we could not demonstrate linear correlation between leptin and AG, however in multiply regression model leptin negatively influenced AG ( $P = 0.035$ ). We did not find such relationships in obese women with HOMA  $> 2.5$ .

#### Conclusions

Leptin significantly influence AG in normal-weight healthy premenopausal women. In obese women without insulin resistance, different influence of leptin on AG seems to reflect a physiological mechanism of adaptation to the positive energy balance, protecting against hyperglycaemia. This mechanism is abolished in women with insulin resistance.

### P361

#### Insulin receptoropathies are distinguished from other syndromes of severe insulin resistance by elevated plasma adiponectin levels

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Leptin and adiponectin are cytokine-like hormones secreted by white adipose tissue. Plasma leptin correlates closely with total body fat mass, and its secretion is positively regulated by insulin *in vivo* and *in vitro*. Hypoleptinaemia is a key centrally-mediated orexigenic stimulus. Plasma adiponectin, in contrast, correlates negatively with whole body fat mass and insulin resistance in adults, and its expression is regulated negatively by insulin *in vivo* and *in vitro*. We have now determined plasma leptin and adiponectin in subjects with severe insulin resistance due to insulin receptor or AKT2 loss-of-function mutations, in those with syndromes of lipodystrophy due to mutations in PPAR $\gamma$ , Lamin A/C, AGPAT2 or BSCL2, and in those with severe insulin resistance of undefined molecular origin. Subjects with insulin receptoropathies, despite having the most severe degree of insulin resistance, had elevated plasma adiponectin (median 24.4 mg/l; range 6.6–27.6), while all other subjects, including those with defective AKT2 function, had low adiponectin levels in keeping with previous observations (median 2.0 mg/l; range 0.12–11.2). Plasma leptin in all but one subject with defective insulin receptors was low or undetectable (median 0.5 ng/ml; range 0–16), closer to the levels in total lipodystrophy (median 0.1 ng/ml; range 0–0.4) than in partial lipodystrophy (median 5.6 ng/ml; range 0.2–12.4). These findings suggest that high plasma adiponectin with low leptin may be used as a biochemical discriminator of those severely insulin resistant subjects who harbor insulin receptor mutations, and we speculate that this reflects impaired adipocyte maturation in these subjects.

### P362

#### Impaired endothelial function in young non-obese women with polycystic ovary syndrome: Normalization with Ethinyl Estradiol-Cyproterone Acetate treatment

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#### Background

Women with PCOS carry cardiovascular (CV) risk factors, which may account for the increased CV risk. Oral contraception therapy using ethinyl-estradiol & cyproterone acetate (EE-CA) is administered to normalize menstrual cycles and reduce serum androgen levels in women with PCOS. The aim of this study was to assess the effect of EE-CA on endothelial function in young, non-obese PCOS women.

#### Methods

We studied 13 non-obese (body mass index  $< 25 \text{ kg/m}^2$ ), young women with PCOS before and after 6 months of EE-CA therapy and we compared them with 14 age- and BMI-matched women with normal ovarian function. All women were non-smokers and free of CV disease. Endothelium-dependent flow-mediated dilation (FMD) and nitrate-mediated dilation (NMD) was assessed in all women, using high resolution linear array ultrasound in the brachial artery at baseline and at 6 months.

#### Results

At baseline both groups did not differ in fasting glucose and insulin, indices of insulin sensitivity, serum lipids and blood pressure. FMD was significantly lower in women with PCOS at baseline (increase in brachial artery diameter during hyperemia by  $4.67 \pm 2.38\%$ ) than in control women (increase by  $10.12 \pm 3.19\%$ ,  $P < 0.0005$ ). NMD was also lower in women with PCOS ( $18.45 \pm 5.42\%$  vs  $25.04 \pm 4.42\%$ , respectively,  $P = 0.003$ ). Following EE-CA therapy for 6 months, FMD was improved in women with PCOS, increasing by approximately two-fold ( $9.99 \pm 2.11\%$ ,  $P < 0.005$  compared to pre-treatment), and reaching normal values ( $P$  not significant compared to control women). NMD did not change significantly following treatment. EE-CA therapy resulted in a significant decrease in free androgen index ( $14.1 \pm 13.8\%$  vs  $2.3 \pm 1.3\%$ ,  $P = 0.019$ ). EE-CA therapy did not have any effect on BMI, blood pressure, fasting glucose, insulin and indices of insulin sensitivity.

#### Conclusions

Early onset of endothelial dysfunction in young, non-obese women with PCOS may increase their risk for cardiovascular disease. Treatment with EE-CA for 6 months has a beneficial effect on endothelial function, possibly due to reduced androgen levels.

### P363

#### Carbohydrate metabolism in children with Down syndrome

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#### Introduction

Trisomy 21 is often associated with congenital malformation and metabolic abnormalities such as glucose intolerance and an increased risk of developing diabetes mellitus.

#### Aim

The aim of this study was to assess carbohydrate metabolism in children with Down syndrome.

#### Material and methods

Thirty nine children with Down syndrome, aged between 2 months and 16 years (mean 6.5 years), took part in the study. Glycaemia before and after a standard meal, cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides and thyroid hormones were measured. The children were divided into two groups: below and above 2 years of age (ZD-1;  $n = 12$ , ZD-2;  $n = 19$  respectively). We excluded 7 children with glucose intolerance and 1 child with diabetes mellitus type I from statistical analysis. The Ethics Committee of Wroclaw Medical University approved of the study protocol.

#### Results

In both groups flat glucose curves after meal ingestion were observed. No significant differences were found in glucose concentration levels between the two groups. The mean glucose levels measured in mg% (0', 30', 60', 90', 120') in ZD-1 group were: 82,83; 82,58; 88,58; 81,33; 85,71; 81,79; and in ZD-2 group: 83,79; 89,91; 94,83; 93,16; 92,26; 89,90. In both groups a negative correlation was found between fasting glucose concentration and TSH concentration (ZD-1:  $r = -0.58$ , ZD-2:  $r = -0.30$ ) and a positive correlation between glucose and TG concentration (ZD-1: in 30'  $r = 0.53$ ; ZD-2: in 120'  $r = 0.31$ ). The results enabled to make the diagnosis of glucose intolerance in seven children and of diabetes mellitus in one child.

#### Conclusions

Due to the apparent abnormalities, carbohydrate metabolism should be closely and regularly monitored in children with Down syndrome.

### P364

#### Anti-oxidative effect of 17beta-estradiol in human endothelial cells: the role of Bcl-2

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#### Aim

To determine if the endogenous estrogen 17beta-estradiol protected against oxidative stress-induced endothelial cell damage and the mechanism of this potential effect.

#### Methods

Human umbilical vein endothelial cells were isolated and cultured in phenol red free media with 2% charcoal-stripped serum. The following were undertaken (1) Cells were exposed to 0.1 to 1 nM 17beta-estradiol immediately prior to 100  $\mu\text{M}$  hydrogen peroxide added for 24 hours, with and without the anti-estrogen ICI182,780. Cell proliferation, apoptotic DNA

fragmentation and expression of Bcl-2 and Bax were subsequently measured by tritiated thymidine incorporation assay, Cell Death ELISA and Taqman quantitative PCR, respectively. (2) Cell Bcl-2 expression in response to 17beta-estradiol treatment was determined by Taqman quantitative PCR and western blotting. (3) Cells with or without Bcl-2 siRNA transfection (Bcl-2 knockdown) were exposed to 17beta-estradiol and hydrogen peroxide, and cell DNA fragmentation was then detected. The outcome of the Bcl-2 gene knockdown was confirmed by Taqman quantitative PCR and western blotting.

#### Results

Hydrogen peroxide significantly reduced cell proliferation, increased cell apoptotic DNA fragmentation level and caused reduced mRNA expression of Bcl-2 but elevated Bax. All of these were significantly protected against by 17beta-estradiol but reversed by the anti-estrogen ICI182780. 17beta-estradiol dramatically increased Bcl-2 expression at both mRNA and protein levels. Bcl-2 expression was silenced by the siRNA transfection, and within these cells, the protective effect of 17beta-estradiol on hydrogen peroxide induced apoptosis was completely lost.

#### Conclusion

17beta-estradiol had protective effect against oxidative stress induced cell proliferation inhibition and cell apoptosis in human endothelial cells *in vitro*, mediated through a Bcl-2 dependent pathway.

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### P365

#### Emergence of new addictions following gastric reduction surgery for morbid obesity

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Surgery is the most effective way of treating morbid obesity. The laparoscopic gastric bypass (Roux en-Y) is the preferred bariatric surgical procedure performed at our hospital. This combines restriction of intake with a profound sensation of early satiety.

Ideally, multi-disciplinary team involvement (including psychological assessment), over a period of at least six months, is a prerequisite for bariatric surgery referral. The more psychotherapeutic work that has been done before surgery, the better the psychological adjustment after surgery. Unresolved issues can surface in the lives of patients that were not sufficiently addressed prior to surgery. If food was being used to address unmet emotional needs and the patient has not begun to practice appropriate ways to meet these needs, psychological problems will ensue. A patient, whose eating behaviour fits an addictions model, is at risk of substituting food for another substance, such as alcohol, to self-medicate emotional pain.

During the period 2001 to 2004, 40 gastric bypass procedures were performed. A retrospective study of this group demonstrated the emergence of new addictions in 4 patients (eg. alcohol, smoking, shoplifting).

Examination of psychometric test scores of the four patients who developed new addictive behaviours, when compared to the rest of the post bariatric surgery group, revealed some notable differences. These four patients all had higher scores on two facets of the Neuroticism personality domain - depression and impulsiveness. The four also had lower scores on the Conscientiousness personality domain, which includes measures of competence, achievement striving, self-discipline and deliberation.

This highlights the need for ongoing psychological monitoring for the emergence of alternate addictive behaviours in this increasing patient group.

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### P366

#### Is there male androgenetic alopecia the sign of male equivalent of polycystic ovary syndrome or metabolic syndrome?

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#### Introduction

Androgenetic alopecia (AGA) is the most common cause of balding in men. AGA is the risk factor of cardiovascular diseases, glucose metabolism disorders and also the risk of benign prostatic hyperplasia and prostate carcinoma. Polycystic ovary syndrome (PCOS) and metabolic syndrome

are the risk factors of insulin resistance, obesity and diabetes mellitus. The genetic autosomal trait of PCOS initiated a hypothesis about the existence of a male equivalent of PCOS. Premature alopecia was suggested as one of the signs of a male phenotype of this syndrome. However, it can be the sign of metabolic syndrome as well. PCOS is characterized by hyperandrogenaemia while metabolic syndrome is characterized by low androgens.

#### Methods

A group of 30 men (mean age: 31 years), in which premature hair loss begun before 30 years of age was involved in the present study. In all individuals, their hormonal profile was determined and insulin tolerance test was made. Robust Mann-Whitney test and Fisher's exact test were used for statistic analysis.

#### Results

Based on the laboratory findings two subgroups of individuals were shown. The first one revealed similar hormonal changes as women with PCOS, the other had either no anomalies in steroid spectrum. Both subgroups did not differ in either BMI or age. The subgroup with hormonal changes resembling those of PCOS, namely lower SHBG, lower FSH and elevated free androgen index, showed a significantly higher insulin resistance than the group without these changes. Only one man in our group was low in androgens.

#### Conclusions

Based on our results it can be concluded that men with premature alopecia and hormonal changes partially resembling those typical for female PCOS, might probably represent the male equivalent of PCOS, than to be suspected of the metabolic syndrome. The study was supported by grant No. NR/8525 - 5 of the IGA MZCR.

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### P367

#### Increased prevalence of the metabolic syndrome by NCEP, WHO and IDF criteria in women with PCOS

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#### Background

The polycystic ovary syndrome (PCOS) is a condition defined by hyperandrogenism and ovulatory dysfunction. It is also known to be associated with insulin resistance and has many features in common with the metabolic syndrome (MS) - a cluster of risk factors that have been shown to predict a greater risk of future cardiovascular events and type 2 diabetes. While there are studies confirming that the MS is more common in PCOS, none have systematically compared the relative prevalence of the MS in PCOS as defined by the World Health Organisation (WHO), National Cholesterol Education Program Adult Treatment Panel 3 (ATPIII), and the International Diabetes Federation (IDF).

#### Aim

To investigate the prevalence of the MS in women with PCOS and to compare the metabolic and hormonal profiles in PCOS women with and without the MS.

#### Methods

A retrospective chart review of 261 women (as defined by the 1990 National Institutes for Health consensus statement) with comparison to an age matched selection of 584 female subjects from the AusDiab study.

#### Results

Mean age of PCOS subjects was 29 (+/-8.8yrs). The prevalence of the MS in women with PCOS was 31% by IDF, 30% by WHO, and 24% by ATPIII definitions. The average risk ratio of the MS was 4.2, (4.9 by WHO, 3.6 by IDF, and 4.0 by ATPIII definitions, p values < 0.001), compared with the control group. The most frequent components of the MS in women with PCOS were: elevated waist circumference (WC > 79 cm in 80%), reduced HDL (HDL < 1.3 mmol/L in 66%), and insulin resistance (HOMA-IR > 2.2 in 61%). Among the PCOS subjects, those with the MS were older and had lower sex hormone binding globulin (SHBG) levels, compared with those without the MS (p values < 0.05).

#### Conclusion

There is a four fold increase in the prevalence of the MS in a clinic population of women with PCOS compared with age matched controls from the general population, and its presence is associated with older age and lower SHBG. These results imply greater cardiovascular risk in women with PCOS.

## P368

**Estrogen receptor alpha (ER "alpha") gene polymorphisms and first-ever primary intracerebral hemorrhage (PICH)**M Strand<sup>1</sup>, I Söderström<sup>1</sup>, P-G Wiklund<sup>1</sup>, G Hallmans<sup>2</sup>, L Weinehall<sup>3</sup>, S Söderberg<sup>1</sup> & T Olsson<sup>1</sup><sup>1</sup>Department of Public Health and Clinical Medicine, Medicine, Umeå University, Umeå, Sweden; <sup>2</sup>Department of Public Health and Clinical Medicine, Nutritional Research, Umeå University, Umeå, Sweden;<sup>3</sup>Department of Public Health and Clinical Medicine, Epidemiology, Umeå University, Umeå, Sweden.

## Context

Estrogens may have protective properties in the cardiovascular system, linked to the activation of estrogen receptor alpha (ER $\alpha$ ), present in vascular smooth muscle cells (VSMCs) and endothelial cells (ECs). ER $\alpha$ -mediated signaling regulates vasodilatation and atherogenesis, and since hypertension and atherosclerosis are major mechanisms in stroke development, we hypothesized that genetic variations in the ER $\alpha$  gene (*ESR1*) were associated with stroke.

## Materials and methods

We performed a population-based prospective nested case-control study, in which the relationship between the *ESR1* polymorphisms *c.454-397T/C* and *c.454-351A/G* and stroke was examined. Definitive first-ever stroke events ( $n = 388$ ), i.e. ischemic stroke (IS) ( $n = 320$ ), primary intracerebral hemorrhage (PICH) ( $n = 61$ ) and unspecified stroke ( $n = 7$ ) cases, and controls without cardiovascular disease ( $n = 775$ ), matched for age, sex, and geographical region were included. Traditional cardiovascular risk factors were obtained from all participants. Genotyping was performed using restriction fragment length polymorphism (RFLP) technique and allelic discrimination using the 5' nuclease assay.

## Results

The unadjusted odds ratio (OR) for PICH was 2.19 (95% CI: 1.10–4.34) for carriers of the *c.454-397T/T* genotype compared with non-carriers. This association persisted after adjustment for established stroke risk markers (OR = 3.58, 95% CI: 1.25–10.21). Carriers of either one or two alleles of *c.454-397T* also had significantly higher mean systolic ( $P = 0.0046$ ) and diastolic ( $P = 0.0198$ ) blood pressure than non-carriers did. The combinations of genotype *c.454-397T/T* and hypertension (OR = 21.46, 95% CI: 5.20–88.51), high systolic (OR = 18.17, 95% CI: 4.91–67.31) or diastolic blood pressure (OR = 11.94, 95% CI = 3.75–38.03) were strongly associated with increased risk of PICH, with synergy indices (SIs) for these combinations indicating significant positive interactions.

## Conclusions

The genotype *c.454-397T/T* is associated with increased risk of PICH, particularly in combination with hypertension. This implies alterations in ER $\alpha$ -mediated signaling in the pathophysiology of PICH.

## P369

**The metabolic control levels of 548 diabetic outpatients compared with the current guidelines of Polish Diabetes Association (PDA)**

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The current PDA recommendations relating to the good metabolic control of diabetes are: self-controlled fasting blood glucose (FBG) 70–90 mg%, HbA1c  $\leq 6.1\%$ , lipid profile (LP) TChol  $\leq 175$  mg%, LDL  $\leq 100$  mg%, HDL  $\geq 40$  mg%, TG  $\leq 150$  mg%.

## Objective

To evaluate the accomplishment of the proper metabolic control in diabetic outpatients with regard to the PDA guidelines.

## Material and methods

548 diabetics (249M; 299F), aged 18–83 years (mean 56.2  $\pm$  14.9), treated between 2004–2005 have been studied. The patients were classified into 3 groups: with type 1 (16.1%), type 2 (79%) and type 3 diabetes (4.9%). In metabolic control were assessed: self-controlled FBG, HbA1c, and LP. The patients have been evaluated according to HbA1c and the number of the reached parameters.

## Results

Mean levels for whole group and subgroups with type 1, 2 and 3 diabetes were: for FBG 127.4  $\pm$  35.3; 134.8  $\pm$  47.0; 125.8  $\pm$  30.9; 124.8  $\pm$  40.5 mg%; for TChol: 203.1  $\pm$  43.3; 194.6  $\pm$  39.5; 207.2  $\pm$  44.4; 175.9  $\pm$  36.0 mg% and HbA1c 7.35  $\pm$  1.5%; 7.7  $\pm$  1.6%; 7.2  $\pm$  1.4%; and 8.21  $\pm$  1.9% respectively.

Parameter	Whole group (%)	Type 1 (%)	Type 2 (%)	Type 3 (%)
HbA1c $\leq 6.1\%$	25.8	16.1	29.0	8.0
HbA1c 6.2–7.0%	23.8	21.8	24.9	12.0
HbA1c $> 7.0\%$	50.4	62.1	46.1	80.0
3 criteria	3.0	5.7	2.7	0
2 criteria	11.6	12.5	11.4	11.1
1 criterion	30.9	26.1	31.5	37.1
none	54.5	55.7	54.4	51.8

## Comments

The mean values of estimated parameters in all groups of diabetic patients were more higher than those recommended by PDA. In 25% of all cases, HbA1c level was  $\leq 6.1\%$  and moreover in nearly half of them it was "satisfactory" below 7%. Simultaneously, only 3% of the patients reached 3 criteria of good metabolic control, whereas over 50% achieved none criterion.

## Conclusions

In the most of diabetic outpatients, the degree of the metabolic control remain unsatisfactory. In practice, it seems to be difficult to achieve the all criteria recommended by PDA. As for the outcomes considered more attention should be paid to the patient educational programs, life-style counseling, practice guidelines as well as more effective diabetes management overcoming the "clinical inertia".

## P370

**Increased sub-clinical inflammation and markers of vascular injury in obese children**AL Harte<sup>1</sup>, AR Baker<sup>1</sup>, ML Hill<sup>1</sup>, L Gilardini<sup>2</sup>, A Girola<sup>2</sup>, NF da Silva<sup>1</sup>, C Invitti<sup>1</sup>, S Kumar<sup>1</sup> & PG McTernan<sup>1</sup><sup>1</sup>Unit for Diabetes and Metabolism, Warwick Medical School, University of Warwick, Coventry, United Kingdom; <sup>2</sup>Unit for Diabetes and Metabolic Diseases, Instituto Auxologico Italiano, Milan, Italy.

Childhood obesity and its later progression to Type 2 Diabetes (T2DM) are known to be associated with sub-clinical inflammation; although the underlying cause for this is unclear. However, studies suggest that bacterial endotoxin (LPS) derived from commensal bacteria in the human gastrointestinal tract may contribute directly to sub-clinical inflammation. Human adipose tissue expresses toll like receptors that induce an inflammatory cascade in the presence of endotoxin. Hence, with increasing adiposity the inflammatory response is exacerbated producing inflammatory adipocytokines, such as plasminogen activator inhibitor type-1 (PAI-1), IL-6 and TNF- $\alpha$ . This study investigated the pathogenesis of the metabolic syndrome in obese children and its' association with inflammation, with LERC approval. In particular, we examined the role of endotoxaemia in childhood obesity through the application of multiplex cardiovascular disease (CVD) biomarker immunoassays to investigate the levels of a range of inflammatory and CVD risk markers. Serum was obtained from children with varying degrees of obesity; age  $\pm$  SD: 13.9  $\pm$  2.3 yr; BMI  $\pm$  SD: 35.1  $\pm$  5.2 Kg/m<sup>2</sup>;  $n = 60$ . All children underwent an OGTT; insulin resistance was measured by homeostasis model assessment (HOMA-IR) and insulin secretion by Stumvoll index (ISI). BMI correlated strongly with some, but not all, of the inflammatory markers. However, endotoxin levels demonstrated a significant and positive correlation with the majority of markers for vascular injury and atherogenesis (TNF- $\alpha$ , myeloperoxidase;  $P < 0.05$ ; PAI-1, matrix metalloproteinase-9, monocyte chemoattractant protein-1, soluble intercellular adhesion molecule type-1, vascular endothelial growth factor;  $P < 0.01$ ), as well as blood pressure. These relationships remained significant following adjustment for sex, BMI and HOMA-IR. Therefore, we conclude that sub-clinical inflammation in obesity and T2DM may be mediated by endotoxin in serum. Furthermore, that children as young as 11 exhibit the same inflammatory profiles as identified in obese adults and may, as a result of long-term sub-clinical inflammation, have increased risk of diabetes and CVD at an earlier age.



### P371

#### Clinical, functional and polysomnographics parameters in severely obese patients

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Obesity is a major cause of chronic hypoventilation and/or obstructive sleep apnea syndrome (OSAS). Aim of this study was to evaluate the prevalence of pulmonary dysfunction and the relationship between OSAS and several clinical, functional and polysomnographics parameters in severely obese patients. 101 subjects (68 females and 33 males) aged  $49.2 \pm 13.2$  years (mean  $\pm$  SD), with BMI  $46.8 \pm 6.5$  Kg/m<sup>2</sup> were enrolled. Sleep quality and daytime sleepiness were assessed using the Epworth Sleepiness Scale (EPSS). Serum triglycerides, HDL-cholesterol, glucose, leptin, and thyroid hormones were measured. All patients underwent cardio-respiratory polygraphic sleep study and lung function tests. Arterial hypertension was present in 56.4% patients and type 2 diabetes in 30.7%. Snoring was referred by 91% patients, nocturnal awakening by 51% and apneas by 41%. Pathologic sleepiness was present in 61% patients. 70.3% patients had larger than normal neck circumference. Arterial gas analyses showed reduced PaO<sub>2</sub> in 41% patients. An obstructive ventilatory pattern was found in 15% patients, a restrictive pattern in 10% and a mixed pattern in 3%. The expiratory reserve volume and functional residual capacity were significantly reduced in 50% patients. 14% patients had mild, 13% moderate, 13% severe and 21% very severe OSAS. Only 39% patients did not meet the diagnostic criteria for OSAS. A significant positive correlation was found between the apnea-hypopnoea index (AHI) and EPSS ( $P < 0.005$ ), waist/hip ratio ( $P < 0.005$ ), neck circumference ( $P < 0.005$ ) or PaCO<sub>2</sub> ( $P < 0.05$ ). An inverse correlation was observed between AHI and PaO<sub>2</sub>, nocturnal mean or minimum oxygen saturation. No correlation was found between AHI and other respiratory functional, cardio-respiratory, metabolic or hormonal parameters.

In conclusion, our data indicate a high prevalence of respiratory dysfunction in severely obese patients, including diurnal hypoventilation and OSAS. We suggest that pulmonary function should be systematically screened in severely obese subjects even in the absence of overt manifestations of disease.

### P372

#### The role of tumour necrosis factor-alpha in insulin-stimulated endothelial nitric oxide production

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Insulin resistance states are associated with endothelial dysfunction, but the molecular mechanisms underlying this association are incompletely understood. Impairment of insulin stimulated endothelial NO production is proposed to be a key mechanism in this process. In cultured human aortic endothelial cell (HAEC) models, insulin stimulates an intracellular signalling cascade resulting in activating phosphorylation of Insulin Receptor Substrate-1 (IRS-1), Protein kinase B (PKB / Akt) and endothelial Nitric Oxide Synthase (eNOS), with resultant NO release. The pro-inflammatory adipokine Tumour Necrosis Factor Alpha (TNF- $\alpha$ ) is over-expressed in models of insulin resistance, acting in humans to inhibit both insulin-stimulated glucose uptake and endothelial-dependant vasodilatation. Studies in bovine aortic endothelial cells have shown that preincubation with TNF- $\alpha$  inhibits insulin stimulated NO production, with concomitant inhibition of activating phosphorylation of IRS-1, PKB and eNOS but the precise molecular pathway that accounts for the inhibition seen is unclear. Accordingly, we studied the interaction between TNF- $\alpha$  and insulin-mediated NO production in HAECs.

We have demonstrated that pre-incubation with TNF- $\alpha$  inhibits insulin stimulated NO production. In contrast to bovine studies this was not associated with reduced activating phosphorylation of PKB (Ser473) or eNOS (Ser1177). A previously uncharacterised eNOS residue (Ser 617) is phosphorylated in response to insulin and appears to be unaltered by TNF- $\alpha$  preincubation. Phosphorylation of IRS-1 residue Ser 312 has been proposed as a key inhibitory signalling mechanism in insulin resistance in adipocytes and skeletal muscle, however in our model no change was demonstrable. TNF- $\alpha$  activates JNK and IKK, and these kinases may form the intracellular link between TNF- $\alpha$  and insulin signalling. We hypothesise that the

reduction in NO production in this model is not solely due to impairment of the insulin signalling cascade. A potential mechanism of action currently being investigated is a reduction in NO bioavailability through 'quenching' by superoxide, produced in response to TNF- $\alpha$ .

### P373

#### Correlation of WBC and PLT count with parameters of type 2 diabetes mellitus

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#### Aim of the study

To examine the white blood cell count and the platelet count in type 2 diabetic patients vs. age-matched healthy controls, as well as to investigate if the abovementioned counts are associated with parameters of diabetes.

#### Methods

This study included 337 subjects, divided into two groups. Group A comprised 190 type 2 diabetic patients (98 men, mean age  $66.5 \pm 9.6$  years, mean diabetes duration  $11.7 \pm 8.6$  years). Group B comprised 147 healthy controls (76 men, mean age  $65.8 \pm 11.2$  years). Exclusion criteria for both groups were acute or chronic inflammation, infection, malignancy or other systemic disease. In all subjects WBC and PLT count were measured, as well as serum lipids, atherogenic index, uric acid, fasting glucose, HbA<sub>1c</sub>, BMI and waist circumference.

#### Results

WBC count was significantly ( $t = -4.604$ ,  $P < 0.001$ ) higher in Group A ( $7097.5 \pm 1477.6/\mu\text{l}$ ) than in Group B ( $6268.6 \pm 1227.7/\mu\text{l}$ ). No significant difference in PLT count was found between the two groups ( $P = 0.892$ ). In group A, WBC count was significantly ( $r = 0.408$ ,  $P = 0.001$ ) positively correlated with HbA<sub>1c</sub>, while PLT count was positively correlated with HbA<sub>1c</sub> ( $r = 0.475$ ,  $P = 0.001$ ) and negatively with uric acid levels ( $r = -0.262$ ,  $P = 0.023$ ). In the same group, a positive correlation was demonstrated between WBC and PLT count ( $r = 0.346$ ,  $P = 0.001$ ). No association was found between WBC or PLT count and fasting glucose, BMI, waist circumference and lipids.

#### Conclusions

In type 2 diabetic patients, WBC count is significantly higher as compared to healthy controls and it is correlated with HbA<sub>1c</sub>, an index of glycaemic control. PLT count in type 2 diabetic patients was also correlated with glycaemic control, although it was not higher in comparison to healthy subjects. These findings are consistent with the hypothesis that a chronic activation of the leucocytes and platelets may play a role in the pathogenesis of type 2 diabetes.

### P374

#### Field evaluation of a multi-sensor armband in Greek women

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#### Objective

To examine the ability of a multisensor armband (SenseWear Pro 2 Armband™, Body Media, Pittsburgh, PA) to estimate daily energy expenditure (EE).

#### Patients and methods

Eighteen healthy women (mean age:  $29.4 \pm 5.8$  yr and mean BMI: 23.2) participated in the study. Daily EE was estimated by the Sensewear Armband and a physical activity record (PAL) during the same day. Appropriate MET factors were assigned to the different activity categories.

#### Results

The SenseWear Armband significantly underestimated daily EE compared with the PAL,  $2552.7$  vs  $2915.5$  kcal.d<sup>-1</sup>, ( $P < 0.001$ ). Correlations between the SenseWear Armband and PAL was  $r = 0.77$  ( $P < 0.001$ ). When compared SWA and PAL using the Bland and Altman technique we show that they had significant limits of agreement. However, the difference between the methods was not negligible for individual subjects. Compared with the PAL, the SenseWear Armband underestimated time accumulated in active behaviors ( $43.9$  vs  $85.0$  min.d<sup>-1</sup>).

#### Conclusions

These data show that the SenseWear Armband underestimated free-living energy expenditure regarding to a PAL. Multisensor detectors provide a



feasible method for evaluating the physical activities of non-athletes, and could be a common tool for epidemiological research and health promotion despite its limitations. Future studies examining energy expenditure during free-living conditions along with DLW as the criterion measure of energy expenditure will be also of great value.

### P375

#### The acute stimulation of nitric oxide synthesis by rosiglitazone in human aortic endothelial cells is independent of the PPAR gamma receptor but is dependent on the fuel sensing enzyme AMPK

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The PROactive study recently demonstrated that pioglitazone, a thiazolidinedione class drug, reduces macrovascular morbidity and mortality in patients with type 2 diabetes. This class of drug has been reported to exert PPAR gamma receptor dependent as well as receptor independent effects, possibly via AMP kinase activation (AMPK) but the precise mode of action resulting in improved cardiovascular outcome remains uncertain.

We studied the effects of rosiglitazone in cultured human aortic endothelial cells (HAEC). Cell lysates were prepared after pre-incubation of cells with rosiglitazone in the presence or absence of the PPAR gamma receptor antagonist GW9662 or a dominant-negative AMPK construct. Nitric oxide (NO) release was measured with a Sievers NO meter. AMPK was immunoprecipitated from lysates and assayed using the AMARA substrate peptide.

Rosiglitazone caused acute stimulation NO production. This reached a maximum 2.1 fold increase at 60 minutes with 0.2 mM ( $P < 0.05$ ) and was dose dependant. AMPK was activated by 0.2 mM rosiglitazone within 10 minutes, reaching a maximum 10.6 fold stimulation at 60 minutes ( $P < 0.05$ ). Pre-incubation with GW9662 had no effect on rosiglitazone related NO production or AMPK activity ( $P < 0.05$ ). When HAEC's were infected with a dominant-negative AMPK, stimulation of NO by 0.2 mM of Rosiglitazone for 1 hour was significantly reduced ( $P < 0.05$ ).

These data support the hypothesis that the beneficial effects on cardiovascular outcome of thiazolidinediones may be partly explained by a PPAR gamma independent increase in endothelium derived NO. This may be explained by direct and rapid effects on mitochondrial respiration which reduce ATP levels so activating the enzyme AMPK. Further investigation of the way in which thiazolidinediones affect intracellular signalling in vascular endothelial cells may provide an understanding of the relative importance of receptor independent versus receptor dependent actions and facilitate more selective treatments for the management of diabetes and vascular disease.

### P376

#### Dehydroepiandrosterone in relation to obesity, insulin resistance and lipid spectra in Czech non-diabetic population

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Dehydroepiandrosterone (DHEA) and its sulfate derivative (DHEA-S) are major adrenal secretory products in humans, but their biological function is not still fully explained. Some studies reported negative correlation of DHEA(S) with BMI, fat distribution, and correlations with insulin sensitivity or lipid spectra, but the results are often controversial. Our objective was to assess the correlations of DHEA and DHEA-S levels in Czech non-diabetic population with anthropometric, biochemical and hormonal parameters related to glucose and lipid metabolism. The study entered 435 well anthropometrically and metabolically characterized healthy volunteers (women  $n = 290$ , age  $31.6 \pm 11.4$  yrs, BMI  $23.5 \pm 4.1$  kg/m<sup>2</sup>, men  $n = 145$ , age  $32.3 \pm 10.0$  yrs, BMI

$24.7 \pm 3.6$  kg/m<sup>2</sup>) with/without family history of diabetes type 2. Statistics: Spearman correlations without/with adjustment for age and/or BMI. Ancova were used. In both men and women, the correlations of DHEA with anthropometric parameters disappeared after the adjustment for age, only the correlation of DHEA-S with WHR was positive. In women, there was negative correlation of DHEA and DHEA-S with SHBG, triglycerides, total and LDL-cholesterol. In men, DHEA-S after the adjustment for age correlated positively with fasting and stimulated glucose, insulin and C-peptide levels. Surprisingly, DHEA-S negatively correlated with insulin sensitivity and no correlation with lipids was found. Conclusion: Statistical analysis revealed that almost all correlations of DHEA, DHEA-S, resp., with adiposity and fat distribution in men as well as in women disappeared after the adjustment for age. However, there are differences between men and women in the correlations of DHEA(S) with insulin sensitivity, lipid levels and other steroid hormones. In this respect the benefit of the DHEA(S) supplementation seems at least regarding its alleged antiobesity and antiatherogenic effects to be more than controversial. Study was approved by local Ethical Committee, supported by IGA MH CR NR/7809-5, MSMT COST OC B17.10.

### P377

#### Frequency of dyslipidemias in children – results of lifestyle intervention

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Dyslipidemias should be managed from childhood for prevention of early atheromatous vascular lesions and premature cardiovascular disease in adult life.

Aim

We examined the frequency of different types of dyslipidemia in children and the results on blood lipids of lifestyle intervention (diet and exercise). Patients and methods

We studied retrospectively 136 children, 74 boys and 62 girls, mean chronological age  $8.5 \pm 3.5$  years with dyslipidemia. Secondary causes of dyslipidemia were excluded. High levels were considered for total cholesterol (h-TC)  $> 200$  mg%, LDL-cholesterol (h-LDL-c)  $> 130$  mg%, triglycerides (h-TG)  $> 100$  mg%, HDL-cholesterol (h-HDL-c)  $> 60$  mg% and low level for HDL-c (l-HDL-c)  $< 45$  mg%. For statistical analyses Mann-Whitney test and Wilcoxon test were used.

Results

The frequency of different types of dyslipidemia is presented in the table. LDL-c was significantly increased in children with family history of premature cardiovascular disease (FHCD) compared to those without FHCD ( $178.1 \pm 50.4$  mg% vs  $139.3 \pm 33.3$  mg%,  $P < 0.002$ ). Fifty-nine children who were reexamined after  $0.9 \pm 0.9$  years of lifestyle intervention had significantly decreased levels of LDL-c ( $158.4 \pm 35.1$  mg% vs  $133.7 \pm 25.5$  mg%,  $P < 0.001$ , percentage  $-13.8\%$ ). There was not any significant difference in levels of TG or HDL-c.

Table 1

Causes of lipid testing (%)	Patients characteristics (%)	Dyslipidaemias Frequency (%)
Family history of dyslipidemia	33.1	h-LDL-c & h-HDL-c 29.4
Obesity	36.4	h-LDL-c & h-LDL-c & alone 16.2
Obesity	23.1	h-LDL-c & h-Lp (a) 3.7
Incidental finding	19.8	h-LDL-c & h-TG 12.5
Family history of dyslipidaemia	95.1	
FHCD	10.7	h-TC & h-HDL-c 11.8
		l-HDL-c alone 2.9

#### Conclusions

Increased LDL-c is the most frequent lipid abnormality among children with dyslipidemias. Low HDL-c, alone or in combination with h-LDL-c or/and h-TG is frequently observed. Lifestyle interventions are effective in significantly decreasing LDL-c.

**P378****siRNA-mediated depletion of synaptotagmin-11 abrogates insulin-stimulated glucose uptake in 3T3-L1 adipocytes**SCM Miller<sup>1</sup>, P Mitra<sup>2</sup>, JMC Connell<sup>1</sup> & GW Gould<sup>1</sup><sup>1</sup>University of Glasgow, Glasgow, United Kingdom; <sup>2</sup>University of Massachusetts, Worcester, United States.

The fusion of insulin-stimulated GLUT4-containing vesicles (GSV) with the plasma membrane (PM) of adipose or skeletal muscle cells is governed by regulated exocytosis. In all such membrane fusion events cognate t- and v-SNAREs interact in an ordered way to allow vesicles to first dock with, and then fuse with the plasmalemma. In neurons, the best-studied example of this well conserved process, the protein species responsible for directing the final (rate-limiting) fusion step is Synaptotagmin (Syt). Despite the presence of 16 higher eukaryotic isoforms of this well conserved protein family few have been characterised outwith neuronal cells. Manipulations of cultured adipocytes resulting in aberrant GSV-PM fusion are associated with reduced insulin-stimulated glucose uptake. Furthermore, it can be demonstrated that incomplete fusion occurs in adipocytes taken from insulin resistant individuals with Type-II Diabetes Mellitus. These data imply that the control of GSV fusion with the plasma membrane represents a key rate-limiting step in insulin signalling which ultimately governs insulin action and sensitivity. Whilst the v- and t-SNAREs implicated in GSV-PM fusion are well defined, the Syt compliment of insulin sensitive cells has remained hitherto unknown.

Here we demonstrate the Syt family members expressed in 3T3-L1 adipocytes, and show that Syt-11 is the predominant isoform. Syt-11 expression is upregulated as cells acquire their insulin-sensitive adipocyte phenotype, and is further upregulated when cells are treated with Rosiglitazone. We reveal that Syt-11 is found not only in the same intracellular pool as GLUT4, but also that it is present in the plasma membrane of insulin-stimulated cells. Finally, using siRNA we can demonstrate that Syt-11 depletion leads to abrogated insulin-stimulated glucose uptake. Thus we speculate Syt-11 is the species that may direct the final fusion step in the regulated exocytosis of GSVs, so may represent the link between proximal and distal events in the insulin-signalling cascade.

**P379****Chromosome 1p shows significant linkage to steroid metabolism in hypertension in the British Genetics of Hypertension Study**S Padmanabhan<sup>1</sup>, R Fraser<sup>1</sup>, M Ingram<sup>1</sup>, E Davies<sup>1</sup>, PB Munroe<sup>2</sup>, R Dobson<sup>3</sup>, M Brown<sup>3</sup>, N Samani<sup>3</sup>, D Clayton<sup>3</sup>, M Farrall<sup>3</sup>, J Webster<sup>6</sup>, M Lathrop<sup>7</sup>, M Caulfield<sup>2</sup>, AF Dominiczak<sup>1</sup> & JM Connell<sup>1</sup>

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**Background**

Glucocorticoids can affect blood pressure in humans, as demonstrated most strikingly in Cushing's syndrome. We have previously reported that total cortisol metabolite excretion is raised in obese subjects, while other investigations have identified genetically determined changes in glucocorticoid receptor function as contributors to hypertension. Furthermore, in essential hypertension, vasoconstrictor sensitivity to glucocorticoids is increased. Such raised sensitivity could represent the primary stimulus leading to HTN and has been invoked in a fetal programming process that could underlie raised BP later in life. We performed a genome wide scan on the severely hypertensive Caucasian BRIGHT population to identify chromosomal loci affecting cortisol secretion and metabolism that might contribute to the genetic risk of developing hypertension.

**Methods**

We had complete urinary steroid profile on 119 sibling pairs from the BRIGHT population which comprise 2142 severely hypertensive Caucasian affected sibling pairs. A 10 cM genome wide scan was performed using (THF + THE + aTHE) adjusted for age which is a measure of total cortisol metabolite excretion, as a quantitative trait. Linkage analysis was performed using MERLIN QTL.

**Results**

Significant linkage was observed for (THF + THE + aTHE) on chromosome 1 at 106.5 cM near marker D1S230 (LOD score 4.3,  $P < 10^{-6}$ ). The candidate gene encoding sterol carrier protein SCPX lies near locus for (THF + THE + aTHE).

**Conclusions**

We have shown evidence for linkage to cortisol metabolite excretion to a region in chromosome 1p in a cohort of hypertensive sibling pairs. This suggests that corticosteroids provide a useful an intermediate phenotype for hypertension; the genetic determinants for cortisol secretion in hypertensive subjects may lie in chromosome 1p.

**P380****Impaired glucose metabolism in patients with Cushing's Syndrome**GA Arnaldi<sup>1</sup>, TM Mancini<sup>2</sup>, BP Polenta<sup>1</sup>, MC Cardinale<sup>1</sup>, PC Canibus<sup>1</sup>, EF Faloia<sup>1</sup> & MB Boscaro<sup>1</sup>

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Glucose metabolism was investigated in 65 patients with Cushing's syndrome (CS), 19 with cortisol-secreting adrenal adenoma (mean age  $47.2 \pm 4.9$ ), 40 with ACTH secreting pituitary adenoma (mean age  $41.4 \pm 12.48$ ), 4 with cortisol secreting adrenal cancer (mean age  $63.0 \pm 8.4$ ) and 2 patients with ectopic ACTH secretion ( $38.7 \pm 11.9$ ). Eleven patients (17%) were diagnosed as diabetic after abnormal fasting glucose levels ( $> 126$  mg/dl). While diabetes mellitus was diagnosed in other 22 patients (33%) only after abnormal oral glucose tolerance test (OGTT); 13 patients (20%) had impaired glucose tolerance at OGTT. Thus, basal glucose level were normal in 60% of diabetics and diabetes was diagnosed only after OGTT in ~50% of the patients

There were no significant differences in anthropometric parameters, insulin levels, indexes of hypercortisolism and disease duration between patients with normal glucose metabolism (NGM) and patients with IGM.

Mean $\pm$ DS	NGM (n = 19)	IGM (n = 46)	p
Basal Glucose (mg/dl)	82.1 $\pm$ 11.7	107.8 $\pm$ 41.5	0.04*
120' glucose post	104.9 $\pm$ 18.7	219.1 $\pm$ 66.4	0.002*
OGTT (mg/dl)			
Basal Insulin ( $\mu$ UI/ml)	16.5 $\pm$ 8.5	13.1 $\pm$ 7.5	0.20
Insulin peak at OGTT ( $\mu$ UI/ml)	119.6 $\pm$ 75.2	138.8 $\pm$ 59.5	0.63
BMI (kg/m <sup>2</sup> )	27.5 $\pm$ 4.9	28.1 $\pm$ 8.1	0.44

In a regression analysis, basal glucose levels were significantly positively correlated with plasma and urinary cortisol levels. Impaired glucose metabolism was present in 2/4 of the patients with cancers and in all patients with EC. No differences were found in lipid metabolism, blood pressure, sex and aetiology between NGM and IGM groups

In conclusion, our study shows that diabetes mellitus can be underestimated if only fasting blood glucose levels are considered. Thus, we recommend that OGTT be included in the evaluation of patients with hypercortisolism for a better estimation of the diseases complications and a better therapeutic management of the patient.

**P381****Effects of bariatric surgery on preclinical myocardial alterations in severe obesity and the related role of insulin resistance**A Pucci<sup>1</sup>, F Santini<sup>1</sup>, V Di Bello<sup>2</sup>, A Marsili<sup>1</sup>, P Fierabracci<sup>1</sup>, A Di Cori<sup>2</sup>, C Palagi<sup>2</sup>, MG Delle Donne<sup>2</sup>, M Giannetti<sup>1</sup>, M Anselmino<sup>3</sup>, P Vitti<sup>1</sup>, A Balbarini<sup>2</sup> & A Pinchera<sup>1</sup>

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Obesity is a well established risk factor for congestive heart failure. The existence of an "obese cardiomyopathy" has been hypothesized, as the complex combination of the effects of both hemodynamic and metabolic alterations. Aim of this study was to analyze the effect of bariatric surgery

on the early myocardial alterations detected in a group of severe obese patients by high frame rate integrated backscatter (IBS). Twenty severely obese patients (5 males, 15 females, mean age  $31.2 \pm 6$  yr) with no history of diabetes or hypertension were enrolled. All subjects underwent conventional 2D-Color Doppler echocardiography and IBS. The homeostasis model assessment insulin resistance index (HOMA-IR) was used to assess insulin resistance. All subjects were submitted to echocardiographic and biochemical re-evaluation 6 to 24 months after surgery. The mean BMI value decreased from  $47.8 \pm 8.1$  to  $33.0 \pm 6.0$ . A significant amelioration of various left ventricular myocardial functional and structural alterations was demonstrated. The main findings by conventional echocardiography were: a reduction of left atrium dimension ( $39.5 \pm 4.3$  to  $35.2 \pm 4.6$  mm,  $P < 0.0001$ ), a reduction of left ventricular mass indexed by height (LVMh) ( $58.2 \pm 14.2$  to  $40.4 \pm 10.7$  g/m<sup>2.7</sup>,  $P < 0.0001$ ) and an increase of E/A ratio ( $1.15 \pm 0.8$  to  $1.30 \pm 0.4$ ,  $P < 0.0001$ ). The main findings by IBS were: an increase of cyclic variation index at septum level (CVI<sub>s</sub>) ( $16.6 \pm 5$  to  $27.5 \pm 11.2\%$ ,  $P < 0.0001$ ) and a reduction of mean ultrasonic reflectivity (IBSm) at septum level ( $52.8 \pm 9.5$  to  $46.5 \pm 8.8\%$ ,  $P < 0.04$ ). Significant correlations were detected between BMI variations and variations of LVMh ( $R = 0.7$ ,  $P < 0.0001$ ), CVI<sub>s</sub> ( $R = 0.45$ ,  $P < 0.05$ ) or IBSm ( $R = 0.6$ ,  $P < 0.005$ ). A highly significant association was also found between HOMA-IR variations and variations of LVMh ( $R = 0.8$ ,  $P < 0.0001$ ) or IBSm ( $R = 0.54$ ,  $P < 0.05$ ). In conclusion, weight loss achieved by bariatric surgery is followed by improvement of myocardial functional and structural alterations. These changes may be mediated by reduction of insulin resistance.

### P382

#### The effect of an 18 week group exercise education program on weight, physical activity, cardiovascular fitness, quality of life and attitudes to exercise in obese Irish female adults

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Obesity has reached epidemic proportions worldwide. There are still major gaps in our understanding both of overall effective weight management strategies and physical activity alone as a weight management strategy. The aim of this study was to investigate the effect of an 18 week monthly group exercise education program on weight, physical activity, cardiovascular fitness, quality of life and attitudes to exercise in obese Irish females. Eighteen obese females were recruited the waiting list of a Weight Management Service (mean age 37.6 years, mean weight 117.9 kg, mean BMI 43.5 kg/m<sup>2</sup>). The subjects attended 4 education sessions on physical activity over an 18 week period. Subjects' weight/BMI were recorded and subjects completed the Incremental Shuttle Walk test (ISWT), International Physical Activity Questionnaire – Short Form (IPAQ – Short), Impact of Weight on Quality of Life questionnaire, short form (IWQOL – Lite) and an Opinions/knowledge questionnaire at baseline and at 18 weeks. There were non-significant decreases in subjects' weight (0.7 kg,  $P = 0.444$ ), BMI (0.3 kg/m<sup>2</sup>,  $P = 0.407$ ) and non-significant improvements in IPAQ results (104 MET• or •min• or •week<sup>-1</sup>,  $P = 0.496$ ) and IWQOL – Lite scores ( $P = 0.337$ ). Cardiovascular fitness (CRF), measured by ISWT, improved significantly from 18.00 to 19.87 mL• or •kg<sup>-1</sup>• or •min<sup>-1</sup> ( $P = 0.0002$ ). The importance placed on physical activity by subjects increased by 12% and attitudes towards exercise improved as shown by decreased barriers to exercise (shyness and increased energy) and increased enjoyment and participation. The education programme on physical activity did not produce significant changes in weight loss, BMI, self-reported physical activity or quality of life measures. There was a significant increase in CRF which has been shown to have considerable effects on morbidity and mortality even in the absence of weight loss. Further studies could investigate if a longer duration (> 1 year) may allow subjects time to make the necessary lifestyle changes to increase physical activity and achieve weight loss.

### P383

#### Direct effect of cannabinoids and ghrelin on liver and adipose cell metabolism via the AMP-activated protein kinase (AMPK) enzyme

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Cannabinoids and ghrelin exert hypothalamic orexigenic effects and independent peripheral effects have also been reported. We have recently reported that ghrelin and cannabinoids can stimulate hypothalamic and heart AMPK activity, and can inhibit liver and adipose tissue AMPK activity, in rats (Kola *et al.*, JBC, 2005). These data are concordant with the known orexigenic, adipogenic and diabetogenic effects of these compounds as well as with their beneficial effects on the ischaemic heart. However, it is not known whether the *in vivo* observations on metabolic tissues such as liver and fat tissue are direct effects; therefore we have now studied liver and adipose cell lines.

Rat hepatoma cell line H4 and mouse 3T3 adipose cells were treated with ghrelin 100 nM (G) and with the endocannabinoid anandamide 10 μM (A). Changes in AMPK activity were detected using a kinase assay, while activated AMPK content was assessed by immunoblotting.

Liver cells showed an inhibition in AMPK activity (G:  $74 \pm 12\%$ , A:  $41 \pm 23\%$  of control,  $P < 0.005$  and  $P < 0.001$  respectively) and a decrease in pAMPK content (G:  $63 \pm 14\%$ , A:  $56 \pm 1\%$ ,  $P < 0.01$  for both). Adipose cells also showed inhibition of AMPK activity (G:  $42 \pm 3\%$ , A:  $18 \pm 10\%$  of control,  $P < 0.01$  for both).

In conclusion, we have shown that cannabinoids and ghrelin have a direct effect on liver and adipose cells to modulate cell metabolism via the AMPK enzyme. The fact that the liver cell line lacks expression of GHS-R1A implies that ghrelin effects occur via an alternative receptor in the liver. We are currently performing CB1 knockout animal studies to identify if this receptor is involved in the cannabinoid effect. Our data could provide a mechanism for earlier unexplained observations in various cell-based and *in vivo* experiments, including knockout models, regarding the direct peripheral effects of cannabinoids and ghrelin.

### P384

#### Expression and Localisation of human tissue kallikrein in transfected human embryonic kidney cells (HEK-293). Development of a novel panel of monoclonal antibodies (mAbs) against human tissue kallikrein

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Tissue kallikrein is a serine protease involved in the generation of kinins in kidneys, colon, salivary glands, pancreas and blood vessels which have vasodilator roles and influence ion transport. Abnormal renal synthesis and urinary excretion of tissue kallikrein have been linked to diabetes and hypertension. This study's objective was to produce mAbs against native forms of prokallikrein and active kallikrein in order to study the expression of tissue kallikrein in different cell culture conditions and patient samples. A full-length cDNA encoding human tissue kallikrein (hK1) from a human colon carcinoma cell line (T84) was cloned into mammalian expression vector pcDNA5/FRT/V5-His and stably transfected into HEK-293. The transfected HEK-293 cells were adapted to grow in serum-free suspension culture. These cells synthesised and released inactive prokallikrein into the medium. Prokallikrein was activated by treatment with trypsin or thermolysin. Recombinant hK1 was identified by Western blot analysis with a band of approximately 50 kDa using a rabbit anti-hK1 antibody. Transfected prokallikrein in HEK-293 cells were localised to the cytoplasm with a granular distribution by immunostaining with anti-his tag mAb and rabbit anti-hK1 antibody. Prokallikrein and active kallikrein were purified and used to immunise mice. Following lymphocyte and myeloma fusion, resultant hybridomas were screened and a panel of mAbs selected. In sandwich ELISA, all mAbs recognised transfected prokallikrein and active kallikrein in HEK-293 cells. In immunofluorescence microscopy, these mAbs recognised transfected and endogenous hK1 in the cytoplasm of HEK-293 cells, showing the same localisation as the rabbit anti-hK1 antibody.

Following further characterisation, these new mAbs against native hK1 will be useful tools to study hK1 expression and function.

### P385

#### Dietary regulation of peripheral glucocorticoid action: comparison of saturated and unsaturated fats

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Obesity/Metabolic syndrome (insulin resistance, hypertension, cardiovascular disease) has reached epidemic levels in western societies. Aberrantly elevated glucocorticoid amplification by intracellular enzyme 11 $\beta$ -hydroxysteroid dehydrogenase type 1 (11 $\beta$ -HSD-1) within adipose tissue might explain the striking similarities between idiopathic obesity/metabolic syndrome and Cushing's syndrome, caused by plasma GC excess. A major contributor to obesity/metabolic syndrome is increased consumption of high fat foods. Specifically, saturated fats are associated with obesity and insulin resistance whereas unsaturated fats are reported to ameliorate metabolic disease. Chronic high fat feeding decreases adipose 11 $\beta$ -HSD1 in mice, possibly counteracting metabolic disease. Here we compare the effects of diets enriched in saturated and unsaturated fats on peripheral GC metabolism. Male C57BL/6J mice ( $n = 12$ /group) were fed stearate (saturated), oleate (monounsaturated), safflower oil- (polyunsaturated) enriched diets (45% as fat) or control diet. Groups were pair-fed to control diet. Body weight was measured for 4-weeks. Plasma insulin, glucose (6h fasting, a.m., p.m.), corticosterone (a.m., p.m.), adipose and liver 11 $\beta$ -HSD1 (activity, mRNA) and GR (mRNA) levels were determined (mRNA levels determined relative to 18S/18S RNA; reported as arbitrary units (AU)). The stearate group lost weight ( $-14.5 \pm 1.5\%$  BW,  $P = 0.004$ ), whereas oleate ( $+11.1 \pm 2\%$ ,  $P = 0.001$ ) and safflower groups gained weight ( $+6.6 \pm 2.4\%$ ,  $P = 0.008$ ). Stearate lowered morning insulin ( $0.8 \pm 0.1$  pg/ml vs. control  $4.2 \pm 1.0$  pg/ml,  $P = 0.012$ ) but increased corticosterone levels ( $138 \pm 26$  nmol/L vs. control  $13 \pm 4$  nmol/L,  $P = 0.001$ ). In adipose, stearate increased 11 $\beta$ -HSD1 activity ( $41 \pm 3\%$  conversion 11-dehydrocorticosterone to corticosterone vs. control;  $28 \pm 5\%$ ,  $P = 0.03$ ) but decreased GR mRNA levels ( $68 \pm 3$  vs. control;  $102 \pm 6$  A.U.  $P = 0.05$ ). Stearate increased liver 11 $\beta$ -HSD1 mRNA levels ( $5.9 \pm 0.9$  vs. control;  $1.4 \pm 0.6$  AU,  $P = 0.001$ ) with a similar trend in GR mRNA. These data suggest that a diet enriched only in saturated fat increases adipose and liver 11 $\beta$ -HSD1 activity. There appears to be a reciprocal down-regulation of adipose GR, possibly limiting GC action. Stearate diet also perturbed the hypothalamic-pituitary-adrenal axis. These changes would be expected to exacerbate insulin resistance.

### P386

#### The KCNJ11 E23K and UCP2 G-866A SNPs in relation to DM2 in Czech population

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KCNJ11 as well as UCP2 genes are involved via modulation of ATP concentration in pancreatic beta-cells in control of insulin secretion. The aim of study was to compare genotypic distribution of E23K (KCNJ11) and G-866A (UCP2) polymorphisms between diabetics, their offspring and controls and to study the possible association of these polymorphisms with biochemical and anthropometric parameters.

The study entered 302 diabetics, 165 offspring, 241 controls. OGTT and ITT were performed in offspring and controls. The SNPs were detected by PCR-RFLP.

The frequencies of minor alleles in controls, diabetics and offspring: 23K: 37.1%, 38.4% and 43.6%; -866A: 40.3%, 37.3% and 41.2%.

DM2 high-/low-risk haplotypes were assessed in diabetics, offspring and controls. No significant differences in haplotype distribution were found. E23K polymorphism was associated with the insulinogenic index in non diabetic subjects: KK homozygotes had lower insulinogenic index in comparison with EE homozygotes ( $P = 0.005$ ). Stimulated glucose levels were higher in KK homozygotes in comparison with EE homozygotes ( $G_{30}$ :  $P = 0.004$ ;  $G_{60}$ :  $P = 0.001$ ;  $G_{90}$ :  $P = 0.024$ ). G-866A polymorphism was not associated with DM2 markers and body composition.

The association of the KCNJ11 E23K and UCP2 G-866A with DM2 was not found. The association of KK genotype of KCNJ11 gene with beta-cell function was confirmed. This study was approved by Ethical Committee of the Institute of Endocrinology and supported by grants IGA NR/7809-5 and COST OC B17.10.

### P387

#### Androgen regulation of hepatic glucocorticoid metabolism in obese Zucker rats

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Obesity is associated with decreased hepatic reactivation of glucocorticoids (Gc) by 11 $\beta$ -hydroxysteroid dehydrogenase type 1 (11HSD) and increased metabolism of glucocorticoids by hepatic A-ring reductases, which may contribute to activation of the hypothalamic-pituitary-adrenal axis. Androgen action encourages central obesity and increased metabolic complications, possibly by altering Gc metabolism.

This study investigates the role of gonadal androgens in the dysregulation of hepatic glucocorticoid metabolism in obese Zucker rats.

Lean (L) and Obese (O) Zucker rats (9wks,  $n = 10$ /group) were studied 3 wks after gonadectomy (GDX) or sham surgery (SH). Hepatic 11HSD and 5 $\beta$ -reductase activities were measured by conversion of [<sup>3</sup>H]<sub>4</sub>-corticosterone (pmol/hr/mg protein) and mRNA for 11HSD1, 5 $\beta$ - and 5 $\alpha$ -reductases, and 3 $\alpha$ -HSD measured by real-time PCR, corrected for cyclophilin A and 18S endogenous controls (AU). \*Denotes  $P < 0.05$ , and \*\*  $P < 0.01$ .

As reported previously, in obese rats hepatic 11HSD was lower (\*), 5 $\alpha$ - and 5 $\beta$ -reductase higher (\*\*) and 3 $\alpha$ HSD not different ( $P = 0.5$ ) compared with lean rats (see table 1). GDX decreased 11HSD activity and mRNA (\*\*) in lean and obese rats, whereas 5 $\beta$ -reductase was increased only in obese rats (\*\*). GDX increased 5 $\alpha$ -reductase 1 (\*\*) and 3 $\alpha$ -HSD (\*) mRNA in lean and obese rats. Furthermore GDX increased adrenal mass in lean ( $58 \pm 3$  v  $50 \pm 4$  mg \*\*) and obese ( $56 \pm 4$  v  $46 \pm 3$  mg \*\*) rats.

Table 1

	11HSD activity	11HSD mRNA	5 $\beta$ -R Activity	5 $\beta$ -R mRNA	5 $\alpha$ -R mRNA	3 $\alpha$ HSD mRNA
L-SH	85 $\pm$ 11	1.6 $\pm$ 0.3	10 $\pm$ 1	1.2 $\pm$ 0.2	0.1 $\pm$ 0.02	0.5 $\pm$ 0.1
O-SH	72 $\pm$ 11	0.7 $\pm$ 0.1	18 $\pm$ 2	1.0 $\pm$ 0.2	0.4 $\pm$ 0.1	0.6 $\pm$ 0.1
L-GDX	66 $\pm$ 7	0.6 $\pm$ 0.1	9 $\pm$ 1	0.8 $\pm$ 0.1	1.1 $\pm$ 0.1	0.7 $\pm$ 0.1
O-GDX	40 $\pm$ 5	0.3 $\pm$ 0.1	24 $\pm$ 2	1.5 $\pm$ 0.2	1.4 $\pm$ 0.2	0.8 $\pm$ 0.1

In conclusion, while androgens exert potent effects on glucocorticoid metabolism and adrenal mass, removal of gonadal androgens exacerbates, rather than ameliorates, changes in hepatic glucocorticoid metabolism in obese Zucker rats.

### P388

#### Plasma N-terminal pro-brain natriuretic peptide and adiponectin levels increases during weight loss

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#### Objective

The cardiac hormone brain natriuretic peptide (BNP), including the N-terminal fragment of the prohormone (NT-proBNP) have in cross-sectional studies been demonstrated to be inversely associated with BMI. Clearance of these peptides by adipocyt specific receptors have been suggested. We therefore investigated the impact of weight loss on plasma levels of NT-proBNP and adiponectin in obese individuals.

#### Methods

A total of 20 obese women and 2 men (BMI > 28 kg/m<sup>2</sup>), age 61.7 ( $\pm$  6.6) year (mean  $\pm$  SD) were included. All subjects were without cardiovascular disease. NT-proBNP was < 125 pg/mL, which is the recommended cut-off value for rule out a diagnosis of heart failure. The participants were on a low energy diet of 3.4–5 MJ/day for 1 year. Body weight, body composition measured by DEXA scan, as well as plasma levels of NT-proBNP and adiponectin were measured at baseline and after 52 weeks.

#### Results

Mean total weight loss was 15.4% ( $\pm$  1.7%) and DEXA scan revealed, that this primarily was due to loss in body fat of 27.2% ( $\pm$  3.2%), the decrease in lean tissue mass was 3.0% ( $\pm$  1.2%) (mean  $\pm$  SE). Plasma levels of NT-proBNP increased from 50.2 (39.7–83.3) pg/mL at baseline to 90.3 (49.0–118.9) pg/mL after 52 weeks, median (interquartile range) ( $P = 0.003$ ). Plasma adiponectin levels increased from 14.2 (9.0–17.25) to 16.5 (10.4–19.9) mg/L ( $P = 0.004$ ). Furthermore, a significant association between percent weight loss and increase in plasma adiponectin levels ( $R = 0.59$ ,  $P = 0.004$ ), and a trend towards an association with increase in NT-proBNP levels ( $R = 0.31$ ,  $P = 0.15$ ) were found.

#### Conclusions

Plasma levels of NT-proBNP increases significantly during weight loss, suggesting a possible role of adipose tissue in the clearance of this peptide from circulating plasma. The observed increase in adiponectin supports, that plasma levels are regulated by a negative feedback mechanism.



**P389****Multiple signalling pathways are involved in the phosphorylation of ERK1/2 upon activation of human OX1R and OX2R: Evidence for differential modulation by orexin-A and orexin-B**

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Orexin-A (OR-A) and orexin-B (OR-B) play an important role in the regulation of energy balance and the control of sleep-wake cycle. They act via G-protein coupled receptors, namely orexin receptor-1 (OX1R) and orexin receptor-2 (OX2R). OX2R has equal affinity for both OR-A and OR-B, whilst OX1R has a 10-fold greater affinity for OR-A. Orexin-mediated functions have been extensively explored, however, the intracellular signalling pathways remain poorly understood. Using HEK-293 cells, we investigated the signalling pathways involved in extracellular-regulated-kinase 1 and 2 (ERK1/2) were mediated by OX1R and OX2R activation. Full-length human OX1R and OX2R were amplified from human brain by RT-PCR and subsequently cloned and transfected into HEK-293 cells. Using OR-A and OR-B (0.01, 0.1, 1, 10 and 100 nM), phosphorylation of ERK1/2 were examined time course (5, 10, 15, 20 and 30 min). These effects were further dissected by the use of specific inhibitors for PKA and PKC and Gi/o-protein blocker (pertussis toxin, PTX).

The results demonstrated a dose-dependent phosphorylation and activation of ERK1/2 with a maximal activation by OR-A and OR-B (5 and 15 min). Activation of ERK1/2 by OR-A was greater than OR-B in HEK293-OX1R transfected cells, whereas a similar level of ERK1/2 activation by OR-A or OR-B was observed in HEK293-OX2R transfected cells. In HEK293-OX1R and -OX2R transfected cells, OR-A- and OR-B-induced ERK1/2 activation was abolished by PKC inhibition. Inhibition of PKA also attenuated OR-A- and OR-B-induced ERK1/2 activation. However, the effect was weaker than that observed for PKC. This suggests that PKC is mainly involved in OX1R- and OX2R-mediated ERK1/2 activation. In addition, orexin-activated phosphorylation of ERK1/2 was inhibited with pre-treatment of PTX in HEK293-OX2R transfected cells, but not in HEK293-OX1R transfected cells.

In conclusion, activation of ERK1/2 by OR-A and OR-B via both receptors resulted in a dose-dependent response with a maximal activation between 5 and 15 min. These data demonstrated that PKC and PKA signalling pathways are involved in the phosphorylation of ERK1/2 via OX1R and OX2R. Furthermore OX2R activation to ERK1/2 is associated with PTX-sensitive G-protein.

**P390****Association among individual deprivation using the EPICES score and anthropometric factors linked to abdominal and metabolic markers**H Bihan<sup>1</sup>, G Nguyen<sup>3</sup>, R Cohen<sup>1</sup> & C Menu<sup>3</sup>, K Soufi<sup>2</sup>, G Reach<sup>1</sup>, H LeClésiau<sup>2</sup>.<sup>1</sup>Hôpital Avicenne, Bobigny, France; <sup>2</sup>CPSS, Bobigny, France; <sup>3</sup>Gemba Life, Courbevoie, France.

The medical burden of deprived patients is high. In diabetes deprivation status is associated with poor metabolic control and more frequent complications. No data to date existed showing the association between individual deprivation in adults and anthropomorphic factors linked to abdominal obesity. We would determine whether individual index of deprivation were associated with anthropomorphic dimensions, postures or body girths and metabolic parameters. We conducted a cross-sectional study in France, using in a general population of adults a 3 D body scanner consisted of 4 columns each including 2 CCD cameras. More than 50 body dimensions on lengths, circumferences and postures were recorded. Fasting glycaemia and lipid profile were performed. Individual deprivation was assessed by the EPICES score (11 questions validated in more than 500000 French subjects).

A total of 339 adults were enrolled (mean age of 42.89 ± 14.63, 54.3% male). The prevalence of overweight and obese subjects was significantly higher in deprived individuals (respectively 33.8% vs 28.8% and 21.3 vs 13.0%,  $P = 0.03$ ) with higher BMI, weight, clinical waist circumference and waist/hip ratio. Fasting triglycerides ( $1.17 \pm 0.81$  vs  $85.36 \pm 13.18$ ,  $P = 0.03$ ), glycaemia ( $5.56 \pm 1.79$  vs  $5.18 \pm 0.96$ ,  $P = 0.01$ ) and HOMA index ( $2.47 \pm 3.14$  vs  $1.69 \pm 1.28$ ,  $P = 0.001$ ) were significantly higher in deprived subjects. In women anthropometric girths determined by body scanner were significantly higher when associated with deprivation. Higher chest bust ( $96.83$  vs  $94.79$  cm,  $P = 0.04$ ), waist ( $90.10$  vs  $84.86$ ,  $P = 0.03$ ), high hip ( $100.68$  vs  $94.13$ ,  $P = 0.01$ ), hip ( $106.12$  vs  $100.69$ ,  $P = 0.009$ ) and abdomen ( $94.06$  vs  $87.72$ ,  $P = 0.02$ ) girths were observed.

In deprived subjects clinical or measurements by optic body scanner were significantly higher with a gender effect. Prevalence of higher abdominal circumferences associated with higher triglycerides and glycaemia was higher in deprived women evoking the existence of early insulino-resistance status.

**P391****Mapping of adiponectin and adiponectin receptors in the human adult and fetal heart**

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Obesity is linked to the development of insulin resistance, diabetes and an increased risk of cardiovascular disease and the metabolic syndrome. Adipose tissue-derived proteins so termed 'adipokines' have been implicated in cardiovascular disease and the metabolic syndrome although the molecular mechanisms are not fully understood. Adiponectin, unlike other adipokines, has been shown to have anti-inflammatory, anti-atherogenic, and insulin-sensitising effects. Importantly, circulating adiponectin levels are inversely correlated with cardiovascular risk factors such as C-reactive protein (CRP), and hypercholesterolemia. Adiponectin exerts its effects by activating two families of seven transmembrane domain receptors, functionally distinct from G protein-coupled receptors, termed AdipoR1 and AdipoR2. Given the widespread distribution of adiponectin receptors and the observation that adiponectin is synthesised in other tissues, including skeletal muscle we hypothesised that adiponectin is present in the human heart and may therefore act locally. Therefore, in this study we investigated the expression of adiponectin and its receptors across the regions of the human heart.

To assess adiponectin receptor expression in the adult heart, we used a human cardiovascular multiple tissue cDNA panel. RT-PCR analysis revealed aberrant expression of both *adipoR1* and *adipoR2* genes in whole fetal and adult heart and in the following compartments of the adult human heart: aorta, apex of the heart, left atrium, right atrium, right auricle, left auricle, left ventricle, right ventricle, interventricular septum, and atrioventricular node. Interestingly, adiponectin expression was more confined in left atrium and right atrium and in the left ventricle and right ventricle. Immunohistochemical analysis confirmed protein expression of both adiponectin receptors in human adult and fetal hearts. These novel data demonstrate the presence of both adiponectin receptors across the human heart, with interesting regional the adiponectin gene. Our study potentially implicates adiponectin and its receptors in signalling mechanisms in the heart.

**P392****Testosterone replacement therapy reduces insulin resistance, and improves glycaemic control in hypogonadal men with Type 2 diabetes**D Kapoor<sup>1</sup>, E Goodwin<sup>1</sup>, KS Channer<sup>2</sup> & TH Jones<sup>1</sup><sup>1</sup>Centre for Diabetes/Endocrinology, Barnsley District General Hospital, Barnsley, United Kingdom; <sup>2</sup>Department of Cardiology, Royal Hallamshire Hospital, Sheffield, United Kingdom; <sup>3</sup>Academic Unit of Endocrinology, Division of Genomic Medicine, University of Sheffield, Sheffield, United Kingdom.

Type 2 diabetes mellitus is associated with increased prevalence of low serum testosterone levels in men [1]. Testosterone levels in men are known to be positively correlated with insulin sensitivity and negatively with visceral obesity. We performed a double blind placebo controlled crossover study to determine the effect of testosterone treatment on insulin resistance and glycaemic control in 24 hypogonadal men (10 patients treated with insulin) above age of 30 with Type 2 diabetes. Treatment was with intra-muscular testosterone 200 mg every two weeks or placebo given for 3 months in random order, followed by a washout period of 1 month before the alternate treatment phase. The primary outcomes were changes in fasting insulin sensitivity [as measured by homeostatic model index (HOMA)], fasting blood glucose and glycated haemoglobin. The secondary outcomes were changes in body composition, fasting lipids and blood pressure. Statistical analysis was performed on the delta values with the treatment effect of placebo compared against the treatment effect of testosterone using t-test. Testosterone therapy reduced the HOMA index ( $-1.73 \pm 0.67$ ,  $P = 0.02$ ,  $n = 14$ ) indicating an improved fasting insulin sensitivity. Glycated hemoglobin was also reduced as a result of reduction in insulin resistance

( $-0.37 \pm 0.17\%$ ,  $P = 0.03$ ). Testosterone treatment also resulted in a reduction in visceral adiposity as assessed by waist circumference ( $-1.63 \pm 0.71$  cm,  $P = 0.03$ ). Total cholesterol decreased with testosterone therapy ( $-0.4 \pm 0.17$  mmol/l,  $P = 0.03$ ) but no effect on blood pressure was observed.

Our data thus show a beneficial effect of testosterone therapy on integral components of the diabetic state in men. Improvements in glycaemic control, insulin resistance, cholesterol and visceral adiposity together represent an overall reduction in cardiovascular risk.

### P393

#### Functional analysis of cardiac orexin receptors: preferential activation by OR-B

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The family of G-protein coupled receptors (GPCRs) are amongst the best-described regulators of cardiovascular pathophysiology. In rodents, intracerebroventricular administration of orexins increases mean arterial pressure and heart rate. However, important peripheral actions of orexins and their cognate receptors is now increasingly of interest.

In this study we demonstrated that both receptors are present across the rat heart, at mRNA and protein level. Moreover, we also demonstrate that the rat heart is a potential source of orexins, since the prepro-orexin gene appears to be present, as well as the cleaved functional orexins -A (OR-A) and -B (OR-B). Treatment of myocytes with OR-B resulted in a dose-dependent increase of myosin light chain (MLC<sub>20</sub>) and Troponin I phosphorylation. In isolated rat myocytes, acute application of OR-B but not OR-A (10 nM) caused an increase in contractile strength but has little effect on diastolic or systolic calcium levels. A specific OX2R agonist ([Ala<sup>11</sup>, D-Leu<sup>15</sup>]-Orexin B) at 10 nM had the same effect as OR-B, whereas blocking of OX1R using a specific antagonist (SB-408124), did not alter the effect of OR-B.

Activation of ERK1/2 was required for the OR-B-induced activation of MLC<sub>20</sub>, since treatment of myocytes with the inhibitor of MEK1 (U0126) inhibited the OR-B-induced phosphorylation of MLC<sub>20</sub>. Collectively, these data point towards a novel role for OR-B in cardiac function.

Our novel observations provide a new insight into the regulation of cardiac contractions by orexins independent of CNS input, and suggest that these effects are mediated via a dedicated OR-B/OX2R pathway.

### P394

#### Orexin receptor expression in human adipose tissue: differential effects of orexins

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Besides playing a role in energy homeostasis, orexins have been reported to have divergent effects on physiological behaviour, cardiovascular regulation, glucocorticoid release, as well as playing a key role in the aetiopathogenesis of narcolepsy. Recent studies using rodent models suggest orexins may also be involved in the regulation of thermogenesis. For example, mice lacking the prepro-orexin gene are significantly hypophagic but have normal body weight suggesting differences in energy homeostasis and metabolic rate. Furthermore, orexins may regulate both brown adipose tissue energy expenditure and thermogenesis through stimulation of sympathetic nerve activity. There are no data as yet on the expression of orexin system components in adipose tissue. We therefore analyzed the expression and localization of orexin receptor-1 (OX1R) and orexin receptor 2 (OX2R) in intra-abdominal omental (Ome) and subcutaneous (Sc) adipose tissue. In addition the effects of orexin A and orexin B on the expression of key genes involved in adipose tissue metabolism and on glycerol release were measured. The study was approved by the Local Research Ethics Committee and all patients involved gave their informed consent.

Using RT-PCR analysis we demonstrated expression of OX1R and OX2R mRNA in Sc and Ome human adipose tissue, as well as in pre-adipocytes and differentiated adipocytes *in vitro*. Immunohistochemical analysis revealed intense membrane staining for both OX1R and OX2R protein in human Sc adipocytes. Furthermore, treatment of human adipose tissue explants with OR-A, resulted in a significant decrease in glycerol release

from Ome adipose tissue only ( $P < 0.05$ ). In OR-A and OR-B-treated adipose tissue explants (100 nM, 24 h), hormone sensitive lipase mRNA expression was significantly reduced in ome adipose tissue only. Interestingly, orexin B but not orexin A treatment resulted in an increase in PPAR-gamma mRNA expression in Sc but not ome. These findings indicate a direct role for orexins on adipose tissue metabolism and proliferation through PPAR-gamma activation.

### P395

#### Insulin sensitivity and lipid profile in obese and normal weight hypertensive subjects

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The aim of this study was to compare metabolic profile in hypertensive obese and lean subjects. The study population consisted of 76 patients. We measured fasting glucose and insulin levels, triglycerides, total, LDL and HDL cholesterol. We also estimated the BMI, WHR, systolic (SBP) and diastolic blood pressure (DBP). We used WHO criteria for abdominal obesity (WHR  $> 0.90$  men,  $> 0.85$  women), and WHO criteria for diagnosing the metabolic syndrome (triglyceride  $> 1.7$  mmol/l, HDL  $< 1.0$  mmol/l). According to BMI (normal weight if BMI  $< 25$  kg/m<sup>2</sup>, obesity if BMI  $> 30$  kg/m<sup>2</sup>) the population was divided into two groups: A-hypertensive obese (47 subjects, 16 males, 31 females, age  $38.74 \pm 8.75$ , BMI  $43.45 \pm 8.88$  kg/m<sup>2</sup>, SBP  $161.49 \pm 16.42$  mmHg, DBP  $104.89 \pm 9.70$  mmHg); B-hypertensive lean (29 subjects, 12 males, 17 females, age  $40.18 \pm 6.32$ , BMI  $24.02 \pm 2.20$  kg/m<sup>2</sup>; SBP  $169.48 \pm 20.15$  mmHg, DBP  $102.76 \pm 12.14$  mmHg) (mean  $\pm$  SD). Homeostasis model assessment (HOMA-IR) was used to estimate insulin sensitivity. Statistics analysis was performed by Student-T and correlation test. There was no significant difference between two groups in age, sex, SBP, DBP and duration of hypertension. In both groups abdominal type obesity was presented (A-100%men, 83.9%women; B-64.6%men, 64.7%women). There was no significant difference between two groups in HOMA-IR ( $4.56 \pm 1.86$  v  $3.08 \pm 1.96$ ;  $P > 0.05$ ) and in lipid profile. Both groups showed increased triglycerides and reduced HDL cholesterol. In group A, we found significant correlation between SBP, DBP and HOMA-IR ( $P < 0.01$ ), between triglycerides and HOMA-IR ( $P < 0.05$ ), and inverse correlation between SBP and HDL ( $P = 0.0013$ ). Although obese hypertensive individuals were more resistant to insulin than lean hypertensive individuals, there was no significant difference in insulin sensitivity between these two groups.

### P396

#### Human adipocytes attenuate cardiomyocyte contraction. A direct link between heart failure and obesity

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The causal relationship between obesity and heart failure is broadly acknowledged; however, the pathophysiological mechanisms involved remain unclear. Besides hemodynamic changes, an alternative mechanism of cardiomyocytes apoptosis secondary to intracellular lipid accumulation has been proposed. However, this seems to be valid only for severe obesity but may not explain the increase in heart failure risk with mild-to-moderate overweight. Since adipocytes secrete a wide variety of factors with autocrine, paracrine and/or endocrine action, we tested whether human adipocytes produce cardioactive substances that directly and acutely affect cardiomyocyte contractility. We cultivated adipocytes obtained from human white adipose tissue, and incubated isolated rat adult cardiomyocytes with adipocyte-conditioned or control medium. The study was approved by the local ethical committee. This is the first report to demonstrate that human adipocytes exhibit cardiodepressant activity with a direct and acute effect on cardiomyocyte contraction. This adipocyte-derived negative inotropic activity directly depressed shortening amplitude as well as intracellular systolic peak Ca<sup>2+</sup> in cardiomyocytes within a few minutes. The adipocyte-derived cardiodepressant activity was dose-dependent, and was completely blunted by heating. Filtration of adipocyte-conditioned medium based on

molecular mass characterized the cardiopressant activity at between 10 and 30kDa. In summary, our data demonstrate that adipocytes exert a hitherto unknown negative inotropic effect, depressing cardiac contraction by reducing intracellular  $Ca^{2+}$ . These findings suggest a direct involvement of adipose tissue in the pathogenesis of myocardium dysfunction, thus explaining the tight association between obesity and heart failure.

### P397

#### Dietary potassium driven responses in the renal WNK kinase pathway *in vivo*

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WNK1 (With-No-K, lysine) and WNK4 are serine/threonine kinases, mutated in Gordon syndrome (Pseudohypoaldosteronism Type II), a dominant, hypertensive, hyperkalaemic disorder; implicating this novel WNK pathway in normal regulation of blood pressure (BP) and electrolyte balance. Previous *Xenopus* oocyte work implicates WNK4 in regulation of K<sup>+</sup> secretion via ROMK (renal outer medullary K<sup>+</sup> channel) and Cl<sup>-</sup> transport pathways both paracellularly via tight junctions (claudins), and transcellularly via the thiazide-sensitive NaCl cotransporter (NCCT) and NKCC1. WNK1 may in turn inhibit WNK4.

To begin to clarify the role of this pathway we detail renal WNK pathway gene expression in mice, a species closely mirroring these aspects of human physiology. We have identified an important, short, kinase-deficient WNK1 isoform (WNK1-S) is overwhelmingly predominant in kidney. Expression of WNK1-S and WNK4 is strongest in distal tubule dropping sharply in collecting duct and with WNK4 also expressed in thick ascending limb (TAL, including macula densa), extending the spectrum of potential WNK4 targets. Interestingly, the WNK4-NKCC1 interaction is reportedly dependent on SPAK, a kinase which also interacts with NKCC2 (predominantly expressed in TAL).

*In vivo* this novel WNK pathway responds to chronic changes in dietary potassium and aldosterone, particularly upregulation of WNK1-S and WNK4 with high potassium which via inhibition of NCCT and enhanced distal sodium delivery would facilitate enhanced K<sup>+</sup>-excretion. As potassium intake drops the WNK pathway modulation of sodium reabsorption in distal tubule coordinates with H<sup>+</sup>/K<sup>+</sup>-ATPase and striking reciprocal ROMK isoform-specific expression changes.

These *in vivo* findings have important implications for our understanding of the WNK pathway and the regulation of BP, K<sup>+</sup> and acid-base balance.

### P398

#### The *in vitro* and *in vivo* role of diabetic status and insulin sensitisers on the novel adipocytokine, visfatin

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Visfatin, a novel adipocytokine preferentially expressed in omental (Om) adipose tissue (AT), has been shown to exert insulin-mimetic effects in mice and humans. Recent studies in Asian populations show elevated serum visfatin levels in subjects with Type 2 Diabetes Mellitus (T2DM), suggesting a potential role for visfatin in the pathogenesis of this disease. Whilst central adiposity is closely related to IR and T2DM, the role of AT in the development of these conditions remains unclear. Therefore for this study we investigated circulating levels of visfatin in Caucasian non-diabetic (ND) and diabetic (DB) subjects (DB BMI: 35.9 (mean ± SD) ± 7.2 kg/m<sup>2</sup>; Age: 56.0 (mean ± SD) ± 9.8 yrs, n = 36; ND BMI: 26.0 ± 2.7 kg/m<sup>2</sup>, Age: 38.9 ± 12.9 yrs, n = 23). Our studies demonstrated that the T2DM subjects exhibited higher serum visfatin levels compared with ND controls (DB: 21.8 ± 3.6 ng/mL, ND: 13.9 ± 1.5 ng/mL, P < 0.05). Secondly we examined serum visfatin levels in T2DM patients pre- and post-RSG treatment (BMI: 36.1 ± 7.0 kg/m<sup>2</sup>; Age: 57.8 ± 11.2 yrs, n = 8). Post-RSG treated diabetics showed a significant reduction in circulating visfatin and insulin levels compared with pre-treated subjects (pre-RSG: 10.77 (mean ± SEM) ± 1.47 ng/mL; post-RSG: 7.45 ± 1.60 ng/mL, P = 0.04). We then investigated the *ex vivo* depot specific expression of visfatin in human AT (abdominal subcutaneous

(AbdSc) n = 11, Om n = 11 and thigh n = 6) which showed that visfatin protein is expressed in a depot specific manner (Om > AbdSc > Thigh, P < 0.01). Finally we studied the effect of insulin alone and in combination with the insulin sensitiser, rosiglitazone (RSG) on visfatin in isolated human AbdSc adipocytes. This determined that RSG 10 nM/insulin 100 nM reduced visfatin protein expression compared with insulin alone (P < 0.001). In summary, visfatin is expressed in a depot specific manner in human AT. Furthermore, RSG reduces visfatin protein expression in AbdSc adipocytes, as well as circulating levels in T2DM patients. These data indicate that elevated visfatin levels are a feature of T2DM and are regulated by insulin. The finding that RSG lowers serum visfatin in diabetics may contribute to the functional effects of this anti-diabetic agent.

### P399

#### Adrenocortical function in obese Zucker rats

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Adrenal steroids and alterations in steroid metabolism are important factors in the development of obesity in Zucker rats. One might predict, therefore, that the function of the adrenal gland differs between lean and obese Zucker animals. Accordingly, we investigated adrenal gland morphology and function and have considered not only glucocorticoids and HPA activity but also the renin-angiotensin-aldosterone system. Plasma samples and adrenal glands were obtained from young 12 week old rats, at a stage when obesity is becoming apparent. Adrenals (n = 6) were either fixed and stained for morphology or were used to measure gene expression by a real time PCR method. The size of corticosterone-synthesizing cells was increased in glands of obese compared with lean rats; inner and outer zona fasciculata and zona reticularis cells were 15–30% larger (P < 0.05). In contrast, the cells of the aldosterone-synthesizing zona glomerulosa were 15% smaller (P < 0.02). This pattern of difference was even more marked in adrenals from older animals where obesity was fully established. The morphological differences between adrenals from lean and obese rats were compatible with functional changes. Plasma corticosterone and ACTH concentrations were elevated in obese rats and expression of adrenal CYP11B1, the gene encoding 11β-hydroxylase, the final enzyme in corticosterone biosynthesis, was increased (P < 0.01). The expression of CYP11B2, the gene encoding aldosterone synthase, was decreased and plasma renin activity, a major determinant of zona glomerulosa hypertrophy and hyperplasia was reduced (P < 0.01). However, plasma aldosterone concentration was higher in obese compared with lean rats (P < 0.05). These observations of increased mineralocorticoid and glucocorticoid hormone activity, differential effects on adrenocortical zonation and suppression of renin activity are similar to those we and others have made concerning the effects of chronic ACTH excess. We conclude that increased adrenocortical activity may contribute to the hypertension that we have observed in obese Zucker rats.

### P400

#### Recurrence rate of atrial fibrillation in amiodarone-induced hyperthyroidism

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#### Background

Atrial fibrillation occurs in 5–15% of hyperthyroid patients. On the other hand, amiodarone is a highly effective antiarrhythmic agent. Despite antiarrhythmic treatment, iodine-induced hyperthyroidism via amiodarone may be associated with recurrence of initial arrhythmias.

#### Aims

To assess if amiodarone maintained its antiarrhythmic efficacy in the presence of amiodarone-induced hyperthyroidism (AIT).

#### Patients and methods

40 patients with AIT, 19 M/21F, aged 27–78 years, 15 from iodine deficient areas, initially received amiodarone for prevention of atrial fibrillation



( $n = 26$ ), of ventricular tachycardia or ventricular premature beats ( $n = 14$ ). Control group consists of 46 patients with common hyperthyroidism, matched for age and gender with study group. TSH, total T<sub>3</sub>, total T<sub>4</sub>, free T<sub>4</sub> were measured by microenzymatic immunoassay. Resting 12-lead surface ECG assessed cardiac rhythm. Local Ethical Committee approval has been obtained for this study.

#### Results

Despite significantly lower T<sub>3</sub> levels in AIT ( $225.8 \pm 33.2$  ng/dl) versus common hyperthyroidism ( $334.7 \pm 25.7$  ng/dl,  $P = 0.01$ ), recurrence of paroxysmal atrial fibrillation occurred in 13 out 20 patients (65%), significantly more frequent ( $P < 0.001$ ) than in common hyperthyroidism, where the relevant rates were 13 out 46 patients (28.3%). Chronic atrial fibrillation was already present at diagnosis of AIT in 6 patients. Ventricular arrhythmias recurrence occurred in 2 out 14 patients (14.3%) in study group and in 1 out 46 patients in control group (2.1%),  $P < 0.001$ ; however, in AIT recurrence of ventricular arrhythmias is significantly rare than of atrial fibrillation ( $P < 0.001$ ).

#### Conclusion

Amiodarone antiarrhythmic efficacy is surpassed in AIT by the increased arrhythmic susceptibility of both atrial and ventricular myocardial tissue; atria seem to be more sensitive than ventriculi to minimally increased thyroid hormones levels.

### P401

#### The effect of hyperthyroidia on glycemic control in patients with diabetes mellitus

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#### Aim

The aim of the study was to evaluate the effects of hyperthyroidia on glycemic control in patients with diabetes mellitus.

#### Research design and methods

We retrospectively evaluated the patients with diabetes and hyperthyroidism. We analysed clinical data, thyroid ultrasound exploration, laboratory analyses (glycosylated hemoglobin, free thyroxin FT<sub>4</sub>, triiodothyronine T<sub>3</sub>, thyroid stimulating hormone TSH).

#### Results

We evaluated 48 patients (42 women/6 men), mean age was  $55.7 \pm 15.3$  years and duration of diabetes  $8.8 \pm 7.9$  years; 24 patients with Graves diseases (50%), 14 with toxic multinodular goiter (29%), 7 with autonomous hyperfunctioning adenoma (14.5%), and 3 with amiodarona induced hyperthyroidism (6.5%). The treatment used for diabetes: insulin 30 (62.5%) patients, antidiabetic oral agents 11 (23%), diet 7 patients (14.5%). In the context of hyperthyroidism mean value of glycosylated hemoglobin was  $9.4\% \pm 2.2\%$  (poor glycemic control) versus  $7.1\% \pm 1.6\%$  after the treatment of hyperthyroidism. Among insulin-treated patients, the average needs of insulin in the context of hyperthyroidism was  $0.72$  u/kg versus  $0.55$  u/kg in the context of stable thyroid function ( $P < 0.01$ ). We found a significant association between type 1 diabetes mellitus and Graves disease compared to toxic multinodular goiter (80% vs 50%,  $P < 0.01$ ).

#### Conclusions

The presence of hyperthyroidism aggravates glycemic control of the patients with diabetes and increases insulin need in insulin-treated patients. Once the thyroid function was stable, the insulin need decreased significantly ( $P < 0.01$ ). Hypothyroidism must be treated radical to obtain a good glycemic control. Type 1 diabetes is significant associated to Graves disease by autoimmune mechanism.

### P402

#### Flow mediated dilatation and svcam concentration as the markers of endothelial dysfunction in patients with acromegaly

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Acromegaly causes various cardiovascular dysfunctions, and cardiovascular complications are the main cause of increased mortality in non-cured acromegalic patients. The aim of the study was assessment of flow mediated

dilatation (FMD) and the concentration of soluble vascular cell adhesive molecule (sVCAM) as the selected markers of endothelial dysfunction in patients suffering from acromegaly. The study was carried out in 40 patients with acromegaly in various stages of the disease (20 in active acromegaly, 20 cured) and in 20 healthy subjects from the control group. FMD was assessed by means of ultrasound method using Sonoace 9900 with a 10 MHz vascular transducer on the brachial artery. Serum sVCAM concentration was analyzed by ELISA.

#### Results

FMD was statistically significantly lower ( $P < 0.05$ ) in patients with active acromegaly ( $7.5 \pm 3.2\%$ ) than in cured subjects ( $13.9 \pm 4.2\%$ ). Serum sVCAM concentration was statistically significantly higher ( $P < 0.05$ ) in patients with active acromegaly ( $673.8 \pm 156.8$  ng/ml) than in cured subjects ( $530.6 \pm 213.1$  ng/ml).

#### Conclusions

Active acromegaly is associated with the deterioration of arterial reactivity and increased sVCAM concentration. This is due to the impairment of endothelial properties and is connected with increased risk of cardiovascular diseases.

### P403

#### Low endogenous testosterone induces fatty streak formation following cholesterol feeding in the testosterone deficient testicular feminised mouse and castrated male

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Low endogenous testosterone is associated with atherosclerosis in elderly men. The aim of this study was to examine the role of the androgen receptor (AR) in the development of atherosclerosis and determine whether an inactive AR coupled with testosterone deficiency, inherent to the testicular feminised (Tfm) mouse, is associated with atherosclerosis following cholesterol feeding compared to surgically-castrated male littermates with AR intact.

Ten-week-old Tfm mice and XY controls littermates were separated into 4 groups and fed a cholesterol-enriched diet (comprising 42%butterfat, 1.25%cholesterol and 0.5%cholate) for a period of 28-weeks. Group 1, Tfm mice fed diet alone ( $n = 4$ ), Group 2, XY controls fed diet alone ( $n = 4$ ), Group 3, Sham-operated Tfm mice ( $n = 6$ ) and Group 4, castrated XY controls ( $n = 6$ ). Mice were sacrificed, the hearts perfused with saline and frozen at  $-80^{\circ}\text{C}$ . Five, 8 micrometre cryosections were taken at approximately 100 micrometre intervals through the aortic root of each heart. Sections were stained with oil red O and counterstained with haematoxylin, and lipid-stained areas quantified via digital analysis, and expressed as percentage of medial area.

Table 1

	AR/Testosterone Status	Lipid Deposition (% medial area)
Tfm	AR deficient/low testosterone	3.74 (0.72)
XY	AR intact/physiological testosterone	0.31 (0.11)*
Tfm - sham	AR deficient/low testosterone	2.15 (0.61)
XY - castrate	AR intact/low testosterone	4.96 (0.49)*

$P < 0.01$  versus respective control.

Low endogenous testosterone is associated with increased fatty streak formation in testosterone deficient Tfm mice with an inactive AR and AR intact castrated XY littermates compared to controls following feeding for 28 weeks on a cholesterol-enriched diet.

### P404

#### Testosterone replacement reduces aortic fatty streak formation in testosterone deficient Tfm mice following feeding on a cholesterol-enriched diet

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We have previously shown that Tfm mice which have a non-functional androgen receptor (AR) and low endogenous levels of testosterone exhibit increased fatty streak formation within the aortic root. The aim of the present study was to administer varying degrees of testosterone to these animals in comparison to littermate controls to determine the role of the AR in the anti-atherogenic action of testosterone.

Eight-week-old Tfm mice ( $n = 24$ ) and XY littermates ( $n = 16$ ) were placed into 5 groups which received a fortnightly  $10 \mu\text{l}$  i.m injection of either saline, Sustanon<sup>100</sup> (S100) or Sustanon<sup>250</sup> (S250). At ten weeks of age all mice were fed a cholesterol-enriched diet for 28-weeks. Mice were sacrificed, the hearts perfused with saline and frozen at  $-80^\circ\text{C}$ . Five, 8 micrometre cryosections were taken at 100 micrometre intervals through the aortic root of each heart. Sections were stained with oil red O and counterstained with haematoxylin and lipid-stained areas quantified via digital analysis, and expressed as percentage of medial area.

**Table 1**

	AR/Testosterone Status	Lipid Deposition (% medial area)
Tfm – saline	AR deficient/low testosterone	2.86 (0.39)
XY – saline	AR intact/physiological testosterone	0.44 (0.09)
Tfm – S100	AR deficient/physiological testosterone	0.37 (0.14) <sup>a</sup>
Tfm – S250	AR deficient/supra-physiological testosterone	1.25(0.20) <sup>a,b</sup>
XY – S250	AR intact/supra-physiological testosterone	0.22(0.17)

<sup>a</sup> $P < 0.001$  versus Tfm – saline, <sup>b</sup> $P < 0.05$  versus Tfm – S100.

Aortic fatty-streak formation is significantly reduced in AR-deficient Tfm mice receiving physiological testosterone replacement and to a lesser extent supra-physiological testosterone therapy. Testosterone induces anti-atherogenic effects via a non-genomic mechanism.

#### P405

##### Growth hormone and IGFBP-3 but not IGF-1 are independent predictors for insulin sensitivity in healthy subjects: on the role of hGH in the metabolic syndrome

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##### Objectives

Insulin resistance (IR) and obesity are very common metabolic abnormalities, which are often associated with reduced GH secretion. GH reduces intraabdominal fat and its deficiency may contribute to the metabolic syndrome. IGF-1 improves IR but IGFBP3 have a potential role in regulating its bioactivity although only little information exists. We postulate that the elevated insulin levels in IR may persistently suppress GH provided its regulation remains insulin sensitive. We therefore tested the sensitivity of the GH axis to insulin suppression.

##### Methods

181 healthy subjects underwent assessment of fasting total IGF-1, fasting IGFBP3 and fasting and post-OGTT GH and insulin levels (57 men; age  $19-76$ ; BMI  $29.8 \pm 0.5$ ). IR was estimated by calculating three indices: HOMA, QUICKI and ISI.

##### Results

All three indices for IR were well correlated ( $r = 0.6-0.9$ ,  $P < 0.001$ ). IGFBP3 and log-transformed nadir GH but not IGF-1 were significant predictors for insulin sensitivity ( $r = 0.35$ ,  $P < 0.001$ ;  $r = -0.23$ ,  $P < 0.01$  respectively) even after correction for IGF-1 levels. We explain the positive correlation between IGFBP3 and IR through its ability to reduce free IGF-1 levels since a positive correlation between IGF-1/BP3-ratio, as an indicator for free IGF-1 levels, with QUICKI is observed ( $r = 0.16$ ,  $P < 0.05$ ). Moreover, the negative correlation between nadir GH and IR may be due to a higher insulin/glucose-induced GH suppression after glucose ingestion

since a negative correlation between nadir GH and peak post-glucose insulin level is seen ( $r = 0.33$ ,  $P < 0.001$ ).

##### Conclusion

Our data indicate that GH secretion remains sensitive to insulin in IR. IGFBP3 and nadir GH are more predictive than total IGF-1 of IR. We show for the first time that higher IGFBP3 and lower free IGF-1 estimated by IGF-1/BP3 ratio are associated with a higher IR indicating a role of endogenous IGF-1 in glucose homeostasis.

#### P406

##### Dehydroepiandrosterone inhibits differentiation, proliferation and 11 $\beta$ -hydroxysteroid dehydrogenase type 1 activity in human preadipocytes

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The adrenal steroid dehydroepiandrosterone (DHEA) has been shown *in vivo*, to mimic the effects of peroxisome proliferator-activated receptor (PPAR) ligands and oppose those of glucocorticoids, thus producing beneficial effects on insulin sensitivity and adipogenesis in obese and diabetic rodents. Furthermore, DHEA treatment has recently been shown to reduce subcutaneous and visceral fat in humans *in vivo*. However, the mechanism by which DHEA produces these anti-adipogenic effects remains to be elucidated. Glucocorticoids, which play a key role in regulating fat metabolism and distribution, are reactivated locally by 11 $\beta$ -hydroxysteroid dehydrogenase type 1 (11 $\beta$ -HSD1), expressed in adipocytes. Recent literature has shown that DHEA can significantly inhibit 11 $\beta$ -HSD1 activity in differentiated murine 3T3-L1 adipocytes, albeit at levels considered supraphysiological in humans. Utilizing a human adipocyte cell system stably expressing 11 $\beta$ -HSD1 (chub-s7) we have tested if these findings would be replicated in human cells treated with physiological concentrations of DHEA, irrespective of the innate differences between the rodent and human endocrine system. Fully differentiated chub-s7 cells were treated with physiological (10–1000 nM) and supraphysiological (25–100  $\mu\text{M}$ ) DHEA concentrations for 48 hours. Adipocyte differentiation, as assessed by 11 $\beta$ -HSD1 activity and the expression of early (lipoprotein lipase) and terminal (G6PHD) differentiation markers, decreased markedly when treated with 25–100  $\mu\text{M}$  DHEA. In contrast lower concentrations had no effect on differentiation. Similar findings were seen when chub-s7 cells were differentiated in the presence of DHEA. To investigate the effect of DHEA on proliferation pre-confluent proliferating Chub-s7 cells were incubated for either 24, 72 or 96 hours with DHEA. Again, higher DHEA concentrations had a marked effect on proliferation while physiologic concentrations had no effect. In summary, DHEA inhibits proliferation and differentiation of human adipocytes, possibly via the observed significant inhibition of 11 $\beta$ -HSD1 activity. However, this effect was only seen when employing supraphysiological DHEA concentrations.

#### P407

##### Urocortin 2 mediates glucose utilization and insulin sensitivity in skeletal muscle

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Skeletal muscle is the principal tissue responsible for insulin-stimulated glucose disposal and is thus the major site of peripheral insulin resistance. Urocortin 2 (Ucn2), a corticotropin releasing factor (CRF) family member and the type 2 CRF receptor (CRFR2) are highly expressed in skeletal muscle. To determine the physiological role of Ucn2, we generated mice deficient in this peptide. Using glucose and insulin tolerance tests and hyperinsulinemic euglycemic glucose clamp studies in mice fed standard or high fat diets, we demonstrated that mice lacking Ucn2 exhibit increased insulin sensitivity and are protected against fat induced insulin resistance. Administration of synthetic Ucn2 to mutant mice prior to the glucose and insulin tolerance tests restores blood glucose to wild type levels. Administration of a CRFR2 selective antagonist to wild type mice results in a glucose tolerance test profile that mirrors that of Ucn2-null mice. Ucn2-null and wild type mice gain weight and consume food similarly, both on standard or high fat diets. However,

significant increases in blood glucose and insulin levels are observed only in the wild type mice and not in Ucn2 null mice. Body composition measurements of Ucn2-null mice on a high fat diet demonstrate decrease in fat and increase in lean tissue compared to wild type. Cellular mechanisms mediating Ucn2 effects were studied using *in-vitro* and *in-vivo* systems. The null mice display increased glucose uptake in skeletal muscle putatively through the removal of Ucn2-mediated inhibition on insulin signaling. Activation of cAMP/PKA signaling by Ucn2 changes the balance between active and inactive IRS-1 serine phosphorylation. This balance modulates insulin signaling and glucose uptake in skeletal muscle cells. These data support a physiological function for Ucn2 as a local regulator of glucose uptake. Because impaired glucose transport in muscle contributes to the pathogenesis of type 2 diabetes, our results suggest Ucn2/CRFR2 pathway as a potential targets for the management of this disease.

#### P408

##### Elevated serum hs-CRP in the obese – still much to be explained

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Obesity is considered as a state of chronic, systemic low-grade inflammation. Likely causes include infectious factors, leptin, cigarette smoking, low serum HDL-cholesterol and high serum triglycerides. No definitive confirmation on the causative factor is available so far.

The aim of the study was to evaluate serum levels of C-reactive protein (hs-CRP, sensitivity 0.085 ng/L), IL-6, IL-10, TNF- $\alpha$  and its soluble type 2 receptor (sTNF- $\alpha$ R-2) in visceral obesity (VO) as well as to assess relationships between proinflammatory cytokines and adipocytokines, i.e. leptin (LEP), adiponectin (ADN) and the previous Chlamydia pneumoniae (ChP) infection or infectious viral diseases of the childhood. The obese group was composed of 48 women with VO (BMI > 30 kg/m<sup>2</sup>) and the control group of 42 normal-weight (NW) women. The inclusion criteria were: age 20–45 yrs., normal menstruation pattern. The exclusion criteria were: chronic infection, systemic autoimmune disorder, acute viral/bacterial infection, trauma, surgical treatment, treatment with NSAIDs, corticosteroids or statins during the last 6 months. IgG antibodies against Ch. trachomatis, pneumoniae and psittaci were measured. Body composition was assessed by bioimpedance method (BIA). No difference was observed in levels of IL-6, IL-10, TNF- $\alpha$  and sTNF- $\alpha$ R2 between VO and NW women. However, levels of hs-CRP and LEP were higher and levels of ADN lower in VO than those in NW women. 27,1% of the obese had normal hs-CRP (1,14  $\pm$  0,5 ng/L), but the remaining 72,9% had elevated hs-CRP (5,2  $\pm$  2,5 ng/L). Both subgroups of VO did not differ in terms of anthropometric parameters, IL-6, IL-10, TNF- $\alpha$  and sTNF- $\alpha$ R2 levels, percentage of ChP-seropositive subjects and prevalence of viral diseases of the childhood. Although the subgroup with elevated hs-CRP had higher BF and %BF, absolute and adiposity-corrected LEP and AND levels were comparable. Cigarette smoking, low HDL-cholesterol and high TG did not influence mean hs-CRP levels in VO. The answer to the question on possible factors causing CRP elevation in ca. 70% of the obese remains unclear and warrants further study.

#### P409

##### Lipoprotein Lp(a) in patients with systemic lupus erythematosus

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Systemic lupus erythematosus (SLE) is a multifactorial multisystem autoimmune disorder. Although the survival of SLE patients has been improved with the administration of immunomodulatory therapy, patients seem to suffer from complications of the disease such as atherosclerosis. Lipoprotein Lp(a) is a known risk factor for the development of atherosclerosis.

The aim was to evaluate lipoprotein Lp(a) and its relationship with disease activity in patients with SLE.

Patients with SLE, n = 32, aged 23–58 years and healthy controls, n = 32 were included in the study. In patients and controls the levels of hematocrit, hemoglobin, erythrocyte sedimentation rate, C-reactive protein, antinuclear antibodies, complement, anti-DNA antibodies, cholesterol, triglycerides, HDL, LDL and Lp(a) were measured.

The levels of Lp(a) (normal values < 30 mg/dl) were found increased in 9 of 32 patients (28.1%) and in 4 of 32 controls (12.5%). Within the SLE cohort 7 of the 9 patients with increased Lp(a) levels had active disease and renal involvement.

Lipoprotein Lp(a) levels were higher in patients with SLE. Increased Lp(a) levels seemed to correlate with disease activity. Increased Lp(a) levels may contribute to the development of cardiovascular diseases and atherosclerosis in patients with systemic lupus erythematosus.

#### P410

##### Effects of alcoholic extract of walnut (*Juglans regia* L.) septum on serum lipids and Activities of serum aminotransaminase enzymes in streptozocin-induced diabetic rats

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Considering the high incidence of diabetes mellitus in society and related therapeutic problem, we investigated antihyperlipidemic effects of alcoholic extracts of *Juglans regia* septum on serum triglycerids, cholesterol, LDL and HDL in streptozocin-induced diabetic adult male rats. In present study, alcoholic extract of septum of *juglans regia* linn. Was found to have potent antihyperlipidemic activity. supplementation of this alcoholic extract by gavage at dose of 0.1, 0.2 and 0.5 g/kg at 0.5 ml distilled water in diabetic rats result a significant diminution of fasting blood glucose and tryglycerids and increase LDL level after 14 days. Continuous supplementation of this extract for 14 days result no significant difference in serum insulin, cholesterol and HDL in diabetic rats. Elevated levels of of serum aminotransaminase enzymes (AST and ALT) were reported in animals treated with toxic plant derived products. Activities of serum aminotransaminase enzymes alanine aminotransferase (ALT) and aspartate aminotransferase (AST), were decreased significantly in the alcoholic extract supplemented group in respect to control group.

#### P411

##### The ways in which zinc enters beta cells in the pancreas and the influence of zinc blockers on the development of type 1 diabetes

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Pancreatic islet beta cells contain a substantial amount of chelatable zinc (Zn<sup>2+</sup>), where it acts as a structural component of insulin packaging and is also co-secreted with insulin. Zinc at concentrations found in the pancreas is potentially harmful, and like in the brain, its permeation into endocrine cells may trigger the destruction of the islet leading to the onset of diabetes. Despite the potential importance little is known about the permeation pathways of zinc into beta cells.

In the present project, we have studied the influx pathways of zinc into the beta cell line Min6 and thereby possible mechanisms to lower intracellular zinc levels using single cell fluorescent imaging. We show that depolarization of cells is followed by massive influx of zinc, via the L-type Ca channels (LTCC) as indicated by the inhibition of this influx using the channel blocker nifedipine. Zinc influx persists in the presence of physiological concentrations of Ca<sup>2+</sup>, in contrast to Ca<sup>2+</sup> is not pumped out. Indeed blocking of the LTCC prevents zinc induced cell death determined by LDH release. We have further determined the potential of a compound called Clioquinol (CQ) that can chelate intracellular zinc, to chelate the pancreatic pool of zinc and therefore to protect against zinc toxicity. Application of CQ had a protective effect and reduced zinc-induced cell death by 75%. Finally, *in-vivo* chelation of zinc protected against the onset of type 1 diabetes triggered by STZ. Thus our results indicate that the *in vivo* chelation of zinc may serve as therapeutic tool for the treatment of type 1 diabetes.

#### P412

##### Lipoprotein Lp(a) in patients with rheumatoid arthritis

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Epidemiological studies show increased mortality in patients with rheumatoid arthritis (RA). One of the causes of death in RA patients is cardiovascular disease. Lipoprotein Lp(a) appears to be an independent risk

factor for the development of cardiovascular diseases. The aim of the study was to evaluate serum lipid and lipoprotein Lp(a) levels in patients with RA. Patients with RA,  $n = 50$ , aged 24–65 years and healthy controls,  $n = 50$  were included in the study. In patients and controls the levels of hematocrit, hemoglobin, erythrocyte sedimentation rate, C-reactive protein, cholesterol, triglycerides, HDL, LDL and Lp(a) were measured.

The levels of Lp(a) (normal values  $< 30$  mg/dl) were found increased in 12 of 50 patients with RA (24%) and in 6 of 50 controls (12.5%). Within the RA cohort 9 of the 12 patients with increased Lp(a) levels had increased inflammation markers and increased DAS28.

Lipoprotein Lp(a) levels were found increased in patients with RA. Patients with RA appear to be at increased risk for the development of atherosclerosis. Lipoprotein Lp(a) levels seem to be increased in patients with active RA and inflammation may alter Lp(a) levels.

#### P413

##### Polymorphisms in insulin-like growth factor binding protein-1 (IGFBP-1) are associated with altered circulating IGF-I and lower body mass index in type-2 diabetes mellitus

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##### Introduction

Dysregulation of the insulin-like Growth Factor (IGF) system and particularly IGF-I and IGF binding protein-1 (IGFBP-1) has been implicated in the pathogenesis of obesity, diabetes mellitus and its complications such as cardiovascular disease and nephropathy, but relatively little is known about the genomics of the IGF system in health and disease.

##### Methods

Six single nucleotide polymorphisms (SNPs) were genotyped in the IGFBP-1 gene, in a representative sample of 732 Type-2 Diabetes patients from the Salford Diabetes Register, using ABI TAQMAN endpoint PCR.

##### Results

Minor alleles of two SNPs, one in the second intron (C2877T) and one in the 3' flanking region (A5881G) of the IGFBP-1 gene, were associated with altered circulating total IGF-I. One of these SNP's (A5881G), and four others, from the promoter (G-575A), intron 1 (A643G and T555C), and exon 4 (A4403G) were associated with consistently lower BMI over the ten years 1993–2003, and all of these except G-575A were further associated with a reduced rate of increase in BMI over time. Total, non-fasted plasma IGFBP-1 levels were negatively associated with BMI, but not with any genotype.

##### Discussion

Given the importance of IGFBP-1 in modulating IGF bioavailability in relation to nutrient intake, it is quite conceivable that subtle changes in IGFBP-1 expression could have a significant long term impact on an individual's predisposition to weight gain, whether or not they have type 2 diabetes. We propose that genetic variation in the IGFBP-1 gene may have a direct influence on the development of obesity and type 2 diabetes.

#### P414

##### Polymorphisms in insulin-like growth factor binding protein-1 (IGFBP-1) are associated with increased type-2 diabetes mellitus prevalence

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##### Introduction

Dysregulation of the Insulin-like Growth Factor (IGF) system and particularly IGF-I and IGF-binding protein 1 (IGFBP-1) has been implicated in the aetiopathogenesis of obesity, diabetes mellitus and such complications

such as cardiovascular disease and nephropathy. However, relatively little is known about the genomics of the IGF system in health and disease.

##### Methods

Six single nucleotide polymorphisms (SNPs) were genotyped in the *IGFBP1* gene, in a representative sample of 732 Type-2 Diabetes patients from the Salford Diabetes Register and 359 aging healthy controls without diabetes from the Dyne-Steele Aging in Cognition Cohort, also based in Salford. All SNP's were genotyped using ABI Taqman endpoint PCR.

##### Results

Two of these SNPs, T2877C in the second intron and A5881G in the 3' flanking region, were associated with 2.6-fold and 2.7-fold increased prevalence of Type-2 Diabetes, respectively. The effect of these SNPs on odds ratios was not additive, and they were not in linkage disequilibrium, suggesting independent association with disease risk. No functional effect of either SNP could be postulated from the data available.

##### Conclusion

These are the first genetic markers for Type-2 Diabetes prevalence described in *IGFBP1*. Our findings suggest that important genetic polymorphisms within the *IGFBP1* gene, which may be in LD with those described here, exert important biological effects and remain to be characterised.

#### P415

##### Cardiac autonomic neuropathy in relation to some components of metabolic syndrome in newly diagnosed type 2 diabetic patients

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##### Background and aims

Components of metabolic syndrome (MS) carry an increased risk for cardiovascular disease (CVD). People with abnormal glucose regulation are more prone to develop complications. At diagnosis 20-30% of type 2 diabetic (T2DM) patients (pts) already have neuropathy-one of the most dangerous complications. Cardiac autonomic neuropathy (CAN) is associated with five-fold risk of mortality. Our aim was to study possible relation between CAN and some components of MS in newly diagnosed T2DM pts.

##### Materials and methods

We observed 33 T2DM pts with Grade 1/2 arterial hypertension (AH) (ESH/ESC Guidelines), increased BMI, but without known CVD, ketoacidosis, alcoholism and/or liver disease. males/females-21/12, mean age-47.5 ± 4.8 yrs.; mean HbA1c-8.1 ± 2.04%; mean BMI-29.6 ± 3.3 kg/m<sup>2</sup>. Ewing's standard reflex tests were performed; severity of CAN was evaluated according to Jermendy et. al, 1995. Lipid profile, resting blood pressure (BP), pulse pressure (PP) and heart rate (HR) were assessed.

##### Results

Response CAN reflex tests were normal in 15 cases (45.4%); 13 pts (39.4%) had mild, and 5 (15.1%) – moderate CAN. No severe CAN was registered. There was positive correlation between prevalence of CAN and HbA1c ( $P < 0.001$ ); CAN and mean heart rate ( $P < 0.001$ ), CAN and mean SBP/PP ( $P < 0.05$ ). Only 7 pts had normal lipid profile. High total cholesterol levels (mean 5.59 ± 0.84 mmol/l) were registered in 18 cases, elevated LDL levels (mean 3.86 ± 1.10 mmol/l)-in 4 pts; low HDL levels (mean 0.89 ± 0.69 mmol/l)-in 21 pts; high triglycerides (mean 2.91 ± 0.54 mmol/l)-in 8 pts. No statistically evident correlation was observed between CAN severity and lipid profile indices.

##### Conclusion

CAN was registered in 54.5% of patients; compared to general population abnormal lipid profile indices were observed more frequently (78.9%) in the study population. We can presume, that in newly diagnosed T2DM hyperglycemia, that may be asymptomatic and not treated for years, plays more important role in CAN development than lipid disorders.

#### P416

##### Severity of depression in patients with type 1 and type 2 diabetes and its relation to the level of glycemia control and anti-depressive therapy

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##### Background and aims

There are consistent findings about increased prevalence of depressive disorders in diabetes. The aim of the study was to reveal prevalence of



depression in patients (pts) with type 1 (DM1) and type 2 (DM2) diabetes.

**Materials and methods**

Totally, 157 pts with DM were questioned. To assess depression degree Beck's scale was used, and 83 depressive pts (scores > 10), were allocated into 2 groups (Gr.): Gr.1 (n = 44)–DM2 pts (m20/f24), mean age 57.5 ± 8.4 yrs, diabetes duration 6 ± 2 yrs, HbA1c–8.8 ± 1.3%, Gr. 2 (n = 39)–DM1 pts (m16/f23), mean age 30 ± 5 yrs, diabetes duration–7 ± 2 yrs, HbA1c–9.2 ± 1.9%. Assessment scores: Gr.1(m) > 55(7 pts/35%), 30–50(8 pts/40%), ≥ 10(5 pts/25%); (f) > 60 (11 pts/45.8%), 30–50(8 pts/33.4%), > 15(5 pts/20.8%). Gr.2(m) > 50 (8 pts/50%), 30–50(7 pts/43.7%), ≥ 10(1 pts/6.25%); (f) > 60(12/52.2%), 30–50(8/34.8%), > 20 (3/13%). 18 Gr.1 pts were treated with insulins and oral hypoglycemic agents (OHAs); 26–only with OHAs. Intensive insulin therapy was used in 22 Gr.1 pts. Tianeptin 3 tablets/daily was used for depression treatment in both groups.

**Results**

Repeated examination at month 3 post study initiation showed: HbA1c dropped by 7.3 ± 0.9%; P = 0.000 (Gr.1), and by 6.5 ± 0.6%; P = 0.000 (Gr.2). Beck's scores were: Gr.1 (m): 30–40 (7 pts), 20–30 (8 pts), 0 (5 pts); (f): 50–60 (11 pts), 20–30 (8 pts), ≥ 10 (5 pts). Gr.2 (m): 30–40 (8 pts), ≥ 20 (7 pts), 0 (1 pts); (f): 50–60 (12 pts), 20–25 (8 pts), ≥ 8 (3 pts). At entry there was no statistically evident difference in depression severity between the groups (P = 0.177; P = 0.537, respectively). Post treatment there was statistically evident correlation between decline in HbA1c levels and depression severity in both groups, this evidence was relevant for males and females: Gr.1(m) r = 0.948, P = 0.000; (f) r = 0.909, P = 0.000. Gr.2(m) r = 0.915, P = 0.000; (f) r = 0.921, P = 0.000.

**Conclusion**

We conclude: 1. There was no statistically evident difference in depression severity between the DM types. 2. There is statistically evident post-treatment correlation between glycemia control level and depression degree in both groups.

**P417**

**Cardiac autonomic and peripheral neuropathy in newly diagnosed type 2 diabetic patients**

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**Background and aims**

At diagnosis 20–30% of patients (pts) with type2 diabetes (T2DM) have neuropathy, that often goes unrecognized. Cardiac autonomic neuropathy (CAN) is associated with five-fold risk of mortality. As today there is no specific treatment for overt neuropathy, it is important to use prevention and timely revealing. The goal of the work was to evaluate prevalence of peripheral neuropathy and CAN in pts with newly diagnosed T2DM.

**Materials and methods**

We observed 39 pts with newly diagnosed T2DM without known CV disease, ketoacidosis, alcoholism and/or chronic liver disease and non-diabetic nerve damages, not taking medications affecting CAN reflex tests. Ewing's standard reflex tests were performed, severity of CAN was evaluated according to Jermendy, 1995. Standardized evaluation was conducted (Neurometer®, Baltimore, MD), that discriminates between neuropathic and non-neuropathic pts and tests different nerve fiber types. Electric current perception threshold was measured at: 5Hz, 250Hz, 2000 Hz. Measurements were obtained from hallux deep and superficial peroneal nerves. Forced choice method was used to confirm minimum intensity, which was considered as current perception threshold. Clinical data of the study population: males/females-27/12, mean age – 49.3 ± 3.6 yrs.; HbA1c 8.3 ± 1.73%; BMI 24.4 ± 2.6 kg/m<sup>2</sup>.

**Results**

CAN test scores – 0–1–18 cases (46.15%), 2–3 (mild) – 17 pts (43.59%) and 4–6 (moderate) – 4 pts (10.26%) CAN. No severe CAN were registered. CPT abnormalities had 14 pts: 8 (20.5%) hyperesthetic and 6 (15.4%) hypoesthetic, 12 of them had CAN. Positive correlation was demonstrated between prevalence of CAN and HbA1c (P < 0.001); CAN and peripheral sensory dysfunction (P < 0.001).

**Conclusion**

CAN was observed in 53.85% of the patients; peripheral sensory dysfunction prevalence here was higher (35.9%), than in general population. Undiagnosed hyperglycemia that may be asymptomatic and untreated for years, may explain why at diagnosis CAN and peripheral sensory dysfunction are present in many patients.

**P418**

**Rapid effect of repaglinide in combination with metformin in poorly controlled obese patients with type 2 diabetes mellitus**

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**Background and aims**

Combination of drugs, affecting early insulin secretion and peripheral insulin resistance are a good therapeutic option for patients (pts) with type 2 diabetes mellitus (T2DM). The aim of the present work was to compare efficacy of Repaglinide + Metformin and Sulphonilurea + Metformine in obese T2DM pts.

**Materials and methods**

A total of 107 pts with T2DM not well-controlled on monotherapy. Repaglinide – naïve were allocated into 2 groups (Gr.): Gr 1 (n = 65) – Repaglinide 1 mg/meal and Metformin 1000 mg twice/daily. Gr.2 (n = 42) – Glimepiride 4 mg/breakfast and Metformin 1000 mg twice/daily. At entry following data were obtained for Gr.1 and Gr.2, respectively: age – 56.9 ± 9.7 and 54.6 ± 6.9 yrs; diabetes duration – 4.18 ± 2.06 and 4.04 ± 3.1 yrs; BMI – 28.9 ± 5.3 and 29.1 ± 4.9 kg/m<sup>2</sup>; HbA1c – 10.43 ± 2.46 and 9.72 ± 2.72%; fasting glycemia (FG) – 195.5 ± 59.5 and 158.3 ± 83.2 mg/dl; postprandial glycemia (PG) – 285.6 ± 91.17 and 220.1 ± 91.64 mg/dl. Pts were supervised for 3 months.

**Results**

Examinations at month 3 post treatment initiation revealed: BMI decreased to 26.1 ± 4.2 kg/m<sup>2</sup>; P = 0.001 (Gr.1) and 28.8 ± 5.1 kg/m<sup>2</sup>; P = 0.784 (Gr.2); HbA1c dropped to 6.94 ± 1.11%; P = 0.000 (Gr.1) and 7.99 ± 1.46%; P = 0.000 (Gr.2). HbA1c values decreased by 3.49 ± 1.35% (Gr.1) and 1.73 ± 1.26% (Gr.2). FG decreased to 114.6 ± 19.7 mg/dl; P = 0.000 (Gr.1) and 138.8 ± 50.2 mg/dl; P = 0.197 (Gr.2); PG dropped to 123.3 ± 24.18 mg/dl; P = 0.000 (Gr.1) and 178.6 ± 43.9 mg/dl; P = 0.01 (Gr.2). The latter values decreased by 162.3 ± 66.99 mg/dl (Gr.1) and 41.5 ± 17.74 mg/dl (Gr.2). Analysis of the data obtained revealed statistically evident positive shifts in BMI, HbA1C, FG, PG.

**Conclusion**

These data confirm that Repaglinide + Metformin gives rapid effect in reducing glycemia (FG, PG), HbA1c and BMI indices in T2DM pts.

**P419**

**Impact of migration on circulating adipocytokines in an Asian Indian population**

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**Introduction**

Propensity to centripetal adiposity is an ethnic characteristic amongst South Asians, which may underlie the excess risk for both coronary heart disease (CHD) and diabetes in this population. Circulating levels of adipocytokines (hormones which include leptin, adiponectin, tumour necrosis factor (TNF-alpha)) and C-reactive protein (CRP) reflect the adipose tissues endocrine and metabolic activity, and are implicated in CHD.

**Methods**

We compared Gujaratis in Britain with similar, non-migrant Gujaratis in India, to investigate the impact of migration on circulating levels of adipocytokines and CRP. Gujaratis living in Sandwell were compared with age, gender and caste- matched contemporaries remaining in their villages of origin in Navsari, India. Adipocytokines were measured by two-site ELISA with commercially available antibodies, CRP was measured by an automated high sensitivity assay.

**Results**

In UK Gujarati men (n = 94) who displayed greater centripetal adiposity, median (IQR) TNF-alpha levels were significantly higher: 210 pg/ml (20–1330) than Indian Gujarati men (n = 110) 30 ng/ml (20–340), P = 0.001. Serum leptin was also higher in UK Gujarati men 3100 pg/ml (1700–6100) vs Indian Gujarati men 1300 pg/ml (500–3500), P < 0.001. No differences were seen in men for CRP or adiponectin by migration status. Even greater differences with migration were seen for women where in addition hsCRP was greater in UK Gujarati women (n = 92) at 1.39 g/l (0.74–3.45) vs Indian Gujarati women (n = 130) hsCRP 0.87 mg/l (0.38–2.23), P < 0.001. Adiponectin was also higher in UK Gujarati



women 4630 ng/ml (3320–6430) than Indian Gujarati women 3870 ng/ml (2780–5490) ( $P < 0.05$ ).

#### Conclusion

The higher levels of TNF-alpha and CRP (particularly in women) seen with migration in this Gujarati Indian population suggests a role for pro-inflammatory cytokines in the excess CHD risk in this population. The higher adiponectin level in women suggests a possible compensatory mechanism to improve glucose handling and insulin sensitivity as adiposity increases.

### P420

#### Migration to the UK results in significantly lower testosterone levels in South Asian men

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#### Introduction

Compared to ethnically European men, Pakistani origin men living in the UK have a lower circulating total and free testosterone level, corresponding with greater central adiposity and higher insulin resistance. Within a South Asian group we have now examined the effect of migration to the UK on male testosterone level.

#### Methods

Circulating testosterone concentration was measured by automated immunoassay in 97 Gujarati males resident in India and in 79 males from the same villages of origin living in Birmingham UK. The relationship between serum testosterone level and other metabolic indices together with anthropometric parameters was determined.

#### Results

Circulating testosterone was significantly lower in UK Gujarati males (mean:interquartile range) 16.7 nmol/L (13.9–20.3) vs Indian Gujarati males 21.5 (16.1–28.1), ( $P < 0.001$ ). Waist:hip ratio was correspondingly higher in UK Gujarati males (mean: 95% Confidence Interval) 0.92 (0.90–0.94) compared with Indian Gujarati males 0.87 (0.86–0.88), ( $P < 0.001$ ). In univariate analysis a lower total testosterone was associated with elevated waist:hip ratio (Spearman's  $\rho = -0.27$ ,  $P < 0.001$ ), diastolic BP ( $\rho = -0.28$ ,  $P < 0.001$ ), fasting NEFA ( $\rho = -0.29$ ,  $P < 0.001$ ), CRP ( $\rho = -0.19$ ,  $P = 0.014$ ), and leptin ( $\rho = -0.28$ ,  $P = 0.001$ ). Testosterone correlated positively with insulin sensitivity (HOMA-S) ( $\rho = 0.16$ ,  $P = 0.04$ ).

#### Discussion

The lower circulating testosterone in UK Gujarati males and its association with markers of adiposity, suggest a significant influence of body composition change with migration on androgen levels with potentially adverse consequences for male health.

### P421

#### Prevalence of skin manifestations in diabetics

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#### Background

Diabetes Mellitus is one of the endocrine disorders that involved many organs in the body. Many of diabetes patients show cutaneous manifestations. This study is performed to assess the prevalence of different skin manifestations in diabetic patients referred to Ahvaz Jondishapour University Diabetes Center.

#### Methods

All diabetic patients (type 1 and type 2) referred to our diabetes center since 2003 to 2004 enrolled in this study. A questionnaire include: age, sex, type of diabetes, duration of disease, antidiabetic medication and last HbA1C, was filled out for each patient. Careful physical examination for detection of skin disorders was done in each patient. Hematologic, serologic, culture and biopsy of skin were performed when it were necessary.

#### Results

One hundred patients (70 females & 30 males) enrolled in this study. Mean age was 51 years (age range between 9 to 80 year). Duration of diabetes was between one month to 30 years (mean duration 8 year). 83 patients had type

2 and 17 patients type 1 diabetes mellitus. 92% of patients had at least one skin manifestation. The most common skin manifestations were: skin dryness 60%, hair loss 56%, pruritus 46%, diabetic neuropathy (24%), diabetic dermopathy (22%), acrocordon (22%), fungal infections (22%), bacterial infections (7%), nail changes (13%), acantosis nigricans (6%), xantoma (4%), diabetic bulla (3%), skin thickening (3%) viteligo (7%), robeosis (2%) and necrobiosis lipoidica in 1% of patients. No any cases of anular granuloma and perforating disease and cutaneous aduers reactions of oral hypoglycemic agents were seen. Lipoatrophia in site of insulin injection was detected in 2 of 18 patients used insulin. There were no significant correlation between type of diabetes and HbA1C, and skin manifestations.

#### Conclusion

Skin manifestations had high prevalence in studied group. Skin examination is recommended in fallow up visits of diabetic patients.

### P422

#### Correlations between leptin, insulin resistance and lipid profile in postmenopausal women

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Leptin is a circulating hormone produced primarily by the adipose tissue, which controls food intake and energy homeostasis. Important peripheral actions of leptin involve inhibition of insulin biosynthesis and secretion in pancreatic  $\beta$ -cells. In turn, insulin stimulates leptin secretion from adipose tissue, establishing a hormonal regulatory feedback loop: "adipo-insular axis". The aim of our study was to determine the interrelations between leptin, insulin/insulin resistance and lipid profile in pre- and postmenopausal women. We investigated 146 postmenopausal white women, aged between 42 and 78 years and in addition 45 premenopausal white women aged between 22 and 51 years. In both groups we measured plasma levels of leptin, insulin, fasting glucose, glycemia at two hours after glucose load, total cholesterol, HDL- and LDL- cholesterol and triglycerides. The index of insulin resistance was calculated with HOMA formula [(fasting insulin  $\mu$ U/mL  $\times$  fasting glicaeamia mg/dL)/405] and body composition was performed by a Body Composition Analyzer TBF 310 GS (Tanita Corporation) measuring Body Mass Index (kg/sqm) – BMI, Fat%, Fat Mass (Kg) – FM, and Fat Free Mass (Kg) – FFM. Statistical analysis performed was: linear regression analysis (correlation between parameters) and paired t-test for differences among groups. We found statistically significant positive correlations between leptin and fat mass, as well as waist circumference in both groups. Our results further suggest that in addition to adiposity, the hyperinsulinemia and lipid profile modulate leptin levels in pre- and postmenopausal women. Also, plasma leptin levels, mainly in premenopausal women, could be a predictor for the development of the insulin resistance syndrome and the risk for cardiovascular diseases.

### P423

#### Correlation between glycemia levels and mortality rate in patients with type 2 diabetes and prior myocardial infarction

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It is clear that effective control of blood glucose, hypertension and dyslipidemia, may prevent development of vascular complications in type 2 diabetes (T2DM).

The aim of the present work was to reveal correlation between glycemia levels and mortality rate in T2DM patients (pts) with prior myocardial infarction (PMI).

#### Materials

Totally, 131 T2DM pts with PMI were studied (mean age – 57.2  $\pm$  3.1 yrs.; diabetes duration-6.5  $\pm$  2.8 yrs). Pts were supervised for 6 months. According to their glycemia control pts were divided into 2 groups (Gr.): Gr.1,  $n = 72$  – home-blood glucose monitoring (five-point profiles, 3 profile days/weekly). Oral hypoglycemic agents (OHAs) were administered in 53 pts, and 19 were treated with insulins and OHAs. Gr.2,  $n = 59$  – scarce blood glucose control (2–3 times/monthly); 49 pts were treated with OHAs and 10 – with insulins and OHAs.

## Results

Data at entry revealed that glycemia and  $\beta_{17}$  levels were practically identical for both groups: HbA<sub>1c</sub> (Gr.1-7.9 ± 0.4%; Gr.2-8.1 ± 0.6%,  $P = 0.024$ ), fasting glycemia (FG) - (Gr.1-140.5 ± 39.15 mg/dl; Gr.2-139.3 ± 36.1 mg/dl,  $P = 0.857$ ), postprandial glycemia (PG) - (Gr.1-162.5 ± 32.43 mg/dl; Gr.2-169.1 ± 28.6 mg/dl,  $P = 0.197$ ) There was no statistically evident difference between the groups. Repeated examination at month 6 revealed: HbA<sub>1c</sub> (Gr.1-6.0 ± 0.3%; Gr.2-7.5 ± 0.7%,  $P = 0.000$ ), FG - (Gr.1-101.5 ± 39.15 mg/dl; Gr.2-131.1 ± 17.8 mg/dl,  $P = 0.000$ ), PG - (Gr.1-124.7 ± 20.8 mg/dl; Gr.2-158.1 ± 28.6 mg/dl,  $P = 0.000$ ). Totally, during the 6-month follow-up period, five Gr.1. (6.9%) patients died, all of them having repeated MI. In Gr.2, seven out of 10 deaths (16.9%) were caused by repeated MI.

## Conclusion

T2DM patients with PMI, and PG < 130 mg/dl showed lower mortality rate (6.9%), than those with PG > 150 mg/dl (mortality rate - 16.9%). According to our data 30 mg/dl decrease in PG, 30-40 mg/dl decrease in FG and 1.5% drop in HbA<sub>1c</sub> levels result in 10% decline in mortality rate.

## P424

### Left ventricular geometry and contractile ability in type 2 diabetes and non-diabetic patients with mild hypertension

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#### The aim

To reveal relation between altered left ventricular (LV) geometry (G) and contractile ability (CA) in nonbese type 2 diabetic (DM2) patients with mild hypertension (MH).

#### Materials and methods

BP at rest, 24-h ambulatory BP monitoring and echocardiography were investigated in 70 non-obese males, aged 25-50 yrs. They were divided into the following groups: Gr.1,  $n = 15$  - DM2 and normal BP, fasting C-peptide (C-p, ng/ml) 4.3 + 0.95, HbA<sub>1c</sub> 10.5 + 0.75%; Gr.2,  $n = 10$  - DM2 and latent hypertension,  $\leq 190/110$  mmHg, fasting C-p 4.2 + 0.75, HbA<sub>1c</sub> 11.2 + 0.95%; Gr.3,  $n = 15$  - DM2 with overt MH  $\leq 190/110$  mmHg, fasting C-p 3.95 + 1.1, HbA<sub>1c</sub> 10.7 + 0.83. Gr.4,  $n = 10$  - nonDM pts with latent hypertension, C-p 2.1 + 0.54, HbA<sub>1c</sub> 4.5 + 1.1; Gr.5,  $n = 10$  - nonDM pts with MH, fasting C-p 1.95 + 0.62, HbA<sub>1c</sub> 5.2 + 0.95%; Controls (CG),  $n = 10$  - healthy persons. Thyroid pathology was excluded in all cases. Using LVMI and RWT, LVG was divided into: normal (N) - LVMI < 51 g/ht<sup>2</sup>, RWT < 0.44; concentric remodeling (CR) - LVMI < 51 g/ht<sup>2</sup>, RWT > 0.44; eccentric LV hypertrophy (EH-LLVMI > 51 g/ht<sup>2</sup>, RWT < 0.44), concentric LV hypertrophy- (CH) (LVMI > 51 g/ht<sup>2</sup>, RWT > 0.44).

#### Results

Gr.1 shows EH with EDV 127.0 + 2.1 ml, EF 63.9 + 2.5%, FS 34.3 + 0.9%, that did not differ from CG, ( $P > 0.05$ ) Gr.2 and 3 pts. In addition EH shows tendency to worsening of diastolic (EDV = 199.5 + 3.8 ml, 220.0 + 4.5 ml respectively,  $P < 0.01$ ) and systolic functions (FS 28.0 + 0.9%, EF 53.3  $P < 0.05$ ). Gr.4 pts had CR accompanied with significant (as compared to CG,  $P < 0.01$ ) increase in EDV 210.0 + 2.4 ml, but preserved systolic function EF 65.0 + 2.6%, FS 35.0 + 0.9%. Gr.5 revealed CH with elevated EDV up to 230 + 3.2 ml and insignificant ( $P > 0.5$ ) changes in systolic function (EF 61.3 + 2.5%; FS 32.0 + 0.9%).

#### Conclusion

Thus, EH characteristics of insulin resistance syndrome in DM2 pts make favorable background for disturbing heart diastolic and systolic functions in presence of even moderate hypertension.

## P425

### Effect of lacidipine on left ventricular mass and blood pressure circadian rhythm in hypertensive diabetic patients

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#### Background

Impaired circadian blood pressure (BP) variation is a frequent phenomenon in hypertensive type 2 diabetic (T2DM) patients (pts), and is considered a risk factor for development of cardiovascular complication.

The aim - to assess the effect of Lacidipine on left ventricular mass (LVM) and BP in hypertensive T2DM pts.

## Materials and methods

Totally 32 T2DM pts with mild-to-moderate hypertension (20m/12f, mean age 57 ± 9.6 yrs) were allocated to treatment with 4 mg Lacidipine for 3 months. 24-hour ECG; ambulatory BP monitoring (ABPM); echocardiography; plasma lipid, HbA<sub>1c</sub> and microalbuminuria were performed at baseline and the end of the study.

## Results

Mean 24-hour systolic (SBP) and diastolic (DBP) BP were reduced with Lacidipine (151.1 ± 12.3/91.8 ± 10.9 vs 133.7 ± 11.4/82.1 ± 9.2 mmHg;  $P = 0.000$ ). Lacidipine reduced awake (152.5 ± 14.3/92.6 ± 12.4 vs 134.8 ± 13.8/83.9 ± 9.9 mmHg;  $P = 0.000$ ,  $P = 0.004$  respectively), sleep (140.4 ± 12.7/83.2 ± 9.4 vs 125.8 ± 9.7/75.5 ± 10.3 mmHg,  $P = 0.000$ ;  $P = 0.003$  respectively), early morning (146.6 ± 14.3/90.1 ± 8.7 vs 137.4 ± 12.3/82.3 ± 7.6 mmHg,  $P = 0.008$ ;  $P = 0.000$  respectively) SBP and DBP. According to first ABPM data 19 out of 32 pts were non-dippers. At the end of the treatment 15 pts (78.9%) became dippers. Lacidipine reduced LVM (244.7 ± 27.3 vs 231.8 ± 21.3 g;  $P = 0.039$ ), it was due to LVM reduction in pts with elevated baseline LVM; and frequency of silent myocardial ischemia episodes according to Holter ECG data. Heart rate was not altered. Lipids, HbA<sub>1c</sub>, microalbuminuria did not change significantly.

## Conclusion

Treatment with Lacidipine 4 mg daily for 3 months effectively controls BP in mild-to-moderate hypertensive T2DM pts, reduces 24-hour, awake, sleeping and early morning BP, LVM and frequency of silent myocardial ischemia episodes and does not cause disorders of cardiac rhythm, glucose and lipid metabolisms. Thus, it may be indicated for use in T2DM pts with mild-to-moderate hypertension.

## Endocrine disruptors

### P426

#### Prevalence of the phyto-oestrogen urinary metabolites enterodiol and enterolactone in patients under endocrine investigation

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Phyto-oestrogens are plant-derived compounds that have oestrogenic and/or anti-oestrogenic activity. They are constituents of many human foodstuffs but exposure is on the increase due to increasing availability of 'herbal' type medicines. The current study was initiated following the identification of large concentrations of lignan related phyto-oestrogen metabolites in the urine from a girl under investigation for precocious puberty.

A GC-MS procedure for measuring steroid metabolites was adapted to identify the urinary metabolites enterodiol (END) and enterolactone (ENL). A total of 421 (149 Male; 272 Female) urine samples were received over a one-year period for analyses of steroid and related metabolites as part of the investigation of endocrine-related disorders.

END and/or ENL were detected in the majority of samples (75.6%) collected from patients greater than 5 years of age. In children under the age of 5 years only 32% had detectable concentrations. In babes (<1 yr) no metabolites were detected.

In conclusion we have demonstrated that the phyto-oestrogen metabolites END and ENL are detectable in the urine of many children and adults under investigation for endocrine disorders. More detailed quantitative investigations and comparisons with a healthy population are currently in progress to assess more fully the clinical significance of these findings.

## P427

### Comparative review of primary and secondary empty sella syndrome

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It is known from the literature that primary empty sella syndrome (PES) and secondary empty sella syndrome (SES) are distinguished. Empty sella syndrome is a radiographic finding characterized of a herniation of suprasellar cistern into the sella turcica. SES is a result of influence of surgery or radial therapy on hypothalamic-pituitary area. The aim of our study was a comparison the clinical features of primary and secondary empty sella syndrome.

Fifty four patients (47 females, 7 males, range 15-71 years) at an average age 43.4 ± 11.2 years with PES were studied. The results were compared with earlier published data from 12 patients (females, range 23-42 years) at

an average age  $34 \pm 6.4$  years with SES consequence the treatment by proton beam irradiation among 100 patients with Cushing's disease with mean follow up  $7 \pm 2.5$  years.

Both the patients with PES and those with SES equally frequently had a headache (87% vs 83%) and visual disorders (87% vs 91.7%). Among endocrine abnormalities a hyperprolactinemia (24% vs 25%), hypothyroidism (22% vs 33%), type 2 diabetes (20.4% vs 16.7%) were found in PES group and SES group respectively. The patients with SES, compared to those with PES had a higher frequency of hypocorticism (25% vs 5.6%), disorders of menstrual cycle (41.7% vs 19%). In the group with PES compared to the group with SES more frequently were seen obesity (72% vs 41.7%), cranial diabetes insipidus (11% vs 0%), hypopituitarism (3.7% vs 0%).

In conclusion, we suppose that the common clinical features: headache, visual disorders, hyperprolactinemia observed in patients with PES and SES because of the presence of intracranial hypertension which was revealed in the most of the cases. The symptoms predominated in SES were due to earlier preceded hypercorticism and partially to radial therapy.

## P428

### Adult's primary empty sella

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Primary empty sella (PES) is a frequent radiological entity in general population. The herniation of sub arachnoid space in the sella turcica can induce various visual and/or endocrine disorders. In this work we want to study first symptoms leading to diagnosis, clinical and paraclinical aspects of 36 adults with PES. Diagnosis of PES is made by TDM and/or IRM after exclusion of secondary empty sella. In this study empty sella is considered as complete in 51.5% cases and partial in 48.5%. Analyzed factors are: age, sex, BMI, clinical history, visual exam and pituitary exploration.

#### Results

Diagnosis of PES is made after endocrine disorders in 61%, headache and/or dizziness in 22%, visual disturbances in 11% and rhinorrhea in 2.8%. Our population is composed by 28 females and 8 males with a sex ratio = 3.5. Their mean age =  $41 \pm 9$  years. BMI of our group is  $\geq 25 \text{ Kg/m}^2$  in 61%. Systemic hypertension is objectived in 1/3 of our population, a history of autoimmune disease is found in 22% a benign intra cranial hypertension is noted in one subject = 2.8%. 25% of female cases have 7 or more pregnancies. Clinical and paraclinical explorations are as follow: headaches = 66%, meningitis = 2.8% and visual abnormalities = 21%. Pituitary disorders are: hyperprolactinemia = 16% and pituitary deficits = 46%. Post hypophyse is preserved.

#### Conclusion

Primary empty sella is more frequent in mild age women. Endocrine and/or ophthalmological disorders are the first symptoms leading to diagnosis. Subjects with PES have an overweight in 61%. 1/3 of our group have a systemic hypertension and 22% have a history of autoimmune disease. Visual deficits are objectived in 21% and hypopituitarism in 46%. Rhinorrhea with meningitis is observed in 2.8%.

## P429

### The UV filter benzophenone 2 inactivates human recombinant thyroperoxidase *in vitro* and disturbs thyroid hormone homeostasis in rats

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UV filters are produced in high amounts for multiple uses. Their main application is in sun lotions for skin protection against accelerated ageing or cancer, but they are also found in many other cosmetics or in plastic materials to prevent their radiation-induced damage. Regardless of these protective properties, UV filters seem to interfere with both the reproductive and the thyroid endocrine axis. We here screened for effects of the UV filter benzophenone 2 (BP2) on thyroid hormone biosynthesis and serum levels. Possible inhibition of iodide uptake was examined using the sodium iodide symporter-expressing rat thyroid cell line FRTL-5, but inhibition was not observed. Effects on human thyroperoxidase (TPO) were measured using

extracts prepared from human FTC-238 cells stably transfected with human TPO. BP2 inhibited TPO activity with  $IC_{50}$  values of 0.45 and  $0.37 \mu\text{mol H}_2\text{O}_2$  reduced per min and per mg protein in the guaiacol and the iodide oxidation assay, respectively. The values for the well known TPO inhibitor, genistein, were 61.1 and  $2.06 \mu\text{mol H}_2\text{O}_2 \times \text{min}^{-1} \times \text{mg}^{-1}$ , respectively. BP2 in combination with  $\text{H}_2\text{O}_2$  inactivated TPO, an effect that was prevented by adequate iodide concentrations in the reaction mixture. To examine *in vivo* effects of BP2, adult female ovariectomized rats were treated via gavage for 5 days with olive oil (control) or with 10, 33, 100, 333 and 1000 mg/kg body weight BP2 (12 animals per group). Serum total T4 was reduced and TSH was increased; differences were significant for 333 and 1000 mg/kg BP2. The observed decrease in serum total T3 was not significant. Our data indicate that BP2 interferes with thyroid hormone biosynthesis, thereby disturbing thyroid hormone homeostasis. This may have consequences for human health, especially in the context of a still prevailing iodide deficiency in many parts of the world.- Supported by EU grants.

## P430

### Endocrine disorders in thalassaemia – local experience in an inner city hospital in Birmingham, England

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Endocrinopathies are amongst the most common complications of thalassaemia, which is a hereditary disorder of haemoglobin synthesis and excessive iron deposition is thought to be the main reason.

Our hospital serves a multiethnic population and consequently we see a substantial number of patients with thalassaemia who are screened for endocrine complications. Those found to have endocrine problems are reviewed in our joint thalassaemia-endocrine clinic. Between 2003 and 2005, 14 patients were reviewed in the clinic. 8 were males. Their mean age was 32 years (range 25–43). 12 were of Asian origin, 1 patient was Afro-Caribbean and 1 Caucasian. Their mean ferritin level was 2376 ng/ml (normal range 20–300).

Six patients (42%) had diabetes while 2 patients had impaired glucose tolerance as defined by the WHO criteria. 6 patients (42%) had secondary hypogonadism with suppressed gonadotropins and subnormal testosterone levels. 2 patients (14%) had hypoparathyroidism. 1 patient had primary hypothyroidism. 4 patients (28%) had vitamin D deficiency. 5 patients (35%) had osteoporosis as evidenced by a T score of less than 2.5 by bone mineral density measurement.

The prevalence of endocrine complications is quite high, particularly in our multi-ethnic thalassaemic population. In particular, diabetes management is a big challenge given the ethnicity and the multi-disciplinary input required. Determining the exact prevalence of endocrine dysfunction in patients with thalassaemia is difficult because of the heterogeneity of the population and differences in age of first exposure to chelation therapy. However, the high prevalence of endocrinopathies in thalassaemia makes regular follow up essential for the early detection and appropriate treatment of associated complications. Thus, treatment of various endocrine complications along with improvements in protocols of transfusion regime and chelation therapy should improve the quality of life of these patients.

## P431

### Extracts of black cohosh (*Actaea racemosa*) inhibit growth of MCF-7 and MDA-MB-231 breast cancer cells and dose-dependently inhibit oestrone sulphatase activity

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Extracts of black cohosh (BCE) are frequently used as an alternative to conventional HRT to treat menopausal symptoms, particularly in Germany and the United States. In fact, BCE is the 14th most popular herbal product in the US and in 2003 sales were at least \$15.7 million. Evidence for an increased incidence of breast cancer in long-term users of HRT has reduced its use and may increase the use of alternative treatments. These still require rigorous scientific investigation, not only as to their effectiveness but also their safety. We have investigated the effects of an ethanolic extract of black cohosh (BNO 1055) on the growth of an oestrogen receptor (ER)-positive (MCF-7) and an ER-negative (MDA-MB-231) breast cancer cell line and its effects on enzymes that convert androstenedione (17 $\beta$ -hydroxysteroid dehydrogenase (HSD)/aromatase), oestrone (17 $\beta$ -HSD) and oestrone



sulphate (17 $\beta$ -HSD/sulphatase) to oestradiol. Using conventional culture techniques, cell growth was measured over a 4-day period with Almar Blue. BCE inhibited growth but only at the highest concentrations (50 and 100  $\mu$ g/ml) tested. The activity of the enzymes was measured by culturing cells for 48 h in the presence of BCE and appropriate steroid substrates ( $10^{-7}$ M). The conversion of substrates to oestradiol was highest for oestrone and lowest for oestrone sulphate. BCE was ineffective in inhibiting the conversion of oestrone or androstenedione to oestradiol but a dose-dependent effect of BCE on the conversion of oestrone sulphate to oestradiol in both cell lines was observed with significant inhibition ( $P < 0.05$ ) occurring at 10  $\mu$ g/ml. These results will be compared with the effects of genistein, a major constituent of soy, which is also used widely as an alternative therapy to conventional HRT. The results show that BCE has no stimulatory effects on the growth of breast cancer cell lines and may offer some protection against the local production of oestrogens by inhibiting oestrone sulphatase.

#### P432

##### Androgen-dependent Sertoli cell proliferation as a target for endocrine disruptors relevant to human male reproductive health

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Smoking by women during pregnancy can result in a 30–48% reduction in sperm count and testis size in the exposed offspring in adulthood, probably because of a decrease in the number of Sertoli cells. Until recently Sertoli cell proliferation was thought to be androgen independent because fetal Sertoli cells do not express the androgen receptor, but new evidence suggests that androgens may play the lead role in regulating Sertoli cell proliferation in fetal (Tan *et al.* 2005) and early neonatal life (Atanassova *et al.* 2005). We hypothesize that a unifying mechanism via which fetal exposure to environmental chemicals could affect sperm count in adulthood, is through interference with androgen production/action. To test this hypothesis, we have compared the effects of exposing fetal male rats from E13.5 onwards to 7,12-dimethyl benz[a]anthracene (DMBA) or its metabolite DMBA-DHD (as candidate molecules present in cigarette smoke), or *n*-dibutyl phthalate (DBP; a plasticizer shown to lower fetal testosterone levels), on Sertoli cell number, proliferation, apoptosis and functional development, using a combination of *in vitro* and *in vivo* studies. We have also used the AR antagonist, Flutamide, and tissue from mice, in which ablation of androgen action has been induced by transgenesis (ARKOs).

Any effects that androgens do have on Sertoli cell proliferation/number must be indirect, so our studies are also directed at identifying the pathways via which any such effects could be mediated, such as local production of Insulin-Like Growth Factor 1 (IGF1) which has already been shown to increase Sertoli cell proliferation. We have demonstrated that fetal exposure to DMBA, DBP or Flutamide reduces expression of IGF1 mRNA, although preliminary results have so far shown no change in Sertoli cell number in treated animals and Sertoli cell proliferation indices are still under investigation.

Tan KA *et al.* Endocrinology 2005; 146: 2674–2683.

Atanassova NN *et al.* J Endocrinol 2005; 184: 107–117.

#### P433

##### A randomized, open-label, multicenter study to evaluate octreotide LAR with surgical therapy as primary therapy patients with acromegaly

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This is the first prospective study to compare the efficacy and safety of medical therapy and surgery as primary therapy in acromegaly.

A total of 104 patients with untreated acromegaly were enrolled. Eighty-one patients randomized to receive either octreotide LAR 20 mg ( $n = 40$ ) or surgery ( $n = 41$ ) completed the 48 weeks treatment period, and constituted the population used for this analysis, regardless of response to treatment.

Biochemical response was assessed by changes in mean GH and IGF-I concentrations, determined at weeks 12, 24 and 48. Tumor size was assessed by contrast-enhanced MRI. Octreotide LAR-treated patients responding adequately (control of both GH and IGF-1) to treatment at either of the evaluations continued on the same dose, while partial responders (GH or IGF-1 control) had their dose titrated up to 30 mg at the following visit. Patients treated with surgery demonstrating a complete response at weeks 12 and 24 received no additional treatment, but those with a partial response commenced octreotide LAR.

Treatment success rates after 24 weeks of therapy were 43% and 51% for octreotide LAR and surgery groups, respectively. By week 48, these were 40% and 49%, respectively. At week 28, 49% had switched from surgery to octreotide LAR and 33% from octreotide LAR to surgery. Overall, 73% of patients treated with octreotide LAR had significant (>20%) tumor shrinkage over the 48-week study period, with a mean tumor volume reduction of 35% at the end of treatment.

This large prospective study confirms the effectiveness of octreotide LAR in the primary treatment of acromegaly, but also shows that its efficacy is similar to that of surgery. Given the good compliance, and low complication rate resulting from the medical treatment, primary therapy with octreotide LAR could be considered as first-line treatment for patients with acromegaly.

#### P434

##### Origin of dysgenetic areas in testes of rats exposed to Di *n*-butyl phthalate (DBP) during fetal life: A model of human Testicular Dysgenesis Syndrome

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Disorders of male reproductive health, including testicular cancer, cryptorchidism, hypospadias and low sperm counts, are common and may be increasing in incidence. These conditions manifest at different life stages (low sperm counts and testicular cancer in adulthood; cryptorchidism and hypospadias at birth) but are proposed to originate in fetal life. These disorders have therefore been hypothesized to comprise a 'testicular dysgenesis syndrome' (TDS), which results from dysfunction of the Leydig (LC) and/or Sertoli cells (SC) in the fetal testis and is associated with low testosterone levels.

Fetal exposure of male rats to DBP induces testicular changes similar to TDS in humans, including the formation of focal 'dysgenetic areas' within postnatal testes, surrounded by otherwise normal tubules exhibiting complete spermatogenesis. We hypothesize that these dysgenetic areas arise when SCs (and other cell types) get 'trapped' during the abnormal formation of large LC clusters in fetal life and by postnatal d4 these groups of intermingled cells attempt to form seminiferous tubules. It is likely that the malformed tubules that result correspond to the dysgenetic areas evident in later life.

To test this hypothesis, we evaluated the effect of short-term DBP-exposure from e19.5 to e21.5 (after the seminiferous cords have formed). This treatment regime induces similar testicular changes to longer term (e13.5–e21.5) DBP treatment, including decreased fetal testosterone levels, multinucleated germ cells and LC aggregation, though the latter is less pronounced than after longer term treatment. In contrast to the latter, few if any 'extra-tubular' SCs are found at e21.5. By postnatal day 4 there is little evidence of the large groups of intermingled cells attempting to form tubules, and by adulthood there is no evidence of dysgenetic areas within the DBP-exposed animals. These results provide support for our hypothesis as to the origin of dysgenetic regions within testes of DBP-exposed animals.



## P435

**Endocrine disruptors and idiopathic gynecomastia with aromatase gene polymorphism**IK Czajka, S Zgliczynski & W Zgliczynski  
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Gynecomastia is a benign enlargement of the male breast due to the imbalance between androgens and estrogens in the breast tissue. In up to 40% of cases no evident reasons could be found. This idiopathic gynecomastia may be caused by excess aromatisation within breast tissue. Aromatase is a key enzyme in estrogen biosynthesis. Mutations and polymorphisms of aromatase gene could lead to its overexpression. Some endocrine disruptors – chemicals that interfere with hormone function, could induce aromatase activity. Action of the environmental pollutants in individuals with polymorphism might be a cause of idiopathic gynecomastia.

The aim is to evaluate the association between a (TTTA)<sub>n</sub> repeat polymorphism in intron 4 of aromatase gene and gynecomastia.

DNA was extracted from the blood taken from 30 patients with gynecomastia and 8 men from control group. The specific primers marked with FAM fluorescent stain were added and the region of the CYP19 gene containing the polymorphic TTTA repeats at 174 bp was amplified by PCR. The products were electrophoresed in polyacrylamide gel to verify the PCR reaction and sequenced using the ABI3100 to examine the length of the products.

We found 5 different alleles (TTTA)<sub>n</sub> ( $n = 7, 8, 10, 11, 12$ ) and 3 alleles (TTTA)<sub>n</sub> – 3 bp with a 3 bp deletion just 50 bp upstream of the tetranucleotide repeat sequence. Alleles (TTTA)<sub>11</sub> were more frequent in men with idiopathic gynecomastia than in gynecomastia of other reasons and in control group (25.7%; 19.2% and 12.5% respectively) and (TTTA)<sub>7</sub> – 3 bp were more infrequent (14%, 26.9% and 25% respectively). In few men we observed 3 or 4 products amplified by PCR and we are investigating the reason of that findings now.

This preliminary report indicate the possible importance of (TTTA)<sub>n</sub> repeat aromatase gene polymorphism in gynecomastia. We are evaluating this association by examining more samplings.

## P436

**Loss and resumption of ovarian function after mitotane administration**I Kostoglou-Athanassiou<sup>1</sup>, M Goula<sup>1</sup>, P Athanassiou<sup>2</sup> & Ph Kaldrymidis<sup>1</sup>  
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The adrenolytic agent mitotane [o,p'-DDD or 1,1-(o,p'-dichlorodiphenyl)-2,2-dichloroethane] is a derivative of the insecticide DDT and is used for the treatment of adrenal carcinoma. It induces necrosis and atrophy of the adrenal cortex. Estrogenic action after single exposure but intense antiestrogenic action after repeated exposure of porcine ovarian follicles has been observed *in vitro* (Wojtowicz *et al.* 2004).

The aim of the study was to describe the case of a patient with a personality disorder in whom the loss and resumption of ovarian function was observed after use and discontinuation of the drug mitotane.

A female patient, aged 43 years with a personality disorder, was obese. She decided that Cushing's syndrome might have been the cause of the obesity and took the drug mitotane. A year after treatment the patient developed amenorrhea. FSH and LH levels were 40 and 25 IU/l, respectively, suggestive of the presence of menopause. The patient developed adrenal crisis 1.5 year after the administration of mitotane and was treated with hydrocortisone successfully. Blood cortisol levels were 0.1 µg/dl (normal values 5–25 µg/dl) and ACTH was 1000 pg/ml (normal values 9–52 pg/ml). The diagnosis of adrenal insufficiency was made and hydrocortisone 30 mg daily was administered. Mitotane was discontinued. A year after the discontinuation of mitotane FSH was 2 IU/l, LH 3.4 IU/l and estradiol 250 pg/ml. Menses resumed and the patient has regular menstrual function thereafter suggestive of normal ovarian function. It was not possible to discontinue hydrocortisone.

The extremely rare case of a patient with a personality disorder who took the drug mitotane and experienced adrenal and ovarian insufficiency is described. Discontinuation of mitotane resulted in the resumption of ovarian but not of adrenal function.

## P437

**Estrogen receptor alpha and beta (ERα and -β) gene and protein expression in breast cancer cell lines: verification by RT-PCR and Western blotting**MD Al-Bader, CHJ Ford, J Jacob, LJ Jacob & SS Mohan  
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Two estrogen receptor isoforms are known to exist, ERα and ERβ. The expression of ER isoforms in breast cancer cell lines was studied to see whether both ERs, as well as any other variants, are expressed at the mRNA and protein level. Three breast cell lines: two which are known to be ER + ve (MCF7 and T47D) and one reported as ER-ve (MDA-MB231) were used in this experiment. For gene expression studies RT-PCR methodology was applied; primers were used that detect ERα and ERβ isoforms and their variants. For protein measurements Western Blotting was the method of choice and the antibodies used were: two monoclonal antibodies raised against the steroid binding domain or the hinge region of the ERα, named ERα -S and ERα -H, respectively, and a polyclonal antibody against the ERβ. All cell lines expressed the ERα and ERβ gene and protein isoforms, however, the MDA-MB231 cell line showed a different pattern of expression of the variants whereby some variants were expressed only in this cell line and not in the ER + ve cell lines. Cell lines that have been reported to be positive for ERα (MCF7 and T47D) are also positive for ERβ. Interestingly the cell line which has been reported to be ER-ve showed positivity for both ER isoforms. The presence of ER variants in breast cancer cell lines, created by alternative splicing, where entire exons are skipped, has promoted the hypothesis that tamoxifen resistance and estrogen independent tumor growth could be caused by these variants which may explain why many estrogen receptor positive tumors develop resistance to anti-estrogens such as tamoxifen. We are currently working on the expression of these isoforms and their variants in breast cancer tissue to try and highlight what this means in development, progression and treatment of breast cancer.

## P438

**Challenges associated with the diagnosis, differential diagnosis and treatment of 4 cases of ACTH-dependent Cushing's syndrome due to intermittent hypercortisolism**

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Episodic hypercortisolism has been widely described in Cushing's disease (CD) but is a less commonly recognized presentation of ectopic ACTH syndrome (EAS). We describe 4 cases whose diagnosis and management was complicated by episodic hypercortisolism. These cases highlight diagnostic and therapeutic challenges during the differential diagnosis of CS complicated by intermittent hypercortisolism. All 3 cases of EAS required various combinations of ketoconazole, metyrapone and aminoglutethimide as primary medical therapy for control of hypercortisolism. While EAS is a less common cause of cyclic ACTH-dependent CS, it should be considered in cases with extreme hypercortisolism or hypokalemia. In cases with occult EAS, bilateral adrenalectomy (BAD) may be required due to difficulties in safely achieving long-term eucortisolism with medical therapy.

**Table 1**

	Cases (Age, Y; Gender)			
	64 M Occult EAS	53 F EAS	52 F Occult EAS	52 M CD
UFC (interval; days) nmol/d	202–6889 (19)†	58–7080 (568)*	80–5824 (270)*	72–173 (6)*
Imaging	Persistently negative	1.5 cm pancreatic tumor	Lesion: mediastinum	N/A
Surgery (ACTH staining)	BAD	Distal pan createctomy (+)	Thoracotomy (–); BAD	TSS (+)
Challenge	Differential diagnosis	Episodic hypercortisolism	Extreme hypo/ hypercortisolism	Diagnosis

\*Pre-treatment; † On ketoconazole

**P439**

**Hyperprolactinaemia in a series of adults with craniopharyngiomas and Rathke's cleft cysts: what are the upper limits?**

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**Introduction**

Disruption of the hypothalamic dopaminergic inhibitory control of prolactin (PRL) secretion results in hyperprolactinaemia. We have previously shown in a large series of patients with non-functioning pituitary macroadenomas that serum PRL virtually never exceeds 2000 mU/L in the absence of PRL elevating medications. Current data on the effect of other sellar/parasellar masses are limited.

**Objective**

To investigate the range of PRL values at presentation in adults with craniopharyngiomas (CR) and Rathke's cleft cysts (RCC).

**Patients and methods**

All the patients who presented to our Department between January 1990 and September 2005 with histologically confirmed CR and RCC were studied.

**Results**

Forty-one patients with CR [median age 44 years (range 16–83), 28 males/23 females] and 8 with RCC [median age 45 years (range 23–88), 3 males/5 females] were identified. 2% (1/41) and 25% (2/8) of those with CR and RCC, respectively were on medications reported to raise serum PRL. All lesions were located in the sellar/parasellar area and had a suprasellar component. The median PRL in the total number of patients was 442 mU/L (range 12–6050) without a significant difference between the two groups [CR:median 437 mU/L (range 12–6050), RCC:median 679 (range 178–3731)]. Among those not on the above medications 96% (44/46) had PRL < 2000, 4% (2/46) between 2000–3000 and 0% (0/46) > 3000. The serum PRL of the 3 patients on drugs was 212 mU/L (verapamil/omeprazole), 3731 mU/L (sertraline/oestrogens) and 6050 mU/L (oestrogen implants). The median value of the remaining ones was 440 mU/L (range 16–2902).

**Conclusions**

In this series of patients with CRs or RCCs serum PRL > 3000 mU/L was uncommon; such levels were found only in subjects treated with drugs capable of increasing PRL. Provided concomitant PRL elevating drugs are not being taken, PRL levels in pituitary stalk compression syndrome caused by CRs or RCCs do not exceed 3000 mU/L.

**P440**

**Parathyroid scintigraphy for hyperparathyroidism, an assessment of performance**

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**Aims**

To calculate the sensitivity, specificity and positive predictive value of Parathyroid scintigraphy using Technetium-Sestamibi scan in the localization of parathyroid disease.

**Methods and material**

This is a retrospective medical review of 161 patients who underwent the Parathyroid scintigraphy at our hospital in last six years. True positives were defined as patients with confirmed primary hyperparathyroidism (PHPT) on pathological examination. True negatives were patients with negative biochemical evaluation and normal Parathyroid scintigraphy. False positives were patients with positive scans and no biochemical features of PHPT. False negatives were patients with a negative scan and biochemical hyperparathyroidism with a histological diagnosis of PHPT.

**Results**

The sensitivity of Parathyroid scintigraphy (PS) for precise localization of parathyroid adenoma is 76% with a specificity of 78%. The positive predictive value for Parathyroid scintigraphy (PS) for accurate and unambiguous localization is 84.2%.

**Conclusions**

<sup>99m</sup>Tc-Sestamibi remains a valuable tool for localization of preoperative parathyroid disease. In the present clinical setting it may not be sensitive enough to replace bilateral neck exploration with more limited surgical procedures.

**P441**

**Insulinomas – An experience from a tertiary care institution in South India**

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**Background**

Insulinoma is an uncommon disease. The clinical profile of insulinomas among Indian patients is not well known.

**Aim of the study**

To study the clinical characteristics, biochemical and radiological parameters, and treatment aspects of our adult patients with insulinomas.

**Methodology**

We did a retrospective analysis of 18 patients with insulinoma treated from 1992 to 2004 in our institution. The data was analysed using a 11.0 SPSS software package.

**Results**

The mean age of patients was 43.11 years (+/- 15.24) with a male predominance. The commonest symptom was recurrent loss of consciousness. The mean fasting plasma glucose was 34.22 mg % (+/- 5.82). The mean insulin glucose ratio was 1.67 (+/- 1.08). Two patients had features of MEN I syndrome (11 percent). Fourteen patients underwent surgery and four patients opted for medical treatment. All the tumours that were identifiable histologically were palpable intraoperatively. Histopathology was positive in 10 out of 14 patients. All patients except one were found to have benign lesions on histopathology. The most common site of tumour location was in the head and uncinate process of the pancreas. Preoperative tumour localization was possible in 11 out of 14 (79 percent) patients.

Computerised Tomography had a sensitivity of 62.5% and positive predictive value of 100% in localizing tumours. In 12 patients out of 14 patients who underwent surgery sugars normalized. Two out of four patients who are taking medical treatment are asymptomatic and remaining 2 patients are lost in follow up.

**Conclusions**

In our series, Insulinoma has a male predominance and preoperative localization was 79 percent in the patients who underwent surgery with various imaging techniques.

**P442**

**Biological diagnosis of 63 pheochromocytomas and/or paragangliomas**

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Pheochromocytomas are neuroendocrine tumors able to synthesise catecholamines as well as to metabolise them in metanephrines (NM, M). We assayed Adrenaline, NorAdrenaline, Dopamine and metanephrines in serum (p) and urine (u), and the chromogranin A. We measured 11 parameters (Ap, Au, NAp, NAu, Du, MNp, MNu, Mp, Mpu, 3Metu, CgA). Catecholamines and metanephrines were performed using HPLC with electrochemical detection, and CgA with an immunometric assay. The aim of this study was to evaluate each parameter, to adapt the cut-off levels for diagnosis as well as to evaluate the contribution of the CgA. We studied two groups of patients: group 1 consisted of 63 patients with proven pheochromocytomas. They included 49 pheochromocytomas and 14 paragangliomas. Fourteen patients had genetic pathology (22%) (2 NEM2, 5 VHL, 3 SDHB, 2 SDHC, 2 SDHD). The control group included 71 patients. The control group had false positive results for all the serum assays (between 6 and 24% for NMp). We searched the best sensitivity and specificity with ROC curves. The three best tests were NMu, NMp and CgA with areas under curves respectively to 0.992, 0.990 and 0.988. We then raised the cut-off levels for those parameters for decrease the false positive results. The other parameters were Du < Au < Ap < Mp < Mu < 3Met < Nap < NAu. The areas under curves ranged from 0.689 (Du) to 0.946 (NAu). A significant difference was found between sporadic and hereditary diseases only for Ap ( $P = 0.03$ ), Au ( $P < 0.01$ ), Mp (0.015), Mu ( $P = 0.01$ ). The adrenaline pathway seemed to be less often active in hereditary pheochromocytomas. In conclusion, it's important to refine the cut-off levels for these diagnostic tests to ensure they are adequately sensitive. We have confirmed that the sensitivity and specificity of NM, M and 3Met are relatively better than NA, A and D assays. Urinary tests were more specific for detecting sporadic diseases and NMp, Mp were more sensitive for familial diseases. We have also shown that CgA is a useful test which increased the sensitivity of the metanephrines assays.

**P443****Dedifferentiated papillary thyroid cancer. Use of array CGH in searching for prognostic markers**A Gazdag<sup>1</sup>, G Pocsay<sup>1</sup>, R Pocsay<sup>1</sup>, L Puskas<sup>2</sup>, L Feher<sup>2</sup>, E Tarko<sup>3</sup> & M Tarko<sup>3</sup><sup>1</sup>Pandy K County Hospital, Gyula, Hungary; <sup>2</sup>Biological Research Center, Szeged, Hungary; <sup>3</sup>Borsod Abauj Zemplen County Hospital, Miskolc, Hungary.

Papillary thyroid cancer (PTC) is the most common endocrine malignancy. The survival rate is excellent, however in some cases it transforms to dedifferentiated invasive tumor. Tumor cell invasion and metastasis are major causes of cancer mortality. It is a challenging task to identify critical genes controlling metastatic potential and disease recurrence. We sought molecular prognostic markers that could improve diagnostics and planning therapy.

Comparative genomic hybridisation (CGH) allows identification of changes in relative copy number of DNA sequences (gains and losses) and gives image of the primary genetic changes. We worked with a microarray based CGH method. Paraffin-embedded tissues were used. After amplification of genomic DNA with DOP-PCR the labelled PCR products were hybridized on human cDNA microarrays having 3200 gene specific samples, and scanned. Afterwards, confirmatory real-time PCR was applied.

We analysed the case of a 63-year-old man suffering in PTC. Although treated with thyroidectomy and radioiodine ablation, we lost him in 10 months. Two possible genes were identified which might be related to the unfavourable outcome and could possibly be identified already at the beginning of the disease. These two genes may be used as new prognostic markers. Our results must be confirmed in more cases, and the putative genes have to be analysed.

**P444****Stereotactic conformal radiotherapy in pituitary adenomas – outcome in 36 consecutive patients during years 1996–2004**C Schalin-Jääntti<sup>1</sup>, L Valanne<sup>2</sup>, M Kouri<sup>3</sup> & T Sane<sup>1</sup><sup>1</sup>Division of Endocrinology, Department of Medicine, Helsinki University Hospital, Helsinki, Finland; <sup>2</sup>Department of Neurology, Helsinki University Hospital, Helsinki, Finland; <sup>3</sup>Department of Oncology, Helsinki University Hospital, Helsinki, Finland.**Background**

The use of conventional radiotherapy (CR) in the treatment of pituitary adenomas has been associated with adverse effects such as development of hypopituitarism, cognitive impairment and optic nerve damage. Fractionated stereotactic conformal radiotherapy (FSRT) provides a new treatment regimen and was introduced at our University Hospital in 1996.

**Objective**

To evaluate the effects of FSRT on tumour size, hormonal overproduction and development of hypopituitarism and record other possible side-effects. Methods

Between 1996 and 2004, 36 consecutive patients with pituitary adenomas were treated with FSRT. Of these patients, 21 patients had a non-functioning and 15 patients a functioning adenoma (7 GH-adenomas, 6 prolactinomas, 1 ACTH-adenoma, 1 FSH-adenoma). Thirty patients had been treated surgically 1-3 times before FSRT, the other 6 patients had not been operated before FSRT. Follow-up after FSRT ranged from 12 to 84 months. Clinical evaluation at the end of follow-up included use of a dopamine agonist or octreotide treatment, assessment of pituitary function and hormone replacement therapy and change in tumour size assessed by pituitary MRI. Results

Preliminary tumour size data indicates that FSRT prevents further growth or reduces tumour size in most patients. In one patient with clear tumour growth after FSRT, histology of the pituitary adenoma revealed an atypical adenoma. Hormonal activity decreased in all patients with GH-adenomas but not in all patients with prolactinomas. After FSRT, further impairment of pituitary function was rare. FSRT was associated with allergy to the fixing mask in 1 case and a few patients reported tiredness or temporary hair loss. Conclusions

FSRT seems to efficiently prevent further growth or reduce tumour size in most patients treated. FSRT decreases GH secretion in GH-adenomas. FSRT is safe and well-tolerated.

**P445****Intra-ovarian adrenocortical carcinoma**F Chentli, F Yesli, N Meziani, S Alem, N Kesri & S Fedala  
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Malignant intra ovarian tumor secreting glucocorticoids is extremely rare (<5 cases reported in the world). Our aim is to report the first case observed in our practice. We also try to relate different mechanisms which are discussed in literature to explain intra ovarian position of adrenocortical tissue.

**Case report**

KM, 34 years, female, mother of two children, came for lower limbs edema which began one month after last delivery. Clinical exam found systemic hypertension, Cushing's syndrome and manifestations of diabetes mellitus. On biological exploration there was a metabolic and androgenic hypercorticism (cortisol > 500 ng/ml  $N = 50-250$ , testosterone = 4,5 ng/ml  $N = 0,1 - 1,2$ ) non ACTH dependent (ACTH < 10 pg/ml  $N = 0,46$ ). Adrenocortical glands were normal on echosonography and TDM but there was an ovarian tumor with a size of 14 cm.

Surgical exploration confirmed normality of adrenocortical glands, kidney and liver but there was a big ovarian tumor with peritoneal bleeding and ganglia. Histological exam noted a malignant adrenocortical tumor surrounding by small ovarian tissue with peritoneal and ganglia metastasis. Conclusion

In this observation non ACTH dependent Cushing's syndrome with normal adrenocortical glands argue for ectopic glucocorticoids secretion. Radiological and surgical explorations showed an ovarian localisation which is confirmed by histological findings. Malignancy is proved by peritoneal and ganglia metastasis. This intra-ovarian localisation of adrenal carcinoma is very rare and has a bad prognosis.

**P446****Comparative efficacy of therapy with sandostatin and parlodel in patients with acromegalia**

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Efficacy of the combined therapy of somatostatin analogues and dopamine receptor antagonists in patients with growth hormone (GH) producing hypophysial adenomas was compared. 30 patients with acromegalia aged from 28 to 58 with the disease duration from 2 to 5 years receiving radio-therapy (gamma-therapy, 60Gy) within 2001-2002 were examined. 7 of the examinees (1st group) received sandostatin in the dose of 100mg/day and 23 patients received parlodel in the dose of 10-12.5 mg/day (2nd group) for 3 months. Levels of GH, insulin-like growth factor - 1 (IGF-1) and daily GH secretion rhythm as the major criteria of the disease were measured before and 3 months after the therapy.

The basal values of the above in the 1st group patients were as follows: GH  $47 \pm 2.7$  mUI/ml, mean daily GH  $46 \pm 2.8$  mUI/ml, IGF-1  $1446 \pm 76.8$  ng/ml, in the second group the parameters were  $43.8 \pm 5.7$  mUI/ml,  $45 \pm 2.8$  mUI/ml and  $1393 \pm 56.6$  ng/ml, respectively. After the therapy clinical improvement in the form of headache regression was observed in 5(%), edema reduction and extremities growth termination being registered in 4 (%) patients of the 1st group. Significant stabilization and improvement in hormonal parameters have been noted observed too. Thus, basal GH level was  $20 \pm 2.7$  mUI/ml, mean daily GH level and IGF-1  $14.6 \pm 2.8$  mUI/ml  $789 \pm 23.3$  ng/ml, respectively. In the 2nd group headache regression was registered in 5 (%) patients, edema reduced in 4 (%) and no extremities growth termination was observed. The hormonal parameters were  $354 \pm 3.7$  mUI/ml,  $31.4 \pm 2.5$  mUI/ml and  $1323 \pm 76.8$  ng/ml, respectively. As compared with the dopamine antagonists, sandostatin could be seen significantly efficient in pathogenic therapy within early periods (up to 2 months) of the therapy.

**P447****Pituitary tumors in republic of Croatia**M Vrkljan<sup>1</sup>, A Maric<sup>1</sup>, M Matovinovic<sup>1</sup>, V Cerina<sup>2</sup>, D Herman<sup>1</sup> & HI Pecina<sup>3</sup><sup>1</sup>Center for Clinical Neuroendocrinology and Pituitary Diseases; Department of Endocrinology, Diabetes and Metabolic Disaeses, University Hospital Sestre milosrdnice; University of Zagreb, Zagreb, Croatia;<sup>2</sup>Department of Neurosurgery, University Hospital Sestre milosrdnice; University of Zagreb, Zagreb, Croatia; <sup>3</sup>Department of Radiology, University Hospital Sestre milosrdnice; University of Zagreb, Zagreb, Croatia.

Over the 10 percentage of all intracranial tumors are pituitary tumors. Their malignancy depends on their localisation, meanwhile their biological nature is benign. Regarding the size there are microadenomas (smaller than 10 millimeters) and macroadenomas (10 millimeters or more). Clinical manifestations of pituitary tumors depend on their localisation and hormonal activity. They are either hormonally functional or nonfunctional, respectively.

In our Center for Clinical Neuroendocrinology and Pituitary Diseases, Clinical Hospital, there have been examined and treated 504 patients with pituitary tumors, in the last working 10 years. All patients were analysed by tumor type: 182 patients with prolactinomas, 137 patients with acromegaly, 70 patients with Cushing's disease and 115 patients with non-functional pituitary tumors. Patients came from the whole territory of our Republic. They were separated on the regional base: Mediterranean (coast) and continental region. The aim of our study was to see if the natural conditions and different life style (including food, water, or war events) have any impact on appearance of pituitary tumors. In Mediterranean region were found 127 patients with pituitary tumors. Mb Cushing had 19 (15%), prolactinoma 42 (34%), acromegaly 36 (28%), and nonfunctional tumors had 30 patients (23%). In continental region were found 377 patients (because of the largest number of inhabitants). Cushing had 51 patients (14%), prolactinoma had 140 (37%), acromegaly 101 (27%) and nonfunctional tumor had 85 patients (22%). Our results show there is no statistically significant difference among presentation between these two regions. The natural and life conditions have no impact on appearance of some types of tumors. The incidence of pituitary tumors is very similar to data we found in the literature.

#### P448

##### Histological peculiarities of pituitary adenomas

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The work was initiated to study histological characteristics of various pituitary adenomas (PA). We examined 47 patients with various PA undergoing surgery, 18 men (38.8%) and 29 women (61.7%) among them/ 32 patients of those (68.1%) were operated on transsphenoidally, 15 people (31.9%) and 1 patient (2.1%) undergoing the osteoplastic cranial trepanation and the diagnostic biopsy, respectively. Pre-operational CT/MRT of hypothalamohypophysial system detected infrasellar growth of tumor in 7 (14.9%) patients, endosupraparasellar one with optic nerve compression in 18 (38.3%), endosuprasellar and endoparasellar growth being observed in 14 (29.8%) and 7 (14.9%) patients, respectively, one subject (2.1%) having endosuprasellar tumor growth with the brain cistern compression. In 27 patients the formation was removed completely, in 20 people (42.55%) the resection was partial. Post-operationally growth has been observed to continue in 4 patients (8.5%), the tumor recurring in 3 people (6.4%), secondarily empty Turkish saddle formed in 4 (8.5%). Histological investigation revealed 18 (38.3%) cases of chromophobic adenomas, 13 (27.7%) STH-secreting eosinophilic tumours, 5 patients (10.6%) with PRL-secreting tumours and 2 (4.2%) with the mixed adenomas, 1 subject (2.1%) having basophilic cell tumour. Craniopharyngeomas were found in 3 patients (6.4%), cholesteatoma in 1 (2.1%), cystoadenoma in 2 (4.2%), one chondroma (2.1%) and one meningioma (2.1%) being observed too.

The study showed that eosinophilic cell adenomas secreting both STH and PRL were more aggressive, appearing earlier, growing faster and more frequently invading parasellar structures (14.9%) to hinder removal and recur more often (6.4%). Clinical characterization of the examined patients was of functionally inactive pituitary tumour type with prolactinoma picture in 5 cases, 13 and 2 cases being of acromegaly clinical picture and one of mammosomatotropic adenomas, respectively. Three adolescents had large craniopharyngeomas, growing into adjacent structures and accompanied by physical and sexual retardation.

#### P449

##### Surgical treatment in acromegaly: predictors of outcome

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Transsphenoidal surgery is the treatment of choice in acromegaly. However, some patients do not achieve postoperative remission. The aim of our study was to identify factors that predictive of a poor surgical outcome in patients with acromegaly. In our study we included 39 acromegalic patients, who underwent transsphenoidal surgery as initial treatment. We evaluated clinical, hormonal and radiological predictors based on magnetic resonance imaging (MRI). Also we examined immunohistochemical features of removed pituitary adenomas. Spearman's correlation coefficients showed that young age ( $P = 0.04$ ), visual disturbances ( $P = 0.03$ ), symptoms of hyperprolactinemia ( $P = 0.034$ ), high preoperative basal growth hormone (GH) level ( $P = 0.00005$ ), and intracavernous adenoma extension ( $P = 0.048$ ) were significantly correlated with a poor surgical outcome. We used immunohistochemical staining of removed adenomas for proliferation marker (ki67), angiogenesis index (CD31), marker for malignancy potential (galectin-3), and pituitary hormone prolactin to assess the biological tumor behavior. Ki67 was present in 23% adenomas, CD31 — in 41%, galectin-3 — in 28%, prolactin — in 23%. Preoperative basal GH level was shown to be significantly higher (under Mann-Whitney's test) in patients with positive immunostaining for galectin-3 ( $P = 0.026$ ) and for CD31 ( $P = 0.022$ ) than in patients with negative one. Though we did not find significant correlation between remission rate and these markers. Positive immunostaining for ki67 and prolactin was significantly correlated with MRI-predictors of unsuccessful surgical outcome (large size and intracavernous extension of adenomas). In addition, there were no cases of remission in patients with positive immunostaining for ki67 and prolactin. Summarizing above, evaluation of immunohistochemical predictors of removed adenomas we listed before (ki67, CD31, galectin-3, prolactin) in acromegalic patients gives the information which can determine (in combination with other predictive factors) surgical outcome and post-operative adjunctive therapy for such patients.

#### P450

##### Pituitary mass and hypothyroidism. A case report

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Pituitary enlargement with long standing hypothyroidism is well established.

The clinical evolution of pituitary hyperplasia to thyroid hormone should be monitored closely during primary hypothyroidism because of the rare possibility of an incidental non thyrotropic pituitary tumor that will not regress on thyroid hormone. We report a case of a pituitary mass not responding to thyroid hormone therapy.

A 46 years old woman was admitted for investigation for a pituitary adenoma. The patient presented for evaluation of amenorrhoea galactorrhoea and visual field changes.

Initial biochemical profile repeated twice was indicative of primary hypothyroidism with high levels of serum TSH: 100 mIU/ml (normal range (0.4–4 mIU/ml) with a FreeT4: 5 pmol/l (normal range: 12–24 pmol/l). Antithyroid peroxidase (anti-TPO) were negative.

Prolactin level was 120 ng/ml. Dynamic tests of thyroid function included a TRH stimulation test which led to a strong stimulation of TSH to a peak at 300 mIU/ml.

A pituitary MRI revealed a symmetrical enlargement measuring 22 mm in height suggesting a pituitary hyperplasia. The patient was started on LT4 at a dose of 200 µg/day three months later (TSH: 1 mIU/ml while prolactin level remained high at 60 ng/ml two years later, the patient is euthyroid and still on amenorrhoea despite a normal TSH (0.13 mIU/ml). A pituitary MRI revealed a partial regression with persistence of mass of 19 mm suggesting a pituitary macro adenoma.

In conclusion: in patient with primary hypothyroidism complicated by pituitary hyperplasia, thyroid function tests and pituitary imaging may be unable to differentiate between pituitary tumor and hyperplasia.

#### P451

##### Incidence rate of thyroid cancer in Olsztyn region in the years 1993–2004

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##### Background

The Olsztyn region, in the north-east of Poland, is mainly a rural area with the population of 0.77 mln. After the Chernobyl breakdown the region was



classified as one of the most heavily radioactively contaminated areas of Poland. It was also characterized by mild severity of iodine deficiency and endemic goiter before iodine prophylaxis introduction in 1996/7.

**Aim of the study**

To evaluate the incidence rate (IR), current trend and histotype of thyroid cancer in relation to sex and age of patients.

**Methods**

All the diagnosed cases of thyroid cancer were collected according to the following entry criteria: residence in the study area, histopathological verification according to ICD-10. The incidence rate was calculated as the number of newly diagnosed thyroid cancer cases per 100 000 inhabitants annually. The National Statistical Office was the source of all the demographic data (population, residence, age group).

**Results**

In the study period 566 newly diagnosed cases (476 women average age 48.9 and 90 men average age 50.9) of malignant neoplasms of the thyroid gland were registered. In the age group 0–18 there were 10 patients (6 girls, 4 boys). A significant increase in the incidence rate (IR) of thyroid cancer was observed in the last twelve years from 1.7 in 1993 to 8.37/100 000 for all patients and from 3.1 to 14.8/100 000 in women only. The dominating type was papillary carcinoma – 75.9%.

**Conclusions**

1. Between 1993 and 2004 an increase in the incidence rate of thyroid cancer was observed in Olsztyn region. 2. Papillary thyroid carcinoma was the most common cancer type (75.9%).

## P452

### A typical clinical picture of four insulinoma cases

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**Introduction**

Insulinomas are usually singular adenomas < 1 cm. Less than 5% have diameter > 3 cm. Multiple adenomas frequency is estimated on 3 do 9%. Malignant insulinomas are diagnosed in 3–6% patients. The oldest patient with insulinoma described in literature was 95 years old. About 30–50% of patients are previously misdiagnosed. **The aim of the study** was presentation of patients with rare clinical characteristics of insulinoma.

**Material and Methods**

Four patients, (2F;2M), aged 45–94, mean 59.7 ± 23.2 years were treated in 2003. Analysis of clinical picture and biochemical and visual investigations have been performed.

**Results**

Duration of symptoms varied from 5 to 9 years (mean 7.5 ± 1.7) before diagnosis was established. The mean BMI value for the whole group was estimated as 29.0 kg/m<sup>2</sup>. The lowest glycaemia in particular patients ranged from 18 to 29 mg% (mean 22.0 ± 5.3) and HbA<sub>1c</sub> from 4.0 to 4.2% (mean 4.1 ± 0.1). The serum insulin measured during hypoglycaemia < 45 mg% was 4.3–13, mean 9.0 ± 3.6 µIU/ml (normal range 0); C peptide – 0.78–3.5, mean 1.9 ± 1.3 ng/ml. In 1 pts tumour was detected by CT (Ø – 3 cm), in others by endoscopic ultrasound. 3 of pts were operated, 1 woman (94 y) died from pneumonia. Tumours were solitary in 3 cases, in 1 – double. MEN1 was recognised in none case.

**Summary**

Our study group was consisted of the following patients: 1 patient with tumour with 3 cm in diameter, 1 with double as well as malignant tumour, 1 patient was 94 years old. One patient was previously misdiagnosed and treated for 8 years for an epilepsy.

**Conclusion**

Every patient has been characterized by at least one very rare and unusually occurred clinical feature in comparison with typical clinical picture.

## P453

### What is the best glycaemic index for the diagnostic of insulinoma?

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We present the observation of a patient in whom the diagnostic of insulinoma was difficult in front of normality on the classically used indexes. A 63-year-old patient, without any antecedent or drug used, was

admitted in our service after a hypoglycaemic coma (glycaemia 1.21 mmol/l, insulinemia not measured). Clinical examination was normal. We performed a fasting testing. Results are listed in this Table:

**Table 1**

Time	7 am	1 pm	1 am
Glycaemia (Gly) mmol/L	2.9	2.1	2.50
Insulinemia (Ins) mU/L N (2 17)	4.12	3.29	4.38
Ins/Gly N < 20	10.34	11.2	12.78
Turner index N < 50	18.72	47	29.2

Glycaemia indexes were normal, as well as the initial interpretation of abdominal scan, but, the diagnosis of insulinoma was suspected. So we performed an echo endoscopy which shows a mass of one centimeter in the pancreatic isthmus. Transgastric ponction of this mass shows a neuro-endocrine proliferation cells compatible with an insulinoma. In spite of a treatment by octreotide, hypoglycaemias was frequent and sever. The patient was sent to a surgeon who practiced a enucleo-resection with immediate result on hypoglycaemias. Insulinoma was confirmed by the anathomo-pathologist. The interest of this well-known observation is in one hand to discuss the validity of glycaemia indexes. With the new technics who detect only insulinemia and not pro-insulinemi, they are inadequate and should be left. With the new technics any insulinemia superior to 2 mU/ml concomitant of a glycaemia under 2.2 mmol/l allows the diagnostic. In the other hand to show the interest of transgastric biopsy during echo-endoscopy when evaluating pancreatic mass. Finally the enucleo-resection with coelioscopy which reduces the morbidity.

## P454

### Sustained correction of hypercortisolism with a low dose mitotane regimen in a young woman with PPAD and Carney Complex

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A 27-year-old woman with Carney Complex, with inactivating mutation of the PRKAR1α gene [(a 2-bp deletion of nucleotides T and G at positions 576 and 577 with frame-shift mutation beginning with aminoacid residue threonine 163 (ΔFsterThr163)], bearing a GH secreting microadenoma, Cushing syndrome from PPAD and with high operator risk because of the neurological sequelae of cerebral embolism from atrial myxomas, underwent a low-dose mitotane (MT) regimen.

In the first 12 weeks period, the MT daily dose was progressively increased from 0.5 to 4.0 g/day. This dosage was maintained for additional 16 weeks (cumulative dose 602 g, plasma MT 12 µg/ml) and then stopped because of sustained signs of hypoadrenalism requiring prednisone replacement. Profound decrease of both serum cortisol (from 615 to 220 nmol/l) and urinary free cortisol (UFC) values (from 1498 to 477 nmol/day) was noted after 16 weeks of treatment (cumulative dose 314 g, plasma MT 8 µg/ml). MT treatment was associated with mild gastric discomfort and reversible increase of cholesterol plasma levels. Normal serum cortisol and UFC were still observed 57 weeks after MT was discontinued (plasma MT 0.2 µg/ml), although cortisol bio-rhythm was still abnormal. 60 weeks after discontinuation, mitotane treatment was replaced because of mild increase of serum cortisol and DHEAS levels and onset of oligomenorrea. Now we achieve normal serum cortisol and UFC levels with a MT daily dose of 750 mg, not requiring prednisone replacement, without any side effects.

Our report demonstrates that low dose MT treatment may be a safe and effective modality for a sustained correction of hypercortisolism by PPAD in subjects with CNC with high risk for both adrenalectomy and the consequent trouble from total dependency on corticosteroid substitutive therapy.

## P455

### Hypocorticism after radiosurgery (protonotherapy) in patients with normal MRI and in patients with confirmed tumor for Cushing's disease

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In some patients with Cushing's disease (CD), magnetic resonance imaging (MRI) fails to detect small pituitary ACTH-secreting adenomas despite the results of endocrine evaluation indicating Cushing's disease.

The aim of this paper is to study frequency of hypocorticism after radiosurgery – protonotherapy (PT) for patients with normal MRI for Cushing's disease and for patients with confirmed adenomas.

#### Methods

Between 1997 and 2002, 125 patients with CD underwent PT at our institution: 59 patients with normal MRI (group 1) and 66 patients with confirmed adenomas (group 2). Their results were analyzed retrospectively.

#### Results

Clinical improve occurred in 88.6% pat. of group 1 and in 95.9% pat. of group 2; complete hormonal remission was achieved in 81.1% patients with normal MRI and in 70% pat. with confirmed adenomas. Recurrence after PT was documented only in two patients of group 2. Radiologically-induced hypocorticism have occurred in 20.3% (12/59) pat. of group 1 and in 12.3% (8/66) pat. of group 2 ( $P = 0.33$ ). These data is comparable with results of selective adenomectomy

#### Conclusion

PT can be performed for Cushing's disease with normal MRI. Patients with no histological confirmation of tumor after PT for CD are likely to have a good outcome. The results do not differ significantly from reported hypocorticism rates in patients with confirmed adenomas.

### P456

#### Prognostic value of Ki-67 expression in the cytologic identification of pancreatic endocrine tumors: preliminary data

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#### Background

Organ infiltration and metastasis are the only parameters defining malignancy of pancreatic endocrine tumors. Several studies had demonstrated the prognostic value of Ki-67 expression measured on histological sections obtained from the removed pancreatic endocrine tumors. We postulate that Ki-67 expression measured on fine needle aspiration cytology provides pre-operative indications of the malignancy of pancreatic endocrine tumors.

#### Aim

Aims of this study were: 1) to examine the feasibility of measuring expression of Ki-67 on fine needle aspiration cytology; and 2) to compare the measurements of Ki-67 expression obtained on fine needle aspiration cytology and histological sections from the removed tumor.

#### Methods

We measured Ki-67 expression on endoscopic ultrasonography fine needle aspiration cytology performed for diagnostic purposes and histological sections obtained after surgical removal of the tumor in ten patients with pancreatic endocrine tumors.

#### Results

Adequate aspirate volumes were available on 6 out of 10 patients. Expression of Ki-67 was 20%, 15%, 1%, < 1%, 1–2%, < 1% in cytology and 15%, 19%, < 1%, < 1%, 2%, < 1% in histology, respectively.

#### Conclusions

The results of this pilot study suggest that measurement of Ki-67 expression is feasible on fine needle aspiration cytology and there is a good category agreement of Ki-67 expression between endoscopic ultrasonography fine needle aspiration cytology and histological sections of the tumor. If our results are confirmed in a larger study, Ki-67 expression on cytology may help to discriminate between low and medium/high proliferative tumors and used for prognostic and therapeutic pre-operative evaluation of endocrine pancreatic tumors.

### P457

#### Difficulties in diagnosing persistent acromegaly using current consensus criteria for cure after transsphenoidal surgery

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Criteria to define biochemical remission of acromegaly following surgery have changed over the years, but still the controversy about current consensus exists. We assessed seventy newly diagnosed patients with acromegaly (43 females and 27 males, mean age 46.9 ± 1.4 years, range 17–66 yrs) 8 weeks after transsphenoidal pituitary adenomectomy done by the same neurosurgeon. Current consensus criteria were used to define postoperative remission: glucose-suppressed (nadir) GH less than 1.0 µg/l, and a normal sex- and age-adjusted IGF-I level. GH was assayed by fluoroimmunoassay, and IGF-I by radioimmunoassay. The short-term remission rates in 70 patients as determined by nadir GH, and IGF-I level were 64%, and 56% respectively. The discrepancy was due to a group of patients (No = 6) with discrepant remission criteria who, despite adequate suppression of GH after glucose administration, i.e. the postglucose GH nadir less than 1 µg/l, had repetitively elevated IGF-I.

In conclusion, transsphenoidal adenomectomy is effective and safe initial, and often definitive, treatment for the majority of patients with acromegaly, especially when done by experienced neurosurgeon. Normalization of the IGF-I level seem to be the principal criteria for documenting biochemical remission in acromegaly, because post-glucose nadir GH as measured by fluoroimmunoassay could be misleading. The discrepancies between different consensus criteria confirm the need for their critical reevaluation and further refinement and standardization.

### P458

#### Carbohydrate metabolism in patients with hypercorticism

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#### Aim

To study the carbohydrate metabolism in patients with Itsenko-Kushing's disease (IKD) and Itsenko-Kushing's syndrome (IKS).

#### Materials and methods

Twenty-three patients with hypercorticism were investigated. From which in 8 patients IKD was diagnosed, and in 15 – IKS. In patients with IKD the amount of cortisol was at 8.00–273.3 ± 56.8 ng/ml (N- 50–250 ng/ml); 20.00–343.3 ± 43.8 ng/ml (N- 50–250 ng/ml); the value of adrenocorticotrophic hormone was – 89.01 ± 7.3 pg/ml (N- 4.7–41.0 pg/ml). In patients with IKS the amount of cortisol was at 8.00–246.3 ± 23.8 ng/ml (N- 50–250 ng/ml); 20.00–287.7 ± 32.5 ng/ml (N- 50–250 ng/ml); the value of adrenocorticotrophic hormone was – 3.6 ± 2.7 pg/ml (N- 4.7–41.0 pg/ml). For the study of the carbohydrate metabolism we determined the fasting glucose and glycosylated hemoglobin (HbA<sub>1c</sub>).

#### Results

In patients with IKD the level of HbA<sub>1c</sub> was > 7.5 mcm/frghb (N- 3.5–7.5), and glucose ranged in – 120–150 mg/dl in 3 patients. In patients with IKS the HbA<sub>1c</sub> was > 7.5 mcm/frghb in 9 patients, and the glucose – > 120 mg/dl in 12 patients. Against a background of the treatment of the primary disease the carbohydrate metabolism was normalized in 2 patients with IKD and in 10 patients with IKS. In the rest of the patients we observed the type 2 diabetes.

#### Conclusions

About the state of the carbohydrate metabolism we have to judge not only by the determination of the fasting glucose, but also by HbA<sub>1c</sub> (every 3 month). The normalization of the carbohydrate metabolism is related with hypercorticism normalization.

### P459

#### Role of family history for diabetes mellitus in determining insulin resistance in acromegalic patients

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#### Objective

Acromegaly is a rare pituitary disease due to excessive secretion of GH. Insulin resistance, impaired glucose tolerance (IGT) and diabetes mellitus (DM) are common features in acromegaly. Seventy-four active acromegalic patients were retrospectively evaluated in order to determine the impact of family history for diabetes mellitus on glucose tolerance, insulin resistance and beta-cell function.

#### Patients and methods

We studied 74 patients with active acromegaly (mean IGF-1 value: 576.8 ± 35.6 ng/ml ES; mean GH value on OGTT nadir 11.8 ± 1.8 ng/ml

ES), 29 males and 45 females, aged  $50.4 \pm 14.1$  years, body mass index (BMI)  $28.1 \pm 4.3$  (kg/m<sup>2</sup>). All patients underwent an oral glucose tolerance test (OGTT): GH, blood glucose and insulin were sampled at 0, 30, 60, 90, 120 minutes. Insulin resistance was evaluated with HOMA2-IR, Quicki and ISI<sub>0,120</sub>; beta cell function was evaluated with HOMA2%-β index.

#### Results

Acromegalic patients with a positive family history for diabetes mellitus showed a higher BMI ( $P < 0.05$ ), an higher fasting insulin ( $P < 0.05$ ); insulin evaluated at 90' ( $P < 0.005$ ) and 120' ( $P < 0.005$ ); and an higher blood glucose levels evaluated at 120' ( $P < 0.05$ ) respect to patients with negative family history. No difference was found about GH and fasting blood glucose levels. Moreover patients with positive family history were more insulin resistant showing an higher HOMA2-IR ( $P < 0.05$ ) and Quicki Index ( $P < 0.05$ ), a lower ISI<sub>0,120</sub> ( $P < 0.005$ ). No difference was found about HOMA beta-cell function (HOMA-%β). Acromegalic patients with diabetes mellitus (n.7) had an higher ( $P < 0.05$ ) prevalence of positive family history (71.4%) compared with patients (n.44) with normal glucose tolerance (29.5%).

#### Conclusions

According to the present study, we found that family history for diabetes mellitus may have a role as an independent risk factor in promoting insulin resistance and diabetes mellitus in acromegalic patients.

### P460

#### Familial acromegaly

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Acromegaly is usually regarded as a disease which occurs sporadically. Familial occurrence of acromegaly due to pituitary adenoma without any other endocrinopathy in a family is extremely rare. These patients should be evaluated for multiple endocrine neoplasia type 1 (MEN 1) and Carney complex. We report here two cases of acromegaly due to pituitary macroadenomas. Subject 1 is a 50-year-old woman and subject 2 is her 23-year-old son with elevated growth hormone (GH) levels during the oral glucose tolerance test (OGTT) and pituitary macroadenomas in Magnetic Resonance Imaging (MRI). Subject 1 was diagnosed nine years ago and had underwent transphenoidal extirpation. But she was lost to follow up after the operation and she had not received any medical treatment. During her evaluation at the surgery unit for toxic multinodular goitre it was noticed that her acromegaly symptoms were persisting, and she had recurrent pituitary macroadenoma in MRI. Transphenoidal reoperation was not successful and medical treatment was applied. She also underwent for thyroid operation after euthyroidism was achieved and pathological examination was compatible with papillary carcinoma of follicular variant. Subject 2 was diagnosed as acromegaly coincidentally when he was visiting his mother. He had high GH levels during OGTT and MRI revealed a macroadenoma of  $10 \times 15$  mm. He was also treated with transphenoidal surgery. Both of the patients and the unaffected siblings underwent extensive investigation and no features of MEN 1 or Carney complex were found. There was no history of consanguinity. We conclude that isolated familial acromegaly is an exceptional clinical entity, it may be more common than it has been realised. Genetic studies are needed to identify the responsible genes for this distinct syndrome.

### P461

#### Role of complex Cdk4/Cyclin D1 in somatostatin subtype 2 receptor-mediated inhibition of cell proliferation of a medullary thyroid carcinoma cell line *in vitro*

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Somatostatin (SRIH) inhibits cell proliferation by interacting with five distinct SRIH receptor subtypes (SSTR) by several pathways in many tissues. We previously demonstrated that SRIH, by activating SHP-1, inhibits cell proliferation of the human Medullary Thyroid Carcinoma (MTC) cell line, TT, which expresses all SSTR. However, the effects of SRIH on cell cycle proteins have not been investigated, so far. We therefore investigated the effects of SRIH and of a selective SSTR2 agonist on cell cycle protein expression, mainly focusing on Cyclin D1 and its associated

kinases. TT cells were serum starved for 48 h and then treated with SRIH or with a selective SSTR2 agonist, BIM-23120 for up to 60 h. Cell proliferation was verified by a colorimetric assay and by [<sup>3</sup>H]thymidine incorporation. Moreover, cell cycle progression was studied by cytofluorimetry. Cell cycle protein expression at different time points was investigated by Western blot. Cyclin D1 expression was also investigated by quantitative RT-PCR.

Our data show that SRIH and the selective SSTR2 agonist, BIM-23120, reduce cell proliferation and DNA synthesis, as well as induce a delay of the cell cycle in G2/M phase. Moreover, treatment with SRIH or with BIM-23120 decreases Cyclin D1 and cdk4 protein levels, with a parallel reduction in Rb phosphorylation levels at Ser-780. These data indicate that the subtype 2 receptor-mediated antiproliferative effect of SRIH on TT cell proliferation may be exerted through a decrease in Cyclin D1 levels, and further underline the importance of SSTR2 in mediating the antiproliferative effects of SRIH, indicating a direct and strong effect on Cyclin D1-cdk4 complex

### P462

#### Selective cyclo-oxygenase 2 inhibitors revert chemoresistance in medullary thyroid carcinoma by a mechanism mediated by P-gp and PGH<sub>2</sub>

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Medullary thyroid carcinoma (MTC) is a malignant tumour deriving from parafollicular C cells, with a highly chemoresistant phenotype. Failure of medical therapy has been ascribed, at least in part, to multi drug resistance (MDR1) gene over-expression. MDR1 encodes for a transmembrane glycoprotein, P-gp, which hampers intracellular accumulation of cytotoxic drugs. It has been demonstrated that P-gp expression and function depend on cyclo-oxygenase 2 (COX-2) expression, which is elevated in many human tumours. We have previously demonstrated that a human MTC cell line, TT, expresses MDR1 and COX-2. In this model, selective COX-2 inhibitors, such as Rofecoxib and NS-398, sensitize TT cells to the cytotoxic effects of doxorubicin (a well known chemotherapeutic drug), reducing P-gp expression and function. The aim of our study was to verify which prostaglandin mediate the effect of COX-2 on TT cells. TT cell were therefore treated with a selective COX-2 inhibitor (NS-398) with or without two COX-2 products, PGE<sub>2</sub> and PGH<sub>2</sub>. P-gp expression was then evaluated by Western blot. P-gp levels were reduced by treatment with NS-398, while PGE<sub>2</sub> and PGH<sub>2</sub> did not significantly modify protein expression. Combined treatment with NS-398 and PGE<sub>2</sub> did not restore P-gp expression, while combined treatment with NS-398 and PGH<sub>2</sub> restored P-gp protein to normal levels.

Our data suggest that the effects of COX-2 inhibitors on P-gp expression are mediated by PGH<sub>2</sub>, an intermediate product in prostaglandin synthesis, and not by PGE<sub>2</sub>, a final product. Moreover, these results confirm the hypothesis that selective COX-2 inhibitors might be employed to inhibit P-gp expression and function, reducing chemoresistance in MTC.

### P463

#### An intrasellar germinoma with normal cerebrospinal fluid β-HCG concentration misdiagnosed as hypophysitis

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Intracranial germinomas are malignant neoplasms which are supposed to arise from primitive germ cells failed to migrate to the genital crest during embryonic development. Most of them are located in the intra-suprasellar region and may cause anterior and particularly posterior pituitary hormone deficits. Early establishment of the histological diagnosis is important for optimum treatment planning and a successful outcome. Intracranial germinomas are radiosensitive and potentially curable. In this study we



report a case of an intrasellar germinomas leading to infundibular thickening with lymphohistiocytic reaction and normal  $\beta$ -HCG concentrations in the cerebrospinal fluid (CSF).

#### P464

##### Response to medical treatment in male macroprolactinomas

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Male prolactinomas are rare and considered as bad responders to medical treatment compared to female cases. Our aim was to evaluate response of pituitary macroleions secreting prolactin (PRL) to dopamine agonists. Our population was composed of 34 men (mean age = 36.6 years) with a diagnosis of macroprolactinomas: mean tumor size = 35.5 mm (12–118) and mean PRL = 2505 ng/ml (132–19996). Bromocriptine was used with a mean dose of 22.94 mg/day (3.75–80) and a mean duration of = 48.5 months (1–240). After medical treatment our patients underwent a reevaluation of clinical symptoms, hormonal exploration, ophthalmological exam, CT scan and/or MRI.

Our results are: treatment was considered as irregular in 57%. Side effects were present in 45% (hypotension and / or gastro intestinal troubles). Headaches disappeared totally in 88% and sexual complaints improved in 34%. Mean PRL after Bromocriptine was significantly reduced = 268 ng/ml vs 2505,  $P < 0.01$ ; lactotropic hormone was totally normalized in 58.8% and Bromocriptine was inefficient on PRL secretion in 5.9%. Tumor size was significantly reduced: 17.9 mm vs 35.5,  $P < 0.001$ . For individual responses: pituitary process disappeared totally in 22%, was reduced in 59.5%; tumor size was not modified in 14.8% and increased in 3.7%. Testosterone was normalized in 50% and others anterior pituitary deficits were improved in some subjects. Post pituitary function was normalized in one from two subjects with diabetes insipidus. Ophthalmological disorders disappeared in 55%.

##### Conclusion

In spite of irregular treatment and side effects response to Bromocriptine in male macroprolactinomas seems good because PRL was normalized or has decreased in 94% and tumor size decreased or disappeared in more than 80%.

#### P465

##### Non functioning pituitary tumors

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Non functioning pituitary adenomas (NFPA) are considered as rare (about 20% of all tumors of pituitary area) but severe regarding to their complications.

In this retrospective study we have analyzed 60 subjects with NFPA in order to study clinical, hormonal, visual and radiological abnormalities.

Diagnosis of NFPA is made when there is a pituitary tumor demonstrated by CT-scan and or MRI with normal or discreetly elevated PRL ( $\leq 120$  ng/ml) but without hypercortisolic or hypersomatotropic signs, negative immunohistochemistry and no response to dopaminergic agonists (for those with elevated PRL).

##### Results

Our population is composed of 26 males and 34 females (sex ratio = 1,30 vs 1,4 in literature). Mean age at diagnosis is  $45,6 \pm 11$  years (range 21 – 74). These patients came to our unit for headaches and or visual disturbances and rarely for gonadal abnormalities.

There is a diagnosis retardation of  $2 \pm 1$  years (1 month to 19 years) regarding to first ophthalmological symptoms.

For clinical signs pituitary insufficiency is in the first position. There is no diabetes insipidus. Neurological and or psychiatric disorders are observed in 17% (10/60). Hormonal results show a total or partial ( $\geq 2$ ) pituitary deficits in 75% (45/60), high prolactin in 29%. Visual abnormalities are seen in 75% with blindness in 20%. For radiological results we have 5 microadenomas ( $< 10$  mm) = 8% and 55 macroadenomas ( $\geq 10$  mm) = 92%. Among macroprocessus 22% are giant ( $\geq 4$  cm).

##### Conclusion

In this study NFPA are observed with equal frequency in men and women. 92% are macro or giant adenomas, so they have many complications as neurological or psychiatric troubles (17%), pituitary deficits (75%) and visual damages (75%). Our results agree with those reported in literature.

#### P466

##### Diabetic retinopathy in acromegaly

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The GH/IGF axis has been implicated in the pathogenesis of retinopathy in diabetic subjects, the aim of this study is to evaluate the prevalence of diabetic retinopathy in hypersomatotropic patients.

##### Subjects and methods

Twenty four hypersomatotropic patients (12 females, 12 males mean age = 49 years, mean hypersomatotropic duration = 11 years) have diabetes for 7,25 years in average. To search for diabetic retinopathy a funduscopy was performed in all patients.

##### Results

Among the 24 patients, only four have retinopathy (prevalence = 16,6%) at an early stage in all cases (background retinopathy). There is no correlation between the prevalence of retinopathy and GH levels or hypersomatotropic duration nor was a correlation with diabetes duration, in contrast there is a significant correlation with glycemic control.

##### Conclusion

We conclude from this study that in acromegalic diabetic patients only glycemic control has an effect on the occurrence of retinopathy and that growth hormone seems not to have a direct effect on retinopathy pathogenesis.

#### P467

##### Malignant catecholamine-secreting paragangliomas of retrocardial localization

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We present a case of 32-year old male patient who was admitted to hospital because of newly discovered hypertension with symptoms of 4P (pain, pallor, perspiration and palpitation). During hospitalization the patient presented with polyuria, polydipsia, hyperglycemia and psychotic disorders. The suspicion of secondary hypertension was made.

Catecholamine examination test was done and revealed very high levels of adrenalin (five times more than normal), noradrenalin (20 times more than normal), vanillylmandelic acid (VMA) 2.5 times more than normal and metanephrines (10 times more than normal). The abdominal ultrasound was normal without suspicion of adrenal mass as well as abdominal CT and magnetic resonance imaging (MRI). Lung X-ray was normal. Radionuclide scintiscan after administration of the radiopharmaceutical (131-I) metaiodobenzylguanidine (MIBG), 37 mBq was done. There was pathologic accumulation of radioisotope in distal part of mediastinum. CT of thorax showed unusual deformation of left atrium. MRI of thorax discovered tumor,  $6 \times 5,5 \times 5$  cm in diameter, paravertebrally localized at the level of 9<sup>th</sup> thoracic vertebrae which was in contact to the left atrium.

Patient was treated with phenoxybenzamine  $3 \times 60$  mg orally and propranolol  $3 \times 40$  mg orally 14 days prior to surgery. He was transferred to the department of cardiac surgery. At the surgery, retrocardial extirpation of pheochromocytoma was done with plastic "patch" of left atrium.

Six months after surgery, metanephrines as well as vanillylmandelic acid were normal. In 2 years follow-up patient's condition is excellent, without hypertension.

#### P468

##### Short-term evaluation of quality of life in acromegalic patients

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Active acromegaly is associated with significant comorbidity and reduced quality of life. Cross-sectional studies have shown that quality of life did not significantly change in long-term cured acromegalic patients. However, the effect of medical treatment on the quality of life in the short term is unknown.

We evaluated the quality of life by a disease-specific questionnaire in a longitudinal study before and after a 6-month course with somatostatin



analogs (SMSa) in 20 acromegalic patients (11 male and 9 female, mean age  $48 \pm 13$  yr).

We evaluated various physical and mental aspects of quality of life by the disease-specific questionnaire, Acromegaly-Quality of Life (ACRO-QOL). The questionnaire comprises two different scales: a physical performance scale and a psychological well-being scale. The psychological well-being scale is further subdivided into appearance and personal relations subscales. Parameters are expressed as percentage, from 0 (very bad) to 100 (very good).

Adjustment of SMSa dosage was done every three months, when necessary.

The ACRO-QOL total score was  $54.7 \pm 23.0$  before and  $62.2 \pm 19.0$  after a SMSa treatment ( $P < 0.03$ ). Items of the ACRO-QOL ranged from  $54.8 \pm 22.2$  to  $63.3 \pm 18.7$  on the psychological well-being scale and from  $36.6 \pm 24.3$  to  $53.5 \pm 21.5$  on the appearance subscale. These scores significantly improved after a 6-month course with SMSa therapy ( $P = 0.007$  and  $P = 0.001$  respectively). On the contrary, the physical performance scale and the personal relation subscale did not change after SMSa treatment.

In conclusion, these data suggest that a significant improvement of the quality of life occurs in acromegalic patients after short-term SMSa therapy.

#### P469

##### Octreotide as alternative to surgically adrenalectomy

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Thirty-five year old female patient with bilateral pheochromocytoma and operated medullary carcinoma of thyroid gland (MEN II B sy.) is put in the octreotide therapy as she doesn't accept operation as a therapy because of religious reasons (transfusion of blood) and attacks of artery hypertension couldn't be control with alfa-adrenergic receptors antagonists.

12 MONTH 2004

ADRENALIN-urine 890 nmoldU/L (10.2–65.5); NORADRENALIN-urine  $> 5000$  nmoldU/L (32.5–271.9); VMA  $> 1000$  mcoldU/L (15.6–31.8); CALCITONIN 310 pg/mL ( $< 13$ ); CHROMOGRANIN A 1635 ng/mL ( $< 60$ ); NMR scan: right suprarenal gland  $4 \times 3$  cm; left suprarenal gland  $11 \times 9$  cm.

Artery pressure RR 200/150 mmHg.

09 MONTH 2005

ADRENALIN-urine 651 nmoldU/L (10.2–65.5); NORADRENALIN-urine 760 nmoldU/L (32.5–271.9); VMA 410 mcoldU/L (15.6–31.8); CALCITONIN 292 pg/mL ( $< 13$ ); CHROMOGRANIN A 1307 ng/mL ( $< 60$ ); NMR scan: right suprarenal gland  $4 \times 2$  cm; left suprarenal gland  $11 \times 7$  cm. Artery pressure RR 130/90 mmHg.

In comparison with test results from 12 month 2004 is evident decrease of chatecholamine, calcitonin and chromogranin A secretion with decrease size of bilateral adrenal tumors. Artery pressure is satisfactory regulated. The octreotide therapy is acceptably alternative to surgically treatment in cases where the operation is not possible.

#### P470

##### Inhibition of IGF-II signal transduction improves chemosensitivity in human adrenocortical cancer cells

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Treatment of adrenocortical carcinoma (ACC) is still unsatisfactory. As IGF-II and IGF-I receptor are frequently overexpressed in human ACC and inhibitors of the IGF system are currently under preclinical investigation, interference with IGF-signaling might have an additive effect in antitumour treatment. We have therefore analyzed several cytotoxic agents (etoposide, doxorubicine, cisplatin, streptocozin;  $0.01$ – $100$   $\mu$ M) on adrenal cell proliferation *in vitro* and further investigated, whether inhibition of IGF-II signaling or downstream inhibition of mTOR via rapamycin affects chemotherapy response in the human adrenocortical cancer cell line NCI-h295 which is known to overexpress IGF-II. IGF-signaling was inhibited by specific antibodies against IGF-I receptor or IGF-II or by the small molecule IGF-I receptor antagonist H-1356. Etoposide, doxorubicine and cisplatin inhibited cell proliferation with IC50 values of  $1.2$   $\mu$ M,  $11$   $\mu$ M and  $9.6$   $\mu$ M, respectively, whereas streptocozin was inactive in NCI-h295 cells.

Inhibition of IGF-signaling reduced adrenal cell proliferation and significantly increased response to the chemotherapeutic agents at cytostatic doses within therapeutic ranges (e.g. inhibition by cisplatin, etoposide or doxorubicine  $10$   $\mu$ M alone or in combination with anti IGF-II  $10$   $\mu$ g/ml were  $61\%$  vs  $49\%$ ;  $60\%$  vs  $50\%$  or  $48\%$  vs  $44\%$  compared to controls, respectively;  $P < 0.01$ ). Rapamycin only slightly inhibited proliferative activity by  $10.5 \pm 5\%$  at  $0.2$   $\mu$ M. In adrenocortical cancer cells, IGF-II appears to be a protumorigenic growth factor reducing susceptibility to apoptosis and chemotherapeutic treatment. Interference with IGF-II activity may improve response of ACC to chemotherapeutic agents. However, effects detected in NCI-h295 cells after blockade of the IGF system were clearly weaker than the effects described in other non-adrenocortical cancer cells and the clinical relevance *in vivo* remains to be elucidated.

#### P471

##### The new TNM classification is inferior to the Lee classification in predicting outcome in patients with adrenocortical carcinoma

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##### Objectives

The TNM classification is a worldwide benchmark for reporting the extent of malignant disease and is intended as a prognostic tool to predict the outcome in patients with cancer. Until 2004, no TNM classification was available for adrenocortical carcinoma (ACC) and different staging systems were used. Due to the rarity of this malignancy, the prognostic value of different staging systems has never been compared directly in a large series of patients.

##### Methods

We compared the new WHO stages based on a new TNM classification published in 2004 with the 2 most commonly used staging systems (Sullivan and Lee) using the German ACC Registry consisting of 256 patients (follow-up completeness index 92%). The WHO classification, which is close to the Sullivan system, differs from Lee in two major points: 1) any invasion into the surrounding tissue results in stage III, whereas Lee III indicates tumors with positive lymph nodes or invasion in neighboring organs or into the renal vein or ICV. 2) WHO IV includes both tumors that involve adjacent organs or metastatic tumors, whereas Lee IV is reserved for patients with distant metastases.

##### Results

Survival as assessed by Kaplan-Meier analysis differed significantly between all four Lee stages ( $P < 0.05$ ), whereas in the WHO and Sullivan systems survival in stage II was not significantly different from survival in stage III. 21/101 patients stage Lee II were classified as WHO III and 9/85 WHO II as Lee III leading to a trend towards improved survival in WHO III (5-year survival:  $46\%$  vs  $33\%$  in Lee III), whereas survival in stage II was comparable at  $54\%$ . Of note, tumor(thrombus) in the ICV was strongly associated with tumor recurrence in our series, but is not a criterion for WHO III. Due to the stricter criteria in Lee only 67 patients were classified as Lee IV but 80 as WHO IV leading to an estimated 5-year survival in Lee IV of  $11\%$  in contrast to  $17\%$  in WHO IV.

##### Conclusion

As the major objective of staging classifications is to predict the outcome of patients our data indicate the need for revision of the new WHO system.

#### P472

##### Stimulated and spontaneous growth hormone release in irradiated acromegalic patients

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Growth hormone deficiency frequently occurs as consequence of radiation therapy in pituitary disorders. However, it not known whether radiation therapy in acromegaly results in comparable diminution of GH secretion. We therefore hypothesized that stimulated and spontaneous GH release is ultimately indistinguishable in irradiated acromegalic patients and irradiated patients with other pituitary disorders. For this study 10 acromegalic patients and 10 patients with other pituitary disorders (8 patients with NFA and 2 with Cushing's disease) were investigated. Mean time after radiation treatment was 17 and 18.7 years, respectively. Stimulated GH was evaluated by insulin tolerance test (ITT), GHRH, GHRH-arginine and arginine tests and spontaneous GH by measuring blood samples obtained at 10 min intervals during 24 h. The 24 h profiles were analyzed with Cluster and approximate entropy. No group differences in stimulated GH concentrations in any test were found. Maximal GH concentration by ITT was below 3 µg/L in all patients, compatible with severe GH deficiency. Spontaneous GH secretion was similar in both patient groups, including basal release, pulsatility and regularity. Pulsatile secretion was lost in two acromegalic patients and in 3 patients of the control group. IGF-I was below 2 SD-score in 9 patients of either group. We conclude that irradiation in acromegaly finally leads to loss of normal GH response to various stimuli and to diminished spontaneous release. The stimulated and spontaneous GH patterns are indistinguishable from those of GH deficiency patients encountered in other irradiated pituitary disorders.

#### P473

##### WNT4 expression in normal human adrenals and adrenocortical tumours

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Members of the secreted WNT glycoprotein family are important in embryogenesis and adult tissue homeostasis, and defects in the signal transduction of several WNT ligands including WNT4 have been linked to a variety of clinical conditions. Deletion of Wnt4 gene in mice leads to improper development of many organs including the adrenals in which the zona glomerulosa forms imperfectly. The objective of this study was to investigate the expression of WNT4 in human adult and fetal adrenals and adrenocortical tumours by immunohistochemistry and quantitative real-time RT-PCR. The effects of ACTH and angiotensin II on WNT4 mRNA expression were evaluated by using primary adrenal cell cultures. Immunohistochemistry of normal adult adrenal revealed WNT4 expression in the medulla and to a lesser extent in all adrenocortical zones, the staining being strongest in the zona glomerulosa. The mRNA expression was significantly lower in fetal adrenals as well as in virilizing carcinomas and Cushing's adenomas ( $P < 0.05$ ), and higher in Conn's adenomas ( $P < 0.01$ ) compared with normal adult adrenals. ACTH induced significantly WNT4 expression after 24 and 48 hours while angiotensin II had no effect on the expression. These data show that WNT4 is expressed in human adrenals and the WNT4 mRNA expression levels are inconstant during the development of the adrenals and in different adrenocortical tumours. The expression is regulated long term by ACTH but not by angiotensin II. The results suggest a possible role for WNT4 in human adrenal function and pathophysiology.

#### P474

**The clinical particularities, medical possibility of prolactinomas in men**  
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The aim of the present investigation was to study first signs of disease, effectiveness of the cabergoline treatment for prolactinomas in men. We studied 53 patients with pituitary adenomas. Patients are divided into 2 groups depending on size of the tumor: group I – microadenomas (11), group II – macroadenomas (42). PRL level before treatment in group I on average was  $5890 \pm 7.4$  mU/L; in group II –  $29790 \pm 18.2$  mU/L. Testosterone level in group I was  $9.45 \pm 0.6$  nmol/L; in group II –  $6.25 \pm 0.2$  nmol/L. From 35 patients studied sexual dysfunction and libido reduction (66%), 14 had visual disturbances (27%), 4 had gynecomastia (7%). PRL concentration, cerebrum magnetic resonance imaging and ophthalmological study was defined for all patients during investigation. All patients received in medium dose 2.5 mg per week during 6 months.

##### Results

PRL level significantly reduced in group I to  $437 \pm 8.4$  mU/L ( $P < 0.001$ ), in group II to  $790 \pm 6.3$  mU/L ( $P < 0.005$ ). Testosterone level significantly increased in group I to  $19.8 \pm 0.04$  nmol/L ( $P < 0.002$ ), in group II to  $16.6 \pm 0.08$  nmol/L ( $P < 0.001$ ). Positive dynamics of the tumors volume is noted in 10 patients in group I (91%): 8 men had reduction of the size of adenoma (more than 30%), and adenoma has disappeared in 2 men. No dynamics observed in 1 man (9%). Positive dynamics of the tumors volume is noted at 38 patients in II group (90.4%): adenoma has reduced in 35 men, and disappeared in 3 men. No dynamics observed in 5 men (9.4%). All 35 patients with sexual dysfunction and libido reduction had libido reinforcement, erectile function is normalized with positive effect on sexual life.

##### Conclusion

The first clinical manifestations of prolactinomas in men are the disturbances of sexual function. Cabergoline is the most effective drug (of available in present) for the treatment of prolactinomas: the individual dose normalizes PRL level in 100% of events, in 82% of them it promotes the reduction of the sizes of tumors up to its disappearances.

#### P475

##### Management of thyroid cancer in children

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The purpose of the study was to assess the peculiarities of diagnostic and treatment of thyroid cancer in children.

##### Material and method

Between 2000 – 2005 we screened 234 patients age 3–18 years that presented for various complaints suggesting possible thyroid disease (thyroid enlargement, dysphonia, dysphagia, delayed growth). They were submitted to clinical examination, ultrasonography, T4 and TSH test. The solitary nodules and polinodular goiters performed 99Tc-pertechnetate scintigraphy and in suspicious cases FNAB.

##### Results

Eight of these patients were diagnosed with cancer on histopathological exam (75% papillary cancer, 12.5% medullar carcinoma, 12.5% follicular carcinoma). 1 patient aged 3 years and a mother diagnosed with MEN2b syndrome, had slightly elevated calcitonin levels. The extemporaneous pathological exam showed multiple foci of medullar carcinoma and lymph node invasion. We decided to perform total thyroidectomy and cervical dissection. From the 6 cases of papillary carcinoma, 2 were diagnosed in 2002 and 3 in 2003 (aged 2, respective 1 year in 1986, suggesting possible relation to Chernobyl disaster). From 6 cases of papillary carcinoma 66% had lymph node metastases and 16.6% had pulmonary metastases. All these cases benefit from near-total thyroidectomy.<sup>131</sup>I therapy was used for recurrent local disease (1 case) and for pulmonary metastases.

##### Conclusions

The distribution of histopathological types was similar to that of adults. The near total thyroidectomy with cervical dissection was the intervention practiced in most cases.

The patient needs to be reevaluated once a year by ultrasonography,<sup>131</sup>I scintigraphy, thyroglobuline levels for detection of residual/recurrent disease and metastases. All the patients survived until now without secondary side effects of <sup>131</sup>I therapy or severe surgical complication (only one transient hypoparathyroidism).

#### P476

##### Diagnosis difficulties in insulinomas

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We have studied 5 cases of insulinomas presented in our clinic in 2004–2005 with typical signs and symptoms of hypoglycemia correlated with high insulinemia. The medium insulin value in the morning was 57.9 mU/ml, but we found values ranging from 10–98 mU/ml for medium and severe hypoglycemia. There was no correlation between severity of hypoglycemia and insulin level, proven by repeated insulin dosages.

The purpose of our analysis was to identify the best-suitable diagnosis imaging methods for location of insulinomas, by comparing abdominal ultrasonography, CT, MRI and echographic endoscopy.

None of the cases had shown positive results on abdominal (pancreatic) ultrasound.

Four of the cases had negative abdominal CT scan, in one of this cases the tumor was identified on MRI abdominal scan, with 1.2 cm diameter, but all of them were visualized using echoendoscopy.

The fifth case who underwent a previous unsuccessful pancreatic resection for insulinoma had negative abdominal MRI, CT scan, echoendoscopy, with high level insulinemia and metastatic lesions in the liver found on echoendoscopy. Selective celiac artery catheterization shown tumor in second part of duodenum. All tumors were successfully operated.

#### Conclusions

Repeated insulin dosage is necessary to confirm insulinoma as well as multiple imaging methods. Echoendoscopy is still the gold standard for insulinoma detection, CT and MRI are useful in planning the surgery by indicating the exact location regarding the adjacent structures.

## P477

### Insulin-like growth factor-i signalling in tamoxifen sensitive and tamoxifen resistant breast cancer cells

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#### Background

Due to its cross-talk with estrogen receptor (ER) signalling, increased IGF-I signalling through the IGF-I receptor (IGF-IR) has been postulated as an important factor in the development of tamoxifen resistance in breast cancer.

#### Aims

To investigate the importance of IGF-I signalling in tamoxifen resistant (TR) and wild-type (WT) MCF-7 breast cancer cells by (i) assessing cell proliferation in response to IGF-I and tamoxifen and (ii) using small interfering RNA (siRNA) to silence IGF-IR expression.

#### Methods

We have previously established a TR cell line from a parent MCF-7 cell line. (i) WT & TR cells were cultured in media  $\pm$  50 ng/ml IGF-I  $\pm$   $10^{-7}$ M 4-OH tamoxifen. Cell proliferation was assessed every 24 hours using the MTS assay. (ii) WT & TR cells were transfected with 200 nM IGF-IR-specific siRNA for 15 days. Cell proliferation was assessed every 3 days. Previous optimisation has verified >90% reduction in IGF-IR levels in siRNA transfected cells.

#### Results

(i) WT cell proliferation showed a significant increase when cultured with IGF-I and decrease when cultured with tamoxifen. In contrast TR cells showed no significant response to IGF-I or tamoxifen (mean cell number  $\pm$  SD expressed as % of media only).

**Table 1**

	WT + IGF-I	WT + Tam	TR + IGF-I	TR + Tam
24 hrs	146.3 $\pm$ 16*	65.3 $\pm$ 6*	97.5 $\pm$ 13	89.9 $\pm$ 7
48 hrs	206.9 $\pm$ 11*	61.1 $\pm$ 3*	110.2 $\pm$ 8	100.1 $\pm$ 3
72 hrs	259.3 $\pm$ 28*	56.7 $\pm$ 3*	103.7 $\pm$ 8	100.4 $\pm$ 9

(ii) Silencing of the IGF-IR caused a rapid decline in WT cell number but significantly less effect was seen in the TR cells (47.2  $\pm$  10% vs. 93.4  $\pm$  7% at day 3\*\*, 23.8  $\pm$  8% vs. 59.6  $\pm$  9% at day 9\*\* and 14.0  $\pm$  5% vs. 49.2  $\pm$  7% at day 15\*\*). (\* $P$  < 0.0001; \*\* $P$  < 0.0001).

#### Conclusions

In contrast to expectation, development of tamoxifen resistance appears to also involve resistance to the effects of IGF-I and IGF-IR signalling. This may reflect the close interaction between the ER and IGF-IR signalling pathways.

## P478

### Functioning adrenocortical carcinoma and the clinical endocrinologist: toxic treatments and poor prognosis

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Adrenocortical carcinoma (ACC) is a rare and highly malignant tumour with a poor prognosis with an incidence of 1–2 per million population per year. We report 2 cases of functioning ACC.

#### Case 1

An 80 yr old with a history of hypertension, paroxysmal atrial fibrillation and cerebrovascular disease presented with leg ulcers, peripheral oedema, tiredness, weight gain, plethora and proximal myopathy. ACTH-independent Cushing's syndrome was confirmed. CT scan revealed a 4  $\times$  4.5 cm right adrenal mass and he underwent laparoscopic adrenalectomy. Histopathology demonstrated ACC, but biochemically he appeared "cured". 5 months later he had a recurrence of his disease, evidenced by clinical and biochemical features of steroid excess and CT scan showing multiple lesions in the right adrenal bed. Laparotomy revealed inoperable disease with widespread metastases and commenced on ketoconazole, metyrapone and spironolactone. Although a degree of biochemical control was initially achieved, drug side effects limited both the dosage and adherence, and he deteriorated and died 6 months later.

#### Case 2

A 67 yr old man presented with a long history hypertension, anxiety, migraine, blurred vision and panic attacks, with recent-onset palpitations of 4 months duration. He had clinical and biochemical features of ACTH-independent Cushing's syndrome, with elevated adrenal androgens. MRI identified a 6.3 cm right adrenal mass. He underwent open right adrenalectomy, but was found to have previously unsuspected liver metastases. Histopathological examination of both tumour and metastases confirmed adrenocortical carcinoma. Cortisol levels remained markedly elevated post-operatively. He was commenced on ketoconazole, mitotane and aminoglutethamide, but his cortisol levels failed to come under control and he deteriorated and died 3 months later.

#### Summary

These cases demonstrate the rapidly progressive nature of functioning adrenocortical carcinoma, toxic side effects of adrenolytic medication limiting compliance and efficacy and the poor prognosis associated with the disease.

## P479

### Post-operative serum cortisol for prediction of long-term remission from Cushing's syndrome

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Cushing's disease (CD) may recur despite early remission following transphenoidal surgery (TSS). We evaluated whether early post-operative serum cortisol (sF) levels on post-operative days 3,4,5 predict recurrence.

#### Methods

Patients with CD treated at NIH completed a questionnaire regarding remission status, treatment, and recent biochemistry. We analyzed patients with adenomectomy or hemihypophysectomy at initial TSS, with ACTH-staining tumor and at least one year follow-up. Pituitary irradiation was an exclusion criterion.

#### Results

Follow-up data was available on 322 patients (age at TSS 36.1  $\pm$  0.8 yrs, 80% female, 83% Caucasian). 87.9% of patients had apparent long-term remission (4.8  $\pm$  0.5 yrs). sF on post-operative days 3,4 and 5 was < 55 nmol/l in 77.4%, 76.2 and 72.0% and was < 138 nmol/l in 93.0%, 91.9% and 89.6% respectively. 277 of 319 patients (86.8%) achieved at least one sF < 55 nmol/l. Of 277 patients with at least one sF < 55 nmol/l, 250 (90%) achieved long-term remission vs. 27 (10%) who recurred. Conversely, 73.8% (31/42) of patients with higher sF had long-term remission while 26.2% (11/42) recurred. The predictive value for long-term remission of a sF < 55 nmol/l was 90% (250/277; 95% CI 86–94%) and the predictive value for recurrence of higher sF was 26% (11/42; 95% CI 14–42%). No sF value excluded all patients with recurrence.



#### Conclusion

An early post-operative sF < 55 nmol/l has high predictive value for long-term remission from CD. Immediate re-operation is not required for all patients with higher values, as most achieve long-term remission.

#### P480

##### An in-frame complex germline mutation in the juxtamembrane intracellular domain causing *RET* activation in familial medullary thyroid carcinoma

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Activating mutations of the *RET* proto-oncogene, encoding a tyrosine kinase receptor, are associated with inherited syndromes, MEN2A and MEN2B, and with both familial and sporadic medullary thyroid cancer (MTC). Single base-pair missense mutations in the extracellular cysteine-rich domain are responsible for the majority of MEN2A and familial MTC (FMTC) cases. Rarely, somatic deletions and germline duplications of variable segments of the gene have been reported in sporadic MTC and in FMTC. We report the detection and functional studies of a deletion/insertion (c.2646del-GinsTTCT) in exon 11 associated with FMTC. This in-frame complex rearrangement leads to the substitution of an Asparagine for a Lysine (Lys666Asn) and to a Serine insertion. The mutation was found in the proband, who had a diagnosis of metastatic MTC at 41 years, and in her son, who presented diffuse C-cells hyperplasia at 4 years of age. After site-directed mutagenesis, pRC-CMV constructs were transiently transfected in 293T cells. Immunoprecipitation and Western blot with anti-Ret and anti-Ptyr specific antibodies demonstrated that the Ret9-delGinsTTCT mutant protein was significantly more phosphorylated than the wild-type. Therefore, even in the absence of ligands, Ret9-delGinsTTCT can be phosphorylated. Computational analysis performed by means of the PredictProtein server predicted significant changes into the protein folding as a result of this in-frame mutation. In particular, the transmembrane  $\alpha$ -helical acquires a different conformation, which could impair the flexibility of the receptor at that level. Moreover, the content and distribution of  $\beta$ -strands is modified and the proportion of solvent-exposed residues decreases drastically.

In conclusion, the functional studies on a complex germline *RET* mutation lying in the juxtamembrane region of the receptor are reported. This mutation is predicted to significantly change the secondary structure of the protein and can activate Ret in a ligand independent manner.

#### P481

##### Macroprolactinoma: 5 cases

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Prolactinoma represents 27 to 35% of pituitary adenomas and 40 to 75% of secreting adenomas. However, the macroprolactinomas are not frequent. We report clinical, biological and therapeutic aspects of 5 cases of macroprolactinomas. It concerns 3 women and 2 men. The average age is 43.4 years (19–68). The reason for seeking medical advice was amenorrhea – galactorrhea syndrome in 2 women and infertility in one case. The diagnosis of pituitary adenoma is incidentally imaged when computed tomographic scan is done because of stroke in 2 patients. Clinically, complete impotence and gynecomastia are present in only one man. The average concentration of prolactin is 2171.2 ng/ml (200–9134). There is no symptoms of acromegaly. Corticotropin deficiency is noted in 3 cases. Two patients present somatotrophic deficiency. Thyrotropin deficiency concerns one case. The pituitary MRI shows a large tumor that extends beyond the confines of the sella turcica in four cases. This tumor is responsible of headache and visual abnormalities in 2 cases. All our patients are treated with bromocriptine, the only dopamine agonist available in Tunisia. No one experienced nausea and postural hypotension. The average dose was 12.37 mg/day. The serum prolactin level is normalised in 4 patients. Surgical resection is considered in one patient who fails to respond to medical treatment. Some degree of tumor shrinkage occurs in 3 cases. One patient was dead after 3 months of beginning of treatment. The treatment should lowering serum prolactin levels, reducing tumor size and improving visual fields.

Successful treatment of macroprolactinoma is most often accomplished with administration of a dopamine agonist. The results of dopamine agonist treatment in patients with macroprolactinomas are similar to those in patients with microprolactinomas. Surgical therapy and pituitary radiation may be used as adjunctive therapy.

#### P482

##### 48 h appears as sufficient duration of fasting in the diagnosis of insulinoma – a single centre experience with 23 cases

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Insulinoma causes fasting hypoglycemia due to inappropriate insulin secretion. The diagnosis of insulinoma is based on Whipple's triad (1. symptoms precipitated by fasting with 2. associated blood sugars of 50 mg/dl or less and 3. relief of symptoms by glucose administration) during a supervised 72 h fasting test. After introducing reliable assays for measurement of insulin and proinsulin, there is an ongoing debate whether a 48 h fasting test is sufficient for diagnosis or a 72 h fast is necessary to detect patients with insulinoma. The aim of our study was to evaluate the positive fast within 48 h in a large series of patients with insulinoma.

In a retrospective study (1970 – 2004) we identified 39 patients (24 females, 15 men; average age 47 years [range 12–78 years]) with insulinoma. Surgical pathology confirmed the diagnosis in 34 cases: 24 patients had a benign tumour, 4 had malignant insulinoma and 6 patients had multiple endocrine neoplasia type 1. The average body mass index (BMI) was 28.5 (range 17.3–39.1). 16 patients were diagnosed by spontaneous hypoglycaemia. 23 patients were tested with a 48 h fasting test.

The fast was terminated due to neuroglycopenic symptoms in 4 patients (17.4%) by 12h, in 17 patients (73.9%) by 24h, and in 22 patients (95.7%) by 48 h. One patient had no neuroglycopenic symptoms, but was diagnosed by glucose and insulin levels during the 48 h fast.

In conclusion, the 48 h fasting test was successful in the diagnosis of insulinoma in our patient cohort, especially if even subtle signs of neuroglycopenia were recognized by the medical personnel. In this series we did not observe a need for fasting beyond 48 h, and we therefore established the 48 h fasting test as standard protocol resulting in cost reduction.

#### P483

##### A case of a patient with differentiated thyroid carcinoma and metastases to the kidneys

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#### Introduction

Characteristic localisations of metastases of differentiated thyroid carcinoma (DTC) include local lymph nodes, the lungs and bones, while the incidence of DTC metastases onto the liver, the brain and skin is clearly lower. The localisation of metastases in other organs, including the kidneys, is very rare.

#### Material and methods

A case of a 73-year-old patient with thyroid oxyphilic carcinoma is reported, the case being diagnosed in 1997, after thyroidectomy and thyroid remnants ablation with <sup>131</sup>I. Since the year 2000, recurrent cervical lymph metastases have been observed. The metastases were surgically treated (five subsequent lymphadenectomies) and the patient received <sup>131</sup>I therapy (the total dose, administered during the years 2000–2002, amounted to 31 GBq); after the last operation, performed in August 2002, the patient presented with hyperthyroglobulinaemia (454 ng/mL). In November 2002, the patient received 8.4 GBq of <sup>131</sup>I, following preparation with cis-retinoic acid, because of the lack of iodine uptake foci in whole body scintigraphy, performed after previous radioiodine administration. No iodine avid foci were found on post-therapeutic scintigrams, nor was any decrease in Tg concentration found in later observations. During follow-up (December 2003), focal changes were observed in both kidneys (US, CT). Fine-needle aspiration biopsy of those changes indicated their DTC metastatic character. The patient did not agree to nephrectomy.



## Conclusions

Maintained increased Tg concentrations, despite the lack of clinically overt features of the disease activity, require a determined search for sources of this marker, also in areas which are rarely targeted by DTC metastases.

## P484

**Development of a non-isotopic immunoassay for the measurement of aldosterone in saliva**

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The purpose of our study was to establish an assay for the measurement of aldosterone in saliva which utilises a simple, non-invasive sampling technique on an out-patient basis. For this we have developed a non-isotopic, time-resolved fluorescence immunoassay. The assay only requires a volume of 100µl for each duplicate value obtained and involves an overnight incubation after extraction of samples. Cross-reactivity with potentially interfering steroids is below 0.01%. The assay has a dynamic range of 30 to 2000 pg/ml. The protocols used for clinical validation of the assay in healthy volunteers as well as patients were all approved by the local ethics committee of the medical faculty. The intra-assay coefficients of variation were between 8.5 and 15.4% for values between 209.2 and 46.2 pg/ml respectively. We found a significant correlation between plasma and saliva values ( $n = 125$ ), ( $r^2 = 0.797$ ,  $P < 0.0001$ ). Saliva values were around 22% of those observed in plasma. In 5 out of 10 healthy volunteers who were tested for a day profile by simultaneous saliva and plasma aldosterone sampling, there was corresponding variability throughout the day although a distinct circadian rhythm was not apparent. The remaining 5 subjects had values close to or below the limit of quantification. In 5 patients undergoing ACTH stimulation test, a significant increase in salivary aldosterone concentration from  $68.3 \pm 30.1$  pg/ml to  $212.3 \pm 68.8$  pg/ml was observed after 60 minutes of administration. In two patients with confirmed Conn's syndrome, mean salivary aldosterone values were higher ( $153.1 \pm 55.8$  pg/ml) throughout the day compared to those of healthy volunteers ( $49.9 \pm 31.3$  pg/ml). The preliminary findings in two subjects with confirmed Conn's support the idea of detecting hypersecretion of aldosterone by multiple sampling rather than a single random aldosterone determination. In conclusion, this assay provides a non-invasive, easy screening method to analyse aldosterone secretion.

## P485

**Thyroid autoimmunity disorders and breast cancer (BC): absence of morphological autoimmune changes in breast malignant tissue**

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Thyroid autoimmunity and BC are strongly related, but the cause of this association is unknown. In BC an increase of lymphoid cell infiltrates may be detected very early during tumor development. BC cells share some antigenic properties similar to those detected in thyroid tissue, as NIS and peroxidase activity and it is possible that in patients with BC and thyroid autoimmunity, should be present serum immunoglobulins reacting with these specific antigens.

The aim of this study was to evaluate frequency and amount of LI in malignant and normal peritumoral breast tissues, in BC patients with autoimmune thyroid disorders. We suppose that an increased LI in breast tissues of this group of patients, may contribute to explain the association between BC and thyroid autoimmunity.

Study group: 26 BC patients with ductal infiltrating carcinoma (DIC) (aged 26–88 yr,  $54.3 \pm 12.5$  mean  $\pm$  SD) and serum thyroperoxidase antibodies positivity (TPOAb +): 14/26 (53.8%) had evidence of Hashimoto's thyroiditis (HT). Control group: 30 aged matched patients with DIC and no evidence of thyroid autoimmunity. Malignant and surrounding normal breast tissues were assessed for LI.

LI was scored as absent or scanty (LI A) and moderate or marked (LI M). LI A was detected in 19/26 DIC (73.1%) with TPOAb + and LI M in 7 (7/26; 26.9%). All the 14 DIC with HT had LI A. LI A was detected in 25/30 (83%) and LI M in 5/30 (17%) DIC with no thyroid autoimmunity. The difference in LI of DIC with or without thyroid autoimmunity was not significant. LI was generally absent in remote breast tissue in all cases.

The results of the study indicate that in breast cancer the presence of humoral and/or clinical evidence of thyroid autoimmunity is not associated to significative autoimmune morphological changes of malignant or peritumoral breast tissue. In conclusion, a role of breast lymphocytic infiltration in the progress of breast tumorigenesis in BC patients with autoimmune thyroid disorders seems unlike.

## P486

**Pituitaryoma – a case report**

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Pituitaryoma is a rare hypophyseal tumour with very few case reports in literature so far. We report a 76 years old gentleman, with known hypertension, hypercholesterolemia and T2DM, who presented initially with features of hypogonadism and a bilateral inferior quadrantanopia. Initial Hormonal assessments revealed Testosterone of  $< 0.05$  nmol/L, FSH 2 U/L, LH 2 U/L and Prolactin 586 mU/L, confirming hypogonadotropic hypogonadism. He was started on testosterone replacement using Striant SR 30 mg every 12 hours. Formal visual field assessment demonstrated bilateral inferior hemianopia and CT scans revealed a suprasellar lesion in keeping with a craniopharyngioma, above a normal looking pituitary gland. MR scans revealed a suprasellar enhancing lesion, extending posteriorly to the optic chiasm which appeared to be compressed. The pituitary appeared compressed but was otherwise normal.

A presumptive diagnosis of Craniopharyngioma was made. He underwent a transphenoidal hypophysectomy, which was uneventful. Post-operatively tests revealed pan hypopituitarism with LH  $< 1.0$  U/L, FSH  $< 1.0$  U/L; Testosterone  $< 0.5$  nmol/L, Prolactin 298 mU/L, TSH 0.12 mU/L, free T4 of 9 pmol/L, and a short synacthen test with baseline 144, 30 minute level 293. He was started on full replacement therapy of thyroxine 50 mcg once a day and hydrocortisone 10/5/5 mg and Sustanon 250 mg. Histopathologically, the tumour was noted to be a pituitaryoma which are rare benign primary tumours of neurohypophysis. Pituitaryomas are spindle cell tumours with little nuclear pleomorphism. They are benign, low-grade, non-infiltrative neoplasms of unique glial elements referred to as pituitaryocytes, arising in the neurohypophysis and within the sella turcica. Clinically, they may present as non functional pituitary tumours with mass effects, as in our patient. Little is known about the clinical course of these histologically benign tumours. Total resection may cure the tumour; however, subtotal resections are known to be prone to local recurrence and high vascularity and location make them surgically challenging.

## P487

**PTTG promotes a novel VEGF-KDR-ID3 autocrine mitogenic pathway in thyroid cancer**

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VEGF exerts its effects by binding to two tyrosine kinase receptors, KDR and VEGFR1. KDR is critical for transmitting signals for proliferation and migration of endothelial cells but is also expressed in several non-endothelial cells, and is elevated in some human tumour cells. We investigated KDR expression in human thyroid epithelial cells *in vitro* and thyroid cancers compared with normal thyroid samples examined *ex vivo*. Expression of KDR was demonstrated using Taqman RT-PCR and Western blotting in a human follicular thyroid cancer line (FTC133) and primary thyroid epithelial cells. KDR expression in these cells demonstrated VEGF-specific and dose-dependent activation of KDR-dependent downstream MAPK signalling and KDR-dependent mitogenesis. KDR mRNA expression was elevated in thyroid cancers (2.5 fold,  $n = 38$ ,  $P < 0.001$ ), and immunohistochemistry demonstrated stronger phosphorylated-KDR staining in thyroid cancer cells compared with normal thyroid cells. Further, we showed that PTTG, a multifunctional protein known to promote angiogenesis, up-regulates KDR (2.2 fold,  $n = 7$ ,  $P = 0.006$ ) and VEGF (2.4 fold,  $n = 4$ ,  $P < 0.001$ ) expression in primary thyroid cells. Next, we examined expression of Inhibitor of DNA binding-3 (ID3), known to be important in VEGF-dependent angiogenesis, and VEGF mRNA expression in a series of thyroid cancers. We observed a strong positive correlation

between expression of these genes ( $R^2 = 0.62$ ,  $n = 38$ ,  $P < 0.001$ ). Stimulation of FTC133 cells with exogenous VEGF increased ID3 expression (2.1 fold,  $n = 6$ ,  $P < 0.001$ ) compared with control, an effect abrogated by a KDR-specific inhibitor, suggesting VEGF regulation of ID3 is KDR-dependent. We suggest the presence of a VEGF-KDR-ID3 dependent autocrine pathway in thyroid cells. By up-regulating both VEGF and KDR expression, we propose that PTTG may promote this autocrine proliferative pathway which may in turn be critical to thyroid cancer progression.

#### P488

##### Multiple Endocrine Neoplasia Type 1 (MEN 1) is associated with insulin resistance and an increased prevalence of diabetes and impaired fasting glucose

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MEN 1 is an autosomal dominant syndrome associated with benign and malignant neuroendocrine neoplasia. It is characterised by primary hyperparathyroidism, pituitary neoplasia and foregut lineage neuroendocrine tumours. It has also been associated with premature cardiovascular death. Since diabetes is associated with an increased risk of cardiovascular mortality we investigated the prevalence of diabetes (DM) and impaired fasting glucose (IFG) in a large cohort of patients with MEN 1.

##### Methods

Records for MEN 1 gene positive and gene negative siblings (control) in Tasmania were reviewed. 72 MEN1 and 133 control patients were eligible for inclusion. Fasting glucose and insulin were compared between groups using WHO criteria. All MEN1 patients had received evaluation of parathyroid, enteropancreatic and pituitary neoplasia by structural imaging and endocrine testing.

##### Results

13 (18.1%) patients with MEN 1 compared to 5 (3.8%) control patients were diabetic. 6 (8.3%) patients had IFG compared to 4 (3%) of controls. The age of onset of IFG or DM in MEN 1 was younger than in the control group ( $49.6 \pm 2.56$  v.  $56.03 \pm 5.85$  years). Glucose/Insulin ratio was reduced in MEN 1 patients compared to controls with DM/IFG ( $0.72 \pm 0.24$  v.  $1.04 \pm 0.41$ ). MEN 1 patients with DM/IFG had a significantly higher gastrin ( $P < 0.05$ ) and elevated PP, GIP and CgA (but not glucagon) and were more likely to have a history of pancreatic lesions than patients with normal glucose tolerance.

##### Discussion

Compared to controls there is a 4 and 2.5 fold greater prevalence of diabetes and IFG respectively in MEN 1. It is possible that insulin resistance may be induced by circulating pancreatic cytokines associated with gastrin hypersecretion. We suggest MEN1 patients may have an increased cardiovascular risk because of diabetes and IFG and should be screened and managed for metabolic risk factors.

#### P489

##### Evaluation of a standardized protocol for the collection and storage of adrenal tumor samples - preparation for an European adrenal tumor bank (ENS@T)

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Tissue samples from adrenal tumors provide the basis for standard diagnostic procedures such as pathological examination. In addition, these tumor samples have been an invaluable source for the discovery of novel molecular pathways involved in adrenal tumorigenesis. Information on the molecular phenotype are based on DNA mutation analysis and epigenetic changes, RNA and protein expression pattern and sub-cellular localization as well as post transcriptional protein modification. However, storage and tissue handling of surgical adrenal tumor samples has not been optimized or

standardized which might affect reproducibility and comparability between different laboratories. To examine different handling and storage procedures with regard to RNA, protein, and DNA quality and subsequent morphological examinations, we subdivided each surgical adrenal tumor sample into six pieces which were either snap frozen or treated with RNAlater (Ambion), after defined storage time at room temperature (up to 60 min). As we could show, DNA and protein recovery as well as integrity of DNA by means of pulse field electrophoresis and long range PCR (MC2-R locus) was not affected by snap freezing or the use of RNAlater after different storage intervals. Moreover, morphology of sectioned tissue samples was comparable between the groups, while overall staining intensity was decreased after RNAlater pre-treatment. In addition, western blotting did not reveal differences in the expression levels of 3BHSD protein between the groups. However, levels of pERK as an example for a phosphorylated protein was significantly decreased by processing the tissue with RNAlater (snap vs. RNAlater after 15 min storage,  $100.0 \pm 17.6\%$  vs  $57.7 \pm 6.4\%$ ,  $P = 0.02$ ). In summary, recovery and integrity of DNA and protein is not significantly affected by the investigated handling conditions while protein phosphorylation is dependent on pretreatment. Investigations of subtle differences in the RNA integrity and expression pattern are under way and will further define the optimal handling protocol of a scheduled European adrenal tumor tissue bank.

#### P490

##### Value of overnight dexamethasone suppression test (1 mg-dst) in subclinical Cushing's syndrome

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Adrenal incidentalomas are referred as clinically silent adrenal masses discovered incidentally during diagnostic testing or treatment for conditions not related to suspicion of adrenal disease. Certain percent of them are hormonally active and autonomous cortisol secretion is reported with increasing frequency. The aim of the study was to assess value of 1 mg-DST in subclinical Cushing's syndrome (CS) compared to the other forms of adrenal incidentalomas. We have evaluated 164 patients with adrenal incidentalomas confirmed by CT or MR imaging. After endocrine evaluation, by multiple criteria, 25 of them were classified as subclinical CS, subsequently confirmed by histology. Dexamethasone 1 mg was administered at 11<sup>PM</sup> and blood samples for cortisol were taken at 8<sup>AM</sup> following day. Statistic analysis was done using ROC curve analysis. *Subclinical CS*: 25 patients (21 females, 4 males), mean age  $54.0 \pm 8.9$  years, BMI  $27.3 \pm 3.8$  kg/m<sup>2</sup>, post 1 mg-DST cortisol  $233.0 \pm 161.8$  nmol/L; midnight cortisol  $238.2 \pm 137.9$  nmol/L and basal ACTH  $6.6 \pm 2.8$  pg/mL. *Other forms of incidentalomas*: 139 patients (92 females, 47 males), mean age  $55.6 \pm 10.9$  years, BMI  $29.5 \pm 4.9$  kg/m<sup>2</sup>, post 1 mg-DST cortisol  $48.7 \pm 22.7$  nmol/L; midnight cortisol  $93.8 \pm 45.0$  nmol/L and basal ACTH  $17.8 \pm 11.7$  pg/mL. Area under ROC curve was  $0.9885 \pm 0.0154$  (95% CI 0.9340- 0.9980). For cortisol cut-off level of 80 nmol/L test sensitivity is 95.9% and specificity is 91.0%. 1 mg-DST remains reliable tool in diagnosis of subclinical hypercorticism.

#### P491

##### Effect of catecholamine uptake inhibition on catecholamine content in human adrenal chromaffin cells and pheochromocytomas

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Abstract withdrawn.

#### P492

##### PTTG binding factor (PBF) can transform cells independently of interaction with PTTG

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We have previously shown PTTG and PBF to be over-expressed in differentiated thyroid cancer and to be prognostic indicators for recurrence. Subsequently we reported PBF to be a transforming gene *in vitro* and tumorigenic *in vivo*. Since over-expression of PTTG results in the same findings, we examined whether PBF-induced tumorigenesis was an independent effect, or else a result of increased PTTG activity. Two HA-tagged mutants of PBF were generated, firstly substituting the five basic residues of the nuclear localisation signal (NLS -) and secondly deleting the C-terminal 30 amino acids responsible for PTTG interaction (C-term). Both PBF mutants were unable to interact with PTTG as demonstrated by immunoprecipitation, and using subcellular fractionation we were able to show that neither mutant could enter the nucleus. Stable over-expression of PBF and both mutants in NIH3T3 cells resulted in anchorage-independent growth in soft agar, with the formation of large and abundant colonies compared with vector-only (VO) (VO = 9.1 colonies  $\pm$  1.4; PBF = 201  $\pm$  33.2,  $P < 0.001$ ; NLS - 159  $\pm$  33.2,  $P < 0.001$ ; C-term 177  $\pm$  23.4,  $P < 0.001$ ). There was no significant difference in the number of colonies between wild-type PBF and the two mutants. A mutant of PTTG, in which the PBF interaction domain (amino acids 123-154) was deleted (BD -), was over-expressed in NIH3T3 cells and its ability to transform cells assessed in the same assay. Over-expression of wild-type PTTG resulted in the formation of large colonies compared to VO; however removal of the region which interacts with PBF abrogated its ability to transform cells (PTTG = 1501  $\pm$  91,  $P < 0.001$ ; BD - = 15.7  $\pm$  2.84,  $P = n/s$ ). We conclude, therefore, that PBF is able to transform cells independently of PTTG, whereas PTTG-induced cell transformation may be dependent on interaction with PBF.

#### P493

##### Carney's complex with acromegaly as the leading clinical condition

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Carney's complex was first identified as the association of primary adrenal nodular dysplasia, lentiginos and cardiac and skin myxomas. Several other endocrine and non-endocrine disorders were subsequently added to the complex, including pituitary tumors and melanotic Schwannomas. We herewith describe a kindred with Carney's complex featuring acromegaly as the common denominator.

##### Patients and methods

A 42-year-old woman first presented to our attention with acromegaly and died some years later after removal of a melanotic Schwannoma. Her 43-year-old brother was diagnosed with acromegaly and cutaneous angiomyxoma prompting investigation for Carney's complex. Blood samples were collected from affected subjects, their mother and their offspring as well as their apparently healthy siblings for sequencing of the *PRKARIA* gene. All subjects were also submitted to OGTT, measurement of IGF-I, prolactin, UFC and ACTH, sonograms and thorough clinical evaluation.

##### Results

The brother and the two daughters of the index case presented a G > A substitution upstream to exon 4 of the *PRKARIA* gene. The mother as well as two siblings presented normal *PRKARIA* sequence. Both daughters of the index case (24 and 21 yr) presented abnormal GH responses to OGTT and lentiginos in typical sites. The elder daughter also presented increased IGFI levels and a pituitary microadenoma and was started on GH-suppressive therapy. No lesions were detected at cardiac, abdominal and testicular sonogram in family members harbouring the *PRKARIA* mutation; adrenal function was normal.

##### Conclusions

Carney's complex is the association of rare and unusual disorders, initially characterized by adrenal, heart and skin lesions. Acromegaly is known to affect some 10% of patients with the complex but is rarely the premier clinical condition. Our kindred demonstrates that familial acromegaly may be the first sign of Carney's complex and that identification of affected subjects by genetic screening allows early detection and treatment of GH hypersecretion.

#### P494

##### Daily profiles of aldosterone secretion in primary aldosteronism

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In supine subjects aldosterone daily variations are determined by spontaneous PRA rhythmicity and ACTH secretory bursts. The aim of this study was to analyze diurnal and pulsatile aldosterone secretion in two patients with primary aldosteronism (PA): a 33-year-old woman with aldosterone producing adenoma (APA) and a 40-year-old woman with idiopathic adrenal hyperplasia (IAH). Blood samples were taken hourly during 24 hours at the time of the diagnosis and three months later, after tumour removal in the first case. Aldosterone was also analyzed after dexamethasone suppression in second case. Patients were supine except from 8 AM to 6 PM when they were allowed unrestricted ambulation. PRA levels were suppressed in both patients, but normal after tumour removal. In APA no circadian variations of aldosterone levels were registered before tumour removal. After that circadian variations were found with amplitude 0.074 nmol/l, period 12.25 h and maximum at 12.40 h. In patient with IAH aldosterone exhibited amplitude 0.148 and 0.297 nmol/l, period 8.67 and 15.59 h and maximum at 10.00 and 15.10 h respectively, but circadian rhythm was lost on dexamethasone. There was no correlation with PRA levels. Aldosterone pulsatile secretion was preserved in both patients: APA 6 and 8 pulses, 4.2 and 2.9 interpulse interval, 2.48 and 0.38 amplitude, 6.86 and 0.59 pulse mass before and after tumour removal; IAH 8 and 7 pulses, 3.14 and 3.67 interpulse interval, 0.79 and 0.99 amplitude, 1.22 and 1.76 pulse mass. We demonstrated that aldosterone pulsatile secretion is present in patients with PA. Mean serum aldosterone levels are increased due to higher pulse amplitude and concentrations between pulses. Reproducibility of aldosterone circadian rhythm existence is confirmed in IAH. The lost of aldosterone daily rhythmicity in PA due to aldosteronoma and IAH on dexamethasone may underline greater dependency on ACTH control.

#### P495

##### Analysis of succinyl dehydrogenase (SDH) subunits gene mutations in patients with paragangliomas

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Tumors derived from chromaffine tissue include pheochromocytomas (tumors located in adrenal medulla) and paragangliomas (extraadrenal tumors). These tumors are in 20-25% inherited. Paragangliomas are even rarer and are presented either as familial disease or pheochromocytoma-paraganglioma syndrome (PPS). The mutations in SDH genes (*SDHB*, *SDHD*) are suspected for causing the syndrome.

The aim of present study is to look for germline mutations in *SDHB* and *SDHD* genes in patients with pheochromocytomas and/or paragangliomas. DNA was isolated from peripheral blood leukocytes obtained from patients with pheochromocytomas and paragangliomas. The polymerase chain reaction (PCR) was performed for *SDHB* (exons 2, 3, 4, 6, 7) and *SDHD* (exons 1, 2, 3). The PCR product was then analyzed with the use of MSCP (Multiplex Single-Strand Conformation Polymorphism). When the change in the conformation of DNA strand was found, it was then identified by sequencing.

We have so far analyzed DNA from 12 patients with diagnosed paragangliomas; we have also examined 72 patients with pheochromocytomas only in order to seek for pheochromocytoma-paraganglioma syndrome. We have found two types of mutations of *SDHD* in 6/12 (50%) of paraganglioma cases. 33 TGC-TGA substitution was associated with benign tumors, whereas N 721 G-A occurred in the only patient with malignant paraganglioma.

##### Conclusions

(1) Mutations in the *SDHD* gene appear to be frequent in patients with paragangliomas. (2) Among inherited cases of pheochromocytomas no correlations of the type of *SDH* mutation with malignancy have been found so far.



**P496**

**Neuropeptide Y-Y1 receptors mediate the effects of neuropeptide Y on prostate cancer cell growth**

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Neuroendocrine molecules play a significant role in the progression of human prostate cancer (PCa) and its neuroendocrine differentiation has been associated to a worse prognosis. Among these molecules, the pleiotropic peptide neuropeptide Y (NPY) was found to be expressed in the human prostate and may show some relevance in PCa progression. In this study, we evaluated the direct effect of NPY on the growth of the human PCa cell lines LNCaP (androgen dependent) and DU145 and PC3 (androgen independent). The Y1-R subtype was found to be expressed in all PCa cell lines at the gene and protein level. Moreover, Y2-R and Y4-R genes were found to be expressed in PC3 cells and in LNCaP/DU145 cells, respectively. Treatment with  $10^{-8}$  M NPY reduced the proliferation of LNCaP and DU145 cells and increased that of PC3 cells. The specific Y1-R antagonist BIBP3226 ( $10^{-6}$  M) abolished such effects, suggesting a mandatory role of Y1-R in this process. LNCaP cells showed elevated constitutive levels of phosphorylated extracellular kinase RK (ERK)1/2, which were not affected by NPY. In DU145 cells, treatment with  $10^{-8}$  M NPY stimulated a long-lasting (> 6 h) ERK1/2 phosphorylation, whereas, in PC3 cells, this effect was rapid, transient and mediated by protein kinase C, since it was abolished by pretreatment with the specific PKC inhibitor GF109203X ( $10^{-7}$  M). In DU145 and PC3 cells, NPY-induced ERK1/2 phosphorylation was prevented by a pretreatment with BIBP3226, further suggesting the involvement of Y1-R. Treatment with  $10^{-8}$  M NPY reduced forskolin-stimulated cAMP accumulation only in PC3 cells, and did not change intracellular calcium concentration in any PCa cell line. Our data suggest that Y1-R activation by NPY represents an important regulator of the proliferation different PCa cell lines, with stimulatory or inhibitory effects depending on the peculiar intracellular signalling pattern activated in each clone.

**P497**

**Differential expression of neurogenins by human pituitary adenomas**

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The beta-HLH transcription factors NeuroD1 and ASH1 are frequently expressed by pituitary adenomas, both being present in all corticotroph, most clinically non-secreting (CNS) and a subset of GH and/or PRL-secreting adenomas. We wished to investigate the expression of the related beta-HLH factors neurogenins (Ngn) 1, 2 and 3 in the pituitary ( $n = 4$ ) and in a series of pituitary adenomas ( $n = 45$ ). RT-PCR was performed at different amplification cycles (up to 45) in all cases. Ngn1 was undetectable in all samples. Ngn2 transcripts were detected in 56% of pituitary adenomas by RT-PCR (33/45) and up to 73% after Southern blotting (33/45). Low levels of Ngn2 transcripts were also detected in some normal pituitary samples. Preliminary data from immunohistochemistry are confirming Ngn2 protein expression and its nuclear localization in pituitary adenomas and a subset of normal pituitary cells. Ngn3 transcripts were undetectable in normal pituitaries and present in a subset of pituitary adenomas: 25% by RT-PCR (11/44), and 30% after Southern blotting (12/40). Overall, Ngn2/3 expression did not significantly correlate with tumour aggressiveness or with NeuroD1/ASH1 expression at a transcriptional level. Neither was their expression significantly influenced by tumor phenotype: Ngn2 was detected in all ACTH-secreting adenomas (100%) and in most CNS and PRL/GH/TSH-secreting adenomas too (67% and 74%, respectively), whereas Ngn3 was detected in a subset of all adenoma phenotypes (25% of ACTH-, 25% of CNS and 37% of GH/PRL/TSH-secreting adenomas, respectively). The expression of Ngn2 and to a lesser extent of Ngn3 in pituitary samples is consistent with their ontogenetic role in other neuroendocrine (NE) tissues, such as neural crest (Ngn2) and gastroenteropancreatic NE cells (Ngn3), and suggest a possible role in pituitary ontogenesis too. At the moment, there is no evidence for a significant role Ngn2/3 in pituitary tumorigenesis or in NeuroD1/ASH1 regulation.

**P498**

**Evidence for an attenuated 11  $\beta$ -hydroxysteroid dehydrogenase type-1 (11 $\beta$ HSD1) response to an inflammatory stimulus in primary cultures of epithelial ovarian cancer**

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Epithelial Ovarian Cancer (EOC) is the leading cause of death from gynaecological malignancy in the developed world. Epidemiological evidence indicates number of lifetime ovulations as being a major risk factor for the development of the disease. As ovulation is an inflammatory process, it is thought that repetitive inflammation-associated damage to the ovarian surface epithelium (OSE) leads to oncogenic events within these cells. Previous studies have demonstrated that healthy OSE cells express 11 $\beta$ HSD1 in response to inflammatory cytokine stimulation, leading to regeneration of cortisol from cortisone, and this system is suggested to provide anti-inflammatory steroid cover to protect OSE from inflammatory damage. Moreover, a feed-forward mechanism exists in OSE, whereby cortisol, in the presence of inflammatory cytokines, augments the inflammation-driven increase in 11 $\beta$ HSD1 expression. The aim of the current study was therefore to investigate whether or not such an anti-inflammatory response was dysregulated in primary EOC cells.

Primary cultures of OSE and EOC were established from solid tumour and ascites obtained with informed consent and with Local Research Ethics Committee approval. Cells were treated with Interleukin 1-alpha at 0.5 ng/ml (IL1), in the presence and absence of 1  $\mu$ M cortisol (F) for 48 hours. RNA extraction was performed and mRNA expression of 11 $\beta$ HSD1 and 2 isotypes assessed by quantitative RT-PCR.

In cultures of human OSE cells ( $n = 4$ ), the mean fold rise in 11 $\beta$ HSD1 was significantly greater than in the EOC samples ( $n = 11$ ) when treated with IL1 (27.2 vs 4.0,  $P < 0.05$ ) and with IL1 and F (63.9 vs 11.1,  $P < 0.05$ ). Levels of 11 $\beta$ HSD2 were not significantly altered by any of the treatments in cultures of EOC or OSE.

These data provide evidence that EOC cells have impaired ability to mount an anti-inflammatory response. This may be an important feature of disease development.

**P499**

**Long-term somatostatin analog octreotide treatment in insulinoma patients**

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The usefulness of long-term medical treatment with somatostatin analogs has not been evaluated in insulinomas. 22 patients with hypoglycaemia related to endogenous hyperinsulinism ( $62 \pm 21$  years,  $M \pm SD$ , 27–88 years) were not treated by surgery: aged patients ( $n = 10$ ), patients with malignant unresectable insulinomas ( $n = 6$ ; locally invasive,  $n = 1$ ; multimetastatic,  $n = 5$ ), multiple insulinomas ( $n = 2$ ), diffuse beta cell pathology ( $n = 4$ ). 12/22 patients underwent an Octreoscan scintigraphy. Octreotide was effective in controlling hypoglycaemia in 13/22 patients (59%): 7 aged patients, 1 patient with an invasive insulinoma, 2 with multiple insulinomas and 3 with diffuse beta cell pathology. Octreoscan scintigraphy was positive in 6/12 patients: 2 were responsive and 4 unresponsive to octreotide. Octreoscan scintigraphy was negative in the other 6/12 patients: however 3 of these patients were responsive to octreotide. Subcutaneous octreotide treatment was prolonged for more than 6 months (6 months–12 years,  $5.3 \pm 4.0$  years) in 10 of the 13 responders (6 elderly patients, 1 invasive insulinoma, 2 multiple insulinomas, 1 nesidioblastosis) at a dose of 50–2000  $\mu$ g/day ( $495 \pm 600$   $\mu$ g/day). No escape to therapy was observed. However, the dose of octreotide had to be increased in 2 patients after 2 months ( $n = 1$ ), and after 7 years ( $n = 1$ ). In a patient with multiple insulinomas, subcutaneous octreotide was replaced by a long-acting somatostatin analog preparation with the same efficacy. The malignant invasive insulinoma remained asymptomatic and unchanged on CT-scan evaluation after 2 years of follow-up. Only 1 patient suffered from a symptomatic biliary lithiasis after 3 years of treatment. In conclusion, Octreoscan scintigraphy is not predictive of the efficacy of octreotide on hypoglycaemia in insulinoma patients; long-term octreotide treatment can be used to control hypoglycaemia in patients with insulinoma who cannot be cured by surgery.



## P500

### Development of dopamine agonist resistance and progression from microadenoma to macroadenoma in two women with hyperprolactinaemia

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Dopamine agonist therapy is an effective long-term treatment in >90% of patients with hyperprolactinaemia, controlling both prolactin secretion and tumour growth. We describe the unusual late emergence of resistance to high-dose dopamine agonist treatment in two recent female patients. Both women presented with secondary amenorrhoea, were shown to have high prolactin levels and a pituitary microadenoma. Each had an excellent initial response to bromocriptine but years later developed a cabergoline-resistant macroadenoma.

#### Case 1

This 57 year old, first seen in 1985, responded well to dopamine agonist treatment over a fifteen year period. However from 2000 onwards, elevation of prolactin levels became unresponsive to cabergoline dose escalation and a macroadenoma developed. Transsphenoidal surgery was performed in 2003 with radiotherapy a year later. On continued cabergoline, prolactin levels are <40 mU/L.

#### Case 2

This 38 year old presented in 1993, tolerated cabergoline well at low doses and conceived in 1996. Persistent galactorrhoea one year post-partum prompted re-introduction of cabergoline. However despite dose escalation, prolactin levels continued to rise. CT pituitary confirmed the presence of an enlarging adenoma and the patient had transsphenoidal surgery in May 2005. Prolactin levels on continued cabergoline therapy (1.5 mg/week) are normal. Late onset of dopamine agonist resistance with progression of microadenoma to macroadenoma is most unusual and raises concerns regarding pituitary carcinoma. This diagnosis can only be made definitively in the presence of metastases. While histology cannot differentiate between benign and malignant pituitary tumours, immunohistochemical techniques may be useful, with carcinoma cells staining strongly for Ki67 and p53. Although tumour tissue from case 1 showed brisk Ki67 activity, this test in case 2 and p53 staining in both, was equivocal. In these cases neuropathological techniques provide only limited characterisation of the tumour and the need for careful ongoing clinical assessment in hyperprolactinaemia is emphasised.

## P501

### The rare RET mutation (insTTCTdelG) at codon 666 is associated with a low penetrance of medullary thyroid carcinoma and pheochromocytoma

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Early 2005 we reported on a new insertion-deletion mutation (insTTCTdelG) at codon 666 (exon 11) of the RET proto-oncogene. The index patient, a 12 year old boy with locally metastasized MTC, inherited the mutation from his maternal grandfather. His mother had a basal calcitonin of 30 ng/L and underwent a prophylactic total thyroidectomy at age 48, showing C-cell hyperplasia in both lobes and early MTC with a maximum size of 1.5 mm. At age 82, his grandfather did not have clinical MTC. The early presentation in the index patient was explained by the additive effects of the maternal RET mutation and a functional coding polymorphism (G691S) in his paternal RET gene.

Since then, the codon 666 mutation was identified in 3 other unrelated families. The 3 index patients were a female with locally metastasized MTC at age 70; a 46 year old female with a 0.5 cm MTC incidentally found in a thyroid lobe, that was resected because of a large colloid cyst, and a male with a pheochromocytoma at age 48 and 2 small (2 and 8 mm) foci of MTC following screening for MEN-2a at age 54.

In all cases the family history was negative for MTC and pheochromocytoma. At present (10-2005), genetic testing in 3 of 4 families has identified 7 living carriers without clinical or biochemical evidence of MTC even at high age (range 23-83 yrs). All families originate from the same geographical region, suggesting a common founder for this unique MEN-2a mutation. Haplotype analysis to confirm this hypothesis is currently in progress.

The insertion-deletion mutation at codon 666 of the RET oncogene is thus associated with a low penetrance of MTC (<40%) and of

pheochromocytoma (<10%). These observations suggest that early prophylactic thyroidectomy in asymptomatic carriers has to be evaluated in the context of the type of RET mutation.

## P502

### Use of TRH in addition to CRH stimulation during bilateral inferior petrosal sinus sampling in Cushing's syndrome – long term experience

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#### Aim

We previously reported preliminary evidence that use of TRH with CRH during inferior petrosal sinus sampling (IPSS) in Cushing's syndrome gave additional information about quality of pituitary venous sampling and significance of gradients. We now report long-term experience of this technique in 40 patients investigated 1993-2005. We used standard central:peripheral ratios (C:P) of 2:1 basally and 3:1 after stimulation to indicate pituitary secretion and intersinus gradient (ISG) of >1.5:1 to indicate lateralisation of ACTH secretion or TSH/PRL after TRH. We attempted to mathematically 'correct' the levels and gradients of ACTH for presumed unequal sampling of pituitary blood evidenced by TSH/PRL ISGs.

#### Results

37 had results consistent with Cushing's disease: 33 patients had basal ACTH C:P >2 and peak C:P >3; three had basal ACTH C:P <2 but peak C:P >3 after CRH.; one patient had C:P >2 basally but <3 after CRH peak. 3 had no significant C:P gradient consistent with ectopic ACTH secretion but TSH/PRL ISG indicated the possibility of inadequate pituitary sampling on one side in 2 cases.

Only 9 patients (22.5%) had equal pituitary venous sampling confirmed with maximum TSH/PRL ISG <1.5; ACTH ISG was >1.5 in 6 of these and ISG did not change significantly after 'correction' in any patient.

31 patients (77.5%) had unequal sampling with maximum TSH/PRL ISG >1.5; in 10 cases ACTH ISG did not change even with 'correction' but in the remainder the conclusion regarding presence of absence of ACTH lateralisation was significantly altered by 'correction' for unequal sampling.

#### Conclusion

IPSS involves unequal sampling of pituitary venous blood in a majority of cases as evidenced by TSH/PRL ISG before and after TRH. Correction of ACTH ISG using TSH/PRL ISG alters the conclusion on presence or absence of ACTH lateralisation in 52.5% of cases and we believe increases accuracy.

## P503

### Glucagonoma syndrome – treatment with intensive insulin and high dose vitamin B

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Malignant glucagonomas are slow-growing tumours which more commonly cause symptoms through glucagon hypersecretion than through effects of tumour bulk. The glucagonoma syndrome includes necrolytic migratory erythema (NME), diabetes mellitus, weight loss, anaemia, chelitis, venous thrombosis and neuropsychiatric symptoms. The syndrome probably results from unrestrained proteolysis<sup>1</sup>, and rapid consumption of B-vitamins through accelerated intermediate metabolism<sup>2</sup>. Here we present a patient with metastatic glucagonoma whose symptoms responded to treatment with intravenous insulin and high dose vitamin B.

A 47-year old lady presented with anorexia, lethargy, weight loss and rash. She was recently diagnosed with diabetes mellitus. Glucagonoma was diagnosed following biopsy of a 6 cm pancreatic mass. She had a distal pancreatectomy and splenectomy. A diffuse maculo-papular rash affecting both legs was retrospectively diagnosed as NME. She had unresectable liver metastases and was commenced on the somatostatin analogue, lanreotide. Her diabetes was easily controlled with gliclazide.

She was admitted following recurrence of symptoms of cachexia, glossitis and NME. In view of the putative pathophysiology of the glucagonoma syndrome, she was commenced on a regime of intravenous insulin and

B-vitamins (Pabrinex®). There was a marked improvement in glossitis, rash and in her overall sense of wellbeing. To our knowledge this is the first case in which a treatment strategy designed to counteract the metabolic effects of hyperglucagonaemia has been shown to ameliorate the symptoms of the glucagonoma syndrome.

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## P504

### Headaches cured by surgery

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Headaches associated with acromegaly are common even after hypophysectomy. Medical treatment with dopamine agonists and Somatostatin analogues often help. We report two cases when pharmacological therapy failed but further surgical removal of residual pituitary tissue cured the headaches.

A 38 year-old lady presented with an eight year history of headaches, unresponsive to simple analgesia. Acromegaly was confirmed by a raised serum growth hormone (GH) 33 mU/l, not suppressed by an oral glucose tolerance test (OGTT). A magnetic resonance imaging scan (MRI) showed a right pituitary macroadenoma. She underwent a hypophysectomy, but two weeks later the headaches reappeared. She was treated with adjuvant external beam radiotherapy but the headaches continued. An MRI scan showed residual tumour in the right side of the pituitary fossa. Further pituitary surgery was performed. Within 20 hours there was complete remission of headaches. Histology failed to demonstrate a growth hormone secreting tumour. An OGTT confirmed biochemical cure. The patient remains symptom free for the past seven years.

A 40 year-old man presented with a five year history of headaches, not relieved by simple analgesia. Acromegaly was confirmed by a raised GH 10.1 mU/l not suppressed by an OGTT. A MRI scan showed a right pituitary macroadenoma. He underwent surgery, one day post operatively GH was less than 0.5 mU/l and the headaches had resolved. Three months on the headaches returned. Octreotide relieved the headache but he soon developed tolerance. Further surgery was carried out a year later, with immediate post operative resolution of the headache. Histology of the pituitary tissue confirmed sparsely granulated somatotrophic cells. Eight months on, he remains symptom free.

These cases provide further evidence that the mechanism of the headache associated with acromegaly is complex and in patients in whom all other therapies fail, further pituitary surgery may be of benefit.

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## P505

### Chromogranin-A in adrenal incidentalomas – marker for tumour secretion

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Adrenal incidentalomas are incidentally discovered adrenal tumors by imaging methods without any prior suspicions of adrenal disease. Most of these tumors are functionally inactive but thorough investigations can reveal discrete hormone excess. Chromogranin A (CgA) is expressed in neuroendocrine cells throughout the body, including large and small intestine, adrenal medulla and pancreatic islets. It is a marker for carcinoid tumors, paragangliomas, and other neuroendocrine tumors. As a member of granin family, it was used in some studies as a marker of pheochromocytoma among patients with adrenal incidentaloma. The aim of this study was to determine CgA in patients with adrenal incidentaloma. We have evaluated 35 patients (28 female and 7 male) with adrenal incidentaloma, mean age  $52.42 \pm 13.62$  years. During inpatient testing blood was sampled and CgA was determined by commercial RIA kits with normal range: 19.4–98.1 ng/ml. In eleven patients, 31.42%, CgA were above upper normal level. Six of them had nonfunctional adrenal tumors with size under 3.5 cm and no

other carcinoma and neuroendocrine tumor were detected. After endocrine testing five patients underwent surgical procedure and pathology confirmed two pheochromocytomas, one cortical adenoma, one macronodular adrenal hyperplasia and one secondary deposit of pancreatic carcinoma. In twenty four patients, 68.58%, CgA level was in normal range. Five of them, were surgically treated and one was MEN II b. Our study showed that CgA might be useful in endocrine testing in patient with adrenal incidentaloma since one third of tested patients had elevated values. Further investigations must be done to confirm its sensitivity and specificity.

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## P506

### A novel germ-line mutation gly321arg in the exon 5 of the ret proto-oncogene detected in a family with familial medullary thyroid carcinoma

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Familial medullary thyroid carcinoma (FMTC) is an autosomal dominant inherited disease, characterized by germ-line mutations in the *RET* proto-oncogene, mainly in exons 10 and 11, but also in exons 13, 14 and 15. Recently, there were described also mutations in exon 8 and 16 associated with FMTC. In our laboratory the screening of six risk exons of the *RET* proto-oncogene in 141 families with MTC was performed. 10 families were classified as clinically FMTC. In 4 of them mutations in exons 11, 13 and 14 were detected. In the remaining families the screening of other *RET* exons has been started. For rapid *RET* proto-oncogene mutation screening we used single strand conformation polymorphism (SSCP) method and fluorescent sequencing method. Here we report a novel missense point mutation of the *RET* proto-oncogene in exon 5 that substitutes glycine for arginine at codon 321 in the cadherin-like domain in the extracellular region of ret protein found in one Czech family with FMTC. It is likely that this mutation causes familial medullary thyroid carcinoma (FMTC), because no other mutation was found in classical risk exons (10, 11, 13, 14, 15 and 16) of the *RET* proto-oncogene. The mutation cosegregates with medullary thyroid carcinoma (MTC) or C cell hyperplasia (CCH) in two patients, two other family members are mutation carriers but still without clinical signs of MTC. Three other relatives are not mutation carriers and they are clinically unaffected. It seems that this newly described mutation belongs to less aggressive mutations with regard to later onset of the disease in index patient. To improve the diagnosis of FMTC, analysis of exon 5 of the *RET* proto-oncogene should be considered in families with unidentified classical *RET* mutations. The work was supported by grant IGA MZ CR NR/7806-3 and was approved by local Ethical committee.

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## P507

### Contemporary management of macroprolactinomas

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Contemporary management of macroprolactinomas relies heavily on the use of dopamine agonist (DA) therapy. However, historically a proportion of patients may have undergone surgery and/or pituitary radiotherapy. We aimed to determine the long-term outcome in terms of tumour control and prolactin normalisation in a large cohort of patients with macroprolactinoma who received various treatment modalities. 80 patients (54 male) with macroprolactinoma (tumour diameter >10 mm, prolactin >6000 mU/L) were identified as being treated at this centre between 1980–2005. Mean ( $\pm$  SE) age at diagnosis was  $42.7 \pm 1.8$  years (men) and  $40.4 \pm 3.6$  years (women). Median (range) duration of follow-up was 8.0 (0.67–31.0) years. Serum prolactin at diagnosis ranged from 8,000–1,160,000 mU/L. Visual fields were abnormal in 58%. DA therapy was used at some point in 79 patients, however 22.5% also had pituitary surgery, 7.5% radiotherapy, and 10% combination surgery and radiotherapy. In all those patients who had

initial surgery, prolactin levels remained high post operatively, necessitating initiation of DA therapy. In addition, 71.4% of those who had pituitary radiotherapy required long-term and on going treatment with a DA to control prolactin hypersecretion. At the most recent clinic visit 94% of patients were taking a DA and 61% had achieved a normal prolactin. Five patients (6%) were not taking a DA, but only one (treated by radiotherapy) had normal prolactin levels. One patient had evidence of uncontrolled tumour growth despite receiving radiotherapy followed by DA treatment. No patients treated with initial DA therapy alone had uncontrolled tumour growth. Despite utilising surgery and/or radiotherapy in the early management of patients with macroprolactinomas, most required long-term use of DA therapy. From this large cohort, there appears to be no rationale for treating macroprolactinomas with anything other than first line DA therapy.

## P508

### Cushing's paraneoplastic syndrome secondary to recurrent ovarian carcinoma

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Obesity is a major cause of chronic hypoventilation and/or obstructive sleep apnea syndrome (OSAS). Aim of this study was to evaluate the prevalence of pulmonary dysfunction and the relationship between OSAS and several clinical, functional and polysomnographic parameters in severely obese patients. 101 subjects (68 females and 33 males) aged  $49.2 \pm 13.2$  years (mean  $\pm$  SD), with BMI  $46.8 \pm 6.5$  Kg/m<sup>2</sup> were enrolled. Sleep quality and daytime sleepiness were assessed using the Epworth Sleepiness Scale (EPSS). Serum triglycerides, HDL-cholesterol, glucose, leptin, and thyroid hormones were measured. All patients underwent cardio-respiratory polygraphic sleep study and lung function tests. Arterial hypertension was present in 56.4% patients and type 2 diabetes in 30.7%. Snoring was referred by 91% patients, nocturnal awakening by 51% and apneas by 41%. Pathologic sleepiness was present in 61% patients. 70.3% patients had larger than normal neck circumference. Arterial gas analyses showed reduced PaO<sub>2</sub> in 41% patients. An obstructive ventilatory pattern was found in 15% patients, a restrictive pattern in 10% and a mixed pattern in 3%. The expiratory reserve volume and functional residual capacity were significantly reduced in 50% patients. 14% patients had mild, 13% moderate, 13% severe and 21% very severe OSAS. Only 39% patients did not meet the diagnostic criteria for OSAS. A significant positive correlation was found between the apnea-hypopnoea index (AHI) and EPSS ( $P < 0.005$ ), waist/hip ratio ( $P < 0.005$ ), neck circumference ( $P < 0.005$ ) or PaCO<sub>2</sub> ( $P < 0.05$ ). An inverse correlation was observed between AHI and PaO<sub>2</sub>, nocturnal mean or minimum oxygen saturation. No correlation was found between AHI and other respiratory functional, cardio-respiratory, metabolic or hormonal parameters.

In conclusion, our data indicate a high prevalence of respiratory dysfunction in severely obese patients, including diurnal hypoventilation and OSAS. We suggest that pulmonary function should be systematically screened in severely obese subjects even in the absence of overt manifestations of disease.

## P509

### Moleculargenetic and clinical characterisation of neurofibromatosis type 1 – associated pheochromocytoma

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Familial pheochromocytoma is inherited as a component of one of 5 pheochromocytoma – related syndromes such as von Hippel-Lindau disease, multiple endocrine neoplasia type 2 and the paraganglioma syndromes type 1 and type 4.

Neurofibromatosis type 1 is often cited as the fifth of these syndromes but a clinical-genetic characterisation does not exist. 0.1 to 5.7% of patients with neurofibromatosis type 1 have pheochromocytoma. The NF1 gene as the susceptibility gene for neurofibromatosis type 1 is considered to be one of the largest genes in the human. Mutation detection is a considerable challenge because of the large size of the gene, the lack of any mutational clustering and the presence of 36 pseudogenes.

The background of this study was a registry of 24 patients with neurofibromatosis type 1 and pheochromocytoma. The search for intra-

exonic mutations and exon-specific deletions was performed for all 57 exons of the NF1 gene. An inactivation of the NF1 wildtype allele in tumor tissue was tested by loss of heterozygosity analysis.

22 of the 24 patients showed germline mutations of the NF1 gene. The mutation hit rate was 92%. A loss of the wildtype allele was found in 67% of the paired lymphocytes and tumor samples. Patients showed an average age at diagnosis of 42 years. All NF1 – related pheochromocytomas were adrenal.

The wildtype inactivation found in 67% of the pheochromocytomas suggests the causative role of NF 1 in these tumors. Pheochromocytoma as a classic tumor of neural crest derived origin is therefore a true component of neurofibromatosis type 1. The mutation rate was 92%. 78% of the detected mutations were germline point mutations 14% of them were large deletions affecting one single exon or almost the entire gene.

## P510

### AcroBel: a registry and survey on acromegaly in Belgium

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To evaluate the epidemiology and global quality of care of acromegaly in Belgium, all endocrinologists treating patients with acromegaly were invited to participate in a nationwide survey extending from 15-6-2003 till 30-9-2004, aiming to include all patients (including deaths) that were in follow up or newly diagnosed after 1-1-2000. The project was ethically approved and written informed consent obtained. Retrospective data on demographics, pathology, complications and treatment were anonymously collected through Palms and stored in an Access-based database. A symptom score and the ACRO-Qol questionnaire were used to evaluate current quality of life. The level of hormonal control was determined by central measurements of serum GH, IGF-1 and IGFBP-3.

The participation of 64 physicians working in 37 centres provided a 'real life' picture of acromegaly. Of the 419 patients (51% men) in the dataset, 80% were followed in 12 university or large regional hospitals (9–65 patients each), the care of the remaining 20% was dispersed over 25 hospitals (1–6 patients each).

There were 96 new cases of acromegaly reported, giving a global incidence of 2 cases per million inhabitants (c.p.m.) per year. The prevalence was globally 41 c.p.m. but varied between 21 and 61 c.p.m. among the different areas. Mean age at diagnosis was  $42 \pm 13$  y in male and  $48 \pm 14$  y in female patients, with a mean follow up of  $12 \pm 9$  y (range 0–45). Twenty-seven deaths were reported at a mean age of  $65 \pm 14$  y in men and  $70 \pm 12$  y in women. Central lab assays were available in 316 patients (75%). Mean GH was  $< 2$   $\mu$ g/l in 65% of patients (excluding 11 on GH replacement therapy and 4 on pegvisomant) while 56% had an IGF-I Z-score  $< +2$ .

Conclusion: Despite the available treatment modalities (pre-pegvisomant era), less than 60% of patients with acromegaly in Belgium are cured or controlled according to currently admitted criteria.

Acknowledgement: this AcroBel survey was made possible with the generous support of Novartis Belgium.

## P511

### The latest safety and efficacy data of patients treated with Pegvisomant I Schreiber, K Forssmann, M Buchfelder, M Droste, K Mann, B Saller & CJ Strasburger

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The German Acrostudy is at present the largest database on acromegalics ( $n = 184$ ) treated with pegvisomant (Somavert<sup>®</sup>). 91% pts underwent pituitary surgery, 45% received radiation therapy, and previous medical therapy included dopamine agonists (56%), octreotide (90%) and/or lanreotide (10%). Common concomitant diseases at baseline were hypertension (47.0%), diabetes mellitus (31.7%), and gallstone disease (25.6%). Efficacy analysis was performed in 134 pts with a follow-up after 6 months, in 99 pts after 12 months and in 22 pts after 2 years. Mean duration of pegvisomant therapy was  $42.2 \pm 30.0$  wks. IGF-I was



normalized in 65.4% at 6 months with a median dose of 15.0 mg/d and in 90.2% of pts treated for at least 2 years. The fasting glucose levels improved significantly from  $117.3 \pm 49.5$  mg/dl to  $105.0 \pm 44.9$  mg/dl after 6 months and  $100.7 \pm 34.0$  mg/dl after 12 months respectively. General physical condition measured by specific sign and symptom score improved significantly. Elevated liver function tests (LFTs)  $>3$  times above normal occurred in 9 pts receiving octreotide before. In 6/9 pts, LFTs spontaneously normalized during continued treatment, in 3 pts (1.6%), levels normalized after discontinuation. With exception of one patient with transient LFT elevation due to alcohol excess, ALT was the enzyme most prominently elevated. Progression of remnant pituitary adenoma was reported in a total of 9 pts. 7 cases were re-evaluated by an independent neurosurgeon. In 3 pts tumor progression could not be verified at re-evaluation. In 2 pts, the tumor continuously grew already on somatostatin analogues. In one case, a slight and clinically non-significant growth was confirmed during pegvisomant therapy and one case showed a tumor growth due to rebound from somatostatin-induced shrinkage. In conclusion, Pegvisomant is generally well tolerated with a safety profile similar to that reported in clinical trials, and can effectively reduce IGF-1 in pts refractory to conventional therapy.

## P512

### Mutation at codon 804 detected in a Greek kindred by screening of the RET gene in patients with medullary thyroid carcinoma

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Medullary Thyroid Carcinoma (MTC) is a rare cancer that arises from the thyroid C-cells and occurs as sporadic in 75% of the cases. In 25% of MTC cases, mutations of the RET proto-oncogene are responsible for the development of three dominantly inherited neoplastic disorders including multiple endocrine neoplasia (MEN) 2A, MEN 2B and familial medullary thyroid carcinoma (FMTC). Since 2–8% of MTC cases considered sporadic conceal germline mutations, direct analysis of the RET gene should be performed in all MTC patients.

In this study we have analysed RET gene for mutations in 30 Greek patients, belonging in unrelated families, who had previously undergone surgery for sporadic MTC. Informed consent was obtained and exons 10, 11, 13, 14, 15 and 16 were screened in all of the patients. A 51-years-old female patient carrying the mutation V804L was identified. Genetic testing in 6 members of her family revealed the presence of 3 more heterozygote carriers without clinical symptoms. The index-patient's mother refused surgery. Her 31-years-old son underwent prophylactic surgery and diffuse C-cell hyperplasia has been detected. His 4-years-old son is a mutation carrier too. All mutation carriers do not present any clinical and laboratory evidence of pheochromocytoma and primary hyperparathyroidism. The index-patient, 2 years after surgery, is still without evidence of MTC, as assessed with pentagastrin stimulated calcitonin levels.

Two types of mutations have been observed in codon 804: V804L and V804M. Presently the V804M mutation has been associated only with FMTC, whereas pheochromocytoma has been reported in a family with the V804L mutation. There is wide clinical variability and there is not yet agreement as to therapeutic strategies for mutation carriers. We recommended annual evaluation for both adult patients with PTH, calcium and metanephrines; additionally a pentagastrin stimulation test every two years for the child.

## P513

### A rare RET gene mutation is found in two apparently unrelated Greek kindreds with familial medullary thyroid carcinoma

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Familial medullary thyroid carcinoma (FMTC) is caused by germ-line mutations in the RET proto-oncogene. These mutations concern mainly exons 10 and 11, whereas mutations in exons 13–16 are rare. Mutations in exon 8 have been reported in the literature only twice.

We performed direct analysis of exons 7–19 and 21 of RET gene in two apparently unrelated Greek index-patients with FMTC, presenting negative initial screening for mutations in exons 10–16. We have found the same exon 8 mutation in both. Informed consent was obtained from all members of both families. The mutation was detected in heterozygosis, in seven MTC patients as well as in twenty-four asymptomatic relatives of both families. An additional MTC patient was found to be homozygous for the mutation, due to parental consanguinity. The same point mutation has been reported only once in the literature in another family with different country of origin. It is the first time that a biallelic carrier of this mutation is described.

It is most likely that this mutation causes FMTC, as no other mutation was found, the mutation co-segregates with FMTC, and family members without the mutation are clinically unaffected. The mutation shows wide clinical heterogeneity, as patients' ages at diagnosis ranged from 23 to 88 years. Because of the rarity of this point mutation, there are no specific recommendations regarding the age of malignant progression and therefore, appropriate timing for prophylactic surgery. Since the earliest age at diagnosis of MTC reported up to date for this mutation is 21, we recommended pentagastrin stimulation test in all carriers of the mutation and total thyroidectomy (with or without lymph-node dissection of the central neck compartment) in all adults. Two of them were operated recently, a 35-year-old male presenting C-cell hyperplasia and a 25-year-old female presenting a microscopic MTC focus.

## P514

### Surgical cure with preserved pituitary function is rare in acromegalic patients. Results from the Preoperative Octreotide Treatment of Acromegaly (POTA) study

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#### Background

In many acromegalics neurosurgery is unable to control GH secretion while it may harm the normal pituitary.

#### Objective

To investigate the effect of pre-treatment with octreotide on surgical outcome in acromegaly.

#### Methods

Previously untreated acromegalics were randomised directly to surgery or octreotide for six month before transphenoidal surgery. Three months postoperatively patients underwent OGTT and Insulin Tolerance Test (ITT). Cure was defined as IGF-1  $\leq$  upper reference value and GH-nadir during OGTT  $\leq$  2 mIE/l.

#### Results

Twenty-five of 61 patients (41%) were cured by surgery. In 7 patients ITT were contraindicated. Among the 54 patients performing ITT, 23 had a normal cortisol response ( $>550$  nmol/l), 39 had a normal GH response ( $\geq 9$  mIE/l) and 19 had a combined normal response. Twenty-three of 25 patients cured by standard criteria underwent ITT. Only 8 (3 pretreated; 5 direct surgery) who were cured by standard criteria had preserved pituitary function in terms of normal GH and cortisol response.

#### Discussion

This is probably the first study investigating the effect of octreotide pre-treatment and transphenoidal surgery for acromegaly on both cure by standard criteria and on indices of pituitary function. We propose the concept of "extended cure" meaning cure by standard criteria combined with preserved pituitary-adrenal function and GH-secretion. In our series only 8 out of 54 (15%) achieved "extended cure" while 15 out of 23 (65%) of those cured by standard criteria had indications of pituitary insufficiency. Pre-treatment with octreotide did not affect the results. We believe our data mirrors the clinical reality. Hence, we question whether or not surgery should remain the first line therapy for acromegaly. Future studies on the effect of both medical therapy and surgery in acromegaly should include evaluation of pituitary function and preserved pituitary function should be included in the definition of cure.



**P515****Different scintigraphic patterns between sympathetic and parasympathetic paragangliomas**

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Paragangliomas (PGLs) are neuroendocrine tumors arising from the neural crest and localized from the skull base to the pelvic floor. In particular, parasympathetic PGLs arise in the head/neck region (carotid body, vagal nerve, jugular and tympanic glomus) and more rarely in the anterior thorax, while sympathetic PGLs are localized in the posterior thorax and in the abdomen and secrete catecholamines.

Localization of paragangliomas might be performed by anatomical (CT and MRI) and functional (nuclear medicine) imaging studies. The latter are useful to confirm the diagnosis, when CT/MRI are negative in spite of positive biochemical data or to detect multifocal or metastatic disease.

In this study we evaluated 15 patients affected by PGL-1, an hereditary syndrome caused by SDHD gene mutations and characterized by multiple PGLs and/or pheochromocytomas.

CT and MRI detected 27 PGLs: 11 glomus tumors, 6 vagal PGLs, 3 jugular PGLs, 1 tympanic PGL, 3 thoracic PGLs and 3 abdominal PGLs. All patients underwent scintigraphy with <sup>111</sup>In-pentetreotide (octreoscan), while scintigraphy with <sup>123</sup>I-metaiodobenzylguanidine (MIBG) was performed in 8 patients. Octreoscan identified the majority of head/neck PGLs (18/21) and all thoracic PGLs, but it was negative in all abdominal PGLs; on the contrary MIBG resulted positive in each abdominal PGLs, in 2/3 thoracic PGLs and only in 1/12 PGLs in the head-neck region.

In conclusion, PGLs show a differential tracer uptake confirming their different nature: PGLs which are parasympathetic in origin are detected by Octreoscan, while PGLs which are sympathetic in origin are preferentially detected by MIBG.

The presence of a high concentration of somatostatin receptors in parasympathetic PGLs suggests to further evaluate their functional role in these tumors in the hope that somatostatin analogues might represent an alternative treatment to surgery in patients whose tumors cannot be surgically removed because of their dimensions or location.

**P516****mRNA expression of somatostatin receptor subtypes in pheochromocytomas/paragangliomas**

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Somatostatin (sst) receptors are expressed in many tissues and multiple subtypes are often present in the same cells. Their activation by endogenous sst as well as by sst-analogues leads to inhibition of secretion and growth in some tumor, such as the neuroendocrine ones.

Pheochromocytomas (Pheos) and paragangliomas (PGLs) are neural crest-derived tumors which can be sympathetic or parasympathetic in origin and present themselves as sporadic or familial. Their therapy is surgical but in some cases (metastatic pheos/PGLs or large Head/Neck PGLs) their removal is impossible and an alternative medical therapy is desirable.

We evaluated mRNA expression of the five different sst receptors in 25 Pheo/PGLs obtained at surgery in patients affected by a sporadic (17) or familial (8) tumors. Familial tumors were due to VHL (1), Ret (1), NF-1 (1) and SDHD (5) mutations.

Measurement of sst receptors was performed by quantitative real-time PCR (TaqMan™). Primers and probes for each sst receptors were selected by the proprietary software Primer Express (Applied Biosystem Inc.). Standard curves were made by cloning in PCR<sup>II</sup>-TOPO vector (Invitrogen) a specific amplicon for each receptor. Results, expressed as copies/ug total RNA show similar median values with a wide range of distribution for each receptor class. Median (M) and range (R) values were as following: sst-1: M 3.20E + 04; R 7.40E + 02/2.00E07. sst-2: M 2.56E05; R 1.30E + 03/9.90E + 05. sst-3: M 1.70E + 04; R 8.20E + 01/2.30E + 06. sst-4: M 2.68E + 04; R 1.40E + 03/3.10E + 05. sst-5: M 5.98E + 03; R 4.00E + 01/1.60E + 07.

All the 5 sst receptors subtypes were expressed in the tumors. sst-5 showed the highest variability while sst-2 and sst-4 the lowest. We found no differences among sporadic and familial tumors.

The expression of all the sst receptors subtypes is the first step towards the possible therapeutic use of different sst-analogues in patients with non operable pheos/PGLs.

**P517****A retrospective review of the effect of pituitary radiotherapy after transphenoidal surgery for non-functioning pituitary adenomas**

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**Background**

The role of post-operative radiotherapy (RT) in the treatment of non-functioning pituitary adenomas (NFA) remains controversial. We compared the difference in outcome between patients receiving transphenoidal surgery (TSS) alone and TSS plus RT in 93 patients treated for NFA between 1986 and 2005. 64 (69%) had TSS alone whilst 29 (31%) received TSS plus RT (45GY, 25Fr, 3 field). All patients were followed up by CT and then MRI scans repeated at increasing intervals. Tumor recurrence was defined as a significant increase in pituitary size on CT/MRI.

**Results**

Follow up (median + range) was 6.4 (0.1–17.8) years in TSS and 5.7 (1.8–18.7) years in TSS + RT groups. Tumor recurrence occurred in 14 (22%) TSS at 4.1 (1.6–10.3) years and 2 (7%) TSS + RT at 6.3 and 14 years. In TSS group the recurrence rates were 18% at 5 years and 41% at 10 years. Only 4 of 14 recurrences in TSS group involved suprasellar extension (SSE; 3 in era of CT for post-op scan) and recurrence was intrasellar in the remainder; only one SSE caused worsening of visual field defect and this improved after repeat surgery. Second treatment in the TSS group included RT alone (n = 10), repeat surgery plus RT (n = 2), repeat surgery alone (n = 1) and observation alone (n = 1). Both TSS + RT received repeat surgery. Regarding endocrine replacement, 37.5% of TSS required 3 or more hormone products, compared with 62% in TSS + RT.

**Conclusion**

Our results confirm that NFA recurrence after TSS is reduced in patients treated with RT, but show no evidence of harm prior to treatment of recurrence in the TSS only patients. Given the increased morbidity and cardiovascular risk associated with hypopituitarism, and recent evidence that dopamine agonists may inhibit tumor recurrence, we suggest that this expectant approach as regards RT is justifiable and potentially advantageous.

**P518****Biochemical characteristics of "silent" ACTH-secreting pituitary adenomas: Preoperative serum and urine hormone studies**

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"Silent" ACTH-secreting pituitary adenomas are not characterized by specific clinical features that would suggest the presence of hypercortisolism. In contrast, they present as non-functioning pituitary macroadenomas with either visual compromise or hypercortisolism. However, immunostaining reveals ACTH-secreting cells.

In order to characterize the laboratory features of these tumours, we have preoperatively assessed 56 patients with either clinically non-functioning pituitary adenomas or Cushing's disease with a standardized protocol. They had plasma measurements of cortisol and ACTH, low-dose (2 mg) dexamethasone testing and 24-hour urine corticosteroid determinations. All tumour specimen underwent immunohistochemistry. We compared the laboratory findings in "silent" ACTH-secreting (SACTH; n = 6) and "silent" gonadotropin-secreting (SGON, n = 14) tumours to those who did not immunostain for any hormone (INACT, n = 22) and patients with Cushing's disease (CD, n = 13).

Mean plasma cortisol varied from 555.8 nmol/l in patients with CD, 358.3 nmol/l in patients with SACTH, 389.6 nmol/l in patients with SGON and 409.3 nmol/l in patients with INACT. In contrast, mean plasma ACTH was highest in patients with CD (30.6 pg/ml) and SACTH (22.3 pg/ml), but much lower in patients with SGON (14.2 pg/ml) and in INACT (12.3 pg/ml). Likewise, mean dexamethasone-suppressed cortisol levels were much lower in patients with SGON (49.7 nmol/l) and INACT (48.7 nmol/l) than in patients with SACTH (216.2 nmol/l) and of course, those with CD (441.5 nmol/l). While all mean steroid hormones in the 24-hour-urines of patients with CD were clearly elevated, there was no significant difference in the urine steroids between patients with SACTH, SGON and INACT. Mean free urinary 24-hour-cortisol was 304.8 µg in CD, 38.7 µg in SACTH, 70.5 µg in SGON and 44.2 µg INACT.

We thus conclude, that biochemistry allow us to preoperatively differentiate the subtypes of "non-functioning" pituitary adenomas.

However, plasma ACTH-levels and dexamethasone suppression testing in this context seem to be superior to urine steroid measurements.

## P519

### Radiotherapy and o,p'-DDD induce an inhibition of growth and interfere in the cell cycle in H295-R adrenocortical cell line

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#### Background

Mitotane (o,p'-DDD) is a compound with potent adrenotoxic effect and is able to block cortisol synthesis by inhibiting 11 $\beta$ -hydroxylation and cholesterol chain cleavage. For these reasons, mitotane was widely used in the treatment of adrenocortical cancer. Nevertheless the biological mechanism induced by these treatments in this cancer cells remain unknown.

#### Aim

To study whether the o,p'-DDD could increase the susceptibility to the radiation exposure in adrenocortical cancer cells, we investigated the cell growth inhibition, cell cycle perturbation and cell cycle related molecules at 24, 48, 72, 96 and 120 hours of post-irradiation (post-IR).

#### Results

The analysis of cell growth inhibition showed that o,p'-DDD/6 Gy/min combined treatment enhanced the inhibitory effect induced by 6Gy alone (83% and 50%, respectively). The cell cycle analysis performed at 24 hours post-IR showed that 6 Gy/min alone and o,p'-DDD/6 Gy/min combination induced the same accumulation of cells in the G2 phase, with a concomitant depletion from the G1 phase, as compared to untreated cells. However, a significant difference between the two treatments is observed during the cell cycle progression. In fact, at 120 hours post-IR, the H295-R cells are able to recover the 6 Gy/min induced G2 block (27%) while the cells treated with o,p'-DDD/6 Gy/min are still arrested in the G2 phase (43%). The inability of the cells to recover the G2 block could be related to cyclin B1, A and cdk2 modulation. Cortisol levels in pharmacological treatments were undetectable, while in irradiated cells its values are included in normal range. We found an increase both the irradiated and treated with adrenolytic therapy in a time dependent manner. Proteomic study are in progress for determination the proteic pattern induced by o,p'-DDD in the same cell line.

#### Conclusions

The adrenolytic compound o,p'-DDD renders adrenocortical cell line more susceptibility to radiotherapy determining a G2 irreversible block.

## P520

### Role of PKA regulatory subunit 2B protein on cortisol-secreting adrenocortical cells proliferation

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The two regulatory subunits (R1 and R2) of PKA are differentially expressed in several cancer cell lines and studies indicate distinct roles for these subunits in growth control. Recently, mutations of the regulatory subunit 1A of PKA gene have been identified in patients with Carney complex (CNC). The aim of this study was to evaluate the expression of the different PKA regulatory subunits (R1A, R2A, R2B) in adrenocortical tumors not associated with CNC, as well as the effects of subunit activation on cell proliferation. Immunohistochemistry demonstrated an absent expression of R2B in all 10 cortisol-secreting adrenal tumors studied, while both R1A and R2A were expressed at high levels. Conversely, in all the adrenal carcinomas studied all the 3 regulatory subunits were expressed at high levels. Sequencing analysis of the R1A and R2B genes revealed a wild type sequence in all tumors. The effect of R1/R2 ratio on proliferation was assessed in mouse adrenocortical Y-1 cells that showed a similar pattern of R subunits expression compared to human normal adrenocortical cells. The R2-selective cAMP analogue 8-Cl cAMP dose-dependently inhibited Y-1 cell proliferation on one hand and stimulated apoptosis on the other. The

anti-proliferative effect of R2B was further confirmed by the observation that R2B silencing by siRNA technique was associated an increase on Y-1 cell proliferation. Conversely, the R1-selective cAMP analogue 8-HA-cAMP stimulated the proliferation of the same cells. Finally, no effect of cAMP analogue was observed in NCI-H295R, a human adrenal carcinoma cell line. In conclusion, although a high R1/R2 ratio promotes cell proliferation in adrenocortical cells, the loss of R2B seems to be associated with a benign phenotype.

## P521

### Outcome of management of craniopharyngiomas – a contemporary series

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The optimum management of patients with craniopharyngiomas is controversial. Evidence relies on a small number of retrospective outcome surveys that encompass time periods that are not necessarily representative of current surgical techniques and imaging modalities. We have reviewed outcome in a more contemporary group of patients managed at a single centre.

Case-notes and electronic data were reviewed of all patients treated for craniopharyngioma since 1980.  $N = 66$ , 53% female; average age:  $40.3 \pm 20.3$  yrs (59 patients >16 years); average follow-up:  $10.1 \pm 6.6$  yrs. 67% of patients were treated with surgery alone and 33% received radiotherapy (22 surgery + radiotherapy, 2 radiotherapy alone). One patient received neither. Overall 5-year survival was 80% and age at time of first surgery was an over-riding factor: 5-year survival <30 years was 100%, <50 years 89%, and >50 years 59% ( $P = 0.004$  for <30 yrs/>30 yrs;  $P = 0.002$  for <50 yrs/>50 yrs). Application of radiotherapy did not influence 5 year survival: 83% who received radiotherapy survived 5 yrs vs 77% who did not. In the subgroup of those with tumour remnant post-operatively who were administered radiotherapy, figures were 82% and 67% respectively ( $P = N/S$ ). Rate of tumour re-growth was lower in those who received radiotherapy (20% vs 43%,  $P = N/S$ ). 31% of patients had no evidence of residual tumour on initial post-operative scan and 5 year survival in this group was 100% compared with 72% for those with an identified residuum ( $P = 0.042$ ). Hormone deficiencies were more common in those who had received RT (91% of axes tested vs 73%,  $P = 0.002$ ).

In this contemporary series of patients with craniopharyngioma, major determinants of mortality were age at surgery and presence of residual tumour on initial post-operative scan. The reduction in re-growth after radiotherapy did not reach statistical significance and the administration of radiotherapy did not significantly reduce mortality. Pituitary hormone deficiencies were more common in those who had received radiotherapy.

## P522

### Anatomo-clinical features in multihormonal prolactinomas

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It has been generally believed that clinically functioning pituitary adenomas are highly specialized neoplastic lesions, which produce a unique hormone. Increasing body of evidence argue against this concept, since about half of prolactinomas (PRM) and approximately 60% of TSH-secreting adenomas are multihormonal and 30–40% of GH-secreting adenomas co-secrete PRL. Most of classically considered null cell adenomas have been proved to be immunohistochemical positive for gonadotropins and with ultrastructurally morphologic evidence of plurihormonality.

#### Aim

To evaluate the immunohistochemical hormonal profile of PRM and to establish a potential correlation between multihormonality and their biologic behaviour.

#### Subjects and methods

After obtaining local Ethical Committee approval, we performed a retrospective study on 19 PRM operated between 1994–2005. Measurements of serum PRL level and tumor size were carried pre- and postoperatively, with serial determinations during dopamine agonist therapy. The surgical specimens were fixed in PFA and immunohistochemically stained by the avidin-biotin peroxidase complex method. The

diagnosis was considered PRM when more than 50% of cells stained positive for PRL and multihormonality was defined when more than 10% of cells stained positive for other hormone than PRL.

#### Results

9 out of 19 PRM were multihormonal (47.4%)- 5 women and 4 men, aged 21–56 yrs, mean 31 yrs. There was no significant difference between mono and multihormonal PRM concerning gender, age, tumor size and preoperative PRL level. Postoperative mean serum PRL level was significantly higher than in monohormonal PRM ( $P < 0.05$ ). Resistance to DA therapy was encountered only in 3/9 multihormonal PRM.

#### Conclusions

Plurihormonality in prolactinomas is not a marker of aggressive biological behaviour or predictor of treatment efficiency.

### P523

#### Glucocorticoid receptor gene N363S variant in patients with clinically inapparent adrenal adenomas

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N363S polymorphism of the human glucocorticoid receptor gene has been detected in the heterozygous state in approximately 3–9% of general European population. This variant has been associated with increased sensitivity to glucocorticoids, increased insulin response to dexamethasone, a tendency towards lower bone mineral density, increased body mass index, and unstable angina. However, other reports found no associations with these pathological conditions. We assessed the prevalence of N363S in a group of 85 healthy subjects recruited between medical students and in a group of 51 patients with adrenal adenoma of incidental detection referred from 2000 to 2005 (29 women and 22 men, aged between 37–80 yr, median 60 yr). Aims of the present study were to compare the prevalence of N363S in the 2 groups, to investigate whether the presence of N363S in patients with adrenal adenoma was associated with some phenotypic characteristics. DNA was extracted from peripheral blood leukocytes using polymerase chain reaction (according to Qiagen protocol). The PCR product was digested with 1 U of *TaqI* (Fermentas) at 65 °C overnight, and the accuracy of genotyping was confirmed by sequence. Patients with adrenal adenoma underwent a thorough evaluation of the HPA axis. Eight patients (15.7%) had subclinical Cushing's syndrome (defined by the presence of at least 2 altered endocrine tests). The frequency of N363S at heterozygous state did not differ between patients and controls (4% vs 2.4%,  $P = \text{NS}$ ). One of the two heterozygote patients qualified for subclinical Cushing's syndrome, the other one had a normal function of HPA axis. Our data confirmed the prevalence of N363S variant described in literature. Other studies are needed to establish if N363S is associated with an increased sensitivity to glucocorticoids and may modulate the clinical phenotype.

### P524

#### Characteristics and follow up of thyroid cancer in patients with hyperthyroidism

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Thyroid cancer in patients with hyperthyroidism is considered to be a rare event and its course seems to be more aggressive in patients with Graves' disease. The coexistence of medullary thyroid cancer and hyperthyroidism is very rare and there are only 14 cases reported in the literature.

The aim of this retrospective study is to assess the clinical and histological characteristics and the evolution of thyroid cancer in patients with different types of hyperthyroidism.

#### Patients/Methods

Among 652 patients with thyroid cancer, 52 (11 males and 41 females), with mean age  $51 \pm 13$  years, were operated because of hyperthyroidism between 1988–2004, due to: toxic multinodular goiter 26(5%), toxic adenoma 19(36.5%) and Graves' disease 7(13.5%). Pathology revealed differentiated thyroid cancer (DTC) in 49 patients (94.2%) and medullary Ca in three (5.8%). The tumor was multifocal in 15/52 patients (28.8%) and bilateral in 5/15 (33.3%) and its size ranged from 0.2–6 cm. Extrathyroidal disease was observed in 6/26 with multinodular goiter, 5/7 with Graves'

disease and 3/19 with toxic adenoma. After surgery, 25 patients received ablation therapy with radioactive iodine  $^{131}\text{I}$ . The mean duration of follow up was 50 months. During follow up 4 patients presented with local recurrence or multiple metastases (1 with medullary and 3 with DTC). Two of these patients had a history of multinodular goiter and 2 of toxic adenoma. None of our patients with Graves' disease presented with local recurrence or metastases.

#### Conclusions

Thyroid cancer may occur in any type of hyperthyroidism. Its course was not found more aggressive in patients with Graves' disease. The coexistence of medullary thyroid cancer and hyperthyroidism is not a rare event. Given the coexistence of both diseases, we recommend surgery instead of radioiodine treatment, mainly in patients with suspicious nodules.

### P525

#### Investigation and surgical management of pancreatic neuroendocrine tumours

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#### Introduction

Diagnosis of pancreatic neuroendocrine tumours (NETs) is often a challenge and involves biochemical characterisation and anatomic localisation of the tumour. Wherever feasible, curative surgical excision is the treatment of choice.

#### Materials and methods

Twenty four consecutive patients with pancreatic NETs were referred for consideration of surgery over a six-year period (1999–2005). Following use of multi imaging modalities to localise, all were operated on by the same surgical team.

#### Results

Twenty-four patients underwent surgical resection (11 women, 13 men; age 21–77 years, median 53). Sixteen tumours secreted known hormones (11 insulinomas, 1 somatostatinoma, 1 gastrinoma, 1 glucagonoma, and 2 multiple islet cell tumours) and 8 were non-functioning. Three tumours were part of the MEN 1 Syndrome. All 24 tumours were successfully localised prior to resection. Operations performed included 7 pylorus preserving pancreatoduodenectomies, 7 tumour enucleations, 7 distal pancreatectomies, 1 duodenum-preserving resection of pancreatic head, 1 excision of retropancreatic gastrinoma, and 1 liver segment resection were performed. There were no fatalities. Six patients (25%) had perioperative complications. These included wound infection (2), pancreatic leak managed conservatively with drainage and octreotide (2), and peri-pancreatic collection requiring percutaneous drainage (2). One late complication involved a duodenal stricture after a duodenum-preserving resection of the pancreatic head. Complete excision with symptomatic cure was documented in 20 patients. Of the remaining 4, two had MEN 1 with multiple islet cell tumours. One patient with neuroendocrine carcinoma (positive resection margins) received adjuvant chemotherapy. One patient had MIBG avid liver metastases and received targeted radiotherapy for these.

#### Conclusion

Accurate pre-operative localisation can be achieved in pancreatic NETs and facilitates pancreas sparing surgery. Surgical resection can be performed in high-volume centres with a very low mortality and acceptable morbidity, and carries a high likelihood of cure.

### P526

#### Estrone and cortisol co-secreting adrenocortical carcinoma in a man presenting with hypogonadotrophic hypogonadism and painful gynecomastia

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#### Case report

A 56-year old man presented with a 3 month history of painful bilateral gynaecomastia and sexual dysfunction. Investigations revealed hypogonadotropic hypogonadism with markedly elevated serum E2, but otherwise normal baseline anterior pituitary function. Tumour markers were negative and adrenal androgens and LFTs were normal. Imaging identified a ~6 cm right adrenal mass. Preoperative workup excluded pheochromocytoma, but identified low-grade, ACTH-independent Cushing's syndrome. There were no signs of metastases at laparotomy and a 145 g adrenal tumour was removed. Post-operative cortisol and reproductive axis remain to be assessed, but E2 level normalised almost immediately (109 pmol/L), with early improvement in his gynaecomastia. Histology confirmed a completely excised adrenocortical carcinoma.

#### Results

LH 2.5, FSH < .0 U/L & Prolactin 113 mU/L (NR < 450), Testosterone 4.1 nmol/L (NR: 9–25), E2 694 pmol/L (NR < 80), E1 821 pmol/L (NR < 330), A4 5.7 nmol/L (NR < 13), DHEAS 1.9 umol/L (NR: 1.1–10.9), Cortisol (post 7 mg IV DST) 330 nmol/L.

#### Discussion

Estrogen-secreting adrenal tumours are exceedingly rare in both men and women. Although co-secretion of cortisol has been previously described, this case is virtually unique in that adrenal androgen levels were entirely normal, effectively ruling out peripheral aromatisation of androgens as the source of the elevated circulating E1 & E2 levels. It is therefore likely that the tumour itself had unusually high-level aromatase activity and was thus able to secrete E1, which was in turn further aromatised both locally and peripherally to E2. However, results of tissue aromatase expression studies are still pending.

The hypogonadotropic hypogonadism exhibited by this patient at presentation nicely illustrates how the physiological feedback inhibition of LH & FSH secretion is predominantly mediated by E2 in men as well as in women.

#### P527

##### Origin and spread of the SDHD p.Y114C mutation causing head and neck paraganglioma in Trentino, Italy

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Head and neck paragangliomas (HNPs) are tumors derived from the neuroectoderm. HNPs are generally hormonally silent and often asymptomatic. They can be part of the Pheochromocytoma/Paraganglioma Syndrome, due to mutations of SDHB, SDHC and SDHD genes. SDHD founder effects have been described in the Netherland, USA, Italy and Spain.

We identified a new founder effect in a small geographic area (Valsugana-Trentino, actually 50,000 inhabitants), with high anecdotic incidence of HNP. Twelve unrelated kindreds with HNP presented the SDHD c.341G > C p.Y114C mutation.

We extended the genealogies and draw some of the family tree up to 1750 without identifying a common ancestor.

Haplotype have been studied using 5 polymorphic markers spanning 360 kb around SDHD gene. A conserved haplotype was identified in SDHD c.341G > C chromosomes from the Trentino families suggesting the occurrence of a common founder.

The SDHD c.341G > C mutation was present also in 4 German and 1 Polish families. Two German families presented the same haplotype, instead it was different in the Polish family and in other two German families. The three haplotypes probably derived from a single ancestral haplotype, as the differences among them could be explained with one independent recombination event in each.

There could be an ancient link between Italian, German and Polish families: one of the three valley in which we identified this founder effect, named Val dei Mocheni, was settled in the 13th century by German farmers, originary from Baviera and Boemia. This community never integred in the social environment, and the dialect they currently speak derive from the ancient German language.

We hypothesize that a German ancestor with the SDHD c.341G > C mutation was part of the group who settled the Val dei Mocheni, and that the cultural and geographical isolation caused the bottleneck effect, explaining the spread of SDHDc.341G > C-linked HNPs in this population.

#### P528

##### Characterization of familial non-syndromic pheochromocytoma

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Hereditary forms account for 30–40% of pheochromocytomas (pheo). The role of germ-line mutation of VHL, RET, SDHB, SDHD gene has been largely elucidated. However, genotyping a group of 172 sporadic or familial pheo, we have characterize five unrelated probands with familial pheo without any sequence variants of RET (7 exons), or of the entire coding sequence of VHL, SDHB, SDHC or SDHD.

The proband #1 had a bilateral pheo when 32 and a local recurrence at 48. His brother died of malignant pheo and his nephew died suddenly for an undiagnosed pheo. The proband #2 had a 5 cm benign adrenal pheo at 34, her cousin had a monolateral pheo at 42. The proband #3 had a bilateral pheo at 66. Her sister had a bilateral pheo and a breast cancer at 54. Several other tumors have been recorded in this family, including larynx cancer, leukaemia and medullary thyroid carcinoma (MTC). MTC was excluded in the proband and in her sister. The proband #4 had a bilateral pheo at 46 and few years later liver metastasis. Her brother had a monolateral benign pheo. The proband also had a melanoma and bilateral renal cysts. In this case a VHL sequence variant IVS2 + 43 A > G was found. The same sequence variant was found in one other unrelated sporadic pheo. The proband #5 had a monolateral pheo at 50 and breast cancer at 49; her mother had a pheo at 61.

Despite other molecular mechanisms, as particular intronic variants and partial gene deletions, cannot be excluded, we think that the description of families with non syndromic pheo with any mutation of RET, VHL, SDHB, SDHC or SDHD may argue in favor of the presence of others pheo related genes.

#### P529

##### Endostatin and VEGF levels in serum of patients with pituitary tumors

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Endostatin, a cleaved fragment of collagen XVIII, is a potent endogenous angiogenesis inhibitor. Elevated serum endostatin levels have been recently reported in patients with various types of neoplasms. The purpose of our study was to evaluate serum concentrations of endostatin in patients harbouring various types of pituitary adenomas and to examine the relationship of serum endostatin levels to circulating vascular endothelial growth factor (VEGF) levels. Preoperative serum endostatin and VEGF concentrations were measured using competitive enzyme immunoassays in 71 patients with pituitary adenomas (20 somatotropinomas, 3 corticotropinomas, 6 prolactinomas and 42 clinically nonfunctioning pituitary adenomas – CNFPAs) and compared with the levels from age-matched controls. In case of 35 patients postoperative immunohistochemical investigations were performed. Serum endostatin concentrations were significantly higher in all investigated types of pituitary adenomas, except for prolactinomas (somatotropinomas: 123.58 ± 16.26; P < 0.02, corticotropinomas: 156.91 ± 41.87; P < 0.02, prolactinomas: 140.8 ± 37.26; P > 0.05, CNFPAs: 168.91 ± 11.06 ng/ml; P < 0.000005 vs 72.97 ± 9.6 ng/ml in the controls). There was a significant positive correlation between endostatin and VEGF serum levels in patients with pituitary adenomas (r = +0.322; P = 0.006). In the control group a significant negative correlation between circulating endostatin and VEGF was found (r = -0.653; P = 0.00975). The simultaneous elevation of endostatin and VEGF may attenuate the proangiogenic action of the latter and be responsible for rather weak intensity of neovascularization of pituitary adenomas. Prospective studies are required to assess the usefulness of circulating endostatin and VEGF as markers of progression or recurrence of pituitary tumors.

#### P530

##### 18F-deoxy-D-glucose positron emission tomography (FDG PET) increases the detection rate of recurrent or residual medullary thyroid cancer

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Surgical treatment is the first therapeutic option in patients affected with medullary thyroid carcinoma (MTC). However, cure-rates are often low due to the high frequency of loco-regional metastases and recurrences. Therefore, post-operative hyper-calcitoninemia is a common feature in CMT. Despite these findings, traditional imaging techniques are often unable to localize tumour foci. In the last years the availability of new morpho-functional techniques might offer new chances for localization of occult MTC.

The aim of this study was to evaluate the ability of postoperative FDG-PET and OctreoScan to detect residual or recurrent MTC after surgery in comparison with conventional imaging techniques. Twenty-three patients had persistently elevated and progressively increasing calcitonin levels after standard surgical treatment for MTC. Conventional imaging techniques (including neck ultra-sonography and computed tomography of neck and chest in all patients, magnetic resonance and bone scintigraphy when appropriated) detected tumour foci in 7 of 23 patients (30%), identified as neck or mediastinal lymph node metastases in all but one with lung metastases. FDG-PET and OctreoScan were performed in 18 patients each detecting tumour foci in 8 (44%) and 5 (28%), respectively. Among the 16 patients undergone to both conventional imaging techniques, FDG-PET or OctreoScan, MTC foci could be identified with at least one of the imaging procedures in 8 patients (50%). New MTC foci, previously unrecognized by conventional radiologic techniques, were found at the FDG-PET in 3 patients who underwent surgery and consequent histological confirmation of MTC relapse. In all cases but one OctreoScan positivity was found in MTC foci previously revealed by the conventional imaging techniques.

In conclusion, in patients with occult loco-regional MTC which persists after surgery, FDG-PET shows the highest diagnostic performance. Its combined use with conventional imaging techniques makes possible to detect residual tumour in 50% of the patients with occult postoperative MTC.

## P531

### Papillary thyroid microcarcinoma: a low risk neoplasia?

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#### Introduction

Papillary thyroid micro-carcinoma (PTMC) is a well-differentiated thyroid cancer  $\leq 10$  mm in diameter. The recent higher prevalence of PTMC is due to the frequent detection of micro-carcinomas as an incidental finding in surgical specimens, but especially during routine imaging of the neck with highly sensitive techniques.

#### Aim

To review the clinical course of PTMC so to reach its optimal management by adapting therapy and follow-up schemes to the risk of persistent or recurrent disease.

#### Methods

We selected 160 PTMC patients and retrospectively analyzed their case history, surgical therapy [total thyroidectomy (TT), lymphnode dissection (LL), near-total thyroidectomy (NT), partial thyroidectomy (PT)],  $^{131}\text{I}$ -remnant ablation ( $^{131}\text{I}$ -RMT), post-surgical follow-up (serum thyroglobulin evaluation, neck ultrasonography,  $^{131}\text{I}$ -WBS, other radiological test results). Results

Out of 160 pts, 143 were affected by sporadic PTMC (sPTMC), 11 by familial neoplasia (fPTMC). Mean follow-up time: 6 yrs.

83 sPTM (group A) were incidentalomas, identified during thyroidectomy for nodular goiter, and 60 sPTM (group B) were preoperatively identified with FNA.

In group A (67 pts underwent NT and 16 pts PT) 4 cases had a loco-regional relapse. In group B, among 28 subjects who underwent TT + LL +  $^{131}\text{I}$ -RMT, 5 presented loco-regional and mediastinal secondary localizations and 1 pt died of distant metastases. 32 pts of group B underwent TT  $\pm$   $^{131}\text{I}$ -RMT and 2 of them showed local recurrence and distant metastases.

3 out of 11 pts affected by fPTMC underwent NT: 1 died of pulmonary metastases, 1 had cervical lymphnodal metastases and 1 had a good clinical course. 1 pt who underwent PT developed loco-regional recurrence, 3 pts who underwent TT  $\pm$   $^{131}\text{I}$ -RMT had a good clinical course, 1 out of 4 pts treated with TT + LL +  $^{131}\text{I}$ -RMT showed loco-regional recurrence.

#### Conclusions

Our observations suggest that prognosis is less favourable in subjects with locoregional metastases at the baseline control and in those with fPTMC; a more careful management and accurate follow-up are suggested in high risk patients.

## P532

### The value of fluorine-18 fluorodeoxyglucose PET during follow-up of patients with medullary thyroid carcinoma

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#### Introduction

Fluorine-18 fluorodeoxyglucose positron emission tomography ( $^{18}\text{F}$ -FDG PET) is an innovative method for the detection of primary tumours or metastases of thyroid cancer; however, recurrence or metastases of medullary thyroid carcinoma (MTC) are still difficult to detect.

#### Aim

The objective of this study was to evaluate the clinical use of  $^{18}\text{F}$ -FDG PET in patients with MTC.

#### Patients and methods

We enrolled 11 patients with MTC, presenting elevated serum calcitonin levels during post-surgical follow-up. They underwent  $^{18}\text{F}$ -FDG PET and conventional imaging techniques: ultrasonography (US), magnetic resonance (MR), computerized tomography (CT),  $^{111}\text{In}$ -DTPA-Phe-octreotide (Octreoscan),  $^{131}\text{I}$ -MIBG scintigraphy.

#### Results and discussion

$^{18}\text{F}$ -FDG PET was positive in 7 cases: 2 of them were false positive results - 1 due to pneumonia and 1 to post-surgical fibrosis in the thyroid bed. In the remaining 5 patients,  $^{18}\text{F}$ -FDG PET detected tumour foci, confirmed also by CT, MR, Octreoscan and  $^{131}\text{I}$ -MIBG scintigraphy in 4, and by histology in 1 of them. 4 patients showed completely negative  $^{18}\text{F}$ -FDG PET scans: 2 results were true negative, as confirmed also by other techniques, whereas 2 were false negative (in the first one a secondary lesion localized in the pulmonary region was detected only by CT, while in the second loco-regional lymph node metastases were correctly identified by Octreoscan).

$^{18}\text{F}$ -FDG PET was able to identify the metastatic foci as efficiently as the conventional imaging techniques and to localize a previously unknown tumour relapse in one patient; nevertheless, 2 false positive and 2 false negative results were recorded.

#### Conclusion

$^{18}\text{F}$ -FDG PET appears to be a valuable and useful tool when used in association with other morphological and functional diagnostic imaging techniques during the follow-up of MTC patients with increased serum tumour markers.

## P533

### N363S and BclI variants in the glucocorticoid receptor gene and their associations in Cushing's syndrome and Addison's disease

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#### Objective

Interindividual variation in glucocorticoid sensitivity can be partly explained by polymorphisms in the GC receptor gene. The most frequent polymorphisms of the GR gene (N363S and BclI) are associated with increased BMI, less lean mass, higher cholesterol and insulin levels. In the present study we investigated the role of the N363S and the BclI polymorphisms in patients with adrenal disease to evaluate their prevalence and the possible clinic and hormone correlations.

#### Patients and method

We studied a cohort of 156 patients divided into three groups. Group I: 44 patients with Cushing's syndrome. Group II: 40 patients with Addison's disease; Group III: 72 healthy subjects. In order to identify genetic variations, the GR gene was screened for nucleotide variations using a PCR and digestion.

#### Results

In the cohort of 156 patients, 3 subjects (two control and one with Cushing's syndrome) were heterozygous for the N363S variant (allelic frequency 1%). For the BclI variant, 61 subjects were heterozygous and 15 subjects were homozygous (allelic frequency 30%). The frequency of these polymorphisms is not statistically different within the three groups. In patients with Cushing's syndrome, the G allele of Bcl I polymorphism, was associated with increased BMI, blood pressure, and elevated total cholesterol/HDL ratio ( $P < 0.05$ ). Within the three groups, only a patient with Cushing's disease was positive for both polymorphisms.

#### Conclusions

These preliminary results suggest that there is not a prevalence of these polymorphisms in patients with Cushing's syndrome and Addison disease regarding to healthy subjects.

Furthermore, these data confirm that the BclI polymorphism is associated with an increased sensitivity to glucocorticoids as well as cardiovascular risk.

#### P534

##### Novel inactivating mutations in four Italian cases of familial hypocalciuric hypercalcemia

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Familial Hypocalciuric Hypercalcemia (FHH) is an autosomal dominant disorder characterized by moderate and lifelong hypercalcemia, relative hypocalciuria, and inappropriately normal serum PTH levels. Loss-of-function mutation of the CaR are responsible for this disease.

In this study we describe three unrelated Italian kindreds (A, B and C) and one patient with FHH. The diagnosis of FHH in the proband was suspected on the finding of hypercalcemia, normal serum, and calcium clearance/creatinine clearance ratio  $< 0.01$ . Genetic analysis of the CaR gene was carried out by polymerase chain reaction amplification and DNA sequencing of exons 2–7, which include the entire coding region and flanking exon-intron boundaries of the gene. The region of interest detected in the probands was also amplified in the family members and in 50 unrelated healthy subjects. In family A, genetic analysis of CaR revealed a novel heterozygous missense mutation (C  $\rightarrow$  T) in exon 7 leading to a substitution of histidine for tyrosine at codon 595 (H595Y) in extracellular domain of CaR. The same mutation was identified in affected family members (sister's and father's proband). In family B, a heterozygous mutation was found in the proband at codon 748 with a substitution of C  $\rightarrow$  A in exon 7, leading to conversion of proline to histidine. The same mutation was identified in the affected son. This mutation is located in the second extracellular loop. In family C, nucleotide sequencing revealed that the proband had a novel heterozygous mutation substituting cysteine for tryptophan at codon 765 (C765W). The absence of three mutations in 50 unrelated healthy subjects could exclude their polymorphic nature. In last case, direct sequencing showed a substitution of G to C in the donor splice site of intron 2 (IVS2 + 1G > C).

In conclusion, we describe three unrelated Italian cases of FHH with a loss of function mutation of CaR. Two are missense and located in the extracellular domain, one is located in intracellular domain and one is a splice site mutation.

#### P535

##### Genetic analyses of familial isolated primary hyperparathyroidism: implications for clinical assessment and surgical management

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Familial isolated primary hyperparathyroidism (FIPH) can result either from incomplete expression of a syndromic form of familial primary hyperparathyroidism [multiple endocrine neoplasia types 1 (MEN 1), hyperparathyroidism-jaw tumor syndrome (HPT-JT), or familial hypocalciuric hypercalcemia (FHH)] or still unrecognized causes.

We investigated the involvement of *MEN1*, *HRPT2* and *CASR* genes by direct sequencing of germline DNA in seven well-characterized Italian kindreds with FIPH, with negative clinical features for MEN 1, HPT-JT and FHH. The mean age at diagnosis was  $45 \pm 17$  yr (mean  $\pm$  SD; range 18–70 yr) in the probands and  $42 \pm 18$  yr (range 15–69 yr) in the other affected. Germline *MEN1* mutations were detected in three kindreds. Multiglandular involvement was found in all but one affected subjects belonging to the three kindreds with *MEN1* mutation. In these patients persistence/relapse of the disease was observed unless an extensive parathyroidectomy (excision of  $3\frac{1}{2}$  glands) had been performed, with the exception of one patient, who is currently normocalcemic 168 months after excision of two glands. No mutations of *MEN1*, *HRPT2* and *CASR* genes were identified in the remaining four families.

*MEN1* genotyping appears worthwhile in FIHP families, since the finding of mutation(s) may predict multiglandular involvement and therefore have practical surgical implications, and prompt further investigation in the family, with the possibility of identifying new cases and beginning a program of periodic surveillance for emergence of tumours in all carriers.

#### P536

##### Non-pancreatic carcinoid tumours – prognostic value of proliferative index (Ki67%)

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Carcinoid tumours are the commonest neuroendocrine neoplasms (NET) with a prevalence of less than one person per 100,000 population. The proliferative index (Ki67%) has a prognostic relevance for pancreatic neuroendocrine tumours, however its value in non-pancreatic NET tumours is unclear. Our retrospective audit was done to look at factors affecting survival. Medical records of 96 patients referred to our clinic since 1999 with a diagnosis of NET tumour (excluding pancreatic primaries) were reviewed in October 2005. 81 patients were alive, 15 were deceased. Analysis of 45 alive and 10 deceased patients, with documented Ki67% was done. *Cohort of the alive patients* (n = 45): 25 (56%) men, mean age at diagnosis  $57.6 \pm 14.5$  years, median survival from diagnosis was 2 years. Tumour sites: foregut 5 (11%), midgut 24 (53%), hindgut 8 (17%), unknown sites 8 (17%). 36 (80%) had metastases at diagnosis (37%-liver, 49% lymph node, 18% in peritoneum, bone, spinal cord.) Immunohistochemistry: Ki67% of  $< 5$  13 (64%) 5–10% – 7(16%) and  $> 10$  – 9 (20%) patients. Management: Surgical resection of primary 24 (53%), long acting somatostatin analogues 17 (38%), interferon 10 (22%), chemotherapy 6 (13%), hepatic embolisation 5 (11%), MIBG therapy in 1 (2%) patient. *Cohort of deceased patients* (n = 10): 8 (80%) men, mean age at diagnosis  $62.1 \pm 11.5$  years and median survival since diagnosis 1.5 yrs. Tumour sites: foregut 2 (20%), midgut 5 (50%), unknown 3 (30%). All patients had metastases at diagnosis (80%-liver, 20%lymph node, 20% ovary, 30% bone, 10% peritoneum and brain). Immunohistochemistry: Ki67% of  $< 5$  4 (40%), 5–10% – 2(20%) and  $> 10$  – 4 (40%) patients. Management: Surgical resection of primary 4 (40%), long acting somatostatin analogues 5 (50%), interferon 6 (60%), hepatic embolisation 2 (20%), chemotherapy 2 (20%), radiotherapy 2 (20%) patients. Conclusion: A trend (not reaching statistical significance) towards an association between increased age, metastases at diagnosis and Ki67  $> 5\%$  and poor survival was noted.

#### P537

##### Somatostatin receptor subtypes 1–5 in pituitary tumors of various etiologies: investigation by immunohistochemistry

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For somatostatin, five receptor subtypes (*sst1-5*) have been identified that are widely distributed in various endocrine tissues and tumors. Potent somatostatin analogs like octreotide, lanreotide and the new multiligand SOM230 – with different binding properties to the receptor subtypes – have been developed. We examined somatostatin receptor protein expression in 134 pituitary tumors of various etiologies. Immunostaining was performed with specific polyclonal antibodies for *sst1-5*. Seventeen growth hormone-producing adenomas (GH), 23 ACTH-producing adenomas (CUSH), 36 prolactinomas (PROL) and 47 non-functioning adenomas (NFA), as well as 11 atypical adenomas (AA) were investigated. Staining pattern, distribution and subcellular localization of *ssts* were evaluated. A tissue known to stain positively for the respective *sst* was used as a positive control. About 70% of GH were positively stained for *sst2A*. About 20% expressed *sst1* and 6% were positive for each *sst3*, 4 and 5. *Sst5* was detected in  $> 60\%$  of positively stained tumor cells, while *sst1*, 3 and 4 were found in  $< 60\%$  of tumor cells. About 11% of PROL expressed *sst2A*. *Sst4* was found in about 6% of PROL and about 3% were positively stained for each *sst1*, 3 and 5. Seventy percent of CUSH were positively stained for *sst2A*. Interestingly,

*sst4* was detected in 40% of CUSH. *Sst1* and *3* were found in about 10% and *sst5* was detected in 5% of CUSH only. About 40% of NFA were positive for *sst2A*, about 30% for *sst3*, 20% for *sst1* and *4*, and 11% for *sst5*. AA revealed an *sst2A*-immunostaining in approx. 30%. *Sst3* and *4* were expressed in 20% of AA, whereas *sst1* and *sst5* were not detected. In pituitary tumors, Somatostatin receptor proteins are expressed with tumor-specific distribution pattern. This may offer new diagnostic and therapeutic possibilities with multiligand or subtype specific somatostatin analogs.

### P538

#### High performance liquid chromatography (HPLC) in the follow-up of mitotane therapy in a patient with adrenal carcinoma

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Adrenal carcinoma is a rare neoplasm with poor prognosis. Mitotane (o,p'-DDD) is the only known therapeutic agent with action on the adrenal. Although it has been used for many decades, its pharmacological properties and exact mechanism of action are still debated. It has been suggested that its therapeutic effect is dose-dependent (Baudin *et al* 2001). High performance liquid chromatography (HPLC) has been used for the exact measurement of mitotane dose.

The aim of the study is to describe the case of a patient with adrenal carcinoma in which HPLC was used for the follow-up of mitotane blood therapeutic levels.

A female patient, aged 36 years, presented with a large nodule in the left adrenal and a cold thyroid nodule. The adrenal nodule was not hormone producing and was removed surgically. The size of the nodule was 10.3 × 5 × 19 cm and on histology it was proved to be an adrenal cortex carcinoma. A near total thyroidectomy was also performed and on histology a benign thyroid adenoma was found. On follow up 6 years after removal of the adrenal carcinoma the patient presented with metastatic disease in the liver and the lungs. An effort was made to surgically remove the metastases and mitotane was administered. Mitotane blood levels were monitored by HPLC. When the patient was on 2.5 g mitotane daily the respective blood levels of the drug were 4.629 µg/ml. The dose was increased to 3.5 g daily and the blood levels of the drug increased to 12.157 µg/ml.

Mitotane administration is necessary for the management of adrenal carcinoma. The use of HPLC contributes to therapy monitoring and the administration of the proper therapeutic dose while minimizing the danger of side effects.

### P539

#### Multiple endocrine neoplasia type 1 and angiomyxoma

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The presence of lipomas is a characteristic of the syndrome of multiple endocrine neoplasia type 1. The presence of myxomas, however, is a characteristic of Carney syndrome.

The aim of the study is the description of a patient with the syndrome of multiple endocrine neoplasia type 1 who presented with a malignant angiomyxoma.

A male patient, aged 42 years, presented with intense gastric complaints and was diagnosed with a gastroduodenal ulcer. A lung carcinoid tumor was found and was surgically removed. In the course of the disease an increase in calcium and parathyroid hormone blood levels was observed and primary hyperparathyroidism was diagnosed. Parathyroidectomy was performed and on histology parathyroid hyperplasia was diagnosed. On further follow-up a pituitary adenoma was found. It was removed transsphenoidally. Imaging studies were performed and a neoplasm in the perianal region was found. In the course of the disease a genetic screening was performed. An analysis of MEN 1 gene was negative for the presence of mutations. A polymorphic genetic variation was found without consequences in protein expression. The neoplasm in the perianal region increased in size, so that the patient had difficulty sitting. The perianal neoplasm was removed and on histology it was found to be a malignant angiomyxoma measuring 14 cm in diameter.

The case of a patient with the syndrome of multiple endocrine neoplasia type 1 is described. On genetic screening a polymorphic variation of the MEN 1 gene was found. The patient presented with a malignant

angiomyxoma. This is the first case described of a malignant angiomyxoma in a patient with the syndrome of multiple endocrine neoplasia type 1.

### P540

#### Novel L30LR heterozygous mutation of the menin gene in a Hungarian MEN 1 family

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We report a family with a novel heterozygous mutation of the menin gene. This gene encodes a tumor suppressor protein which is responsible for multiple endocrine neoplasia type 1. The index female patient presented with symptoms of mild gastric hyperacidity and recurrent kidney stones. Family history revealed, that the daughter, as well as two sisters of the index patient had operations for primary hyperparathyroidism (PHPT). Clinical studies in the index patient showed elevated serum calcium and alkaline phosphatase, low serum phosphate, and increased urinary calcium. Serum parathyroid hormone concentration was increased and osteodensitometry indicated osteoporosis. 99mTc-MIBI scintigraphy revealed isotope accumulation in 3 parathyroid glands. Peptic ulcer was excluded. The patient underwent parathyroidectomy, which resulted in a normalization of serum calcium and parathyroid hormone levels. Two months after surgery the patient developed headache and visual disturbance, and pituitary MRI indicated a large macroadenoma with suprasellar extension measuring 21 × 15 mm in size. The pituitary adenoma was removed by supraciliary craniotomy and histological examination indicated an FSH/LH-producing adenoma. Bidirectional sequence analysis of exons 2–10 of the menin gene in peripheral blood of the index patient indicated the presence of two known polymorphisms in exon 3 (R171Q) and in exon 9 (D418D) as well as a novel L301R heterozygous mutation in exon 6. Family screening confirmed the presence of this novel mutation in each of the three relatives affected by PTHP, while the mutation was absent in other family members. We conclude that multiple endocrine neoplasia type 1 in this family was associated with a novel L301R mutation of the menin gene.

### P541

#### Differences in the presenting biochemical and imaging data in patients with acromegaly caused by pure GH adenomas, adenomas with GH and PRL cell differentiation and plurihormonal adenomas

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#### Introduction

Pituitary adenomas causing acromegaly are immunocytochemically divided into 3 main groups: growth hormone (GH) cell (A), those with GH and prolactin (PRL) cell differentiation (B) and plurihormonal (C). Recent large series comparing the hormonal and imaging features of these tumours at diagnosis are lacking.

#### Objectives

To investigate differences in the presenting hormonal and imaging data associated with the above groups of adenomas.

#### Patients and methods

The patients were recruited from the Acromegaly Database of our Department. Those treated pre-operatively with somatostatin analogues or dopamine agonists were excluded.

#### Results

Ninety-eight patients were identified [A:27, B:42, C:29, median age 48.5 years (range 17–79), males/females 49/49]. There was no significant difference in the basal, nadir and mean GH values (mU/L) during the oral glucose tolerance test [basal median: A 52.6 (6.4–100), B 30 (4.5–100), C 40.6 (6.9–100) – nadir median: A 35.2 (6–100), B 27 (3–100), C 29.6 (6.4–100) – mean median: A 42.5 (5.1–100), B 31.3 (5.3–100), C 39.9



(8.9–100)] or in the PRL levels (mU/L) [A median: males 206 (100–734)-females 294 (190–638), B median: males 394 (61–3674)-females 359 (162–8335), C median: males 310 (64–4980)-females 301 (87–2430)] among the 3 groups. All but 2 subjects (group B) had insulin-like growth factor-I levels above the reference range. In contrast to tumours of groups A and B, those of C were more frequently microadenomas (micro-/macroadenomas: A 29.6%/70.4%, B 26.2%/73.8%, C 57.1%/52.9%,  $P = 0.02$ ). No difference in the presence of suprasellar extension (A 48%, B 55%, C 43%) or cavernous sinus invasion (A 22%, B 20%, C 7%) between the 3 groups was found.

#### Conclusions

In this large series of the 3 major immunocytochemical subtypes of adenomas causing acromegaly apart from the size of the tumour, no differences in the investigated hormonal and imaging data at presentation were found.

### P542

#### Management of thyrotrophin-secreting adenoma with octreotide therapy

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A 69-year-old female presented in 2002 with symptoms of hyperthyroidism. Thyroid function tests confirmed thyrotoxicosis with elevated  $fT_4$  and  $fT_3$  but with an elevated TSH (TSH 5.8 mU/L,  $fT_4$  31.7 pmol/L,  $fT_3$  8.1 pmol/L). Pituitary function tests other than the TSH were normal. Pituitary MRI showed a mass in the left half of the pituitary fossa, displacing the stalk to the right with no suprasellar extension. A  $T_3$  suppression test was normal excluding thyroid hormone resistance. An octreotide scan showed a well-defined lesion in the pituitary fossa that demonstrated intense octreotide uptake. A TRH test dose produced a flat TSH response ( $t = 0$  min TSH 2.1,  $t = 60$  min TSH 1.9 mU/L) which was highly suggestive of a pituitary TSH-secreting adenoma. The patient declined surgery. Therefore a novel test octreotide infusion was administered. After 24-hours of octreotide, the TSH fell from a baseline of 3.5 mU/L (with  $fT_4$  20.3 pmol/L) to 1.4 ( $fT_4$  17.4). Subsequently she was commenced and maintained on octreotide LAR 20 mg subcutaneous injections monthly. Initial MRI follow-up scans showed no significant change. However, a reduction in size of the pituitary tumour was observed on an MRI scan in September 2004. Thyroid function tests have remained within the normal ranges on octreotide therapy (August 2005: TSH 2.48 mU/L,  $fT_4$  16.2 pmol/L,  $fT_3$  5.3 pmol/L). In conclusion, this case illustrates that TSH-secreting adenomas may be successfully treated medically with octreotide therapy leading to normalisation of thyroid function tests and tumour shrinkage. Thus octreotide could obviate the need for surgical intervention in some cases.

### P543

#### Tumor size and gonadotrophin immunoreactivity of pituitary adenomas

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At least one fifth of pituitary adenomas exhibit plurihormonality when using immunohistochemistry for anterior pituitary hormones. However, the correlation with clinical features is weak, without an agreement upon pathological predictors of tumour behaviour.

The aim was to determine the immunoreactivity for anterior pituitary hormones and alpha subunit in 276 consecutive pituitary adenoma patients, aged 22–79 years ( $44.3 \pm 8$ ), 154F/122M: 83 acromegalics (ACM), 173 nonfunctioning adenomas (NFA) and 20 prolactinomas (PRM) submitted to surgery via transfrontal (81) or transsphenoidal (195) way along 10 years (1995–2005). In addition, clinical data, hormonal secretion and tumour size were evaluated before pituitary surgery. Local ethical committee approved the study design. The immunoreactivity performed by the avidin-biotin-complex method was evaluated for beta FSH, LH, TSH, alpha subunit, PRL and GH, using a semiquantitative scale of stained cells: strong (>20%), positive (10–20%), weak (5–10%) and negative (<5%). CT or MRI tumor size (under 1 cm, 1–2 cm, 2–4 cm and over 4 cm on maximal diameter) were considered together with the Hardy neuroradiological stage. The results showed that 16/83 ACM, 53/173 NFA and 4/20 PRM exhibited

immunoreactivity for beta FSH and LH. Tumour size in the gonadotrophin – positive group (>10% of stained cells) was between 1–2 cm in 6 ACM, 21 NFA and 2 PRM, while positive bigger tumours (2–4 cm) were in 7 ACM, 24 NFA and 2 PRM. Giant, over 4 cm tumours were positive in 3 ACM, 8 NFA and no PRM. A similar trend of the tumour size distribution was observed in the monohormonal or null cell adenomas.

In conclusion, tumour size and gonadotrophin plurihormonality are independent factors in the management of pituitary adenomas.

### P544

#### Carotid arterial intima-media thickness (IMT), a marker of atherosclerosis, does not differ in patients with acromegaly compared to healthy controls.

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Without adequate treatment patients with acromegaly die prematurely from cardiovascular disease (CVD); however the contribution of atherosclerosis in this process is controversial. Increased carotid IMT is an early morphological marker of atherosclerosis and predictor of subsequent cardiovascular events. Contradictory data exist regarding IMT in patients with acromegaly.

We measured carotid IMT in 79 patients with acromegaly (47 male, mean age  $55 \pm 14$  years) and 22 age-matched healthy controls (12 males,  $57 \pm 11$ ) [ $P = 0.5$ ]. 32 patients had HT, 16 DM, 8 IHD, 2 PVD, 19 hyperlipidaemia (on treatment) and 16 smoked; 8 controls smoked. Three measurements were taken bilaterally: at the carotid bifurcation and 1 cm above and below, the mean was calculated for each side.

Median IGF-I was 255 ng/ml (62 – 1155) and SDS 2.0 (–2.85 – + 5.76) in patients and 148 ng/ml (67–201) and 0.54 (–1.68 – + 1.70) respectively in controls [ $P < 0.0001$ ]. Median BP was similar in patients (130/78) and controls (139/69). Total cholesterol and LDL were 5 mmol/L (3–8) and 3 mmol/L (1–6) in patients and 6 mmol/L (4–7) and 4 mmol/L (2–4) in controls respectively [ $P = 0.07$ ,  $P = 0.06$ ].

Median IMT did not differ in patients: 0.75 mm (0.43–1.17) compared to controls: 0.71 mm (0.45–1.03) [ $P = 0.3$ ]. When comparing IMT in patients with high IGF-I levels and patients with normal IGF-I levels no difference was found [ $P = 0.6$ ]. No correlation was found with IGF-I or BP and IMT. 17 (21.5%) patients had 1/more plaques present and 4 (18.2%) controls [ $P = 1$ ].

In summary, carotid IMT does not differ in patients with acromegaly when compared to controls. The lack of increase in IMT is against the development of premature atherosclerosis in this patient group.

### P545

#### Acute biliary tract problems are common on discontinuation of somatostatin analogue (SA) therapy

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The prevalence of gallstones (GS) is increased in acromegaly and is further increased by somatostatin analogue (SA) therapy. The incidence has variously been reported to be between 10 and 63%, but they are often asymptomatic and rarely require definitive management. However, there is evidence suggesting that discontinuation of SA therapy may precipitate acute biliary problems.

We have analysed our experience of symptomatic gallstones in all 44 patients (28 male, mean age  $55 \pm 16$  years) in our centre being treated with SA problems on 1<sup>st</sup> of January 2003. Since that time 14 patients (11 male, age  $51 \pm 14$  years) have discontinued SA therapy with 3 going on to develop acute cholecystitis and 2 biliary colic. The mean interval between discontinuing SA and the development of symptoms was 3.6 months (range 3–5 months). All 5 patients were male and went onto have a cholecystectomy. 2 (both male, mean age 25 years) of the 30 patients who have continued SA therapy experienced biliary colic necessitating cholecystectomy. These data indicate a highly significant increase in episodes of acute biliary problems in patients on discontinuing SA therapy (2 in 99 patient treatment years v 5 in 16.75 patient 'off-treatment' years, Chi Square  $P < 0.001$ ). All seven patients experiencing problems were male.



In summary this analysis demonstrates the high incidence of symptomatic GS following SA withdrawal, particularly in men. No common abnormality of liver enzymes was evident to aid as a predictor of future symptoms. We recommend all patients due to stop SA therapy undergo gallbladder ultrasound and if GS are present are warned of the risk of biliary colic and acute cholecystitis. Further work is required to confirm if there is a gender related difference in the incidence of acute biliary problems on discontinuing SA therapy.

#### P546

**Cell proliferation and outcome of GH-secreting pituitary adenomas**  
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In order to investigate the correlations between immunohistochemical picture with proliferative index (Ki-67), the clinical course and outcome of GH-secreting pituitary adenomas not cured by neurosurgery, we studied 41 consecutive acromegalic patients (M 12, F 29; aged 43 ± 10.8 yr) previously undergone neurosurgical resection of adenoma. Two patients underwent neurosurgical intervention at least twice. Post-surgical follow-up ranged from 6 to 36 months and all patients received medical treatment with somatostatin analogs because relapse or persistence of disease. Ki-67 determination was effectuated on 43 surgical specimens. Patients were divided in 2 groups according to Ki-67 value of the surgical specimen ( $\leq 1\%$  or  $> 1\%$ ).

The first group (Ki-67  $\leq 1\%$ ) was composed by 23 patients (M8; F15; aged 45 ± 2 yrs.; 20 Macro- and 3 microadenomas), one of which underwent two neurosurgical resections. The mean value of IGF-I before surgery was 634 ± 52 ng/ml, while during follow-up was 280 ± 31 ng/ml. At last neuroradiological control, 5 patients (21.7%) showed residue/relapse of disease, while 18 (78.3%) of them had a negative magnetic resonance imaging (MRI). The second group (Ki-67  $> 1\%$ ) was composed by 19 patients (M 4; F 15; aged 41 ± 3 yrs. 18 Macro- and 1 microadenomas), one of which underwent two neurosurgical resections. The mean value of IGF-I before surgery was 814.2 ± 58 ng/ml, while during follow-up was 456 ± 59 ng/ml. At last neuroradiological control 11 (61.1%) patients showed residue/relapse of disease, while 7 (38.9%) of them had a negative MRI. Our results showed that ki-67 index seems to be related to prognosis of GH-secreting adenomas in terms of both neurosurgical outcome and biochemical response to somatostatin analogs, independently from size of adenomas and age at diagnosis.

#### P547

**Efficacy and safety of high doses of long-acting somatostatin analogues for treatment of well differentiated functioning neuroendocrine tumors**  
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##### Introduction

Somatostatin analogues (SSA) represent the main therapeutic option in patients affected with functioning well-differentiated neuroendocrine tumors (NET). However, after a variable time from the start of the therapy tachyphylaxis may cause lack of control of clinical syndrome and progression of the disease.

##### Aim

The aim of this preliminary study was to evaluate efficacy in terms of reduction of circulating markers, control of the clinical syndrome and stabilization of disease with the use of high doses of SSA in a group of functioning NET with progressive disease during standard therapy with SSA. Safety was also assessed.

##### Patients and methods

Long-acting SSA (Octreotide LAR 30 mg) was administered every 21 days in 16 well-differentiated functioning NET with progressive disease (lack of control of associated clinical syndrome and progression of the disease in course of therapy with standard doses (20 or 30 mg every 28 days). Control of symptoms and secretory pattern were evaluated basally and after three month of therapy.

##### Results

High doses therapy results in controls of associated clinical syndrome in 100% of cases. A reduction of at least 30% of secretory pattern was achieved

in 85% of cases. Stabilization of disease was obtained at 3 month in 95% of cases. No further adverse effects were registered comparing high doses with standard therapeutic regimen apart from 2 cases with asymptomatic cholelithiasis.

##### Conclusion

High doses SSA therapy showed high efficacy in controlling clinical associated syndromes and reduce secretory neuroendocrine pattern when compared to standard therapy in patients with progressive disease in course of therapy with standard doses. No significant addictive adverse effect were linked to the increase of dosage. We conclude that high doses therapy should always be considered in the management of NET patients with progressive disease.

#### P548

**Surgical debulking of GH secreting adenomas improves control of acromegaly by lanreotide – a prospective study**

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It has been suggested that primary medical treatment of patients with acromegaly using somatostatin analogues (SSA) is as effective at controlling GH levels as post-operative SSA therapy.

We have carried out a prospective study in patients harbouring GH secreting macroadenomas to see, in a within-patient comparison, whether debulking pituitary surgery improved GH control on lanreotide compared with that obtained pre-operatively. Local Ethical Committee approval was obtained. We studied 27 patients (11 males) with acromegaly and macroadenomas treated with lanreotide for 4 months. At week 8, if necessary (mean GH  $> 5$  mU/L), the dose was titrated up to obtain maximal GH suppression. Four months post-operatively the patients were re-evaluated and, if not cured, were treated with lanreotide, as pre-operatively.

Before commencing on lanreotide, mean GH ranged from 3–343 mU/L (median 59.2). On lanreotide 9/27 subjects (33%) suppressed mean GH to  $< 5$  mU/L. Post-operatively 21/27 patients (77.8%) had mean GH  $< 5$  mU/L. Six had persistently abnormal GH secretion (mean GH 7–94 mU/L, median 35). Re-treatment with lanreotide resulted in lower GH values than those obtained pre-operatively with lanreotide in all six subjects (pre-operatively 12.2–183.3, median 19.6 vs post-operatively 3.1–52.6, median 6.4,  $P < 0.01$ ). In 3 out of these 6 patients (50%), lanreotide post-operatively resulted in mean GH  $< 5$  mU/L, which was not the case in any of them while on lanreotide pre-operatively.

In this first prospective study using lanreotide pre- and post-operatively, we show that surgical debulking of GH secreting macroadenomas, even if not curative, improves response to SSA therapy and the combined therapy (SSA + surgery) improves cure rates.

#### P549

**Diagnostic utility of dexamethazone suppression tests in the work-up of Cushing's disease**

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##### Background

Cushing's disease (CD) may be associated with equivocal results on biochemical investigations.

##### Aim

To evaluate the usefulness of dexamethazone suppression tests in the diagnostic work-up of CD.

##### Patients and methods

Seventy patients with CD [median age 38 yrs(16–76), 53 females] presenting between 1976–2005 were studied. 24-hr urinary free cortisol (UFC), overnight (oDST) (1 mg at 23:00 h), low dose (LDDST) (2 mg daily over 2 days), high dose (HDDST) (8 mg daily over 2 days) dexamethazone suppression tests were assessed. Statistical analyses were based on subjects with available data on each occasion.

##### Results

At initial evaluation 96.4%(53/55) had UFC above normal [median 674 nmol/24hr(195-5220)]. Failure of suppression of serum cortisol

(<50 nmol/l) was found in 0%(0/30) on the oDST [median 465 nmol/l(151-1396)] and 97.7%(42/43) on the LDDST [median 346 nmol/l(49-899)]. 29.2%(14/48) suppressed serum cortisol <50% on the HDDST [median 81.7%(0-92.7%)]. There was no difference in the UFC among subjects suppressing serum cortisol >50% or <50% on HDDST (median 597 vs 1620 nmol/24hr,  $P = 0.2$ ). There was significant correlation between UFC and serum cortisol on oDST ( $r = 0.6$ ,  $P = 0.04$ ), serum cortisol on LDDST ( $r = 0.5$ ,  $P = 0.03$ ), but not percentage fall of serum cortisol on HDDST ( $P = 0.09$ ). No significant correlation between percentage fall of serum cortisol on the HDDST and serum cortisol values on the oDST ( $P = 0.1$ ) or on the LDDST ( $P = 0.1$ ) was found. There was a significant correlation between the percentage fall of serum cortisol on the LDDST and the HDDST (48hours) ( $r = 0.4$ ,  $P = 0.03$ ).

#### Conclusions

In our series, the LDDST was overall satisfactory for the diagnosis of hypercortisolism. The HDDST showed high rate of false negative results rendering it unsatisfactory in the diagnostic work-up of CD. The correlation between percentage fall of serum cortisol on the LDDST and HDDST suggests that the LDDST may be useful in predicting the pituitary origin of Cushing's syndrome.

## P550

### Descriptive epidemiology of thyroid cancer occurring in the population living in the Rhône Alpes region: 1998-2004

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In the Rhône Alpes region, that hosts more than 5.6 million inhabitants; thyroid illnesses are still rather common with 3,600 operations on the thyroid gland per year, comprising 650 incident cases of cancer. A progressive increase in the incidence of thyroid cancer was suspected in the 1990s and a population based register was initiated. The collection consists of histological data validated and obtained directly from pathologists, surgical wards and controlled through hospital claims databases from care units. The register (1998-2004) comprises 3,800 cases, 77% were women. The incidence rates standardized for European and World populations were 5.97 and 4.89/100 000 for men, and 17.63 and 14.12/100 000 for women respectively. Most of these cancers were papillary (85%). pT1, pT2, pT3, pT4 states represented 51%, 25%, 17%, 6.4% respectively. This distribution varied in function of age groups with a higher proportion of pT2 in younger ages and of pT4 after 50 years. 14% had lymph nodes (N1a = 11%, N1b = 3%). A large proportion (37%) was microcancers, 52% of them having a diameter less than 0.5 cm. As a whole, the proportion of cancers incidentally discovered was 25%. The proportion of cancers discovered in goiters varied from 5% in the youngest group to 44% in the oldest one. Considering all goiters operated in our region, the mean proportion of cancers in this setting was 13%. The geographic distribution of incidence rates by canton showed a significant heterogeneity with higher rates in urban versus rural plus semi rural cantons, mainly for men. The influence of the density of general practitioners, and of the geographic distribution of the incidence rates of benign thyroid pathologies that had led to surgery, is under study.

## P551

### In vitro effects of som230 on primary cultured pheochromocytoma cells

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The stable somatostatin analog octreotide has been successfully used for imaging and treatment of a variety of human tumors. Octreotide treatment has been reported of limited value in pheochromocytoma (PHEO). PHEOs often express more than one somatostatin receptor, and it is uncertain by which receptor subtype the functional responses of octreotide are mediated. A recent study showed by immunohistochemical staining that vast majority

of tumors (90%) were positive for sst(3), immunoreactive sst(2A) receptors were only seen in 13 tumors (25%). All other somatostatin receptor subtypes were less frequently detected. These data suggest that these tumors may represent a potential target treatment with somatostatin receptor agonists with improved sst(3) activity. Octreotide is able to bound with high affinity SSTR2 e 5 while SOM230, a new synthetic analogue is active on SSTR1, 2, 3, 5. In this study we investigated the effects of the new synthetic SST analogue SOM230 on the control of growth and apoptosis in primary PHEO cell cultures obtained from a patient underwent to adrenalectomy.

#### Methods

Primary culture of cells were cultured in HAM F 12/dMEM, with 10% FCS. The cells (Pheo-c) characterized by immunohistochemistry were positive for chromogranin and NSE. Pheo-c were starved without FCS for 2 days, then treated with 10nM, 100nM SOM230 and octreotide for 48h. For the analysis of proliferation and apoptosis, cells were harvested after treatment, and analyzed by MTT and TUNEL techniques.

#### Results

Pheo-c, after 48h treatment with 100nM OCT or SOM230, showed a significant cell growth reduction ( $P < 0.05$  and  $P < 0.001$  respectively) and this effect increased after 72h. Induction of apoptosis was detected after 72h of 100nM OCT (8%) or SOM230 (16%) exposure of cells.

#### Conclusions

Our data suggest that SOM230 could be useful to improve diagnostic imaging evaluation, and for the long-term treatment of patients with malignant or recurrent PHEO.

## P552

### Fourier transform infrared spectroscopy (FTIR) of parathyroid pathology

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Surgical management for parathyroid disease worldwide is on the rise. Hand in hand, parathyroid surgery has evolved from the classical bilateral approach to the more focussed unilateral approach. The failure rate in the best of hands continues to be 3-4% but this figure may rise as Minimally Invasive Surgical techniques are universally adopted.

Accurate pathological diagnosis to differentiate parathyroid adenomas from hyperplasia continues to be difficult for the pathologist and is essential for successful parathyroid surgery. We evaluated the ability of FTIR to accurately differentiate between parathyroid adenomas and hyperplasia and present our preliminary results.

Eight glands were analysed, 4 hyperplasias and 4 adenomas.

The sensitivity of FTIR for adenomas was 99.25% and hyperplasias 99.17%, thus making it an excellent tool to differentiate the two.

## Growth and development

### P553

#### Prevalence of the main GH receptor polymorphisms in GH deficient children and in the general population

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GH acts at the target cell through the GH receptor (GHR), stimulating a cascade of events that leads to target gene transcription. Polymorphisms of the GHR (GHRPs) have been reported in the general population and have been described in exons 3, 6 and 10, although their prevalence is not well defined. It is conceivable that GHRPs might affect the growth response to exogenous GH in children with short stature of different origin. To evaluate the prevalence of the different GHRPs in the general population and in GH deficient (GHD) children, we analysed the GHR gene sequence in 54 prepubertal GHD children (11 females) (mean age 7.8 years (SD 3.96); mean height (Ht) SDS -1.95 (SD 0.66) and in 50 non-GHD subjects with normal stature. Informed written consent was obtained by the study subjects as well as approval by the local Ethical Committee. GHR exons were amplified by PCR using pairs of intronic primers. The presence of single or multiple mismatches in the PCR products was revealed by DHPLC. For

exons in which mismatches were found by DHPLC, direct sequencing was performed by automatic sequencer. We found a high frequency of GHRPs in normal subjects, and in particular: Exon 3 deletion (del-3) in 37 subjects (74%); Polymorphism 504 A > G at codon 168 of exon 6 (Poly6) in 44 subjects (88%); Polymorphism 1576 A > C at codon 526 of exon 10 (Poly10) in 33 subjects (66%). In most subjects these different polymorphisms recurred in association. In GHD subjects the polymorphism prevalence was slightly different: del-3 in 26 subjects (48%;  $P = 0.01$ ); Poly6 in 44 subjects (82%; NS); Poly10 in 35 subjects (65%; NS). In GHD patients too the different polymorphisms recurred in association. In conclusion, the main GHRPs show a similar prevalence in GHD subjects and in normal controls, except for del-3, that seems to be more frequent in the general population.

#### P554

##### Developmental control of tissue deiodinases by cortisol in fetal sheep during late gestation

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Deiodinase enzymes have an important role in thyroid hormone metabolism. Type I 5'-monodeiodinase (D1) converts thyroxine (T<sub>4</sub>) to triiodothyronine (T<sub>3</sub>), while type III 5-monodeiodinase (D3) inactivates T<sub>3</sub> and produces reverse-T<sub>3</sub> (rT<sub>3</sub>) from T<sub>4</sub>. In fetal sheep, plasma T<sub>3</sub> rises towards term in association with the prepartum cortisol surge. This study investigated the effect of cortisol on tissue deiodinase activities and plasma thyroid hormone concentrations in fetal sheep during late gestation.

Eighteen sheep fetuses were chronically-catheterised under general anaesthesia at 115-118d of gestation (term 145 ± 2d) and, from 125d, were infused intravenously for 5d with either saline (0.9% NaCl,  $n = 8$ ) or cortisol (3-5 mg/kg/d,  $n = 10$ ). Arterial blood was taken before and during infusion. On the fifth day of infusion, tissues were collected after maternal euthanasia. Tissues were also collected from 15 fetuses at 141-146d: 7 were intact and 8 were adrenalectomised under general anaesthesia at 116-119d. Umbilical arterial blood was taken at delivery. Plasma cortisol and thyroid hormones, and tissue deiodinase activities, were measured by radioimmunoassay and radiometric enzyme assay. Data were analysed by t-test and two-way ANOVA ( $P < 0.05$ ).

Exogenous cortisol infusion increased plasma cortisol and T<sub>3</sub>, but not T<sub>4</sub> or rT<sub>3</sub>. On the fifth day of infusion, hepatic and renal D1 activities were higher, and renal and placental D3 activities were lower, in the cortisol-infused fetuses compared with the saline-infused fetuses. Fetal adrenalectomy abolished the prepartum rises in plasma cortisol and T<sub>3</sub>, without any change in T<sub>4</sub> or rT<sub>3</sub>. At 141-146d, hepatic and renal D1 were lower, and renal and placental D3 were higher, in the adrenalectomised fetuses compared with the intact fetuses.

Therefore, in fetal sheep, the prepartum cortisol surge stimulates hepatic and renal D1, and suppresses renal and placental D3 activities. These changes in tissue deiodinase activity may be responsible for the rise in plasma T<sub>3</sub> concentration near term.

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#### P555

##### Significance of neonatal steroid imprinting and of peripubertal growth hormone excess for the development of prostatic hyperplasia in the rat

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The benign prostatic hyperplasia (BPH) represents an enlargement of the epithelial and fibromuscular parts of the prostate. Although androgens per se do not cause BPH, an intact androgen metabolism is a prerequisite for the normal fetal and pubertal development of the prostate. Furthermore, growth factors play a role in the development of BPH. Animal experiments have shown that the prostate – similar to other organs of the reproductive tract – undergoes a neonatal steroid imprinting. In the present study the role of this neonatal steroid imprinting as well as the role of a peripubertal growth hormone excess for the development of the prostatic size and for the development of the tissue features with special regard to the ratio

epithelium:stroma is investigated with the aim to create an animal model for human BPH. Newborn rats were injected with estradiol benzoate, dihydrotestosterone, testosterone propionate, finasteride, fadrozole or solvent. From day 45–59 of life part of the animals received 1IU/d human growth hormone. At day 60 all animals were sacrificed for investigation of prostatic histology. Pronounced fibromuscular hypertrophy, comparable to that seen in human BPH, was observed in neonatally androgen + GH treated rats, especially in the dorsal part of the prostate, but not in animals which had only received neonatal steroid treatment without additional peripubertal GH. Neonatal androgens appear to increase the susceptibility of the rat prostate to growth factor stimuli as provided by GH. Furthermore, with the administration of peripubertal GH in neonatally androgen treated male rats for the first time an animal model was elaborated which is histologically comparable to human BPH.

#### P556

##### Adrenal (interrenal) development in the zebrafish

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To clarify the role of the pituitary in zebrafish interrenal development we investigated wildtype (wt) zebrafish and 3 pituitary mutants: aal (with lactotrophs only), and pit1 (with corticotrophs only), fgf3-lia (lacking all adenohipophysial cell types) and in MC2R knockdown embryos (MC2R morphants).

The interrenal primordium (IP) is detectable at 22hpf (hours post fertilization) as bilateral clusters of ff1b expressing cells which fuse to one domain at 24hpf. Only after this fusion, additional specific genes (e.g. P450scc, StAR, 3β-HSD and MC2R) become detectable. The adrenomedullary primordia expressing dopamine β-hydroxylase (dβh) migrate into the region of the IP at 2dpf and later develop into a bilobal organ. Interrenal and chromaffine cells are then highly intermingled. In the lia and aal mutants we observed normal development up to 2dpf compared to wt with unaltered expression of P450scc, MC2R, StAR, and the medullary gene dβh. However, at day 5 expression of interrenal genes was significantly reduced. In MC2R morphants we found changes similar to mutants lacking pituitary POMC with dramatic reduction of expression of interrenal markers demonstrating that ACTH plays a key role for the observed changes in mutants, whereas POMC expression was 4-fold increased in the anterior pituitary domain at day 5 indicating feedback regulation by impaired interrenal steroidogenesis. Significant reduction of dβh expression was also observed in lia and aal mutants, demonstrating the functional interaction between interrenal and medullary cells. In pit1 mutants normal interrenal development was observed indicating that only corticotroph cells are required for normal development.

Taken together, interrenal development and expression of interrenal key proteins initially take place in a pituitary independent manner. However, POMC influences further interrenal development by regulating the expression of P450scc, StAR, MC2R in later stages and feedback control is clearly established at day 5. Interrenal steroidogenesis is essential for normal development of chromaffine tissue.

#### P557

##### Comparison of dietary pattern and food habits in urban and rural adolescent girls in Gilan/IRAN

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Adolescence is specific periods with physical, physiological, behavioral and social changes. Dietary intakes and food habits are important factors in determining those well-balanced changes during this period of life).

Three hundred thirty-six high school girls (168 urban and 168 rural) in 14–18 years old were randomly selected to determine their daily dietary patterns, in comparing the differences between rural and urban adolescent girls.

Data were collected by 24 hour recall questionnaires for 3 consequent days and analyzed by N3 food analyzer and its significance determined by SPSS.

The percent age of energy from carbohydrate, fat and protein were 64.3, 24.6, 11.1 in urban and 65.8, 23.2, 11.0 in rural, with no significant differences. Zinc, Ca, P and vit A, B2 intakes showed lowered intake among urban and rural in comparison with RDI. The results suggested that the dietary patterns of urban and rural were not satisfied the needs of daily micronutrient intakes with particular inadequacies in zinc, ca and vit A intakes. Food behavior should be considered in both urban and rural girls.

## P558

### Effective factors on status of nutritional education in primary school children, Tehran, Iran: A Focus Group Discussion (FGD)

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Nutritional education and improvement of nutritional knowledge is an effective intervention that improves School children health. With consideration to few accurate and successful nutritional teaching programs directed at our schoolchildren in this research we evaluated the status of nutrition education system in primary school children and mothers' point of view. The study was a qualitative study as a Focus Group Discussion (FGD). The research team consisted of a coordinator, one or two observers and two note takers. Each FGD was held with 6 to 8 person and each session lasted about 60 minutes. The field work activities of the study included 20 FGD sessions, 16 for male and female primary school children from grades 1 to 4 and 4 for mothers of two district (urban and rural) of Tehran, capital of Iran, were held. At the end of each group discussion all notes were completed, coded analyzed and classified by team members.

Findings show that children were eager to learn effects of bad and good foods on the body. According to their point of view TV, is the most important and effective teaching medium. In addition to TV, food advertisements, school staff especially teachers were named as the most effective factors on children's nutritional behaviors. Most of children preferred to learn nutritional subjects from father, mother and other family members. The mothers preferred teachers, health instructors parents and classmates as source of knowledge and schools as the most effective place for teaching/or education nutritional subjects.

Results of the study confirm the fact that children need to learn, more about nutrition and foods. So that, designing of longitudinal nutrition education projects aiming at children through TV or other effective media is essential.

## P559

### Morphological changes of pituitary region in primary GH deficiency

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#### Purpose of study

The study was focused on MRI of morphological changes of pituitary gland, pituitary fossa and surrounding structures in patients with primary growth failure. We evaluated MRI, clinical status and hormonal changes in 18 patients with primary growth failure referred to University Hospital.

#### Methods and Patients

We examined 18 patients (10 male, 8 female), average age 18.3 (2–34) years. All patients had a severe growth hormone deficiency.

MRI was performed by axial T2 weighted images of whole brain and by coronal and sagittal gradient echo T1 weighted images (T1GE) 1 mm slices of pituitary region without intravenous injection of contrast agent and in indicated patients also after intravenous injection of contrast agent. We evaluated size of pituitary fossa, pituitary gland, localization of pituitary gland, homogeneity, enhancement, and changes of parasellar structures.

#### Results

In all patients with primary dwarfism the adenohypophysis hypoplasia was found (18/18). The other most common findings were: very small sella – phthisis of sella or microsella in 89% (16/18), supradiaphragmatic ectopic neurohypophysis in 89% (16/18) and interrupted or hypoplastic pituitary stalk in 83% (15/18). The most frequent pathology of the surrounding structure was hypoplasia of sphenoid sinus, which was found in 67% (12/18) of our patients. Others not so frequent findings were partially empty sella, pituitary stalk elongation, pituitary gland trapped into small sella,

dysgenesis of corpus callosum, remnant basipharyngeal canal, multiple neurohypophysis – "bright spots" and hyperplasia of sphenoid sinus.

#### Summary

Primary pituitary dwarfism shows rather typical imaging of pituitary fossa and the pituitary gland: Small hypoplastic adenohypophysis, ectopic neurohypophysis, stalk hypoplasia, or interruption, multiple neurohypophysis, hypoplasia of pituitary fossa and sphenoid sinuses. The most common cause of secondary dwarfism in our patients was craniopharyngioma.

## P560

### Growth without growth hormone: normal final height of four patients with multiple pituitary hormone deficiency

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Normal linear growth in children is highly complex process, regulated by interaction of genetic, environmental and hormonal factors.

We present four growth hormone deficient patients which despite severe growth hormone deficiency according to the provocative tests (ITT or GHRH + GHRP-6) and decreased IGF I level grew normally. This study was approved by Ethical Committee of University Clinical Center Belgrade. Three of our patients had idiopathic multiple pituitary deficit (No 1,3,4) while one patient (No 2) was operated and irradiated due to glioma n. optici in childhood. All patients were on complete conventional hormonal replacement. In patients No 1 and No 3 MRI showed normal pituitary, patient No 2 a tumor rest and huge frontal higrma while patient No 4 had stalk disconnection on MRI. In patient No 1 Prop 1 gene mutation was confirmed. We measured insulin and ghrelin levels during OGTT and leptin pool in all. Auxological and hormonal characteristics of our patients are presented in a Table.

Table 1

Sex and age (yrs)	height (cm)	BMI (kg/m <sup>2</sup> )	OGTT-insulin peak (mU/l)	OGTT-ghrelin basal and through (pg/ml)	leptin pool (ng/ml)	PRL (mU/l)
F;22	177	30.7	166	1218; 1112	48	299
F;24	178	24.0	129	937; 1039	27	292
M;25	184	29.5	245	1042; 898	66	73
M;26	181	28.7	93	482; 529	38	338

Some studies speculate that insulin, leptin, PRL and ghrelin could play a role in linear growth without GH. Our patients showed hyperinsulinemia during OGTT which can act as growth stimulus. High leptin levels might contribute to linear growth. Although three patients were obese they did not show decreased ghrelin levels as in simple obese patients and nor suppression during OGTT. This report shows that other factors besides GH and IGF 1 have an important role in growth.

## P561

### RAD21-dependent effects of securin/PTTG and separase on fetal neuronal NT2 cells

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During the metaphase to anaphase transition of mitosis, destruction of the human securin, pituitary tumor-transforming gene (PTTG), and subsequent activation of the cysteine endopeptidase separase, leads to the cleavage of RAD21, a component protein of the cohesin complex. The developing fetal brain has rapidly proliferating neuronal cells, whilst adult neurons no longer proliferate and are maintained in G0 status of the cell cycle. We have previously investigated the expression of PTTG and separase in ontogeny of the human fetal brain. PTTG expression was significantly increased and



separase reduced during fetal life. We have now defined expression of mRNA encoding the cohesin subunit RAD21, and compared this with expression of PTTG and separase in the fetal and adult human brain. mRNAs encoding PTTG and RAD21 showed a negative correlation in fetal brain ( $n = 59$ ,  $R^2 = 0.1$ ,  $P = 0.013$ ) but no correlation in adult cortex ( $n = 11$ ). A strong positive correlation was also apparent between separase and RAD21 mRNAs in fetal brain ( $R^2 = 0.1$ ,  $P = 0.005$ ) but no association was observed in adult samples. Subsequently, human embryonal neuroprogenitor cells (NT2) were used to examine expression of RAD21 following transfection of PTTG and separase cDNAs *in vitro*. A reduction of RAD21 mRNA resulted both from overexpression of PTTG (65% reduction,  $n = 8$ ,  $P < 0.05$ ) and from separase (63% reduction,  $n = 7$ ,  $P < 0.001$ ). Transient transfection of a mutant separase, incapable of cleaving RAD21, also significantly reduced RAD21 mRNA (50% reduction,  $n = 8$ ,  $P < 0.05$ ), albeit to a lesser extent. Reduced RAD21 levels were associated with a repression in cell proliferation ( $R^2 = 0.867$ ,  $P = 0.007$ ). This decrease in RAD21 mRNA expression was accompanied by a parallel induction of apoptosis. We postulate that the influence of the key mitotic regulators, PTTG and separase in fetal embryonal NT2 cells reflects a RAD21-dependent mechanism, and that RAD21 plays an integral role in the development of the fetal brain.

## P562

### Leukemia inhibitory factor promotes the chemomigration of immature GnRH neurons

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Leukemia inhibitory factor (LIF), a pleiotropic cytokine of the interleukine-6 superfamily, is involved in several functions including the control of reproduction at the embryonic-endometrial interface and the regulation of energy homeostasis. LIF activates a cell-surface receptor complex (LIF-Rs) composed of one ligand-specific low affinity LIF receptor  $\beta$  (LIFR- $\beta$ ) subunit and the gp-130 subunit. Since little is known about the involvement of LIF in the modulation of the neuroendocrine circuitry governing the reproductive function and, specifically, of the migration of gonadotrophin releasing-hormone (GnRH) neurons from the olfactory placode to the hypothalamus, we tested whether LIF could exert a chemoattractant or chemotropic action on GN11 immortalized cells, an *in vitro* model of immature and migratory GnRH neurons. GN11 cells were found to express LIFR- $\beta$  and gp130 subunits at both gene and protein level. Exposure to LIF (100 ng/mL) activated Janus kinases (Jak)-signal transducer and activator of transcription 3 (STAT3), mitogen-activated protein kinase (MAPK)-extracellular regulated kinase 1/2 (ERK1/2) and phosphatidylinositol 3-kinase (PI3-K)-Akt pathways. The selective inhibition of Jaks, MEK, and PI3-K indicated that in GN11 cells the three signalling pathways were activated independently and that Jak2 is not the main Janus kinase involved in LIF signalling. LIF stimulated chemotaxis at a concentration-dependent manner, reaching a plateau at 100 ng/mL both after 3 and 20 h of incubation. A 3-h treatment with 100 ng/mL LIF also induced chemokinesis. All the three signalling pathways activated by LIF in GN11 cells were independently involved in LIF-induced cell migration. In conclusions, the present results indicate that LIF promotes the chemomigration of immature GnRH neurons, and suggest that LIF might modulate the development of the reproductive axis by directly influencing the migration of GnRH neurons to the hypothalamus.

## P563

### The study of nutrition with different salt concentrations in prenatal period on BP, tissue changes Na and aldosterone level in next generation

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#### Introduction

Salt is the major environmental factor for a lot of hypertension problems. The effect of different salt consumption during prenatal period has been noticed little. In this study, the role of nutrition with different salt concentrations on BP, tissue changes were studied and Na and aldosterone level in next generation were evaluated.

#### Method

Eight groups of rat, six in each group, (one male and five female) having  $200 \pm 20$  grams of weight were selected, salt solutions were prepared in

concentrations 0.5, 1, 1.4, 1.6, 1.8 and 2 percent. Rats were deprived of drinkable water and above-mentioned salt solutions were given to test groups during pre-pregnancy, pregnancy and lactation period. Control group used tap water of Isfahan and one another of test group used distilled water as potable water during these period. All groups were fed with same diet and all other living conditions for all groups were alike. Birth weight, weights and BP in third months, tissue changes, Na and aldosterone levels were studied.

#### Results

Using 0.5 and 1% salt solution as potable water did not affect SBP and DBP of next generation significantly, additionally birth weight and weight in third months in comparison with control group show an increase, but SBP and DBP in-groups, whose their mothers used higher salt solution during pregnancy increased significantly, additionally vascular response to epinephrine increased and vascular response to acetylcholine decreased and cardiac hypertrophy was observed. Serum sodium level and salt appetite increased in these groups, but aldosterone level decreased.

#### Discussion

NA necessity increases during pregnancy, innate tendency to consume excessive amount of salt during pregnancy was shown in most species of animals. Nutrition with sufficient sodium during pregnancy in animals give birth to more and healthy infants, but nutrition with excessive amount of salt creates harmful effect.

## P564

### Sodium, birth weight, adult weight and Bp in adulthood

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#### Introduction

Many epidemiological studies have shown that people who are small at birth tend to have higher blood pressure latter in life. In this study we evaluated the relation between salt consumption in pregnant rat with birth weight, weight changes during breeding period, weight in third Months and blood pressure.

#### Method

Eight groups, each containing 6 mature rats with  $200 \pm 20$  gr weight were selected, Each group contain 5 non-pregnant rats and one male rat, Six concentrations of salt, 0.5, 1, 1.4, 1.6, 1.8 and 2 percent were prepared and used during the period of pre-pregnancy and pregnancy by test groups another test group used distilled water and control group used tap water of Isfahan. Birth weight, weight changes during breeding period, weight in third months were measured exactly.

#### Result

Birth weight and weight changes process and weight in third month of infants whose mothers had used 0.5 and 1% salt solution showed an increase as compared with control group and other test groups and BP showed a reduction in these groups but 1.4% and higher salt solutions causes low birth weight and weight gain process in these group were slowly and weight's rats in third months in these groups as compared with control group and above test groups were lighter and infant's BP in these groups were higher.

#### Discussion

Nutrition with enough salt in prenatal period has a positive effect on birth weight and weight in third month, pregnant rats which consumed enough salt during pregnancy give birth to more and healthy infants and infant's BP was normal or lower than normal. But nutrition with excessive amount of salt leads to a reduction in birth weight and weight gain process will not be at normal level in future, weight in third month was lowered and BP increased.

## P565

### Embryonic gene expression affected by different sodium concentrations

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#### Introduction

The type of Maternal electrolyte taken during prenatal period may change the homeostasis of body fluids which genetically sensitizes individuals during maturation. So in this experience, we studied the effects of different NaCl concentrations in distilled water used by pregnant rats on their offspring.

#### Method

Eight groups, each containing 6 Mature rats with 200 gr weight were selected, Each group contain 5 non pregnant rats and a male during the

period of pre-pregnancy and till the end of pregnancy. Six concentration of salt 0.5, 1, 1.4, 1.6, 1.8 and 2 percent were used for six groups and the other test group used distilled water and control group used tap water of Isfahan. Birth weight, weight in third months and BP of infant were measured.

#### Result

Birth weight and weight in third month of infants whose mothers had used 0.5 and 1% salt solution showed an increase as compared with control group and BP showed a reduction but 1.4% and higher salt solution causes low birth weight and weight in third month and increases BP in infant rats.

#### Discussion

Nutrition with enough salt in prenatal period promote activation of a group of gene which causes an increase in birth weight and weight in third month and a reduction in BP and nutrition with excessive amount of salt promote another group of gene which promote a reduction in birth weight and weight in third month and an increase in BP.

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### P566

#### Sodium changes autonomic nervous response

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#### Introduction

Despite early demonstrations that sympathetic activation elevates blood pressure and early clinical inklings that human hypertension may have a psychosomatic component, the pivotal role of the nervous system in human hypertension is only recently being clarified. We studied the effect of nutrition with different salt concentrations on autonomic nervous response.

#### Method

Eight groups of rats, six in each group, (one male and five female) having  $200 \pm 20$  gr of weight were selected, salt solutions were prepared in concentrations of 0.5, 1, 1.4, 1.6, 1.8, and 2 percent. Above mentioned salt solutions were given to test groups during pre-pregnancy, Pregnancy and lactation period. The control group used tap water of Isfahan. Another test group consumed distilled water. In order to study autonomic nervous response, 1% body weight of blood was substituted by the same volume of NaCl 0.9% containing  $1 \mu\text{g/ml}$  epinephrine and in another subgroup 1% body weight of blood was substituted by the same volume of NaCl 0.9% containing  $0.1 \mu\text{g/ml}$  acetylcholine

#### Result

Nutrition with salt solutions higher than 1% during prenatal period lead to an increase in infant's BP during adulthood, Vascular response to epinephrine in groups which their mothers consumed higher salt concentrations were significantly increased in comparison with control group and other test groups and vascular response to acetylcholine decreased in these groups.

#### Discussion

Nutrition with excessive amount of salt during prenatal period leads to an increased vascular response to epinephrine which itself was due to heart and vascular changes, heart hypertrophy and an increase in the diameter of media layer. Additionally sodium could increase sympathetic activity and an increase in adrenal hormone release may help to develop hypertension. Vascular response to acetylcholine in groups which their mother consumed higher salt solution decreased and parasympathetic activity may also decreased.

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### P567

#### Lipid peroxidation, activity of caspase 3 and apoptosis activation in low birth weight children

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Children born with low birth weight (LBW, below 2500 g) exhibit slower development with deficit of height and, in adulthood, an increased risk of developing syndrome X. One possible explanation could be an enhanced elimination of cells by apoptosis. The aim of this study was evaluate of mechanism of lipid peroxidation and activity of caspase 3 in children with low birth weight.

Subjects for study were 10 children with LBW and growth retardation ( $\text{SDSHV} < -1.8$ ) and high frequency of apoptotic cells in cultures of lymphocytes and with the 50 kb domain on the DNA electrophoretic profiles, aged 4–11. Control group was 30 children with birth weight above 2700 g, aged 4–11.

Plasma total cholesterol, HDL- cholesterol, HDL<sub>2</sub>-cholesterol, HDL<sub>3</sub>-cholesterol, LDL- cholesterol, lipid peroxidase (LPO). Activity of caspase 3 was estimated in supernatant blood cells.

#### Results

The levels of HDL<sub>2</sub>-cholesterol, LPO and BCL2 were higher in LBW children than in the control group, so that activity of Caspase 3, the level of HDL<sub>3</sub>-cholesterol was markedly lower, whereas total HDL and LDL concentrations did not differ between LBW and the control group. In children with LBW the concentration of LPO negatively correlated with HDL<sub>3</sub> cholesterol ( $r = -0.77$ ,  $P < 0.05$ ). Activity of Caspase 3 positively correlated with HDL<sub>2</sub>-cholesterol concentration. ( $r = 0.91$ ,  $P < 0.05$ ). No significant correlations were found in the control group.

#### Conclusions

It is known that lipid peroxidation products activate apoptosis in human lymphocyte cultures through the direct stimulation of caspase 2 and caspase 3. Enhanced lipid peroxidation in blood of LBW children could be deduced from our data which showed the increase of LPO activity in those children, accompanied with the decrease of HDL<sub>3</sub>-cholesterol level.

We suggested that both enzymatic and non-enzymatic lipid peroxidation could be elevated in LBW children. These results were in accord with increased frequency of apoptosis, found in cultures of lymphocytes obtained from venous blood of LBW children.

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### P568

#### Growth hormone induced improvement in quality of life does not correlate with reduction in vascular risk in adult hypopituitary patients

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Hypopituitary patients on conventional hormone replacement, excluding GH have a reduced quality of life (QoL) and an increased risk of cardiovascular mortality. NICE guidelines on criteria for initiation and maintenance of therapy rely on QoL score defined by the Adult GH assessment (AGHDA) questionnaire. However, it is uncertain how this score is related to other biological effects such as vascular risk.

#### Methods

Sixteen hypopituitary patients were recruited (peak GH  $< 3 \mu\text{g/l}$  during ITT). Patients were randomised using a cross-over design to either six months of GH therapy or no treatment followed by six months of the other. At baseline, six and twelve months, the AGHDA questionnaire was completed. IGF-1, CRP, lipids, leptin and intercellular adhesion molecules (ICAM-1 and VCAM) were measured and waist circumference (WC), hyperinsulinaemic euglycaemic clamp, pulse wave velocity and 24 hour ambulatory blood pressure were carried out.

#### Results

GH had the expected effect on IGF-1 with a significant increase from  $67.7 \pm 7.2$  to  $166.7 \pm 16.2 \mu\text{g/l}$  ( $P < 0.001$ ). AGHDA fell from 10.4 to 6.4 ( $P = 0.059$ ), waist circumference reduced from 88.1 to 84.7 cm ( $P = 0.09$ ), CRP reduced significantly from 3.78 to 1.99 mg/l ( $P = 0.007$ ), 24 hour mean arterial BP fell from 91.3 to 89.3 mmHg ( $P = 0.006$ ) and PWV reduced from 8.1 to 6.7 m/s ( $P < 0.05$ ). Insulin sensitivity did not alter after GH therapy. There was a strong trend to correlation between improvement in QoL and increase in IGF-1 ( $r = 0.49$ ,  $P = 0.09$ ) but there was no correlation between improvement in QoL and reduction in waist circumference, BP, PWV, CRP, total cholesterol, leptin or intercellular adhesion molecules (ICAM-1, VCAM).

#### Conclusion

Growth hormone replacement induces improvements in QoL and discernible reductions in surrogates of cardiovascular risk. Lack of correlation between QoL and cardiovascular risk may mean patients are given or denied GH therapy inappropriately.

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### P569

#### The response of infant's adrenal gland and salt appetite, aldosterone and Na serum level to mother's salt consumption

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#### Introduction

The tendency to eat salty foods is an individualized matter. Aldosterone plays an important role in this increase in salt appetite. In this study the effects of different salt concentrations during pregnancy on Na and aldosterone level, adrenal gland and salt appetite of rat's infants and the relation of salt consumption with Bp were studied.

#### Method

Eight groups of rats, six rats in each group, (ones of male and five female) having  $200 + 20$  grams of weight were selected, salt solutions were

prepared in concentrations of 0.5, 1, 1.4, 1.6, 1.8, and 2 percent and given to test groups during pre-pregnancy, pregnancy and lactation period and control group used tap water of Isfahan. Another test group used distilled water. Na and Aldosterone level, adrenal tissue histologic changes and salt appetite in infants were evaluated.

#### Result

Na serum level in infants show a direct correlation with the rate of mother's salt consumption, but aldosterone serum level in infants does not show this correlation. Adrenal gland shows an increase in weight in group which their mother consumed distilled water and the groups which consumed salty water show an inverse change. Salt appetite and tendency to eat salty foods and salty water correlated with mother's salt consumption. Bp show a direct correlation with salt consumption.

#### Discussion

An increase in salt consumption during pregnancy increases salt appetite and Na serum level in infants, but aldosterone levels shows a reduction. So we could propose that aldosterone is not responsible for this increase in salt appetite and Na level. Other Neurohormonal mechanisms lead to increase in this salt appetite and Na level. Adrenal atrophy is the response of adrenal gland to this conditions. Bp level in rat's infants also increased, which this increase related to direct effect of Na and indirect hormonal and tissue changes.

### P570

#### Maternal testosterone levels during pregnancy are associated with offspring size at birth

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#### Background

Animal studies have indicated that maternal androgen levels influence the intrauterine environment and development of the offspring. As human data are missing, we investigated the possible association between maternal androgens and offspring size at birth in humans.

#### Methods

Randomly collected parous Caucasian women ( $n = 147$ ) were followed prospectively through pregnancy. Maternal serum levels of dehydroepiandrosterone sulphate (DHEAS), androstenedione, testosterone and sex hormone binding globulin (SHBG) were measured at gestational weeks 17 and 33. The main outcome measures were weight and length at birth. Associations between maternal androgen levels and offspring birth weight and length were investigated using multiple linear regression modelling adjusted for potential confounding by maternal height, pre-pregnancy body mass index, smoking, parity, offspring gender, and gestational age at birth.

#### Results

Increasing maternal testosterone levels at week 17 and week 33 were both associated with lower birth weights and lengths. Accordingly, at week 17 an increase in maternal testosterone levels from the 25<sup>th</sup> to the 75<sup>th</sup> percentile was associated with a decrease in birth weight by 160 grams (95% CI: 29 to 290 grams), while at week 33 that estimate was 115 grams (95% CI: 21 to 207 grams). No similar associations were observed for DHEAS, androstenedione or SHBG.

#### Discussion

Increasing maternal testosterone levels during human pregnancy are associated with growth reduction *in utero*, and the weight difference compares with the impact that traditional factors, such as foetal gender or maternal smoking, have on birth weight. Newborn size characteristics are important predictors of a multitude of disorders in adult life. Hence, our identification of elevated maternal testosterone levels as a new indicator of intrauterine growth restriction in humans is important as it opens up the possibility to better understand the mechanisms involved in intrauterine growth. This in turn, may help to elucidate the mechanism(s) behind the "foetal origins hypothesis".

### P571

#### Mortality in growth hormone deficiency

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#### Aim

We aimed to describe mortality for both gender in children and adults, in Growth Hormone Deficiency (GHD).

#### Materials and methods

Using three national registries: The Causes of Death Registry, The National Patient Registry, and The Cancer Registry, and The International Classification of Diseases as a filter, we identified a primary cohort of 9,131 patients. All had a high a priori risk of having GHD. All available hospital files (representing 90.2% of the patients) were traced, and a verification of the diagnosis of GHD was based on a combination of clinical and biochemical definitions. The final cohort was defined during the period from 1980 through 1999, where 1,823 patients had GHD. The patients were divided into childhood onset (CO) and adult onset (AO), discriminated by an age cut-off below or above 18 years at diagnosis.

We used The Central Office of Civil Registration to match the cases (by gender and age) with up to five controls. In total 8,014 controls were identified. Deaths were registered during the period 1980–2004.

#### Statistics

Survival analyses were performed with Kaplan-Meier and log rank techniques.

#### Results

Six-hundred-and-sixty-three cases and 1,700 patients were deceased. For CO and AO, mortality was higher for both genders in patients with GHD than in controls ( $P < 0.0001$ ). Using log rank stratified on age at diagnosis (five years intervals), there was no difference in mortality between the genders for CO and AO ( $P = 0.57$  and  $P = 0.12$  respectively).

#### Conclusion

We conclude mortality is increased in patients with GHD. Further analyzes are warranted to identify which causes of death are increased.

### P572

#### The cardiovascular risk profile and carotid IMT of adults with partial GH deficiency

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We have previously shown that adults with partial GHD have increased total body and truncal fat mass, an adverse lipid profile, and insulin resistance. In this study we have gone on to analyse surrogate markers of vascular risk and carotid IMT. We defined GH status using the combination of two stimulation tests, the ITT and arginine stimulation test, in to patients with severe GHD (pGH  $< 3$  ng/ml,  $n = 30$ ) or GH insufficiency (pGH  $3 - 7$  ng/ml,  $n = 24$ ). Thirty age and gender matched control subjects were also studied.

There were no differences in age between the GHD, GHI, and control subjects (30.9 vs 31.5 vs 34.2 yrs). IGF-I levels were significantly lower in the GHD adults ( $208 \pm 115$  ng/ml) than the GHI or control subjects ( $P = 0.006$ ,  $P < 0.001$  respectively), and were lower in the GHI subjects ( $295 \pm 104$  ng/ml) compared with control subjects ( $373 \pm 123$  ng/ml,  $P = 0.016$ ). Lipoprotein (a) levels were  $19.4 \pm 20.4$ ,  $23.8 \pm 25.5$ , &  $15.7 \pm 20.6$  ng/ml in GHD, GHI, and control subjects respectively ( $P = ns$ ). Fibrinogen levels ( $201 \pm 138$ ,  $205 \pm 135$ , &  $205 \pm 104$  ng/ml respectively) were not significantly different between the groups. PAI-I levels were significantly higher in the GHD and GHI adults compared with controls ( $72.4 \pm 31.5$  vs  $51.3 \pm 34.3$  ng/ml,  $P = 0.02$ ; &  $75.8 \pm 26.1$  vs  $51.3 \pm 34.3$  mmol/l,  $P = 0.01$ ), with no difference between GHD and GHI subjects. C-reactive protein (CRP) was not significantly different between the three groups ( $9.7 \pm 13.6$  vs  $5.5 \pm 3.3$  vs  $4.6 \pm 2.6$  ng/ml respectively). Carotid IMT assessed by high resolution USS was  $0.586 \pm 0.14$ ,  $0.578 \pm 0.13$ , and  $0.503 \pm 0.08$  mm in the GHD, GHI, and control subjects. Carotid IMT was significantly greater in both patient groups compared with controls ( $P = 0.01$  &  $P = 0.02$ ).

The results show GHI adults, in addition to previous data showing these patients to have excess truncal fat mass, an adverse lipid profile and insulin resistance, have elevated PAI-I levels. PAI-I is an independent risk factor for vascular disease. A direct measure of vascular disease, carotid IMT, was also increased in GHI adults. These data collectively suggest GHI adults are at risk of excess vascular disease.

### P573

#### Double blind placebo controlled study on the impact of RGH replacement on quality of life in patients with severe growth hormone deficiency

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#### Objectives

Conflicting data of the benefits of GH replacement improving psychological and physical well being is available, though open GH studies generally agree on an overall improvement. For growth hormone deficient patients to continue on therapy after evaluation there should be a 7 point improvement in their QoL-AGHDA, as documented in the NICE guidelines. The aim of this study was to determine the impact of 0.4 mg of GH on QoL in severe GH deficient patients in a double blind placebo controlled study.

#### Methods

Seventeen patients (10 males and 7 females) with confirmed severe GH deficiency secondary to hypopituitarism were randomised to subcutaneous recombinant GH (Lilly rGH®, dose 0.004 mg/kg/day) versus placebo (sterile diluent containing glycerol and m-cresol) of 12 weeks duration per arm and then crossed over. Thereafter, patients continued open GH therapy for a further 6 months. QoL-AGHDA questionnaires were completed and assessed at the end of each study phase.

#### Results

All patients completed the questionnaires and compliance was more than 95% for GH administration. All patients had restoration of their IGF-1 to the normal range. Two patients had an AGHDA score in the normal range at baseline (1 & 2 respectively), so were excluded from further analysis. When compared with baseline, only 1/15 patients over the initial three months period improved their scores with rGH by more than 7 points which was no significantly different to placebo scores ( $P = 0.73$ ). However, 6/15 patients improved their scores by more than 7 points after 6 months of continuous rGH replacement in the open phase compared with placebo values ( $P = 0.47$ ).

#### Conclusions

Three months rGH treatment was insufficient to affect the QoL-AGHDA of seven points or more and a minimum evaluation period of 6 months treatment appeared to be required to effect a change.

### P574

#### **Prolonged expression of the ACTH receptor, MC2-R, during 3T3-L1 adipocyte differentiation is dependent upon switching promoter usage to an adipocyte-specific, C/EBP-driven, downstream promoter**

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The peptide hormone ACTH stimulates lipolysis and suppresses leptin production in adipocytes via the G-protein coupled seven transmembrane receptor, MC2-R. We have shown previously that PPAR $\gamma$ 2 is the primary factor responsible for transactivation of the already identified murine MC2-R promoter in the differentiating 3T3-L1 adipocyte cell line. In this study we show that despite the activity of this promoter being transient during differentiation, MC2-R mRNA remains elevated at later time points during adipogenesis. Analysis of MC2-R transcripts in terminally differentiated 3T3-L1 cells reveals that they initiate from a transcriptional start site in the first intron of the murine MC2-R, 1.4 kb downstream of the previously identified start site. Placing the genomic sequences in intron 1 from the end of exon 1 to the end of the novel first exon upstream of the luciferase gene in the promoterless vector pGL3 we showed that this region behaves as an adipocyte-specific promoter activated with late kinetics, ie it becomes active after the decline in activity of the 5' promoter. Introduction of a 5' deletion series of this novel promoter into differentiated adipocytes indicates that a C/EBP site, 87 bp upstream of the transcriptional initiation site, is necessary for activity. Mutation of this site inactivates the promoter in differentiated 3T3-L1 cells and EMSA and ChIP analyses reveal that this site is bound by C/EBP factors. Real time PCR analysis of mRNA initiating from the two start sites shows that there is a switch in promoter usage from the 5' to the 3' promoter around day 5, indicating the complex regulation of murine MC2-R during adipogenesis.

### Neuroendocrinology and behaviour

#### P575

#### **Association between the LRP5 gene and serum post-menopausal follicle stimulating hormone levels**

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The LRP5 gene is believed to be primarily associated with bone metabolism via Wnt signalling. The latter pathway, however, seems to control various other systems outside the skeleton. We identified the C/T (c.4037:A1330V)

polymorphism in the LRP5 gene using a restriction analysis of the PCR product in a cohort of 165 white untreated pre- and post-menopausal women. In a subset of 84 post-menopausal women we analysed the association between the LRP5 genotype and circulating sex-hormones including FSH and LH. All procedures have been reviewed by the Ethical Committee of the Institute.

Results. The distribution of CC, TC and TT allele combinations was 73.9%, 23.6% and 2.4%, respectively, which is comparable with other Caucasian populations. As no TT homozygote was found in the group of post-menopausal women, serum sex-hormone levels were compared between CC and TC genotypes. Carriers of the CT genotype had markedly higher circulating FSH values (mean  $\pm$  SD: 107.9  $\pm$  38.8 U/l) as compared with homozygotes CC (84.7  $\pm$  33.0 U/l) ( $P < 0.005$ , ANCOVA). No associations were found between the LRP5 genotype and serum estradiol, testosterone, dehydroepiandrosterone (or its sulphate), androstenedione and/or SHBG levels.

To conclude, serum post-menopausal FSH, but not LH levels are associated with the LRP5 gene in this study. Functionality of the gene in gonadotrope system remains to be determined.

The study has been funded by the IGA grant NR/7827-3 from the Ministry of Health of the Czech Republic.

### P576

#### **Structural analysis of somatotrophs and lactotrophs in annexin 1 null mice**

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Annexin 1 (ANXA1) is a member of the annexin family of phospholipid- and calcium-binding proteins with a well-demonstrated role in the inhibitory actions of glucocorticoids on the release of ACTH, TSH, GH and PRL from the anterior pituitary. Consistent with these findings plasma PRL content is raised in male ANXA1 null mice vs wild-type controls (Cover *et al* Endocrine Abstracts 8, P69). Here we test the hypothesis that because ANXA1 mediates some of the inhibitory actions of glucocorticoids on GH and PRL secretion the somatotrophs and lactotrophs in ANXA1 null mice would be more active. Anterior pituitary tissue from male ANXA1 wild-type and null adult mice ( $n = 6$  of each) was fixed, examined by immunocytochemistry to determine the number of somatotrophs and lactotrophs, and by electron microscopy to examine the size, secretory granule population and secretory machinery of somatotrophs and lactotrophs. No significant differences in lactotroph or somatotroph number, cell size and density of secretory granules were measured. However, ANXA1 null somatotrophs and lactotrophs demonstrated significantly ( $P < 0.05$ ) increased margination of secretory granules to the plasma membrane and increased ( $P < 0.05$ ) amounts of rough endoplasmic reticulum (ER). Secretory granule diameter was significantly increased ( $P < 0.05$ ) in somatotrophs. Although cell size and amount of secretory granules were unchanged, more rough ER and increased granule margination to the plasma membrane suggest that in the absence of ANXA1 the lactotrophs and somatotrophs are more active. Although ANXA1 has a role in exerting anti-proliferative actions of glucocorticoids it does not appear to regulate lactotroph or somatotroph cell number. These data are consistent with our hypothesis that in ANXA1 null animals there is a reduction in the annexin mediated glucocorticoid inhibition of hormone release from somatotrophs and lactotrophs.

### P577

#### **Dietary supplements for stress and obesity co-morbidities reduction**

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The diet of patients exposed to stress had been shown to be low in fibre, low in fruit and vegetables, low in vitamins C and E, and beta-carotene, high in sugar, and high in saturated fat. The implications of these findings are two-fold: the diet associated with stress is similar to those in patients with features of the Metabolic syndrome (Met-S – visceral obesity, diabetes, hyperlipidemia, hypertension).



The aim of the present study was to assess the effect of anti-stress dietary combination (ASDC) of omega-3 fatty acid (O-3FA), magnesium (Mg) and soybean isoflavones (SIF) on stress parameters, endocrine markers and cardiovascular risk factors.

#### Subjects and methods

From a group of 50 patients with the features of Met-S (IDF definition 2005) were selected 25 with highest stress exposure (The Hassles and Uplifts Scale) during the last week and dietary habits (DHQ) showing high fat consumption. They were randomized on ASDC containing 2000 mg. O-3FA, 500 mg. Mg-aspartate and 100 mg. SIF/daily for 3 months. Lipid status, cortisol, glycaemia, body weight and blood pressure were assessed monthly.

#### Results

After 3 months of ASDC supplementation there was significant reduction in lipid parameters – triglycerides reduced from  $2.8 \pm 0.4$  to  $1.6 \pm 0.2$  mmol/l; LDL cholesterol reduced from  $4.1 \pm 0.7$  to  $3.9 \pm 0.9$  mmol/l. No significant change was observed in body weight, fasting plasma glucose and cortisol levels. Despite that enrolled subject showed improved mood and better stress coping.

#### Conclusions

Overwhelming evidence has shown that magnesium, omega-3 fatty acids and soy consumption reduces stress related major abnormalities associated with cardiovascular disease risk. They may also have other heart health benefits, including the ability to reduce the oxidation of LDL cholesterol and promote vascular relaxation, physiological effects which are emerging as important risk factors for heart disease. Scientific researchers and health professionals should agree that the public should be encouraged to incorporate a variety of anti-stress supplementation products into a heart healthy diet and lifestyle plan.

## P578

### The impact of hypothalamic-pituitary (h-p) irradiation on the TSH Bio/Immuno (B/I) ratio in the fed and fasting states

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The TSH B/I ratio is normally reduced overnight and is substantially reduced in patients with overt central hypothyroidism due to h-p axis tumours before and after treatment. It is unknown if similar changes in the TSH B/I ratio in the absence of overt central hypothyroidism contribute to the increased TSH levels seen in cancer survivors who undergo h-p axis irradiation. Thus, we selected 9 patients with the highest TSH levels out of a previously reported cohort of 37 euthyroid adult cancer survivors cranially irradiated for non-pituitary brain tumours or leukemia, in whom the mean TSH of the 24 hourly samples during 24-hour profiling and the peak stimulated TSH levels during the TRH test were increased. Of the 9 patients, 4 had both fed and fasting (last 24 hours of 33-hour fast) profiles, 2 had fed and 3 had fasting profile only. Pooled day (0900–2000 hr) and night (2100–0800 hr) sera were analysed for the TSH B/I ratio. Patients had significantly higher profile mean TSH and significantly lower free T4 levels compared with 33 normal controls (mean  $\pm$  SEM;  $3.15 \pm 0.2$  vs.  $1.8 \pm 0.1$  mU/L;  $P < 0.0001$  and  $13.7 \pm 0.9$  vs.  $15.5 \pm 0.4$  pmol/L;  $P = 0.04$ , respectively). The ratio (mean  $\pm$  SEM) in the patients was not different from that reported in 26 controls ( $1.8 \pm 0.2$  vs.  $1.5 \pm 0.1$ ;  $P = 0.2$ ). The daytime and night-time ratios in the patients were also similar to those reported in 16 controls ( $2 \pm 0.3$  vs.  $2 \pm 0.06$ ;  $P = 0.8$  and  $1.6 \pm 0.4$  vs.  $1.3 \pm 0.1$ ;  $P = 0.2$ , respectively). Paired observations in 4 patients revealed no impact of fasting on the ratio ( $2.2 \pm 0.2$  vs.  $2.1 \pm 0.5$ ;  $P = 0.8$ ). We conclude that, in the absence of overt central hypothyroidism, h-p irradiation does not alter the TSH B/I ratio.

## P579

### Comparison of the catecholaminergic response to 2 different stressors in healthy men

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Alteration of sympathetic nervous system (SNS) activity may be involved in the development of obesity. Among stimuli which may activate the SNS, stress is often quoted. Responses to stressors include stimulation of the hypothalamo-pituitary-adrenal axis (HPA) and of the SNS, which result in increased plasma cortisol and catecholamines concentrations. Although the stress response seems to be automatic (therefore not subject to regulation), studies in human have highlighted the heterogeneity of the cortisol response, leading to the differentiation of 2 subgroups of high- and low- stress responders. Although this distinction is well documented for the cortisol response, it is less known whether epinephrine and norepinephrine stimulation follows the same distribution. Therefore we decided to evaluate the variability of catecholamine responses to different stimuli, ie. hypoglycemia and mental stress, in 8 healthy volunteers. A 2 steps hypoglycemia (30 minutes at 4 mM, 30 min at 3 mM) was obtained by continuous infusion of human insulin (actrapid HM 100). Mental stress consisted in a 30 min session of arithmetic and Stroop task. Epinephrine and norepinephrine were measured by HPLC. As already documented, hypoglycemia provoked mainly an epinephrine release ( $+794 \pm 125\%$ ) while mental stress essentially produced a norepinephrine release ( $+70 \pm 10\%$ ). There were considerable interindividual variations in these responses (epinephrine response to hypoglycemia: 294–1147% norepinephrine response to mental stress 33–108%). The peak concentration of plasma epinephrine during hypoglycemia was positively correlated to the peak plasma norepinephrine concentration during mental stress:  $r = 0.499$ ,  $P < 0.05$ , (Spearman rank test). The observation that the same individuals who rise a large response to mental stress also respond markedly to hypoglycemia support the concept of high and low SNS responders. The mechanisms underlying these interindividual variations (genetic or acquired), and the possible consequences of high SNS responses on the development of cardiovascular or metabolic disorders remain to be evaluated.

## P580

### Effect of gastric bypass and gastric banding on neurotensin levels in morbidly obese patients

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#### Objective

Neurotensin is an anorexigenic peptide produced mainly in the N-cells of the ileum; levels are decreased in obese subjects and increase after bariatric surgery. This study is the first to compare the baseline and postoperative levels of neurotensin in patients after gastric bypass, gastric banding, and non-operated controls, respectively, during long-term follow-up.

#### Patients and methods

Overnight fasting plasma neurotensin levels were measured with a new sandwich immunoassay in morbidly obese subjects at baseline and 3, 6, 12 and 24 months after Roux-en-Y gastric bypass ( $n = 5$ , mean (SD) body mass index (BMI)  $44 \pm 2$ ), gastric banding ( $n = 8$ , BMI  $42 \pm 1$ ), and in non-operated controls ( $n = 7$ , BMI  $41 \pm 1$ ).

#### Results

After gastric bypass and banding, mean (SD) body weight decreased by  $29.5 \pm 5.5$  kg and  $22.8 \pm 5.9$  kg respectively, with no change in non-operated controls. The decrease after 3 and 6 months was more pronounced after gastric bypass as compared to gastric banding ( $P < 0.05$ ). Plasma neurotensin levels in patients after gastric bypass increased from  $24.8 \pm 19.6$  at admission to  $87.0 \pm 60.3$  after 6 months and  $75.2 \pm 48.3$  U/ml after 24 months ( $P = 0.004$ , repeated measures ANOVA). In contrast, in patients with gastric banding, neurotensin levels remained stable ( $20.8 \pm 6.1$  at admission,  $22.0 \pm 13.3$  after 6 months,  $22.8 \pm 11.7$  U/ml after 24 months,  $P = ns$ ). Neurotensin levels did not change in non-operated controls. There was no correlation of neurotensin levels with plasma ghrelin or leptin levels.

#### Conclusion

Plasma neurotensin levels show a significantly more pronounced increase after gastric bypass as compared to gastric banding. This could be an additional explanation for the more pronounced decrease in appetite and weight in patients with gastric bypass.

**P581**

**Mental and neuropsychological disorders of children with congenital hypothyroidism**

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**Aim**

To study physical, sexual and mental development and neuropsychological disorders of children with congenital hypothyroidism

**Methods and materials**

11.5 ± 0.5 year old 60 children and teenagers with congenital hypothyroidism (CH) have been examined. Radioimmunologic (RIA) study of blood hormone level revealed confident decrease of somatotrophic hormone (STH), thyroid (T3, T4), gonadotropic, sex hormones and increase of thyreotropic hormone (TTG). Growth arrest correlating to STH insufficiency in blood ( $P < 0,05$ ).

**Results and discussions**

The degree of physical, sexual and mental development arrest was respectively high in these patients to be accompanied by decrease in level of FSH, LH, estradiol, testosterone and thyroid hormones. Study of neurological status revealed 1–3 degree of endocrine encephalopathy. In 95% of cases electroencephalography (EEG) showed dysfunction of cerebral trunkal-diencephalic structures. Prevalence of slow EEG waves with no normal cerebral cortex biological rhythms has been revealed to indicate neuro-endocrine regulation delay in cerebral neuron functioning. Investigation of mental development of our patients was included evaluation by D. Veksler, which showed deep disorders of IQ ( $23 \pm 1.5$ ).

All these disorders are accompanied by significant higher nervous activity defects (acad. A.R. Luria's method, Russia) with various cortical centre compartments' dysfunction revealed. Severity of disorders in praxis, gnosis, mentality, operative memory, count and speech correlates to general development arrest degree, EEG data and to thyroid hormones deficiency (T3, T4) revealed.

**Conclusions**

Our investigations showed that between hormonal and neuroendocrine disorders in patients with CH have been direct dependence.

The implication of endocrine regulation in pathogenesis of physical development retardation upon various diseases was confidently confirmed in dynamics of examination of hormonal, anthropometrical, neuropsychological, neurophysiological and roentgenological parameters, direct dependence between degree of physical, sexual and mental development retardation in patients with congenital hypothyroidism being observed.

**P582**

**Hypopituitary patients have a high occurrence of metabolic syndrome markers and an increased prevalence of cardiovascular risk factors**

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**Introduction**

Hypopituitary patients receiving conventional hormone substitution, but without GH replacement, have an increased mortality from cardiovascular diseases. Inadequate hormone replacement is a possible cause of this increased mortality. GH deficiency in adult patients has been associated with several cardiovascular risk factors, including hyperlipidemia, increased abdominal adiposity, and impaired insulin sensitivity.

The aim of the study is evaluation of patients with GH deficiency with no clinical signs of cardiovascular diseases in the course of multihormonal hypopituitarism with special attention paid to occurrence of metabolic syndrome markers.

**Material and methods**

The study included 16 patients (12-M and 4-F), from 21 to 59 years ( $X = 39$ ) with multihormonal hypopituitarism which lasted from 1 to 29 years ( $x = 11.15$ ) and after surgical treatment of a tumour in the hypothalamic-hypophyseal region; patients with acromegaly and Cushing's disease were excluded from the study. In all the studied patients basic constituents of metabolic syndrome were evaluated: body mass index (BMI), waist-hip ratio (WHR), arterial pressure, insulin resistance ratios, HOMA-IR and QUICKI, lipidogram, fibrinogen, homocysteine and echocardiography. The control group consisted of 12 healthy individuals.

**Results**

Hypopituitary patients had an obesity value ( $P = 0.0063$ ), independent of sex and age, with a higher WHR ( $P < 0.0001$ ). Mixed hyperlipidemia was

found in 86% of the studied patients, a higher low density lipoprotein cholesterol ( $P = 0.00293$ ), and triglyceridemia ( $P = 0.003$ ). Serum homocysteine was significantly higher in patients than in controls ( $P = 0.02$ ). Furthermore, the patients had a significantly increased left atrium size ( $P = 0.05$ ).

**Conclusions**

Hypopituitary patients exhibit increased values of the basic markers of metabolic syndrome and an increased prevalence of cardiovascular risk factors.

**Summary**

In patients with hypopituitary, it is necessary to apply simultaneous replacement treatment with regard to all hormonal deficiencies, including GH. Close monitoring for premature development of metabolic syndrome risk factors is important, especially in the young.

**P583**

**Spontaneous GH secretion in the fed and fasting states in cranially irradiated adult cancer survivors with normal peak GH responses to two provocative tests: does GH neurosecretory dysfunction exist?**

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A few studies have suggested that radiation-induced growth hormone neurosecretory dysfunction (GHNSD) exists in children irradiated for leukemia (18–24 Gy). The presence or absence of GHNSD in adult cancer survivors has not been studied before. Thus, 24-hour spontaneous GH secretion was studied by 20 min sampling both in the fed state ( $n = 16$ ; 6 women) and the last 24 hours of 33-hour fast ( $n = 10$ ; 3 women) in adult cancer survivors, of normal GH status defined by two provocative tests. The patients had been irradiated for non-pituitary brain tumours ( $n = 12$ ) or leukemia ( $n = 4$ ), aged 16–53.7 (median, 20.25) yr, and studied 13.1 ± 1.6 (mean ± SEM) yr after irradiation. Gender-specific comparisons with age and BMI-matched 21 normal men (11 fasting) and 9 normal women (3 fasting) were conducted. Using, previously published diagnostic thresholds, all patients had stimulated peak GH responses in the normal range (>5 mcg/L and >16.5 mcg/L to the insulin tolerance test (ITT) and the combined GHRH plus arginine stimulation test (AST), respectively) as well as normal physiological GH secretion (mean profile GH levels >0.35mcg/L and 1.25mcg/L during the fed and fasting states, respectively). However, patients' peak GH responses were significantly reduced compared with normal controls (women responses: ITT, 9 ± 1.2 vs. 19.9 ± 4.3 mcg/L,  $P = 0.04$ ; GHRH + AST, 33 ± 6.4 vs. 77 ± 18 mcg/L,  $P = 0.05$ ; men responses: ITT, 20 ± 3 vs. 34 ± 4 mcg/L,  $P = 0.03$ ; GHRH + AST, 37 ± 7 vs. 64 ± 7 mcg/L,  $P = 0.02$ ), while no differences were seen in the profile mean GH levels. In the women: fed 1.03 ± 0.18 vs. 1.17 ± 0.17 mcg/L,  $P = 0.6$ ; fasting: 2.14 ± 0.9 vs. 2.33 ± 0.8 mcg/L,  $P = 0.9$ . In the men: fed: 0.92 (0.25–3.1) vs. 0.64 (0.22–2.1) mcg/L,  $P = 0.5$ ; fasting: 2.2 ± 0.4 vs. 2.5 ± 0.3 mcg/L,  $P = 0.5$ . One leukemia patient had subnormal fed profile mean GH level of 0.25 mcg/L but achieved normal fasting mean GH level of 2.4 mcg/L. We conclude that radiation-induced GHNSD does not exist in adult cancer survivors. Despite "normal" peak GH responses, radiation-induced damage to the h-p axis is evidenced by the attenuation of the maximum somatotrophic reserve during pharmacological stimulation.

**P584**

**The long-term predictive accuracy of the short synacthen (corticotropin) stimulation test for assessment of the hypothalamic-pituitary-adrenal axis**

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Abstract withdrawn

**P585**

**GH secretion in amyotrophic lateral sclerosis**

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Amyotrophic lateral sclerosis (ALS) is the most common motor neurone disorder in human adults. Scanty data on endocrine abnormalities have been reported. Aim of the present study was to investigate the GH-IGF-I axis in ALS patients.

22 ALS patients (12 men and 10 women), aged 46–77 years, performed GHRH + arginine test: blood samples for GH were collected at baseline, 30 and 60 minutes; IGF-I was determined at baseline. The control group consisted of 25 normal age- and sex-matched subjects (12 men, 13 women; age range, 40–77 yr). No patient was under riluzole therapy.

Mean basal GH levels ( $\pm$ SD) in ALS patients were significantly reduced compared with normal controls ( $0.24 \pm 0.27$  Vs  $227 \pm 3.6$  ng/ml,  $P = 0.01$ ), as well as peak GH concentrations ( $12.6 \pm 8.9$  vs  $39.9 \pm 18.7$  ng/ml,  $P < 0.001$ ). Six patients (27.3%) showed a normal GH response to stimulus; 7 patients (31.8%) displayed moderate GH deficiency; in 9 patients (40.1%) GH response was markedly deficient. IGF-I levels ( $143.6 \pm 63.8$  ng/ml) were significantly reduced in patients compared to normal subjects ( $220.4 \pm 18.6$  ng/ml). No significant correlation was observed between peak GH concentrations and age, BMI, disease duration, severity or clinical form. A higher incidence of GH deficiency was observed in males compared to females (83.3% vs 60%); peak GH response in males was significantly lower than in females ( $8.92 \pm 6.63$  vs  $16.98 \pm 9.62$  ng/ml,  $P = 0.03$ ).

In conclusion, the present data indicate a reduction of GH secretion in ALS patients and particularly in males; the mechanisms behind this finding need to be clarified.

**P586**

**Clinical significance of macroprolactin**

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**Introduction**

Macroprolactin (maPRL) is a high molecular weight variant of prolactin (PRL) which has reduced bioactivity. The purpose of the present study was to determine the clinic-analytical repercussion of the presence of maPRL in patients with hyperprolactinaemia.

**Patients and methods**

A polyethylene glycol (PEG) precipitation test was used to detect the presence of maPRL in all consecutive samples with prolactin levels  $> 50$  ng/ml (1060 mU/L). A recovery  $< 75\%$  was taking as indicating of maPRL. Hospital records of subjects with maPRL were review retrospectively.

**Results**

Over a 24-month period, maPRL was found in 22 (9.6%) of 228 patients with total PRL  $> 50$  ng/ml. All patients with maPRL were women; the mean age was 32 years (12–48). Serum PRL levels ranged from 50.5–158 ng/ml. The most frequent reason for the initial PRL request was menstrual disturbance (45% patients). MaPRL was associated with an increase of monomeric PRL level in 36.4% of patients (group A) and in this group hypogonadal symptoms was presented in 87.5%. MaPRL was associated with normal level of monomeric PRL in 63.6% (group B) and in this group only one patient presented amenorrhoea (7.14%),  $P < 0.05$ . Pituitary adenomas were identified in 2 of 6 who underwent neuroimaging. Dopamine agonist treatment resolved hyperprolactinaemic symptoms.

**Conclusions**

Our results indicate that patients with maPRL only presented symptomatology suggestive of hyperprolactinaemia when monomeric PRL concentration was elevated. MaPRL has limited clinical repercussions but its determination in routine practice is important in order to avoid inappropriate management.

**P587**

**ADH and thirst function in defence with hypernatremia formation**

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**Introduction**

Thirst and ADH secretion are two principle mechanisms for controlling body fluid osmolarity. Even an excessive load of salt can effectively be

handled by these systems. They can control plasma osmolarity in a vast range of water and sodium consumption. We studied the effect of salt solution consumption on serum sodium level and osmolarity and the action of these two systems.

**Method**

Seven groups each consisting of 6 male rats weighing  $200 \pm 20$  gr. were chosen. Salt solutions (1, 3, 5, 7 and 9%) were also provided. The rats were denied tap water and these prepared solutions were given to each group. Our control group used Zanjan potable water and another test group drank twice distilled water. Because the mortality rate in rats consuming higher salt solutions ( $> 3$  percent) began to rise on fifth day, the total water consumption, serum sodium level and osmolality were measured on day 5 in all groups.

**Result**

Water intake in groups using 1 and 3 percent solutions, increased 78 and 36 percent respectively. Their urine output was also considerably increased. The groups maintained on distilled water, 5, 7 and 9% solutions showed respective decreases of 11, 56, 73, and 85% in their water intake. Osmolarity and sodium level significantly increased in groups using 3% and higher solutions. ADH and thirst mechanisms could not control serum sodium level and osmolality when the concentration of salt in distilled water exceeded 1.4%.

**Discussion**

The use of higher concentration salt solutions could not decrease osmolarity and quench thirst. Because higher salt solutions stimulate the neurological protective mechanisms, which act more powerfully in higher concentrations, the animal shows no tendency to drink water and osmolarity will significantly increase. The animal's refusal to drink highly concentrated solutions will keep it alive for some days and if it can get fresh water at this time it will escape death. We could propose that ADH and thirst could not control osmolarity when human or animal uses a large load of salt and increase in osmolarity leads to death in a few days.

**P588**

**The effect of garlic odor on appetite center**

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**Introduction**

Some of the gastrointestinal disorder and diseases reduce appetite and inclination to eating food. In this situation for corresponding to this condition, certain medical management to be accomplished. One of the poultry diseases which reduces the rate of feeding most significantly is influenza. Food consumption decrease more than 80% in a very acute cases, because food consumption reduce significantly, poultry may lose their weight amount to 20–30 per cent in a few days. In these cases in order to stimulate poultry for food consumption, fresh garlic amounting to eight kilogram or more in ton was used. In this study we evaluate the effect of garlic on stimulating appetite center.

**Method**

Three groups of one day young cock, each group having 10 cock were selected, control group used a diet without garlic and bred in an environment without garlic odor, one of the test group used a diet with garlic and the another test group used a diet without garlic but bred in an environment with garlic odor. The rate of food consumption and weight changes were evaluated until the end of breeding period.

In a behavior test, poultry inclination to eating food after changing food dishes was studied.

**Result**

The rate of food consumption and weight gain show a significant increase in tests group in comparison with control group but this parameters in tests group does not show significant difference. All of the poultry used food after changing food dishes in group which garlic in diet, most poultry used Poultry used food in-group, which bred in an environment with garlic odor; the number of poultry, which used food in control group in comparison with other groups, was lower.

**Discussion**

As some people appetite may decrease during diseases or inclination to eating food in some people in comparison with other people are lower and this appetite reduce could induce problem for these people, so we proposed these people try to use garlic in daily diet. Garlic odor stimulates appetite center and increases inclination to food consumption; hence the garlic effective substances could be used in stimulant appetite drugs.



### P589

#### Taste protective mechanism in relation with salt

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#### Introduction

Salt is an essential compound to provide Na, but over-consumption of salt could be lethal. As using high salt solution is a traditional way of committing suicide in china, In this research, the action of to salt sensation during consumption of salt solution is elevated.

#### Method

6 groups, each containing 10 male rat with 200 gr weight were selected. 5 concentration of salt in distilled water were prepared (1, 3, 5, 7, 9%). During this study, rats were deprived of potable water and above salt solution were given to rats. Control group used tap water of Zanjan.

#### Results

Water consumption on 1,3% groups as compared to control group increased 81% and 44%, but for 5, 7 and 9% group as compared with control group was decreased 56, 73 and 85%.

#### Discussion

1%, 3% salt solution could not decrease Na serum level and thirst quench and because taste protective mechanism dose not act strongly, salt solution consumption increased in this concentration, but when animal were used 5, 7, 9% salt solution, the kidney have to use some of body water along with salt solution to be able to excrete the excessive solute load and with a positive feedback loop, this phenomena can lead to the immediate death of animal die. Animal refusal from drinking highly concentrated salt solution is related to taste protective mechanism.

### P590

#### The effect of salt on night sleep

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#### Introduction

Salt in the principal supply of sodium. The needs body to salt changes according to life conditions. In normal conditions the average daily salt intake of Iranian adults is 5–15 gr. 4–6 spoonfuls of salt in a day can be lethal, but for soldiers in equatorial regions 50 grams of salt in a day may be needed. Studying the different effects of salt needs separate research. In this study the effects of salt on night sleep was addressed.

#### Method

Twenty volunteer students were chosen for the study. They had no problem sleeping before the study. They recorded the time of sleep and when they went to bed for five nights before salt eating. In the 6th day they took 0.5 gram of salt per 10 kilos of body weight at 8.00 PM at night We did record time of sleep, times of awakening at night, presence of any disturbances, depth of sleep, duration of fulfilling sleep. amount and times of drinking water at night, their general condition after sleep, effect on REM and NON-REM presence of nightmares. All these parameter about night sleep were studied.

#### Results

Salt delays time to go to bed, during sleep the individual awakens several times, Sleep disturbance is about 2–3 hours. The normal pattern is disrupted and deep sleep is decreased and it is superficial at best. REM and NON-REM cycles are influenced. NON-REM decreases and REM increases so that the individual dreams a lot and even sometimes has nightmares. The day after, they are not satisfied with the sleep. Subjects awaken earlier and during the night repeatedly got up to drink water. The day after they felt drowsy and fatigued.

#### Discussion

Chips, nuts and other foods normally contain lots of sodium (5–10%). Each 100 gram introduces 4–6 gram of salt to the body. Taking these especially in the evenings can disrupt sleep. Thus it is recommended people with sleep problems must not take these in the last hours of the day.

### P591

#### Evaluation of cognitive functions by using p300 auditory event related potentials (ERPs) in patients with growth hormone (GH) deficiency and gh excess

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#### Objective

Impaired cognitive function has been demonstrated in adults with GH deficiency (GHD) and acromegaly by using different neuropsychological tests. P300 ERP application is a well established neurophysiological approach in the assessment of cognitive function. However the comparison of the P300 ERPs between GHD and GH excess have not been done yet. The present study was designed to investigate the effects of GHD and acromegaly on cognitive function by using P300 ERPs.

#### Methods

The study comprised 17 patients with severe GHD (6 male, 11 female), with a mean age of  $47.5 \pm 9.8$  yr, 16 acromegalic patients (6 male, 10 female), with a mean age of  $40.3 \pm 11.2$  yr, and 15 age, education and sex matched healthy controls. ERPs were recorded at the Fz (frontal), Cz (central), Pz (parietal) and Oz (occipital) electrode sites; and P300 latencies and P300 amplitudes were estimated at all electrode sites. Standard Oddball paradigm was used to evoke P300 responses.

#### Results

There was a significant difference between the mean serum IGF-I concentrations in the GHD patients and acromegalic patients ( $51 \pm 13$  ng/ml and  $762 \pm 300$  ng/ml). The mean P300 latencies (at all electrode sites) of the patients with GHD were significantly prolonged when compared with those of normal controls and acromegalic patients ( $P < 0.05$ ). The mean P300 amplitudes (at all electrode sites) of the patients with acromegaly were significantly decreased when compared with those of normal controls and GHD patients ( $P < 0.05$ ).

#### Conclusions

P300 latency is related to stimulus evaluation time, and P300 amplitude is related to decision making and memory processing. The present study therefore clearly demonstrates that different components of cognitive function are impaired in GHD and GH excess. This is an objective electrophysiological evidence for cognitive dysfunction in GHD and acromegaly.

### P592

#### Appetite regulating hormones in constitutionally lean and anorexia nervosa subjects

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Energy balance is controlled by the arcuate nucleus through integration of peripheral hormonal signals such as leptin, ghrelin, peptide YY (PYY) and glucagon like peptide 1 (GLP-1). The commonest reason for young women in the developed world to be underweight is restrictive anorexia nervosa (AN). Constitutionally thinness (CT) has been described in young women who satisfies the WHO definition for moderate to severe underweight (BMI 13–16.9 kg/m<sup>2</sup>). CT women have no psychological or hormonal features of anorexia nervosa which includes normal menstruation, normal thyroid function, normal cardiac function and normal insulin sensitivity. We hypothesized that appetite regulating hormones in CT subjects would be comparable with a normal weight control group. In this study, we measured ghrelin, PYY, GLP-1 and leptin in three groups of young women (normal weight ( $n = 7$ ), CT ( $n = 10$ ) and AN ( $n = 12$ )). Samples were collected every four hours for a period of 24 hours. Standardized meals were served at 08:15, 12:15 and 19:00. The area under the curve for the PYY circadian cycle reached significantly higher levels in CT compared to controls and AN subjects. GLP-1 was significantly higher in subjects with AN compared to CT, while ghrelin was significantly higher in AN compared to controls and CT subjects. CT subject had the lowest ghrelin levels. The area under the curve for the leptin was significantly lower in the AN group. We conclude that the physiology of constitutional thinness is different to the pathophysiology of anorexia nervosa, since the appetite regulating hormones show



significant differences. The variant of anorexia nervosa without amenorrhea recently proposed should only be considered after excluding the diagnosis of constitutional thinness.

#### P593

##### **Differentiation of TSH-oma from thyroid hormone resistance syndrome using thyroid color Doppler sonography**

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Thyroid hormone resistance (THR) and pituitary TSH-secreting adenomas (TSHoma) are both characterised by increased serum free thyroid hormone levels and normal TSH concentrations. Differentiation of the two syndromes is a clinical challenge, and relies on the suppression (TRH) or lack of suppression (TSHoma) of TSH-dependent parameters and on the presence of germinal mutations in the thyroid hormone receptor beta 1 gene in patients with THR. Thyroid blood flow, evaluated using color flow Doppler sonography (CFDS) depends on TSH receptor stimulation; in fact, it is increased in patients with Graves' disease and absent in those with thyrotoxicosis factitia.

The aim of the study was to assess whether CFDS might help in differentiating patients with THR or TSHoma. CFDS was performed in 6 patients with THR and 6 patients with TSHoma during T3 suppression test at 50, 100 and 200 mcg Titre. Patients with THR or TSHoma did not differ as serum TSH levels, CFDS pattern and intraparenchymal peak systolic velocity (PSV). Mean PSV values reduced from  $9.2 \pm 1.6$  cm/s to  $3.7 \pm 0.5$  cm/s in patients with THR and from  $11.3 \pm 3.3$  cm/s to  $7.3 \pm 2.6$  cm/s in patients with TSHoma ( $P < 0.005$ ) during T3 suppression test. PSV values and CFDS pattern reduced to the normal range in 5 patients with THR and in 1 patient with TSHoma ( $P < 0.05$ ).

In summary, the present study shows that (1) thyroid blood flow is increased in patients with THR or TSHoma, (2) CFDS pattern and intraparenchymal PSV values reduced to the normal range in most patients with THR but not in those with TSHoma. In conclusion, thyroid CFDS may represent an adjunctive tool for differentiating patients with THR or TSHoma.

#### P594

##### **Maternal consumption of a high-meat, low carbohydrate diet in late pregnancy and stress responsiveness in the offspring**

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##### **Background**

Consumption of a high-meat, low carbohydrate diet in late pregnancy is associated with fetal growth restriction, and raised blood pressure and glucose intolerance in the offspring. In a recent study in Motherwell, Scotland, where pregnant women had been advised to eat one pound (0.45 kg) of red meat per day during pregnancy and to avoid carbohydrate-rich foods, we found elevated fasting plasma cortisol levels in men and women whose mothers reported higher protein consumption in pregnancy. We aimed to test whether this form of abnormal maternal nutrition (increasingly observed because of the popularity of the Atkins diet) reflects an enhanced stress response by measuring the response to a psychological stress test (The Trier Social Stress Test (TSST)).

##### **Methods**

We carried out the TSST (3 min mental arithmetic test and 5 min public speaking test) on 86 men and women born in Motherwell during 1967–68. Ethical approval and written informed consent were obtained. Blood pressure was recorded, and saliva and venous blood sampled for cortisol measurement before and after each stressor.

##### **Results**

BP and heart rate rose in response to stress (BP by 14 mmHg,  $t = -4.5$ ,  $P < 0.0001$ ; HR by 6 bpm,  $t = -2.35$ ,  $P = 0.02$ ) and fell to baseline values by the end of the recovery period. Plasma cortisol also rose in response to stress (men 371 to 478 nmol/l,  $t = -5.1$ ,  $P = 0.00002$ ; women 348 to 380 nmol/l,  $t = -2.2$ ,  $P = 0.03$ ). Between early and late pregnancy, meat

consumption almost doubled while carbohydrate intake fell to a third. Offspring of mothers who reported greater meat intake during late pregnancy had greatest cortisol response to stress ( $P = 0.03$ ).

##### **Conclusions**

The TSST is a robust method in our hands demonstrating significant changes in BP, heart rate and cortisol in response to stress. Although the specific advice given to mothers in this study precludes direct application to other populations this is the first evidence that an unbalanced maternal diet during late pregnancy influences stress responsiveness in the offspring. These findings add to increasing evidence that adverse maternal factors program lifelong effects in the offspring.

#### P595

##### **Patients with pituitary disease who have had radiotherapy have reduced cerebral vasomotor reactivity**

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##### **Background and aims**

Studies have revealed increased vascular mortality, particularly cerebrovascular mortality in hypopituitary adults. Cranial radiotherapy has been implicated. This study was designed to assess the impact of cranial irradiation upon cerebrovascular function in patients with pituitary disease.

##### **Methods**

Three groups were studied: – Group 1- ( $n = 12$ ), patients with pituitary disease who have had cranial radiotherapy, Group 2- ( $n = 10$ ), patients with pituitary disease who have not had cranial radiotherapy and Group 3- ( $n = 24$ ), normal controls.

Patients with acromegaly, Cushing's and idiopathic pituitary disease were excluded.

The blood velocity in the middle cerebral artery (MCA) was assessed with a Scimed QVL 120 transcranial doppler. Velocities were measured whilst breathing air and then after 3-minute inhalation of 5% carbon dioxide (CO<sub>2</sub>). Using these results the vasomotor reactivity was calculated as the change in velocity in the MCA per unit change in CO<sub>2</sub> before and after inhalation. Expired CO<sub>2</sub> concentration was determined using a Datex TC6 capnograph. The study was approved by Local ethics committee.

##### **Results**

Independent t-tests were performed with SPSS ver 10.0, comparing patient group with controls. There was a significant difference in the vasomotor reactivity between the group 1 and group 3, mean difference 14.87 (95% C.I. 5.40–24.35,  $P = 0.003$ ). No significant difference between groups 2 and 3, mean difference 9.26 (95% C.I. 24.03–5.40,  $P = 0.210$ ). No significant difference between groups 1 and 2, mean difference 5.61 (95% C.I. – 3.73–14.45,  $P = 0.200$ ).

##### **Conclusion**

Cranial irradiation possibly contributes to reduced cerebral vasomotor reactivity in patients with pituitary disease.

#### P596

##### **An open label, controlled study to assess the ability of patients with acromegaly, or their partners, to administer Somatuline Autogel®**

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Somatostatin analogues are the mainstay of medical therapy for acromegaly. The introduction of ready-to-use Somatuline Autogel (Autogel) raised the possibility of self/partner-injection. This study explored whether patients could administer monthly injections of Autogel, without compromising efficacy. After ethical approval, thirty patients treated with Autogel for acromegaly chose either the Control or Test group. Control group patients ( $n = 15$ ) received injections from a healthcare professional (HCP) for 40 weeks. Test group patients ( $n = 15$ ) received training to self inject and participated for between 32 and 40 weeks, depending on training requirements. Test patients had to fulfil

competency requirements prior to unsupervised injections. Plasma GH, IGF-1, and lanreotide were measured at Baseline, Interim Assessment (Week 20) and Completion. Adverse events and symptoms were recorded. Disease control was maintained if GH & IGF-1 values obtained on completion were comparable to baseline. On completion GH levels were maintained in 14 Test and 15 Control patients. Mean GH (mcg/L) levels were  $2.5 \pm 1.8$  at Baseline and  $2.3 \pm 1.8$  on Completion in the Test group and  $2.2 \pm 2.9$  at Baseline and  $1.9 \pm 1.9$  on Completion in the Control group. IGF-1 levels were maintained in 15 Test and 14 Control patients. Mean serum lanreotide levels tended to increase in the Test group, but tended to decrease in the Control group. On completion 14/15 patients were deemed competent to perform unsupervised injections based on completion of training and maintenance of GH & IGF-1 control throughout the study. The pattern of injection site reactions seen at Baseline (HCP injections) did not change significantly in either group.

#### Conclusion

Suitably motivated patients with acromegaly (or their partners) can be trained to administer unsupervised Somatuline Autogel injections without compromise of disease control. This may confer advantages in terms of clinic visits and independence.

### P597

#### The effect of dopamine on neuroendocrine disorders in women with PCOS under chronic stress conditions

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Polycystic Ovarian Syndrome (PCOS) is the most common cause of menstrual and reproductive disorders in women of reproductive age. It is the leading cause of female infertility, up to 15%. Women with PCOS and their hormonal status marked in immune-enzyme analysis under chronic stress conditions and without it were the subject of interest. The role of chronic stress in development of many diseases is well-known. In patients with PCOS dopamine content depends on pathogenic form of the syndrome. It is known that in typical form of the syndrome (Stein-Leventhal) these indices are normal, while in combined form (central adrenal) is marked increased dopamine secretion and in central type of the disease is marked apparent lowering of its content in the blood. The goal of the present study was determination of chronic stressful situation impact on neuroendocrine disorder development and establishment of the role of hypothalamic-dopaminergic system in PCOS development of central genesis.

We have studied 66 women of fertile age (19–34) with PCOS of central genesis and women with normal ovarian cycle. These women were divided into three groups: I group was formed by 34 women, refugees from Abkhazia, which were in severe living conditions from 1992–2005. All women had traumatic disorders caused by stress, they were consulted by psychotherapist; II group consisted of 32 women with PCOS of central genesis living in Tbilisi, and III (control) group was formed by practically healthy women with normal menstrual cycle.

Findings indicate that LH blood level increase in patients with PCOS of central genesis was caused both by dopamine system influence and increased activity of LH-releasing producing neurons which cause chronic stress.

Accordingly, decreased dopamine level in refugees confirm that chronic stress is meaningful risk factor of PCOS development of central genesis. Chronic stress causes abnormalities in central activities of hypothalamic-pituitary system neuroregulation and redoubles female reproductive system disorders.

### P598

#### Measurement of basal cortisol in serum and saliva for the diagnosis of secondary adrenal insufficiency

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#### Objectives

The insulin tolerance test (ITT) is considered the gold standard to evaluate corticotrophic function in patients with suspected pituitary disease, but is limited by several contraindications. As an alternative, we evaluated the diagnostic value of basal cortisol in serum and saliva.

#### Methods

188 volunteers (V) and 61 patients with suspected pituitary disease (P) were enrolled into this study. Basal serum and saliva samples were collected simultaneously between 8 and 9 am.

#### Results

A significant correlation was found between age and SeC ( $r = -0.22$ ,  $P < 0.005$ ) as well as SaC ( $r = -0.16$ ,  $P < 0.05$ ), but not for sex or BMI to either parameter. By using a serum cortisol cut point (CP) of 500 nmol/l during ITT, 35 P were considered adrenal insufficient (AI), whereas 26 P were adrenal sufficient (AS). Applying ROC analysis to SeC, an optimal threshold of 260 nmol/l was found (Sens 74%, Spec 73%, AUC 0.81). By using an upper CP of 382 nmol/l (Sens >95%) and a lower CP of 103 nmol/l (Spec >95%), 22 of 61 P (36%) were correctly identified. Regarding SaC, ROC analysis led to an optimal threshold of 7.6 nmol/l (Sens 54%, Spec 85%, AUC 0.76). With an upper CP of 17.5 nmol/l (Sens >95%) and a lower CP of 5.0 nmol/l (Spec >95%), 21 of 61 P (34%) were correctly identified.

#### Conclusions

Measurement of basal cortisol in either serum or saliva does not require sex- or BMI-dependent reference ranges. Although a weak correlation with age was observed, age-dependent reference ranges calculated by  $X \pm 2SD$  were nearly identical. By consideration of lower and upper CPs with high Sens and Spec, respectively, measurement of SeC or SaC classified about one third of P correctly, thereby reducing the necessity for ITT to 65% of subjects. Saliva samples taken at 8 am may be especially useful in an outpatient setting.

### P599

#### Sensitivity and specificity of different provocative tests for the diagnosis of secondary hypoadrenalism in patients with hypothalamo-pituitary disorders

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Insulin tolerance test (ITT) is considered the golden standard test to evaluate HPA axis in suspected hypopituitarism. Low dose (1 µg) ACTH<sub>1–24</sub> short stimulation test (LDSST) and metyrapone are often used when ITT is contraindicated. The diagnostic reliability of LDSST is, however, controversial, as even this dose has been considered supramaximal, while very low ACTH doses have been suggested more reliable to assess the adrenal sensitivity. Thus, in patients with hypothalamo-pituitary disorders, we compared the reliability of ITT (0.1 U/kg i.v., 28 patients), metyrapone (MET, 30 mg/kg p.o., 28 patients), very low (VLDSST, 0.06 µg ACTH<sub>1–24</sub> i.v., 14 patients), low (LDSST, 1.0 µg ACTH<sub>1–24</sub> i.v., 28 patients) or supra-maximal ACTH<sub>1–24</sub> doses (HDSST, 250 µg ACTH<sub>1–24</sub> i.v., 28 patients) on ACTH and/or cortisol (F) secretion. SROC curve analysis was applied with the ITT as reference test, considering a normal response as cortisol peak > 180 µg/L.

The higher ROC AUC values were found for LDSST, HDSST and MET. Particularly, LDSST sensitivity approached 0.85 with a specificity of 0.80 for cut-off values of F > 210 µg/L; either HDSST or MET sensitivity approached 0.8 with a specificity of 0.85 for cut-off values of F > 220 µg/L and ACTH > 100 pg/ml, respectively. A sensitivity of at least 0.8 for VLDSST was associated to a very low specificity.

In conclusion, this study shows that, if compared with ITT, testing with very low ACTH doses may be misleading if used as a screening test in patients suspected for corticotroph deficiency. On the other hand, both low and high ACTH doses as well as metyrapone stimulation tests are equally reliable for the diagnosis of secondary hypoadrenalism, when used with appropriated cut-off limit.

### P600

#### Pulmonary function in Cushing's disease

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Numerous clinical manifestations have been described in association with Cushing's disease (CD). There was no eligible data on pulmonary function tests in CD patients. We aimed to assess pulmonary function

test included spirometry in a series of patients with active Cushing's disease (2 men, 9 women). Mean age, height, weight, body mass index were  $36.7 \pm 12.6$  (range 22–63 years),  $156.9 \pm 8.4$  cm,  $74.1 \pm 10.7$  kg,  $29.6 \pm 3.8$  kg/m<sup>2</sup>, respectively. Spirometric abnormalities (impairments of FEV1, FVC, FEV1/FVC and FEF 25–75 values) were not found, and not different from referans values. In conclusion, there was detected any spirometric impairment in CD patients. It is to need further analyses.

## P601

### Adenosine stimulates connexin 43 expression and increases intercellular communication in the folliculostellate cells of the anterior pituitary gland

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We previously showed that adenosine stimulates IL-6 secretion from TtT/GF cells suggesting that adenosine is an important regulator of folliculostellate (FS) cell function. Gap-junction communication is also important for FS cell function so the aim of this study was to investigate the action of adenosine on connexin 43 (Cx 43) expression and intercellular communication.

Adenosine production, as measured by reverse-phase fluorescent HPLC, was easily detectable in MMQ and GH3 cells, respectively 6–8 and 1–2  $\mu$ M/10<sup>6</sup> cells/hr but not in TtT/GF cells. Ecto-5'-nucleotidase (CD73) (the enzyme that cleaves AMP to adenosine) in these cells was demonstrated by HPLC analysis of the conversion of exogenously added fluorescent ethenoAMP to ethenoadenosine. The rate of AMP degradation was the same in all three cell lines with a half-life of around 1 hr; however preincubation with AOPCP or levamisole confirmed that the enzyme mediating this reaction in GH3, but not in MMQ or TtT/GF cells is CD73. The identity of the enzyme mediating AMP degradation in MMQ or TtT/GF cells is unknown.

We investigated the effect of NECA (universal adenosine receptor agonist) on Cx43 expression in TtT/GF cells using Western analysis and showed a time- and dose-related stimulation of both non-phosphorylated and phosphorylated forms. Expression of Cx43 reached a plateau after 4 hr exposure to NECA and showed peak stimulation at 1  $\mu$ M. Similar findings were obtained with adenosine, but not with the non-hydrolysable form of ATP, (ATP $\gamma$ S) which failed to have any effect on Cx43 expression.

Gap-junction transmission in TtT/GF cells was investigated by microinjecting Alexa Fluor 488 into individual cells. 10  $\mu$ M NECA treatment for 4 hrs stimulated the spread of the dye into  $10 \pm 2.7$  ( $P < 0.01$ ) cells compared with  $3 \pm 1.7$  cells in the untreated cultures. These data show that adenosine, produced by FS cells or neighbouring endocrine cells, stimulates Cx43 expression and increases gap-junction communication.

## P602

### Complement C5a inhibits the secretion of macrophage migration inhibitory factor (MIF) in anterior pituitary cell lines

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Complement C5a is associated with various pro-inflammatory effects such as chemotaxis, production of superoxides, histamine release, vasodilatation and smooth muscle contraction. In sepsis, excessive production of C5a can lead to multi-organ failure. C5a mediates its actions through the C5a receptor (C5aR) but may also bind to a second receptor called C5L2 that acts as a decoy for removing excess C5a. C5a is also rapidly cleaved to a less active form, C5adesR; C5L2, in contrast to C5aR, binds both C5a and C5adesR with high affinity. In this report we show that the anterior pituitary gland expresses C5a and C5L2 receptors and that C5a activates ERK and AKT phosphorylation but inhibits MIF secretion.

Using RT-PCR, C5aR and C5L2 mRNA were detected in rodent anterior pituitary tissue and in all of the rodent pituitary cell lines used. Immunofluorescent immunocytochemistry showed strong expression of C5aR, but relatively weak expression of C5L2. Western analysis of MMQ (prolactin secreting) and TtT/GF (folliculostellate) cells showed that exogenously added recombinant murine C5a (1–100 nM) but not C5adesR stimulated ERK and AKT phosphorylation with a peak time of around 30 minutes. However C5a and C5adR, dose dependently, inhibited MIF (as measured by ELISA) secretion in MMQ, GH3 and AtT-20 cells; at 70 nM inhibition was respectively 35–45% ( $P < 0.001$ ), 45–55% ( $P < 0.001$ ) and 35–45% ( $P < 0.001$ ). In summary we have shown that functional C5a and C5L2 receptors are expressed in many cell types of the anterior pituitary gland and C5a and C5adR both inhibit MIF secretion by, as yet, an unknown mechanism. The presence of C5a (and of C3a) receptors in the anterior pituitary gland suggest that immune-derived molecules may regulate the HPA axis to limit inflammation.

## P603

### Unrecognized chronic hypopituitarism in patients who survived hemorrhagic fever with renal syndrome

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Hemorrhagic fever with renal syndrome (HFRS) is an acute viral disease caused by Hantavirus. One of the rare complications of this disease is hypopituitarism (only sporadically reported) due to hemorrhage, necrosis and atrophy of anterior pituitary lobe. The aim of the study was to investigate the anterior pituitary function in patients who recovered from HFRS.

We evaluated 31 patients (29 male and 2 female), aged  $38.7 \pm 2.2$  years, with BMI  $25.7 \pm 0.6$  kg/m<sup>2</sup>, who recovered from HFRS  $5.8 \pm 0.8$  years ago, and compared them with 31 age-, BMI- and sex-matched control subjects. At baseline, circulating T4, TSH, FSH, LH, testosterone, prolactin, IGF-I and cortisol levels were measured, and insulin-induced hypoglycemia test (ITT) was used to assess GH response, HPA and prolactin. The study was approved by the local Ethical Committee.

According to peak GH levels during ITT (cut-off for GH deficiency  $< 3$   $\mu$ g/l), four patients were diagnosed with severe GH deficiency (GHD, mean GH peak was  $0.04 \pm 0.02$   $\mu$ g/l). All four exhibited IGF-I values below the normal values for sex and age ( $34.0 \pm 7.8$  ng/ml). All had additional pituitary hormone deficits (hypothyroidism, hypogonadism, hypocorticism) including low prolactin levels, suggesting panhypopituitarism (GHD3) i.e. severe damage to the pituitary. MRI of the pituitary revealed atrophic pituitary gland with an empty sella. All four were completely replaced. The rest had normal anterior pituitary function with GH response during ITT not different from control subjects ( $12.7 \pm 1.3$   $\mu$ g/l vs.  $13.9 \pm 1.7$   $\mu$ g/l,  $P > 0.05$ ).

In conclusion, severe hypopituitarism was diagnosed as a late complication of HFRS in 4 (out of 31) patients (12.9%). The clinical course of the hypopituitarism in all four patients was chronic and undiagnosed for a long period.

## P604

### Ghrelin test for the assessment of growth hormone (GH) status in successfully treated patients with acromegaly

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Posttreatment assessment of the disease activity and definition of cure of acromegaly, by measuring GH secretion, remains problematic. Furthermore, with our efforts to achieve tight biochemical control of

the disease it is foreseeable that a proportion of patients may be rendered GH deficient (GHD). The aim of our study was to evaluate residual GH secretion in cured patients with acromegaly. The study was approved by the Hospital Ethical Committee and after informed consent, blood samples were obtained. At baseline circulating GH, IGF-I, IGFBP-3 and leptin levels were measured in 32 acromegalic patients nine years after treatment with surgery and few with radiotherapy. Two tests were performed: the oral glucose tolerance test (OGTT) and ghrelin test (1 µg/kg iv bolus) and results were compared with 11 age-, sex- and BMI- matched control subjects. According to the consensus criteria (normal IGF-I levels and post-OGTT GH nadir <1 µg/l), 20 treated acromegalic patients were cured, 6 had discordant IGF-I and GH nadir during OGTT, while 6 had persistent acromegaly. After GH provocative test with ghrelin (cut-off for severe GHD <3 µg/l) we detected 11 severely GHD patients among 20 cured acromegalic patients. Mean GH peak (±SEM) response to ghrelin test in GHD acromegalics was significantly lower compared with cured acromegalics with sufficient GH (GHS) secretory capacity (1.6 ± 0.3 µg/l vs 20.1 ± 2.4 µg/l, *P* < 0.01) and control subjects (31.1 ± 2.5 µg/l, *P* < 0.01). Mean IGF-1 and IGFBP-3 levels were not different between GHD and GHS cured acromegalics. Leptin levels and BMI were significantly higher in GHD male acromegalics compared with GHS patients, while in GHD females BMI tended to be higher while leptin levels were not different. In conclusion, the assessment of residual GH secretory capacity is necessary in the long-term follow up of successfully treated acromegalics since a large of proportion of these patients are rendered GH deficient.

#### P605

##### The thickening of the pituitary stem in connection with 06 cases

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The recourse to the imagery by magnetic resonance brings to discover pathological images requiring an adapted exploration and therapeutic. The thickening of the pituitary stem can raise diagnostic difficulties. We report the case of 06 patients: 02 men and 04 women, average age is 31 years (04 years (1/2) at 48 years).

The consultation is justified by an insipid diabetes in 05 cases, an amenorrhea - galactorrhea in a case. The hormonal assessment revealed a pituitary insufficiency in *n* = 5. The insipid diabetes is noted in 05 cases. The imagery revealed a thickening of the pituitary stem in all the cases with a macro adenoma in 01 cases and of multiple cerebral nodular images in a case.

We noted an uveite (*n* = 2), an alteration of the optic nerve (*n* = 2) and an encephalopathy (*n* = 2). The etiologic assessment:

- a neuro sarcoidose in 04 cases.
- an "idiopathic" cause in 02 cases. The startup of a corticotherapie allowed the disappearance of the thickening of the stem in (*n* = 5: total, *n* = 1: partial). And on the endocrinien plan, the disappearance of the insipid diabetes in 04 cases, and of the hyperprolactenemia with persistence of the pituitary insufficiency.

##### Conclusion

The thickening of the stem is a rare lesion and the diagnosis is difficult. Associated or not with pituitary alteration, it must make consider an infiltrative cause (granulomatose...) or tumoral (supra-pituitary-infundibulaire). The "unspecified" causes can appear secondarily and encourage with a clinical, biological and radio monitoring.

#### P606

##### Effectiveness of the association of Bromocriptine and Cabergoline in the treatment of 05 invasif and giants prolactin macroadenoma

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The giant prolactin macroadenomas are generally compress if their treatment is very difficult. The surgical treatment is disappointing and is generally resistant to Bromocriptine.

#### Patients

05 Patients (male) hospitalized for giant prolactin macroadenoma > to 04 cm (4-7 cm). 4/5 had less than 35 years (24-49 years) they had an intracranial tumoral syndrome, a deterioration of vision and visual field (oedema-optical atrophy - blindness). Prolactin was higher than 500 ng/ml (500 - > 10 000 ng/ml). 02 patients were operated by rhinoseptale way. All the patients were treated by only Bromocriptine (25-50 mg/j) initially then associated with Cabergoline (1 mg-3,5 mg/Week).

#### Results

After 06 months, prolactin A decreases with less than 100 ng/ml. All the patients had a reduction of the size of the tumor at least 20% after 01 year. Ophthalmologic deteriorations remained stable with an improvement among 02 patients.

#### Discussion/Conclusion

Cabergoline is 30 times more effective than Bromocriptine, it is generally indicated during prolactin microadenoma little evaluated in the macroadenoma. Some studies brought back the effectiveness of the association of the bromocriptine and the cabergoline during giant prolactin macroadenoma.

Our modest study confirmed these results. This synergistic association can be a therapeutic alternative in this kind of pathology but a more significant number of patients must be evaluated.

#### P607

##### Hypothalamic-pituitary dysfunction following irradiation of non-pituitary brain tumours in adults

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Abstract withdrawn

#### P608

##### Bone mass and metabolism in active acromegaly - no impact of gonadal status, a study of 73 patients

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##### Background

Growth hormone (GH) is known to have stimulatory effects on bone tissue through a complex interaction of GH, IGF-1 and IGFBPs. The gonadal status is also thought to influence bone metabolism. Bone mass in patients with acromegaly reflects the long-time effects of GH excess on bone. However, data on bone status in acromegaly have been conflicting.

##### Objective

The object of the study was to examine the impact of chronic GH excess on bone mass and metabolism, with special respect to gonadal status.

##### Methods

Consent was given from the local ethical committee. Of the 73 patients (40 women) with active acromegaly included, none had received somatostatin analogue or radiation therapy. Gonadal status was defined by menstrual history and hormonal replacement therapy in women, and calculated biotestosterone levels in men. Bone mass was examined by DEXA, and serum IGF-1, IGFBP3, GH, osteocalcin and CrossLaps were measured by immunoassay. A reference population (*n* = 40) matched by sex, age and BMI was used.

##### Results

Osteocalcin and CrossLaps were significantly higher in the acromegalic group (*P* < 0.001). BMC of all segments were unaltered, whereas the area of several segments was significantly increased. Nine men and 19 women were hypogonadal. In both men and women markers of bone metabolism, BMC and area of all segments were unaltered compared to the eugonadal acromegalic group. In multiple regression analysis age and sex, but not gonadal status, were significant determinants of total BMC. IGF-1, but not GH, was significantly correlated to total BMC both in the normal and the acromegalic group.



**Conclusion**

No differences in bone mass were found in the acromegalic group compared to a matched reference population; however, markers of bone turnover were increased. In both groups IGF-1 were correlated to BMC. Gonadal status had no impact on bone metabolism or mineralization in this large study.

**P609****Effect of pegvisomant on glucose metabolism**

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**Aim**

To quantify the effect of pegvisomant on glucose homeostasis, lipid profile and disease activity in acromegaly.

**Methods**

5 patients with active acromegaly after surgery, radiotherapy and somatostatin analogs were included. Somatostatin analogs were withdrawn 6 weeks before starting pegvisomant and it was initially administered subcutaneously at doses of 10mg daily. Doses were progressively increased every 3 to 4 weeks until IGF1 normalization. Glucose homeostasis (fasting glucose, HbA1c, HOMA), lipid profile and biochemical disease activity (IGF1, GH, IGFBP3) were assessed basally and at 3, 6 and 12 months after starting pegvisomant. Vital signs and ring size measurements were also assessed at each visit. Quality of life measured by AcroQol and tumor volume determined by MRI were evaluated basally and after one year of treatment. Non-parametrical tests were used to study statistical significance.

**Results**

5 patients (3 males) with a median of 49 years of age were included. Previous surgery had been performed in all of them and postoperative radiotherapy in four of them. Three patients were diabetic. Median dose of pegvisomant required to normalize IGF1 levels was 20 mg daily. Diabetic patients showed a median reduction of HbA1c of 0.5% after one year of treatment. There were trends toward improvements in HOMA, fasting glucose, blood pressure, total cholesterol, LDL cholesterol, ring size measurement and quality of life, although statistical significance was not reached. As expected IGF1 decreased significantly (see Table).

**Table 1**

Months	IGF-1 (ng/ml)	HbA1c (%)	Glucosa (mg/dl)	HOMA
Basal	910 (p25 615-p75 1219)	6 (p25 4.9-p75 7.7)	143 (p25 104-p75 160)	3.57 (p25 1.96-p75 5.25)
3	601 (p25 376-p75 934)*	5.6 (p25 4.4-p75 7.9)	101 (p35 98-p75 198)	3.57 (p25 2.2-p75 4.3)
6	540 (p25 456-p75 756)*	6 (p25 4.6-p75 7.8)	108 (p25 103-p75 164)	3.52 (p25 0.5-p75 4.9)
12	416 (p25 319-p75 651)*	6 (p25 4.45-p75 7.15)	93 (p25 88.5-p75 146.5)	2.98 (p25 1.65-p75 4.66)

**Conclusions**

Pegvisomant shows a beneficial effect on glucose metabolism reducing insulin resistance, fasting plasma glucose and HbA1c. It could also reduce blood pressure and total and LDL cholesterol. Through these effects the drug might contribute to reduce cardiovascular events in acromegaly.

**P610****Psychological and gastrointestinal problems in anorexia nervosa patients**

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Anorexia nervosa (AN) is a product of complex interactions between physiological and psychological processes. Nausea, gastric pain and fullness are common features of AN patients. The aim of the study was to investigate the effects of therapy and psychological conditions on body weight restoration in patients with AN. In 21 AN patients (age 22.4 ± 0.7 yrs), with gastric problems at low weight (BMI 15.9 ± 2.3 kg/m<sup>2</sup>). oesophagogastroduodenoscopy with gastric biopsy was performed and in 13 chronic gastritis was found. They were tested for Helicobacter pylori infections and were positive. Psychological tests were performed after recovery and depression was assessed by Beck Depression Inventory Second Edition (BEK-BDI-II) and anxiety by Hamilton Anxiety Scale (HAMA).

At low weight, we did not find any statistical differences neither in BMI between the gastritis positive and negative group (15.86 ± 1.3 vs 16.31 ± 1.17 kg/m<sup>2</sup>, *P* < 0.05) nor in serum leptin levels (1.69 ± 0.56 vs 2.38 ± 1.1 ng/ml, *P* > 0.05). They were treated with adequate therapy and with a hypercaloric diet for a period of six to nine months. A statistical differences in BMI and leptin levels is found with weight gain in AN patients gastritis positive (BMI 15.86 ± 1.3 kg/m<sup>2</sup> vs 18.0 ± 1.3 kg/m<sup>2</sup>, *P* < 0.001, ΔITM 1.84 ± 0.17 kg/m<sup>2</sup>, leptin 1.69 ± 0.56 vs 4.01 ± 0.7 ng/ml, *P* < 0.001, Δleptin 2.11 ± 0.56 ng/ml). In gastritis negative AN patients we did not find statistical differences in BMI and leptin levels (BMI 16.31 ± 1.17 kg/m<sup>2</sup> vs 17.25 ± 1.3 kg/m<sup>2</sup>, *P* > 0.05, ΔITM 0.72 ± 0.17 kg/m<sup>2</sup>, leptin 2.38 ± 1.1 vs 3.5 ± 0.7 ng/ml, *P* > 0.05 Δleptin 1.12 ± 0.56 ng/ml).

There was a significant negative correlation between body weight and the score of BEK scale of depression (*P* = 0.04) and body weight and HAMA scale score of anxiety (*P* = 0.03) in both groups of AN. The patients with higher anxiety scores had lower body weight at baseline. Treatment directed at improving gastrointestinal morbidity and also psychological factors (depression and anxiety) are important for the presence of disease in AN and influences their rehabilitation.

**P611****Ocreotide shrinks the cellular rather than the vascular compartment in acromegalic tumours *in vivo***

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**Rationale**

Ocreotide is known to shrink acromegalic tumours *in vivo*. However, the mechanism for this is unclear. To investigate the mechanism of shrinkage we used dynamic contrast enhanced MR imaging (DCE-MRI) to visualise the vascular components within the somatotroph adenomas.

**Subjects and methods**

Six patients with confirmed acromegaly comprising 4 microadenomas (all male) and 2 macroadenomas (1 male, 1 female) were recruited. All patients were treated with 3 times daily subcutaneous ocreotide for a 24-week period. Then all but one microadenoma were treated with monthly Sandostatin LAR for a further 24 weeks. All patients underwent growth hormone day curves at the beginning, 24 weeks and at 48 weeks. DCE-MRI was performed at 0, 24 and 48 weeks. The data was analysed using a two compartment model, providing an estimate of vascular permeability and contrast distribution volume. Study was approved by local ethics committee. Results

All but one macroadenoma achieved a medical cure with a mean growth hormone of less than 5 mU/l at 24 and 48 weeks. In the microadenomas, tumour size decreased by 55% (at 24 weeks for the microadenoma that stopped treatment), 59%, 70% and 83% at 48 weeks. In the macroadenomas, tumour size decreased by 2% and 80% at 48 weeks. Mean maximal enhancement and gadolinium exchange rate, a measure of tumour vascularity, did not differ before and after ocreotide treatment (ME 96 ± 36 v 117 ± 28 respectively; ExCH 662 ± 352 v 420 ± 393, respectively; *P* < 0.05). Distribution volume, which is a measure of extracellular volume, increased significantly by 62% before and after ocreotide therapy (95 ± 58 v 154 ± 81, respectively, *P* < 0.028).

**Conclusion**

The shrinkage of acromegalic tumours by ocreotide is either due to a direct reduction in cell volume or a reduction in cell number, but not due to a reduction in tumour vascularity.

## P612

### Positive correlation between AcroQoL and SF-36 and negative with BDI in sample of 60 acromegalics

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Quality of Life (QoL) is a very important issue in the management of a chronic disease like acromegaly. Recently the AcroQoL questionnaire was developed to evaluate the QoL in different acromegalic populations. We decided to compare the scores of AcroQoL and 2 other different questionnaires: one related to general well-being (SF-36) and the other to evaluate depression (BDI). We performed a cross-sectional evaluation of a sample of 60 Portuguese acromegalics, 20 men and 40 women, with an average age of 52.7 y, and mean disease duration of 11.2 y followed at the Department of Endocrinology. We defined controlled disease (CA) as normal IGF-1 for age and sex, and GH levels lower than 1 ng/mL during OGTT and 75% of the samples of a 12 hours profile lower than 1 ng/mL. 51 patients were submitted to surgery, 31 to radiotherapy and 29 to medical therapy (octreotide  $n = 20$ , and lanreotide  $n = 9$ ), and 4 were naive. Twenty-nine patients presented hypopituitarism. Fifty patients were considered controlled and the other 10 uncontrolled. The results are presented as mean  $\pm$  SD, compared using either Student's  $t$  or Mann-Whitney test as appropriated, and correlation was evaluated using Pearson test. The total score of AcroQoL was  $49.9 \pm 19.3$  and had an alpha Cronbach reliability and internal consistency of 0.92. The total score is significantly correlated with SF-36 score ( $107.7 \pm 11.3$ ,  $r = 0.71$ ) and negatively with the BDI score ( $16.5 \pm 13.1$ ,  $r = -0.76$ ). The AcroQoL total score of CA was not significantly different from the uncontrolled ones (UCA) ( $50.9 \pm 19.4$  vs  $45.2 \pm 19.1$ ). The same was observed regarding the AcroQoL physical scale (CA  $47.6 \pm 25.8$  vs UCA  $44.1 \pm 25.1$ ) and psychological scale (CA  $52.9 \pm 18.5$  vs UCA  $45.9 \pm 20.3$ ), although CA tended to be better than UCA. The discrepancy with the previously presented results may be explained by a high number of patients with hypopituitarism. We conclude that the Portuguese version of AcroQoL is a valuable tool to evaluate the QoL of acromegalic patients but hypopituitarism could be a confounding factor.

## P613

### Quality of life (QoL) and psychiatric sequelae following aneurysmal subarachnoid hemorrhage (SAH): Does neuroendocrine dysfunction play a role?

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QoL is an important outcome criterion in clinical research and is used to monitor treatment effects. Patients who have sustained SAH oftentimes have impaired QoL as do patients with untreated hypopituitarism. Considering the high degree of partial hypopituitarism in SAH survivors, we sought to elucidate the impact of neuroendocrine dysfunction on QoL and the prevalence of psychiatric sequelae in these patients.

QoL, depression and symptoms of posttraumatic stress disorder (PTSD) were assessed in 40 SAH survivors who had undergone endocrine function testing at least one year after the hemorrhage. QoL was assessed using the Nottingham Health Profile (NHP), QoL-AGHDA and SF-36. The Beck Depression Inventory and the Impact of Event Scale were used to evaluate depression and PTSD symptoms, respectively. Severe growth hormone deficiency (GHD) and secondary ACTH deficiency were diagnosed by peak GH levels of  $< 3 \mu\text{g/l}$  and by peak cortisol levels of  $< 500 \text{ nmol/l}$  in the insulin tolerance test, respectively. Diagnoses of secondary hypothyroidism and/or hypogonadism were based on basal hormone levels.

Neither ACTH deficiency ( $n = 16$ ) nor total number of patients with any hormone deficit ( $n = 22$ ) were associated with a worse QoL, more severe depression or PTSD as compared to the other SAH patients. Severe GHD ( $n = 8$ ) was associated with a significantly worse QoL in the NHP subscale energy. In a stepwise multiple regression analysis, basal cortisol level was included as the first and oftentimes only predictor for several psychological QoL domains and depression, whereas physical aspects of QoL were predicted primarily by bodily recovery from the SAH. These results indicate a strong association between low morning cortisol levels and impaired psychosocial well-being in SAH patients.

From these studies, we conclude that neuroendocrine dysfunction plays a role in the pathology of reduced QoL and depression in SAH patients.

## P614

### Pituitary function in patients with detectable serum pituitary autoantibodies

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Pituitary autoantibodies have been detected in patients with endocrine autoimmune diseases as well as in various pituitary diseases but their role in determining pituitary dysfunction is not clear. The aim of the present study was to evaluate anterior and posterior pituitary function in a group of patients with detectable pituitary autoantibodies.

31 patients (29 women and 2 men) were enrolled in the study: 21 patients affected by Hashimoto's thyroiditis (HT), 3 patients with Graves' disease (GD), 6 patients with non toxic multinodular goiter (NTMG) and one with Addison's disease (AD). No patient had previous history of cranial trauma. Pituitary autoantibodies were determined through an immunofluorescence assay. Anterior pituitary function was assessed by dynamic testing, including GHRH + arginine, CRH, TRH, GnRH when appropriate. Posterior pituitary function was assessed by prolonged water deprivation test. MRI of sella turcica was performed in patients when pituitary dysfunction was present.

Severe GH deficiency (peak GH  $< 9 \text{ ng/ml}$ ) was observed in 7 patients (22.6%; 1 AD, 4 HT, 2 NTMG) and moderate GH deficiency ( $9 < \text{peak GH} < 16 \text{ ng/ml}$ ) in 6 patients (19.4%; 3 HT, 1 GD, 2 NTMG). AD patient was found to have partial diabetes insipidus. All patients tested with TRH, CRH and GnRH had normal hormonal response. Four patients performed pituitary MRI: 2 had normal pituitary imaging and 2 had partial empty sella. In conclusion, these results suggest that pituitary autoantibodies could be involved in pituitary dysfunction, in particular we observed a high prevalence of GH deficiency.

## P615

### Prevalence of pituitary autoantibodies in thyroid disorders

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Organ-specific autoantibodies have been detected in patients with autoimmune diseases. The relationship between pituitary autoantibodies and lymphocytic hypophysitis has not yet been established and it is not clear if they are a specific serological marker of the disease.

The aim of the present study was to evaluate the prevalence of pituitary autoantibodies in patients affected by autoimmune and nodular thyroid disorders.

803 patients (670 F, 133 M) were enrolled in the study: 387 with Hashimoto's thyroiditis (HT) (350 F, 37 M age  $42 \pm 15$  yr), 159 with Graves' disease (GD) (126 F, 33 M; age  $44 \pm 14$  yr), 37 with toxic uni-multinodular goiter (TNG) (26 F, 11 M; age  $54 \pm 12$  yr) and 220 with non toxic uni-multinodular goiter (NTNG) (168 F, 52 M; age  $50 \pm 12$  yr). 121 normal subjects (98 F, 23 M, age  $39 \pm 15$ ) served as control group.

Pituitary autoantibodies [anti-neuro- (APA-P) and anti-adenohypophys (APA-A)] were determined by an immunofluorescence method using sections of pituitary baboon as substrate. Serum dilutions were initially done at 1/10 and, if the sample was positive, at 1/32 and 1/100.

Pituitary autoantibodies were found in 46 patients (11.9%) with HT, 10 patients (6.3%) with GD, 5 patients (2.3%) with NTNG. None of the control group had detectable APA.

These data shows the presence of serological markers of pituitary autoimmunity, determined by an immunofluorescence method, in patients affected by thyroid diseases. In the majority of patients pituitary autoantibodies were detectable at low titer. Further studies are needed to assess pituitary function in patients with pituitary autoantibodies to support the hypothesis that they can play a role in pituitary dysfunction.

**P616****Rate of change in size of macroprolactinomas with dopamine agonist therapy – is there any relationship to fall in prolactin concentrations?**CL Miller<sup>1</sup>, A Palalau<sup>2</sup>, AA Toogood<sup>2</sup> & NJL Gittoes<sup>2</sup><sup>1</sup>Department of Radiology, University Hospitals Birmingham, United Kingdom; <sup>2</sup>Division of Medical Sciences, University Hospitals Birmingham, United Kingdom.

The most effective means of treating macroprolactinomas, including those associated with significant visual loss at presentation, is with dopamine agonist (DA) therapy. Improvement in visual function often occurs within days of commencing DA therapy, and usually precedes significant reductions in prolactin (PRL) concentrations and may predate significant evidence of tumour volume reduction by months or years. The temporal association between PRL concentration reductions and tumour volume reduction on DA therapy has been poorly documented. We have made a careful assessment of a contemporary cohort of patients ( $n = 18$ , 12 female) with macroprolactinomas who were treated with DA therapy to determine rates of change in pituitary tumour dimensions (using sequential MRI) in the context of changes in PRL concentrations. Median (range) follow-up (FU) was 3 (1–9) years. All patients had a precipitous fall in PRL concentrations (median PRL 0.3 (0.01 to 7.6) % of initial PRL at 12/12) with 12 (67%) having normal serum PRL after 12/12; only 1 with a (mildly) raised PRL at 12/12 subsequently normalised PRL over the FU duration. All tumours reduced in size over the FU duration with 91% showing evidence of shrinkage by 12/12. The median (range) change in tumour dimensions at 12/12 were  $-19$  ( $+7$  to  $-52$ ) % height,  $-4$  ( $0$  to  $-57$ ) % width,  $-7$  ( $0$  to  $-53$ ) % A-P; at 24/12  $-45$  ( $0$  to  $-76$ ) % height,  $-16.5$  ( $+5$  to  $-35$ ) % width,  $-21$  ( $0$  to  $-57$ ) % depth. There was no association between degree of serum PRL reduction and the extent of tumour shrinkage. No regrowth was noted over the FU duration. DA therapy effectively reduces serum PRL concentrations but this does not correlate with reductions in tumour dimensions over 24/12 FU. All macroprolactinomas shrunk to some extent by 24/12 with DA therapy but the rate of change is variable and unpredictable.

**P617****The levels of ghrelin and leptin and body fat mass in patients treated for thyroid dysfunction**J Brunova<sup>1</sup>, L Karasova<sup>2</sup> & P Kasalicky<sup>3</sup><sup>1</sup>IKEM, Prague, Czech Republic; <sup>2</sup>IKEM, Prague, Czech Republic;<sup>3</sup>Mediscan, Prague, Czech Republic.**Introduction**

Patients successfully treated for hyperthyroidism show often the excessive weight gain and patients with hypothyroidism seldom regain their pre-morbid weight despite satisfactory control of their thyroid function.

**Aim of study**

We compared the levels of hormones of ghrelin and leptin in patients with hyper and hypothyroidism, treated and untreated. We aimed to find if they play a role in a new energy balance in these patients.

**Patients and methods**

We investigated the levels of ghrelin and leptin in 12 patients with newly diagnosed hyperthyroidism (group I), 13 patients with newly diagnosed hypothyroidism (group II), 15 patients with well controlled primary hypothyroidism (group III) and 9 patients cured for hyperthyroidism and now with normal thyroid function (group IV). We also measured the BMI and percentage of fat by DEXA in these patients.

**Results**

Group	FT4 (pmol/l)	BMI (kg/m <sup>2</sup> )	Ghrelin (pg/ml)	Leptin (ng/ml)	Fat (%)
I	42.5 ± 16.4	26.3 ± 4.0	1205.6 ± 591	10.9 ± 7.4	42.2 ± 13.5
II	6.14 ± 2.5	29.5 ± 5.6	1942.6 ± 886	27.8 ± 19.4	41.9 ± 3.9
III	12.7 ± 3.8	27.4 ± 4.4	1923.2 ± 726	22.6 ± 2.8	42.4 ± 6.6
IV	14.0 ± 5.6	28.6 ± 7.5	1687.0 ± 971	14.8 ± 3.5	41.1 ± 12.1

Comparisons: FT4: group I to groups II and III  $P < 0.001$ , group I to group IV  $P < 0.01$ ; leptin: group I to group II  $P < 0.05$

**Summary**

The lowest levels of ghrelin and leptin were found in patients with untreated hyperthyroidism. It seems that the levels of ghrelin and leptin

are more influenced by present thyroid function than by percentage of body fat. Supported by grant IGA NR/7928-3.

**P618****Night sleep affected by salt**

R Samadie &amp; F Heydarpour

Zums, Zanjan, Iran.

**Introduction**

Salt in the principal supply of sodium. In normal conditions the average daily salt intake of Iranian adults is 5–15 gr. 4–6 spoonfuls of salt in a day can be lethal, but for soldiers in equatorial regions 50 grams of salt in a day may be needed. In this study the effects of salt on night sleep was addressed.

**Method**

20 volunteer students were chosen for the study. They had no problem sleeping before the study. They recorded the time of sleep and when they went to bed for five nights before salt eating. In the 6th day they took 0.5 gram of salt per 10 kilos of body weight at 8.00 PM at night. We did record time of sleep, times of awakening at night, presence of any disturbances, depth of sleep, duration of fulfilling sleep, amount and times of drinking water at night, their general condition after sleep, effect on REM and NON-REM presence of nightmares. All these parameter about night sleep were studied.

**Results**

Salt delays time to go to bed, during sleep the individual awakens several times. Sleep disturbance is about 2–3 hours. The normal pattern is disrupted and deep sleep is decreased and it is superficial at best. REM and NON-REM cycles are influenced. NON-REM decreases and REM increases so that the individual dreams a lot and even sometimes has nightmares. The day after, they are not satisfied with the sleep. Subjects awaken earlier and during the night repeatedly got up to drink water. The day after they felt drowsy and fatigued.

**Discussion**

Chips, nuts and other foods normally contain lots of sodium (5–10%). Each 100 gram introduces 4–6 gram of salt to the body. Taking these especially in the evenings can disrupt sleep. Thus it is recommended people with sleep problems must not take these in the last hours of the day.

**P619****ADH and Thirst Function in Defence with Hypernatremia Formation**

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Zums, Zanjan, Iran.

**Introduction**

Thirst and ADH secretion are two principle mechanisms for controlling body fluid osmolality. Even an excessive load of salt can effectively be handled by these systems. We studied the effect of salt solution consumption on serum sodium level and osmolality and the action of these two systems.

**Method**

Seven groups each consisting of 6 male rats weighing  $200 \pm 20$  gr. were chosen. Salt solutions (1, 3, 5, 7 and 9%) were also provided. The rats were denied tap water and these prepared solutions were given to each group. Our control group used Zanjan potable water and another test group drank twice distilled water. Because the mortality rate in rats consuming higher salt solutions (>3 percent) began to rise on fifth day, the total water consumption, serum sodium level and osmolality were measured on day 5 in all groups.

**Result**

Water intake in groups using 1 and 3 percent solutions, increased 78 and 36 percent respectively. Their urine output was also considerably increased. The groups maintained on distilled water, 5, 7 and 9% solutions showed respective decreases of 11, 56, 73, and 85% in their water intake. Osmolality and sodium level significantly increased in groups using 3% and higher solutions. ADH and thirst mechanisms could not control serum sodium level and osmolality when the concentration of salt in distilled water exceeded 1.4%.

**Discussion**

Higher concentration salt solutions could not decrease osmolality and quench thirst. Because higher salt solutions stimulate the neurological protective mechanisms, which act more powerfully in higher concentrations, the animal shows no tendency to drink water and osmolality will



significantly increase. We could propose that ADH and thirst could not control osmolarity when human or animal uses a large load of salt and increase in osmolarity leads to death in a few days.

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## P620

### Taste protective mechanism in relation with salt

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#### Introduction

Salt is an essential compound to provide Na, but over-consumption of salt could be lethal. As using high salt solution is a traditional way of committing suicide in China. In this research, the action of salt sensation during consumption of salt solution is elevated.

#### Method

6 groups, each containing 10 male rat with 200 gr weight were selected. 5 concentration of salt in distilled water were prepared (1,3,5,7,9%). During this study, rats were deprived of potable water and above salt solution were given to rats. Control group used tap water of Zanjan.

#### Results

Water consumption on 1,3% groups as compared to control group increased 81% and 44%, but for 5, 7 and 9% group as compared with control group was decreased 56, 73 and 85%.

#### Discussion

1%, 3% salt solution could not decrease Na serum level and thirst quench and because taste protective mechanism does not act strongly, salt solution consumption increased in this concentration, but when animal were used 5, 7, 9% salt solution, the kidney have to use some of body water along with salt solution to be able to excrete the excessive solute load and with a positive feedback loop, this phenomena can lead to the immediate death of animal die. Animal refusal from drinking highly concentrated salt solution is related to taste protective mechanism.

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## P621

### Cushing's syndrome as a model to investigate the effects on cognition of high circulating levels of glucocorticoid

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Glucocorticoid (GC) excess is associated with significant brain morphological changes including loss of volume and cerebral atrophy. These effects correlate with the degree and duration of GC elevation<sup>1</sup>. The neuropsychological abnormalities associated with GC excess, however, remain poorly understood<sup>2</sup>. Patients with Cushing's syndrome represent a useful model in which to study the effects of severe and prolonged GC excess.

We present the case of a 38 year old male (IQ = 98) with confirmed Cushing's syndrome secondary to a 3 cm adrenal adenoma, having had symptoms for 5 years. Prior to adrenalectomy, he underwent a series of cognitive function tests:

- Key figure – tests spatial memory.
- Rivermead memory test – tests immediate and delayed recall.
- Face-name pairs – tests associative memory.
- Hamilton Anxiety and Depression Scale (HADS).
- N-back Working Memory Task (1- & 2-Back).

The results show marked associative memory impairment with preservation of spatial memory, indicating a dissociation in two hippocampal-dependent functions. The HADS revealed severe anxiety and depression. These findings raise the possibility of different components of memory being processed in different areas of the hippocampus, and that GC excess affects some areas more than others. These preliminary findings provide a rationale for further studies, using cognitive testing and functional imaging, to investigate the effects of GC on hippocampal structure and function.

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## P622

### The influence of growth hormone replacement (GHR) in adults with GH deficiency (GHD) on body composition, basal metabolic rate, physical activity and ingestive behaviour

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#### Objectives

GHR in adults with GHD favourably effects body composition (reduces fat and increases lean body mass) although body weight usually remains unchanged. However the effect of GHR on ingestive behaviour and voluntary physical activity in adults has not been studied.

#### Methods

We studied 10 hypopituitary adults (8 males, 2 females, mean age 49.7 years) with severe GHD (mean peak GH response to glucagon 3.26mU/L, baseline IGF-1 level 14.5 nmol/L) before and after GHR (mean duration 9.4 months; mean GH dose 0.35 mg daily). The following were measured pre and post GHR:

Resting energy expenditure (REE) by indirect calorimetry; voluntary activity for a week (pedometer); hunger and satiety scores (visual analog scales [VAS] fasting and after a fixed calorie breakfast {600 kcal}); energy intake during a buffet lunch; anthropometric measures (weight, BMI, waist hip ratio, body fat percentage by bio-impedance) and quality of life (QoL-AGHDA scores). Local research Ethics Committee approval was obtained.

#### Results

After nine months of GHR, significant improvements in QoL-AGHDA scores (16 vs. 7, p 0.016) and activity scores (2330 vs. 4220, P 0.029) were found, however BMI was unchanged (31.9 pre v 31.5 post GH, P 0.49). After GH, fasting hunger VAS scores were higher (73.1 vs. 54.5, P 0.01) and patients ingested more carbohydrates (436.4Kcal vs. 370.8Kcal, P 0.048). Satiety VAS scores, anthropometric measures and REE were unchanged.

#### Conclusions

GHR improves QoL and activity levels but also increases fasting hunger scores and carbohydrate intake. Adults commencing GH replacement should be counselled about restricting energy intake to mitigate weight gain and achieve weight loss.

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## P623

### Influence of affective changes on cortisol response to ACTH

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In this study we wanted to assess whether affective changes influence cortisol response during low dose (1 microgram) ACTH test. Beck Inventory of Depression and Hamilton Anxiety Rating Scale and low dose ACTH test were performed in five subjects during the prolonged psychological stress induced by war and 18 months later. After the war, Beck Inventory of Depression and Hamilton Anxiety Rating Scale scores were significantly reduced. Suppression of the HPA axis was present during the war but not after. Changes in maximal cortisol response and in area under curve (AUC) of cortisol response were significantly correlated with changes in Beck score (maximal cortisol Pearson correlation = -0.873, P = 0.053, AUC cortisol correlation = -0.885 P = 0.046). However, there was no correlation of Hamilton score with any of the parameters. Therefore, during low dose ACTH test affective changes modulate cortisol response to ACTH.



## P624

**Interest of a single treatment by somatostatin analogues in a macroadenoma secreting TSH and GH**

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We report a case of a 69-years-old woman who complained about progressive tachycardia, sweat and flush. Her single background was a breast cancer five years ago which was considered in remission. A first blood sample analysis showed an elevated rate of T4 whereas TSH was normal. An IgF1 assay was realised because of an acromegalic appearance and was abnormally high for sex and age. Further biological investigations confirmed central hyperthyroidism and acromegalia. Alpha subunit and prolactin were slowly elevated whereas FSH and LH were considered as normal for a menopausal woman. There was no impairment of the corticotrophin function. Glucose assay showed a diabetes mellitus. MRI (magnetic resonance imaging) revealed the presence of a voluminous macroadenoma with supra and left latero sellar extension added with an atypical localisation in the frontal lobe. Campimetric analysis were normal. Sellar diagnosis biopsy was performed to eliminate breast cancer metastasis. Immunohistochemistry was positive for TSH and GH. A treatment by somatostatin analogues (octreotide 20 mg/28 days) was started and led quickly to a normal T4 value. IgF1 was not completely normalized after 3 months of treatment so that an increase of octreotide posology up to 30 mg/28 days was decided. One year after starting octreotide, T4, TSH and GH secretions were perfectly normalized. MRI showed a stability of the adenoma size in the last six months whereas it has been initially decreasing. This observation proves the efficacy of somatostatin analogues (octreotide/28 days) in the treatment of a GH and TSH adenoma. Benefit and risk of neurosurgery or radiotherapy as an alternative to medical treatment were also discussed. Nevertheless, it appears that somatostatin analogues could be proposed in first intention, especially when medical context does not allow invasive investigations.

## P625

**Dehydroepiandrosterone (DHEA) improves psychological well-being in male and female hypopituitary patients in addition to growth hormone replacement (GHR)**

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Hypopituitarism is associated with profound androgen deficiency, even in patients who are adrenocorticotrophic hormone (ACTH) replete. DHEA has been shown to have a beneficial effect on well-being in patients with adrenal failure. We hypothesised that DHEA may be additive to the known effects of GH on psychological well-being in patients with hypopituitarism. In a double blind placebo controlled trial 50 mg DHEA or placebo was added to standard replacement, including growth hormone, over 6 months, followed by open phase 6 months DHEA replacement. Primary end points were quality of life and libido. Thirty female and 21 male hypopituitary patients on stable GH and other hormone replacement as indicated, were enrolled. All males and 18/30 females were on gonadal steroid replacement. Serum IGF-I was maintained constant by 4 weekly GH dose adjustments. Psychological well-being was assessed using the QoL-AGHDA, GHQ, SF36 and EQ5D (Euroqol) and libido using SSES-E. Patients had impaired psychological well-being compared to the British population at baseline. Females showed an improvement after 6 months in QoL-AGHDA [ $-2.9 \pm 2.8$  (mean  $\pm$  SD) (DHEA) vs.  $-0.53 \pm 3$  (placebo);  $P < 0.05$ ; 95% CI 0.06, 4.71], SF36 general health perception [ $9.6 \pm 14.2$  (DHEA) vs.  $-1.2 \pm 11.6$  (placebo);  $P = 0.036$ ; 95% CI  $-20.8, -0.7$ ] and SF36 social functioning scores [ $14.6 \pm 23.1$  (DHEA) vs.  $-4.7 \pm 25$  (placebo);  $P = 0.047$ ; 95% CI  $-38.3, -0.3$ ]. Males showed an improvement after 6 months in GHQ self-esteem [ $-1.3 \pm 1.7$  (DHEA) vs.  $(0.5 \pm 1.5)$  (placebo);  $P = 0.03$ ; 95% CI 0.16, 3.34] and depression [ $-1.6 \pm 2.2$  (DHEA) vs.  $1.2 \pm 2.4$  (placebo);  $P = 0.02$ ; 95% CI 0.5, 5.15] scores. There was no effect on libido evident. 71% of women and 48% of men chose to continue DHEA after the trial period. Our data demonstrate psychological benefit of DHEA in addition to conventional hormone replacement including GH and, in addition, confirm the utility of QoL-AGHDA in a placebo-controlled setting.

## P626

**The influence of a GH receptor antagonist (GHRA) on the relationship between GH and IGF-I in adults with severe growth hormone deficiency (AGHD)**

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Approximately 50% of patients with severe AGHD (defined by the international consensus criteria, peak GH  $< 3$  ng/ml) have a normal age- and gender-related IGF-I. It remains unclear whether in these individuals IGF-I is GH-dependent.

We performed a double-blind, randomised, placebo-controlled, cross-over study on the effect of pegvisomant (20 mg daily for 14 days) on the relationship between GH and IGF-I in 3 age-, gender- and BMI-matched cohorts (Norms: 5 GHD patients with normal IGF-I, Los: 5 GHD patients with low IGF-I and Cons: 6 healthy volunteers. IGF-I was measured prior to and after each limb, and 24 h GH sampling (20 minute interval) was performed at the end of each limb.

GH was measured by a pegvisomant-insensitive immunoradiometric assay (intra- and inter-assay CV  $< 7\%$ , lower limit of detection 0.1 ng/ml). IGF-I was measured by immunoradiometric assay (sensitivity 4.4–5.2%, with specificity of 5.7–7.4%, lower limit of detection 6 mcg/l). Statistical analysis was performed by GraphPad Prism software.

Pegvisomant decreased IGF-I in Cons and Norms (median 158.5 ng/mL (range 101–206 ng/mL) v 103(77–125) ng/mL,  $P < 0.01$ ; 124(81–136) ng/mL v 95(51–113) ng/mL,  $P < 0.01$ ), but not in Los (31( $< 31$ –32) ng/mL v 34.5( $< 31$ –38) ng/mL).

At the conclusion of the placebo limb, mean 24 h GH was higher in controls than in GHD patients (Cons: 0.49(0.12–0.89) ng/mL v Norms 0.1( $< 0.1$ –0.13) ng/mL,  $P < 0.01$ ). Pegvisomant increased mean 24 h GH in Cons (0.49(0.12–0.89) ng/mL v 1.38(0.22–2.45) ng/mL,  $P = 0.03$ ) and Norms (0.1( $< 0.1$ –0.13) ng/mL v 0.17(0.11–0.42) ng/mL,  $P = 0.03$ ), but not in Los, where GH remained predominately undetectable.

Our results indicate that GHD patients with normal IGF-I still have the potential to increase GH secretion in response to a fall in IGF-I, while those with low IGF-I levels are unable to increase GH secretion. Therefore, IGF-I appears to be GH-independent in GHD with low IGF-I, while being partially GH-dependant in GHD with normal IGF-I.

## P627

**Can adults with severe growth hormone deficiency (AsGHD) and a low IGF-I be distinguished from those with a normal IGF-I?**

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Approximately 50% of patients with severe AGHD (defined by the international consensus criteria, peak GH  $< 3$  ng/ml) have a normal age- and gender-related IGF-I. It remains unclear whether in these individuals IGF-I is GH-dependent. We performed a double-blind, randomised, placebo-controlled, cross-over study on the effect of pegvisomant (20 mg daily for 14 days) on the relationship between GH and IGF-I in 3 age-, gender- and BMI-matched cohorts (Norms: 5 GHD patients with normal IGF-I, Los: 5 GHD patients with low IGF-I and Cons: 6 healthy volunteers. IGF-I was measured prior to and after each limb, and 24 h GH sampling (20 minute interval) was performed at the end of each limb.

GH was measured by a pegvisomant-insensitive immunoradiometric assay (intra- and inter-assay CV  $< 7\%$ , lower limit of detection 0.1 ng/ml). IGF-I was measured by immunoradiometric assay (sensitivity 4.4–5.2%, with specificity of 5.7–7.4%, lower limit of detection 6 mcg/l). Statistical analysis was performed by GraphPad Prism software.

Pegvisomant decreased IGF-I in Cons and Norms (median 158.5 ng/mL (range 101–206 ng/mL) v 103(77–125) ng/mL,  $P < 0.01$ ; 124(81–136) ng/mL v 95(51–113) ng/mL,  $P < 0.01$ ), but not in Los (31( $< 31$ –32) ng/mL v 34.5( $< 31$ –38) ng/mL).

At the conclusion of the placebo limb, mean 24 h GH was higher in controls than in GHD patients (Cons: 0.49(0.12–0.89) ng/mL v Norms 0.1( $< 0.1$ –0.13) ng/mL,  $P < 0.01$ ). Pegvisomant increased mean 24 h GH in Cons

(0.49(0.12–0.89) ng/mL v 1.38(0.22–2.45) ng/mL,  $P = 0.03$ ) and Norms (0.1(<0.1–0.13) ng/mL v 0.17(0.11–0.42) ng/mL,  $P = 0.03$ ), but not in Los, where GH remained predominately undetectable. Our results indicate that GHD patients with normal IGF-I still have the potential to increase GH secretion in response to a fall in IGF-I, while those with low IGF-I levels are unable to increase GH secretion. Therefore, IGF-I appears to be GH-independent in GHD with low IGF-I, while being partially GH-dependant in GHD with normal IGF-I.

## P628

### Sleep disorders in acromegaly: preliminary data demonstrating a high prevalence of restless leg syndrome (RLS)

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Sleep related breathing disorders (SRBD), due to bone mass and soft tissue swelling of the upper airways, are frequently reported by acromegalic patients, but sleep disorders other than SRBD were rarely studied in acromegaly. We evaluated 38 acromegalics (14 M, 24 F, age  $52.8 \pm 2.3$  yrs) by a structured interview for insomnia, excessive daytime sleepiness (EDS), SRBD, sleep related movement disorders (SRMD), circadian sleep disorders and parasomnias. Epworth sleepiness scale (ESS) and RLS diagnostic interview were performed in patients reporting EDS and specific sensory symptoms. Grouping by symptoms, snoring was reported in 28, EDS in 20, apneas in 13, RLS in 10, insomnia in 7, periodic leg movements sleep (PLMS) in 4, bruxism in 1, nightmares in 1, delayed sleep phase in 1 cases. We selected 21 cases for video-polysomnography (PSG) and 19 for portable monitoring. So far, 14 standardized PSG have been obtained (4 M, 10 F, age  $60.0 \pm 2.9$  yrs, BMI  $30.6 \pm 1.6$  kg/m<sup>2</sup>). Ten PSG were positive for PLMS (PLMS index  $26.5 \pm 10.0$ , v.n. <5), 6 for snoring, 6 for RLS, 5 for sleep apnea syndrome (SAS) (apnea-hypopnea index  $9.5 \pm 5.0$ , SaO<sub>2</sub>  $96 \pm 1\%$  v.n. 97–99). Sleep efficiency (SE) was overall reduced ( $65.1 \pm 3.8\%$ ), especially in patients with RLS ( $59.0 \pm 6.1\%$ ). In patients with RLS the EDS was generally mild (ESS  $7.5 \pm 2.1$ , v.n. <10), but more severe than that overall reported in non acromegalic patients with SAS. Nocturnal sleep latency (SL) was longer in RLS patients ( $33.8 \pm 12.8$ ) than in other cases ( $18.5 \pm 7.4$ ). In conclusion, acromegalics sleep poorly, and are fatigued during daytime. In our study the prevalence of RLS is dramatically increased (26%) in comparison with that reported in general population. Based on these data, a role for sleep related somatostatinergic and dopaminergic mechanisms could be hypothesized in acromegaly. Moreover, despite snoring is quite frequent, the prevalence of SAS is less than that shown in previous studies and it is usually mild to moderate.

## P629

### Risk factors for the development of atherosclerosis in patients with acromegaly

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Acromegaly is associated with cardiovascular diseases. Accelerated atherosclerosis may have important role in that. The aim of this study was to examine the presence of dyslipidemia, glucose control impairment, inflammation and hypertension as risk factors for the development of atherosclerosis in acromegalic patients.

Study group included 28 patients with acromegaly without lesions of thyroid, adrenal and gonadal axis. The examination was performed before and after treatment of acromegaly. Because of that, this group was its own control. We measured IGF-I, total cholesterol, triglycerides, HDL, LDL, total cholesterol/HDL ratio, fasting and postprandial glucose, fibrinogen (as a reactant of the acute inflammatory phase) and blood pressure.

#### Results

Increased levels of IGF-I before therapy, significant decrease and normal levels after therapy suggested successful treatment of acromegaly. Increased triglyceride levels in active acromegaly were normalised and significantly decreased after treatment ( $1.81 \pm 1.1$  v.s.  $1.3 \pm 0.64$  mmol/l,  $P < 0.01$ ).

Total cholesterol and LDL were above normal ranges before therapy and increased after therapy, nonsignificantly. Decreased level of HDL in active acromegaly significantly increased after therapy ( $1.11 \pm 0.2$  vs  $1.46 \pm 0.76$  mmol/l,  $P < 0.05$ ). Total cholesterol/HDL ratio was above normal before and after therapy, but decreased nonsignificantly. Increased levels of fasting and postprandial glycaemia normalised after therapy, but only changes of postprandial glucose were significant ( $10.28 \pm 4.2$  vs  $7.16 \pm 1.53$  mmol/l,  $P < 0.01$ ). Fibrinogen levels were in normal ranges before and after treatment without significant changes. Normal levels of systolic and diastolic blood pressure significantly decreased after therapy ( $136.9 \pm 21.4$  v.s.  $126.7 \pm 17.3$  mmHg;  $85.9 \pm 12.7$  v.s.  $81.7 \pm 10.5$  mmHg,  $P < 0.01$ ).

#### Conclusion

Dyslipidemia (higher levels of triglycerides, decreased levels of HDL), glucose impairment and blood pressure were acromegaly related risk factors for atherosclerosis in our acromegalic patients. Inflammation wasn't present in this study.

## P630

### Microalbuminuria as well as insulin sensitivity are improved under octreotide-lar treatment in acromegalic patients

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High risk of impaired glucose tolerance and diabetes mellitus is frequently observed in acromegalic patients. Some studies have reported a direct correlation between circulating GH levels and the degree of glucose intolerance. Microalbuminuria clusters with the metabolic syndrome and both conditions predict cardiovascular disease mortality. The reported relationships of microalbuminuria with the individual components of the metabolic syndrome are variable. Aim of this preliminary study was to investigate the endothelial function in acromegalic patients during octreotide-LAR treatment. Sixty five acromegalics aged  $47.6 \pm 11.9$  yrs., BMI  $32. \pm 4.7$ , underwent OGTT and hormonal/biochemical evaluation, in basal condition and after octreotide-LAR treatment. In particular, 24 hours urine were collected to evaluate microalbuminuria as index of endothelial damage. Glucose homeostasis was evaluated with OGTT insulin sensitivity index (ISI). In the whole group, mean pre-treatment GH and IGF-I levels were  $25.3 \pm 3.9$  ng/ml and  $683.2 \pm 24.02$  ng/ml, respectively. After six months GH and IGF-I levels were  $2.6 \pm 0.5$  ng/ml and  $334.9 \pm 23.4$  ng/ml, respectively ( $P < 0.005$ ); data about glucose homeostasis were reported in the following Table 1.

Table 1

	PRE	POST	P <
Insulin	$16.6 \pm 2.1$	$8.8 \pm 0.7$	0.005
Microalbuminuria	$26.4 \pm 2.5$	$18.0 \pm 2.3$	0.005
ISI	$4.4 \pm 0.4$	$6.0 \pm 0.4$	0.05

As far as microalbuminuria is concerned, during treatment with somatostatin analogues, a clear reduction was observed ( $26.4 \pm 2.5$  µg/24h and  $18.04 \pm 2.3$  µg/24h, respectively;  $P < 0.005$ ). After octreotide-LAR treatment, a negative correlation was found between ISI index and microalbuminuria ( $P < 0.05$ ). In conclusion, treatment with octreotide LAR is likely to improve insulin resistance and reduce microalbuminuria in acromegaly; this would therefore preserve kidney function and reduce cardiovascular risk besides normalization of GH/IGF-I hypersecretion.

## P631

### Pro-opiomelanocortin and ACTH, precursors of alphaMSH, are secreted from human melanocytes and keratinocytes and can stimulate melanogenesis

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Pro-opiomelanocortin (POMC) is endoproteolysed to ACTH and alpha MSH which bind to the melanocortin-1 receptor, (MC-1R), and act as principal mediators of human skin pigmentation. In addition there is increasing evidence that alpha MSH has important anti-inflammatory actions in the skin. The aim of this study was to determine the efficiency of processing and the involvement of the different peptides in melanogenesis. To address this question we utilized specific two-site immunometric assays for POMC and ACTH and a sensitive immunoassay for alpha MSH.

Human epidermal melanocytes from five different volunteers had significant quantities of POMC and ACTH within the cells with no detectable alpha MSH. Under basal conditions where there is constitutive secretion of peptides, only POMC was detected in the medium. Matched epidermal keratinocyte cultures contained higher levels of POMC and ACTH and low but significant levels of alpha MSH in the cells. In contrast to melanocytes, the keratinocytes did secrete alpha MSH although much higher levels of POMC were released into the medium. In order to investigate whether POMC was able to bind to the MC-1R and induce melanogenesis, we affinity purified POMC from human small cell lung cancer cells. Using CHOK1 cells stably transfected with the MC-1R and a cAMP reporter construct, we found that POMC could activate the MC1R with a similar potency to gamma MSH but with significantly lower potency than ACTH, alpha MSH and beta MSH. Interestingly, POMC also induced melanogenesis in S91 mouse melanoma cells albeit at 100 fold higher concentrations than alpha MSH. In summary, under steady state conditions there is relatively little secretion of the more potent melanogenic peptides from keratinocytes and none from melanocytes. This suggests that processing of POMC may be a key regulatory event in order to generate potent bioactive peptides involved in human pigmentation and immunomodulation in the skin.

## P632

### Processing and sorting of pro-opiomelanocortin is an important checkpoint in regulating release of ACTH from secretory vesicles in pituitary cells

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Pro-opiomelanocortin (POMC) is endoproteolysed by prohormone convertase-1 (PC1) to ACTH within the secretory pathway in pituitary cells, where the regulation of ACTH release is essential for mediating the stress response. However POMC is present in the human circulation, indicating that not all POMC is processed. This suggests that regulation of trafficking and processing of POMC are important in determining ACTH release. To investigate this, we analysed steady-state release of POMC and ACTH from AtT20 pituitary adenoma cells utilising specific immunometric assays.

In cell lysates, we detected high levels of POMC (110 pmol/well) relative to ACTH (13 pmol/well), which remained constant over 48 h. In medium, we observed greater accumulation of POMC (24–1180 pmol/well) relative to ACTH (2–74 pmol/well) from 1–48 h, suggesting that excess POMC is constitutively secreted.

Stimulation of cells with BaCl<sub>2</sub> (1 mM) caused an acute 5 fold increase in ACTH secretion within 15 min while POMC levels did not change. Repeated stimulation with BaCl<sub>2</sub> at 15 min intervals for 60 min exhausted the secretion of ACTH, but release of POMC was not altered. Similarly, acute stimulation of cells with CRH (3 nM) increased ACTH, but not POMC release. Hydrocortisone (10–1000 nM) inhibited POMC and ACTH secretion but only after 24 h. These results show that the POMC sorted to secretory granules, is processed for dynamic release of ACTH, but that most POMC secretion occurs constitutively.

Stable over-expression of PC1 in cells produced a marked increase in ACTH secretion after BaCl<sub>2</sub> stimulation, suggesting that PC1 upregulation enhances post-Golgi sorting of POMC. Conversely, RNAi knockdown of PC1 resulted in a decrease in ACTH with a concomitant increase in POMC, proving that regulation of processing enzymes can impact on the secretory process.

In summary, PC1 may play a regulatory role in the sorting process and the mechanisms for sorting POMC into secretory pathways appear to provide key checkpoints to regulate secretion of ACTH.

## P633

### Hormone therapy effects on sleep and dreams in menopausal women

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#### Introduction and objective

This study aims to report if hormone therapy modifies sleep architecture and dreams in menopausal women. Women with no hormone therapy for flushes usually present sleep deprivation and daily sleepiness as a result of nocturnal arousals. Estrogen and progesterone brain receptors can influence memory and cognition processes. Dreams modulators are cognition, socio-cultural factors, personality and brain physiology (REM/NREM percents, circadian influences).

#### Methods

18 subjects, 53.5 ± 3.3 yrs old, were recorded 2 nights, one without hormone therapy, second after conjugated estrogens 0.625 mg/day and didrogesteron 5 mg/day, 30 days. Polisomnographic recordings with Bio-Logic digital data acquisition system and Sleepscan II software were followed by sleep scoring in according with Rechtschaffen & Kales criteria. Quantitative dream content was analysed by Hall & van Castle system.

#### Results

There were significant decreasing values for sleep apnea indexes: total hypopneas (mean 10.24 vs. 2.25,  $P = 0.047$ ), hypopneas index (mean 1.77 vs. 0.37,  $P = 0.052$ ), apnea-hypopnea index (mean 2.68 vs. 0.65,  $P = 0.055$ ), apnea-hypopnea REM index (mean 6.65 vs. 1.12,  $P = 0.039$ ), apnea-hypopnea NREM index (mean 1.82 vs. 1.27,  $P = 0.046$ ), even all were included between normal values and the sleep efficiency and sleep architecture were not significantly modified. The percents of family and friends in dreams analysis codes are diminished after hormone therapy, with no sexual specific supplementary connotations. Dreams quantity are not related with REMS percent or with sleep cycles, both in treated and non treated menopausal women.

#### Conclusions

Hormone therapy improves sleep quality by reducing sleep fragmentation and it modifies dreams content as a reflection of the impact on brain physiology.

#### Local ethical committee

Approval has been obtained for this study and the included menopausal women had signed a written acceptance.

## P634

### Glucose-suppressed GH levels compared with IGF-I levels in patients with acromegaly

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Nadir growth hormone (GH) levels after oral glucose tolerance test (OGTT) as well as random insulin-like growth-factor I (IGF-I) levels, given as multiples of the upper limit of normal [xULN], were used to evaluate biochemical activity of acromegalic patients. The development of new, more sensitive GH assays makes it necessary to reevaluate the cut off values of the GH nadir after OGTT.

In a cross sectional study, we evaluated nadir GH concentrations during OGTT and IGF-I levels before glucose load in a series of 66 acromegalic patients (31 f/35 m, median age 53 y (range 20–75)). 56 of the 66 patients underwent surgery, 13 were irradiated. 25 patients were on medication (somatostatin analogue or dopamine agonist). GH levels were measured 3 h following glucose (75 g) administration. Concentrations of GH and IGF-I were determined by a single lab using the same chemiluminescence immunoassays.

45 patients had an xULN ≤ 1 (normal IGF-I). 25/45 patients had nadir GH-levels ≤ 0.3 µg/L, whereas 20/45 patients showed nadir GH levels ranging from 0.4–3.6 µg/L (11: 0.4–1.0 µg/L, 9: 1.1–3.6 µg/L). All 21 patients who's xULN exceeded 1 had a nadir GH-level > 0.3 µg/L (7: 0.4–1.0 µg/L, 14 > 1.0 µg/L). In the 7 patients with a GH nadir between 0.4–1.0 µg/L and an xULN > 1, median xULN was 1.43 (range 1.10–1.82).

In our series, all patients with a GH nadir ≤ 0.3 µg/L showed normal IGF-I reflecting complete biochemical remission. All patients with an xULN > 1 also had a GH nadir > 0.3 µg/L. Patients with a GH nadir of 0.4–1.0 µg/L may have a higher risk of relaps (xULN ≤ 1) or the possibility that a small residual activity of acromegaly persists (xULN > 1).



### P635

#### Loss of rise in awakening cortisol response in prepubertal survivors of leukaemia post bone marrow transplantation and chemotherapy treatment

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#### Objective

The aim was to investigate awakening salivary cortisol response (ACR) and its subsequent rise in normal prepubertal children and prepubertal survivors of leukaemia.

#### Methods

90 patients fulfilling the criteria of > 1 year post treatment for leukaemia, > 6 months off steroids, prepubertal, 5–12 years and in remission were recruited. Group1 Bone Marrow Transplantation (BMT) had Total Body Irradiation ( $n = 26$ ), Group2 Chemotherapy only ( $n = 31$ ) and Group3 Controls ( $n = 33$ ) were siblings or best friends. Ethical approval and informed consent was obtained. Salivary cortisols were collected for 3 days-awakening (T1), 30 minutes post awakening (T30). Samples were analysed using radioimmunoassays.

#### Results

Mean salivary cortisol(T1) in Group1 ( $n = 17$ ) was 4.78 ng/ml, Group2 ( $n = 27$ ) 5.43 ng/ml and Group3 ( $n = 22$ ) 5.12 ng/ml. (NS). The (T30) salivary cortisol was significantly different between groups (Anova  $P = 0.036$ ). Group1 was 3.88 ng/ml ( $n = 14$ ) vs Group3 7.59 ng/ml ( $n = 20$ ) ( $P = 0.032$ ). Group2 ( $n = 26$ ) 7.2 ng/ml vs Group3 ( $P = 0.936$ ) (Dunnets t test) Subsequent paired sample t- test in Group1 and 2 did not reveal any significant rise in (T30) salivary cortisols either in males and females. Group3 showed significant rise in salivary cortisol ( $P = 0.003$ ) mainly mediated by male  $P = 0.006$  but not in females. ( $P = 0.178$ )

#### Conclusion

Healthy prepubertal boys but not girls show a normal ACR and 30 minute rise. Prepubertal survivors post BMT and Chemotherapy (neither boys or girls) demonstrated a significant rise in their 30minute awakening salivary cortisol. Survivors of Leukaemia post BMT also have significantly decreased 30 minute mean salivary cortisol. Prepubertal survivors of Leukaemia may have subtle Hypothalamic pituitary adrenal axis dysfunction as a consequence of therapy which is more pronounced in the BMT group and requires further investigation.

### P636

#### The incidence of spontaneous cerebro-spinal fluid rhinorrhea in a large series of patients with macroprolactinoma

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Cerebro-spinal fluid (CSF) rhinorrhea is a recognised complication of pituitary surgery, and occasionally occurs following pituitary radiotherapy. Macroprolactinomas (MPRL) may result in spontaneous or dopamine-agonist-induced CSF leaks, however no detailed large comparisons have been made with non-functioning adenomas (NFA). The incidence of this phenomenon and the mechanisms underlying it are not understood.

We have performed a retrospective review of subjects with MPRLs ( $n = 114$ ) seen over a 17-year period (1985–2003), and compared them with a group of subjects with non-functioning pituitary macroadenomas (NFA) ( $n = 180$ ). Our aim was to determine the incidence of spontaneous, and dopamine-agonist-induced rhinorrhea in subjects with MPRLs, and to determine underlying mechanisms.

Spontaneous CSF-rhinorrhea, confirmed by measurement of glucose and beta-transferrin levels, occurred in 3/114 subjects (2.6%) with MPRL; and in a further 7/114 MPRL subjects (6.1%) following dopamine-agonist therapy (1 week–6 months). In contrast no subjects with NFA developed non-surgical rhinorrhea.

We hypothesised that prolactin levels at diagnosis and rate of change of prolactin in response to dopamine-agonists may predict CSF-rhinorrhea. There was no significant difference of mean prolactin at diagnosis between groups; MPRLs with leaks 113306 mU/L or without leaks 122896 mU/L ( $P > 0.2$ ). Furthermore the rate of change of prolactin, assessed using the drop in prolactin per month following at least 3 months of dopamine-agonist

therapy was not significantly different between MPRL with leaks 49327 mU/L/month vs. non-leakers 16572 mU/L/month ( $P = 0.07$ ). We also assessed dopamine-agonist resistance, a year following diagnosis, in the two cohorts, and found this to be higher in the leakers than the non-leakers (30% ( $n = 10$ ) vs. 5% ( $n = 104$ )).

#### Conclusion

This is the first large series to ascertain the incidence of spontaneous CSF-rhinorrhea in subjects with Macroprolactinoma (9%). This is not a complication of non-functioning pituitary adenomas. Whether the difference in dopamine-agonist resistance is related to the mechanism of prolactinomas inducing CSF-rhinorrhea requires further studies.

### P637

#### Gonadotropin levels in the cerebrospinal fluid (CSF) as a marker of the gonadotropin secretion from non-functioning pituitary adenomas

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#### Objectives

Pituitary adenomas usually do not over-secrete gonadotropins in the bloodstream, although they may stain for gonadotropins. Our study aimed to assess whether the pre-operative gonadotropin level in CSF could be used as a marker of the pituitary tumour's gonadotropin secretion.

#### Patients and methods

359 patients with pituitary adenomas, diagnosed between 1979 and 2005 in the Institute of Endocrinology, Bucharest, were evaluated before pituitary surgery with the approval of the local ethical committee: 122 non-functioning pituitary adenomas (NFPA), 132 with acromegaly (ACM) and 105 prolactinomas (PRM). Anterior pituitary hormones simultaneously sampled in serum and CSF were measured by fluorimetric assay ( $n = 359$ ); immunohistochemistry (IHC) was performed by avidin-biotin method ( $n = 71$ ).

#### Results

While serum and CSF level of GH and prolactin are strongly correlated ( $r = 0.74$  and  $0.78$ , respectively) in all pituitary tumours, glycoprotein hormones are not. FSH level in CSF is correlated with LH ( $r = 0.7$ ,  $P < 0.001$ ). CSF and CSF/serum levels of FSH and LH were significantly higher in macroNFPA (mean  $\pm$  standard error:  $6.4 \pm 1.0$  and  $4.4 \pm 0.7$ , respectively) than in macroACM ( $2.6 \pm 0.4$  and  $2.0 \pm 0.4$ ) and macroPRM ( $1.2 \pm 0.2$  and  $1.5 \pm 0.2$ ),  $P \leq 0.01$ . FSH levels in CSF were higher in gonadotropin IHC-positive NFPA ( $19.8 \pm 6.2$ ) compared to negative NFPA ( $3.8 \pm 1.0$ ),  $P < 0.01$  and to gonadotropin-positive ACM and PRM. FSH in CSF predominated compared to LH in gonadotropin-positive tumours. A CSF FSH value over 5 U/L has 75% sensitivity and 69.2% specificity in identifying a gonadotropin-staining macroNFPA.

#### Conclusions

FSH level in CSF may be used as a pre-operative marker of the gonadotropin secretion in NFPA, but not in gonadotropin-staining ACM. Predominant FSH secretion in CSF from gonadotropin-staining pituitary tumours suggest a different mechanism for blood-CSF barrier by-passing by glycoprotein hormones compared to peptide pituitary hormones.

### P638

#### Prolonged post-operative follow-up is necessary for unirradiated non-functioning pituitary adenomata

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The optimal post-operative management of patients undergoing trans-sphenoidal adenomectomy for non-functioning pituitary adenoma is uncertain. Since 1994, in our centre, patients receiving immediate post-operative radiotherapy have been monitored with annual visual field assessment. Annual MRI is undertaken for 5 years if radiotherapy has not been given, followed by 2-yearly MRI continued indefinitely.

We aimed to examine the efficacy of our follow-up MRI and visual field policy in detection of significant tumour regrowth, requiring further intervention with either surgery or external beam radiotherapy.

127 (78 [61%] male) had complete follow-up data for at least 12 months (median [range] 5 [1–15] years). Median (range) age at presentation was 58 (26–83) years.



77 (61%) had minimal residual tumour post-operatively therefore did not receive radiotherapy. 10 (13%) required further surgical intervention (median [range] time 3 [0.5–14] years), 9 of whom had tumour regrowth or significant residual tumour detected during routine MRI. 25 (32%) had at least one scan omitted according to protocol, although this only affected the care of 1 patient who underwent repeat surgery following urgent MRI after visual field deterioration. All those undergoing repeat surgery received radiotherapy except one (radiotherapy refused). Radiotherapy was arranged immediately post-operatively in 50 (39%) patients with significant residual, or cavernous sinus, disease, and later in 18 after detection of tumour regrowth on MRI (median [range] time post-operatively 4 [1.25–12] years). 4 required repeat surgery following radiotherapy; 3 following visual field deterioration on routine monitoring; 1 following tumour regrowth detected on MRI for investigation of headaches. Our routine post-operative MRI and post-radiotherapy visual field monitoring policy is effective in detecting significant residual or regrowth of tumour requiring further intervention. Our data highlight the importance of continued monitoring, with further intervention being required even 14 years after initial surgery.

### P639

#### Anterior pituitary hormone dysfunction after traumatic brain injury: less common than previously thought?

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#### Background

Traumatic brain injury (TBI) is a cause of hypopituitarism. Previous reports suggest complete or partial pituitary dysfunction in up to 40% of subjects.

#### Aim

To investigate the presence of anterior pituitary hormone deficits in TBI patients tested at least 3 months after the injury.

#### Patients and methods

The patients were recruited from those who have been assessed by the local Rehabilitation Centre over the last 5 years. The endocrine evaluation included short synacthen test, serum FSH, LH, testosterone, oestradiol/menstrual history, TSH, freeT3, freeT4, prolactin, IGF-I and glucagon test. Subjects with peak GH < 6 mU/l on glucagon test were further assessed by GHRH + arginine test and were diagnosed as GH deficient if the peak GH was < 18 mU/l. The cortisol response was considered adequate if > 580 nmol/l on stimulatory tests.

#### Results

29 subjects consented to take part [24/5 males/females, median age 46 years (range 19–65.5), median BMI 24 Kg/m<sup>2</sup> (range 19.6–37)]. The median interval since the TBI [median GCS on admission 5 (range 3–15)] was 35 months (range 3–276). All but four had post-traumatic amnesia of at least 1 week. No patient had FSH/LH or TSH deficiency. Prolactin was normal in all cases. ACTH deficiency was diagnosed in one subject (3.5%), which proved to be transient (diagnosis 4 months and recovery 9 months following TBI). Peak GH < 10 mU/L on glucagon test was found in 3 cases (10.3%). Severe GH deficiency was found in one patient (3.4%) (peak GH 3.8 and 17.8 mU/l on glucagon and GHRH + arginine tests, respectively), who however, 3 years following a minor TBI suffered intracerebral haemorrhage.

#### Conclusions

In this series of patients with TBI tested at least 3 months after head trauma, anterior hypopituitarism was rare. Using strict criteria, it is possible that other series have overestimated the frequency of pituitary dysfunction after TBI.

### P640

#### Effects of long-term GH treatment in Prader-Willi adults

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#### Objective

Prader-Willi syndrome (PWS) is a complex genetic disorder. Body composition abnormalities, hyperphagia, progressive obesity and diminished growth hormone secretion are common. In a previous one year GH intervention trial in 19 PWS adults, we have shown beneficial effects on

body composition. In the present study we sought to re-evaluate the cohort, with special emphasis on long-term effects of GH treatment.

#### Methods

Fourteen patients, 7 men and 7 women, mean age 32 years, were evaluated. Mean follow-up was 6 years. Nine patients were treated with GH during a mean time of 5 years. Body composition and bone mineral density (BMD) were determined with Dual Energy X-ray absorptiometry (DEXA). Plasma glucose and serum insulin were measured.

#### Results

Only small variations were seen in BMI, except in one woman (not treated with GH) who increased enormously in weight. In the GH treated patients a mean increase in lean body mass of 8 kg ( $P = 0.004$ ) and a mean decrease in body fat of 9% ( $P = 0.017$ ) were demonstrated. BMD was unchanged. Only one non-GH-treated woman developed overt diabetes.

#### Conclusion

In this cohort data show that five years of GH treatment has sustained favourable effects on body composition without significant side effects.

### P641

#### Endocrine abnormalities are common in transsexual individuals

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#### Background

The most significant hypotheses regarding biological causes of transsexualism are based on the role of environmental hormonal exposure on neural development.

#### Aim

This study was to analyse the baseline hormonal results in a population of transsexuals attending a single clinic to identify any abnormalities in hormone levels in these individuals.

#### Method

A retrospective cohort study was carried out using data collected from the clinical notes and MISIS database for patients booked to attend the clinic between 1<sup>st</sup> January 2003 and 31<sup>st</sup> December 2005. 191 patients were included in the study, with 161 of these being male-to-female and 30 being female-to-male. The levels of FSH, LH, Oestrogen and Testosterone were recorded, within a database, for each of these patients, and the number of normal and abnormal readings were counted. Chi Squared testing was then used to analyse these results to determine whether or not there was a significant difference to those expected within the general population.

#### Results

In Male-to-Female transsexuals a significant difference was found for LH, FSH, Oestrogen and Testosterone levels ( $P = 0.001$ ). The Oestrogen was higher than the normal range in all cases. LH and FSH were both twice as likely to be below than high [LH6% low versus 14% high, FSH 14% low versus 27% high]. In female-to-male transsexuals a significant difference was found in LH, FSH and Oestrogen levels ( $P = 0.001$ ). Testosterone was raised in 12% ( $P = 0.05$ ).

#### Conclusion

Within this particular patient population it would appear that there are highly significant abnormalities within their hormone profiles which may play a role in the aetiology of their transsexualism. These patients would need to be investigated more closely in order to establish a mechanism by which these abnormal hormone levels may lead to the development of transsexualism, or whether these findings support any of the current theories.

### P642

#### Glucagon-induced suppression of ghrelin secretion is exerted at hypothalamus-pituitary level and is not mediated by an increase in catecholamine secretion

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#### Objective

The mechanisms underlying the well known glucagon-induced satiety effect are unclear. We showed earlier that glucagon induces a remarkable decrease

in the orexigenic hormone: ghrelin. It was the aim of the present study to further evaluate the effect of glucagon on ghrelin secretion and the possible site of origin of this effect.

#### Methods

We studied the endocrine and metabolic responses to intramuscular glucagon administration in 23 subjects (17 men and 6 women; age 21–68 years; BMI  $27.1 \pm 1.2 \text{ kg/m}^2$ ) with a known hypothalamic-pituitary lesion and at least one pituitary hormone deficiency and in 23 healthy subjects as controls (15 men and 8 women; age 20–65 years; BMI  $25.8 \pm 1.1 \text{ kg/m}^2$ ). We also measured catecholamine levels in 14 healthy subjects (7 men) to evaluate their role in mediating the glucagon-induced suppression of ghrelin.

#### Results

The AUC<sub>240</sub>-ghrelin significantly decreased in controls ( $P < 0.001$ ) but not in patients ( $P = 0.446$ ). The AUC<sub>240</sub>-ghrelin was significantly lower in controls when compared to patients [meanAUC<sub>240</sub>  $\pm$  SEM:  $201.5 \pm 6.1$  vs.  $233.2 \pm 8.8$ ; ( $P = 0.005$ )]. Changes in glucagon, glucose and insulin levels were comparable between both groups. In healthy subjects no significant changes in noradrenaline or adrenaline levels were observed during the first 240 min. after glucagon administration.

#### Conclusion

We show that i.m. glucagon significantly decreases ghrelin levels in healthy subjects. However, in the absence of an intact hypothalamic-pituitary axis glucagon failed to decrease ghrelin pointing out to a modulation at the hypothalamic-pituitary level. The mechanisms underlying these effects unlikely include catecholamines, glucose or insulin variations and need to be further elucidated.

### P643

#### Complications of hormonal therapy in male to female transsexuals is uncommon

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#### Background

Transsexuals in Britain are prescribed hormones as part of Gender Reassignment Treatment. Oestrogen therapy is associated with the development of deep vein thrombosis (DVT) with a 25 fold increased risk, and breast cancer; particularly in the older age group and smokers.

#### Aims

To investigate the risk of developing deep vein thromboses in male to female transsexual patients who are, or have been, on oestrogen therapy, and seeing if this differs with age, smoking history or route of transmission. The effect on changing the dose on hormone treatment after gender reassignment surgery was also explored.

#### Method

A retrospective cohort study using the notes of 108 transsexual patients who attended a gender clinic between the dates of Wed 1 January 2003 and 13 June 2003.

#### Results

3.95% of male to female patients who had history of oestrogen treatment (MTF + O) developed DVTs, whereas 3.13% of patients with no history of oestrogen treatment (MTF-O) developed DVTs. 5.23% of MTF + O who developed DVTs were smokers, while 5.26% were non smokers. 6.25% of the MTF + O group who developed DVTs were over 40, and no patients under 40 developed DVTs. 4.11% of MTF + O who developed DVTs had a history of oral oestrogen use, and no patients who had taken transdermal oestrogens developed DVTs. No patients in this group developed breast cancer or osteoporosis.

#### Discussion

There is no significant difference between DVT rates in MTF + O and MTF-O. The incidence is higher for those who were over 40, on oral oestrogens and in non-smokers. There was no difference in the incidence of breast cancer MTF + O and MTF-O, and no difference in the incidence of osteoporosis in all transsexuals who were on oestrogen therapy and those that were not, regardless of the dose taken post operatively.

### P644

#### Effects of targeted ablation of GHRH neurons in mice on anterior pituitary somatotrophs and lactotrophs

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Animal and clinical models of GHRH excess suggest that GHRH provides an important trophic drive to pituitary somatotrophs. Mice in which GHRH neurons have been ablated using a novel viral ion channel transgene (GHRH-M2 mice) show marked anterior pituitary hypoplasia and GH deficiency although GH cells are present. GHRH-M2 mice are also deficient in prolactin which is surprising as GHRH has little or no direct effect on PRL synthesis or release (Le Tissier, Mol Endo 19). In mice two morphological subtypes of lactotroph have been described: Type 1 contain large irregular shaped granules whereas Type 2 contain smaller spherical granules. It is not known what signals influence the differentiation of Type 1 or Type 2 lactotrophs; however GHRH is one candidate. We have examined further the somatotroph and lactotroph populations in non-transgenic (NT) and GHRH-M2 mice. Anterior pituitary tissue from male and female NT and GHRH-M2 adult mice ( $n = 6$ ) was fixed, immunogold labelled for GH and PRL and examined by electron microscopy to determine the number, size, secretory granule population and secretory machinery of somatotrophs and lactotrophs. Somatotrophs, Type 1 and Type 2 lactotrophs were identified in both genders irrespective of the absence of GHRH. Somatotroph numbers were significantly ( $P < 0.01$ ) reduced in male and female GHRH-M2 mice confirming the trophic drive of GHRH to somatotrophs. Female lactotroph and somatotroph morphology was unaltered compared to non-transgenic controls. However, male somatotrophs and Type 2 lactotrophs were smaller and contained significantly fewer ( $P < 0.05$ ) secretory vesicles. Type 1 lactotrophs in male GHRH-M2 mice were smaller, contained more dilated rough ER but no change in vesicle number. These data indicate (1) that male somatotrophs and Type 2 lactotrophs are less active in the absence of GHRH and (2) that GHRH influences lactotroph activity directly or indirectly. In conclusion, Type 1 and 2 lactotroph differentiation occurs independently of GHRH; however, alterations in male lactotroph morphology in GHRH-M2 mice suggests GHRH influences lactotroph function.

### Reproduction

#### P645

#### Spermatogenesis in autologous testicular transplants in the non-human primate *Callithrix jacchus*

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Immature testicular tissue was successfully xenografted in several species but did not lead to spermatogenesis with tissue obtained from the non-human primate *Callithrix jacchus*. Previous data indicate that the microenvironment provided by the mouse host might cause this failure. We conducted an *in vivo* study performing autologous ectopic transplantation of testicular fragments in newborn anaesthetised marmoset monkeys and compared transplanted animals to sham operated animals. Our study aimed to prove whether meiosis can be started and whether this technique offers a perspective for fertility preservation in prepubertal (oncological) patients. 18 months after transplantation we found matured transplants in 2 out of 4 grafted animals. Histology and morphometry of the grafts exhibited tubular development up to an intermediate state compared to pre-graft and adult controls. Dividing spermatogonia and primary spermatocytes were present in the tubules of the transplants but arrested at early meiosis. Immunohistochemistry revealed normal maturation of Sertoli cells, Leydig cells and peritubular cells. Chorionic gonadotropin, the hormone controlling Leydig cells of marmosets, increased to castrate levels (sham-operated castrated controls;  $n = 4$ ) in the transplanted animals, while serum testosterone values did not reach to the normal range of intact controls. Meiotic arrest could have occurred in the grafts due to the lack of sufficient testosterone concentrations, or due to hyperthermia caused by the position of the grafts under the back skin. We conclude that autologous grafting in the marmoset is suitable to maintain testicular tissue and allows maturation up to meiosis, even after long-term ectopic storage.

### P646

#### Hormonal status of women with benign endometrial hyperplastic lesions

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Retrospective randomized blinded study was carried out to find differences in hormone levels in blood between groups of patients with normal endometrium and with endometrial benign hyperplastic lesions. Hospital cases were randomly selected from journals of admittance from 1997 to 2005 years. Only patient name, age and clinical diagnosis, in which endometrial histology was reflected, could be viewed at the time of cases selection. There were to groups of cases selected, with and without of endometrial hyperplastic lesions. All patients included in the study had similar age, regular menstrual, suffered from primary or secondary infertility and was examined in the hospital by the standard infertility protocol. Their cases therefore contained data on endometrial histology, levels of LH, FSH, PRL, estradiol and progesterone in serum. There were 14 cases with proliferative endometrium and 37 cases with secretory endometrium in group with normal endometrium (51 cases in total). Group with endometrial hyperplastic lesions consisted of 26 cases with endometrial cyst hyperplasia, 2 cases with endometrial atypical hyperplasia and 27 cases with endometrial polyp (54 cases in total). Concentrations of LH, FSH and progesterone in serum were almost similar between the groups. It was surprising that patients with endometrial hyperplastic processes had lower estradiol level than that in women with normal endometrium ( $60.41 \pm 9.81$  vs.  $80.62 \pm 12.75$  pg/ml). Although this difference was not statistically significant ( $P > 0.05$ ). It was also amazing that prolactin level in serum of patients with hyperplastic endometrial pathology was significantly lower than that in patients with normal endometrium ( $400.71 \pm 44.04$  vs.  $590.45 \pm 49.29$  mIU/l;  $P < 0.001$ ). Thus, only one significant difference in levels of reproductive hormones was found between groups of women with and without endometrial hyperplastic lesions. This is lowered PRL level in women with endometrial hyperplastic processes.

## P647

### Dependence of semen characteristics on abstinence time, testicular volume and hormones

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Evaluation of male fertility, based on semen analysis, is complicated by high variability of semen parameters and the lack of evidence-based reference values. The standards are currently set by the WHO laboratory handbook (1999). We investigated whether taking into account abstinence time and testicular volume would reduce variation in sperm concentration / count and thereby consolidate the underlying principles of spermatogenesis. The database of our Institute provided data from 1976 to 2005 on 30,965 semen samples from 11,062 infertile men and their abstinence times, testicular volumes and hormone levels. Their impact on semen parameters was analysed by correlation with each individual factor alone and multiple regression analysis.

A highly significant linear correlation between duration of abstinence and sperm concentration/total sperm count was found. Total sperm counts increased between 2.4 (1 d abstinence) and  $6.9 \times 10^6$  (8 d of abstinence) per ml of bi-testicular volume. Sperm concentration varied with semen volume, which exhibited a linear increase with abstinence but reached a plateau after 8 d. The percentage motility and normal morphology changed only marginally between 1 and 8 d of abstinence. Total sperm counts were inversely related to serum FSH but not to testosterone concentrations.

#### Conclusions

1. Correlations between total sperm count and duration of abstinence, testicular volume and FSH are stronger than with sperm concentration, so total sperm count is the more reliable parameter for indicating testicular function.
2. Clinical evaluation of semen samples should take abstinence and testicular volume into account and the diagnostics can be further improved by assessing FSH levels.
3. Normal values for semen analyses need to be established for different abstinence times and testicular volumes.
4. Recommendations for short abstinence periods before timed intercourse or assisted reproduction are not reasonable, as total sperm counts increase far more than motility and morphology vary.

## P648

### Plasma endothelin-1 levels in patients with male hypogonadism

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Endothelin-1 has various paracrine and endocrine effects on the male reproductive system. It is supposed that the testosterone is probably responsible for the higher endothelin-1 levels in males and this may be

related with the enhanced cardiovascular risk in males of reproductive age. In order to study in more detail the relationship between endothelin-1 and testosterone, we have studied 37 male patients with various forms of hypogonadism (14 with hypergonadotrophic hypogonadism and 23 with hypogonadotrophic hypogonadism). Fourteen age-matched healthy males served as controls. The study was approved by the local ethical committee. Endothelin-1 concentrations were determined in blood samples taken in the morning between 08:00 and 09:00 h after an overnight fast. The basal endothelin-1 levels in patients with hypogonadism ( $0.88 \pm 0.11$  fmol/ml) ( $x \pm SEM$ ) were significantly higher in comparison with the controls ( $0.44 \pm 0.04$  fmol/ml),  $P < 0.05$ . The males with hypergonadotrophic hypogonadism were with significantly elevated endothelin-1 concentrations ( $0.83 \pm 0.14$  fmol/ml),  $P < 0.05$ . The same was true for those with hypogonadotrophic hypogonadism ( $0.91 \pm 0.16$  fmol/ml),  $P < 0.05$ . Twenty individuals of these patients (7 with hypergonadotrophic and 13 with hypogonadotrophic hypogonadism) received testosterone depot 250 mg i.m. every three weeks. The endothelin-1 levels, which were determined at 3 and 6 months of the medication, decreased (from  $0.93 \pm 0.20$  fmol/ml to  $0.76 \pm 0.12$  fmol/ml and to  $0.71 \pm 0.21$  fmol/ml, respectively), but not significantly ( $P > 0.05$ ).

The results of this study suggest that plasma endothelin-1 levels in males with hypogonadism are increased and they have tendency to decrease after testosterone administration. These data show that the testosterone do not enhanced the cardiovascular risk and even though may have a protective effect as far as endothelin is regarded.

## P649

### Outcome of 59 pregnancies in 43 acromegalic women

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Limited data are available about pregnancy in acromegalic women. Forty-three women,  $32 \pm 1$  years, presented GH hypersecretion (GH =  $44 \pm 7$  ng/ml, IGF-1 =  $1009 \pm 82$  ng/ml) due to micro ( $n = 6$ ) or macroadenoma ( $n = 37$ ). Thirty-six women had transsphenoidal incomplete surgical resection and 9 external radiation of the pituitary adenoma performed  $2.8 \pm 0.5$  and  $7.1 \pm 3.3$  years before pregnancy. Women received dopamine agonists ( $n = 6$ ), somatostatin analogues ( $n = 11$ ) or both ( $n = 12$ ), and GH/IGF-1 hypersecretion was considered as cured ( $n = 6$ ), uncontrolled ( $n = 15$ ) or controlled ( $n = 22$ ). Pregnancies were reported after spontaneous conception ( $n = 54$ ) or with fertility treatment ( $n = 5$ ). Women had spontaneous abortion ( $n = 1$ ), early termination of pregnancy ( $n = 2$ ), or delivered single ( $n = 51$ ) or twin ( $n = 5$ ) healthy infants without any malformation. The mean birth weight was  $3250 \pm 85$  grs. Maternal diabetes mellitus, hypertension and eclampsia occur during 4, 4 and 3 pregnancies, and were not correlated with GH/IGF-1 hypersecretion. Headaches were reported by 28% of women. Visual abnormalities were observed in undiagnosed acromegaly before pregnancy ( $n = 2$ ) or invasive ( $n = 2$ ) macroadenoma, and patients required medical ( $n = 4$ ) or surgical ( $n = 1$ ) treatment during pregnancy. Breastfeeding was observed in 18 women without any complication. In conclusion, GH/IGF-1 hypersecretion do not increase metabolic and cardiovascular complications during pregnancy of acromegalic women. Pregnancy is associated with symptomatic tumor enlargement is undiagnosed or invasive macroadenomas. GH/IGF-1 hypersecretion and medical treatment did not increase the risk of miscarriage or congenital malformation. Therefore, pregnancy is not contraindicate in women with acromegaly, but acromegalic women should be clinically evaluated for metabolic, cardiovascular or tumor enlargement symptoms during the course of pregnancy.

## P650

### Difficulties in achieving versus maintaining erection: organic, psychogenic and relational determinants

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#### Introduction and objectives

*Achieving* and *maintaining* a penile erection are two essential components of the male sexual response. It has recently been suggested that distinct molecular mechanism could underlie the two disturbances. The aim of the present study is to verify possible clinical differences on pathogenetic factors underlying difficulties of achieving and maintaining an erection.

#### Methods

We studied a consecutive series of 560 patients (aged  $51.9 \pm 12.8$  years old) reporting erectile dysfunction (ED), using SIEDY Structured Interview. Patients were classified in two distinct categories: those with difficulties in *maintaining*, rather than *achieving*, an erection (sample A) and those with main problems in *achieving* an erection (sample B). A complete physical examination and a series of metabolic, biochemical, hormonal, psychometric, penile vascular tests and nocturnal penile tumescence and rigidity evaluations (NPT) were also performed.

#### Results

Sample B patients showed a higher prevalence of organic conditions related to ED, when compared with sample A as confirmed by higher SIEDY scale 1 scores (3[1–5] vs 1[0.1–3] for sample B vs sample A, respectively;  $P < 0.0001$ ) which explores organic component of ED and higher prevalence of pathological instrumental parameters. No difference among groups was observed for SIEDY scale 2 (relational component) and SIEDY scale 3 (intrapsychic component) of ED.

#### Conclusion

In conclusion this study shows for the first time that patients with difficulties in *maintaining* erection are less likely to be affected by organic disturbances interfering with sexual function, when compared with those unable to *achieve* a valid erection.

### P651

#### Psycho-biological correlates of smoking in patients with erectile dysfunction

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#### Introduction and objectives

Warning labels in cigarette packages often links smoking to a severe impairment in sexual health. To evaluate if this is the case, we studied the psychobiological correlates of smoking behavior in 1150 male patients, seeking medical care for erectile problems.

#### Methods

All the patients have been interviewed using SIEDY, which explores organic, relational and intra-psycho components of erectile dysfunction (ED), and completed a self-administered psychometric test (MHQ). In addition, several biochemical and instrumental parameters were studied in this population, to better clarify the biological components underlying the ED problem.

#### Results

Among hormonal levels, we found that current smokers have a higher activation of hypothalamus-pituitary-testis axis (higher LH, testosterone and right testicular volume) and lower levels of both PRL and TSH than never or past-smokers. Hormonal changes were reverted after smoking cessation. Current smokers showed a higher degree of somatised anxiety and were more often unsatisfied of their occupational and domestic lifestyle. Smoking, as part of a risky behavior, was significantly associated with abuse of alcohol and cannabis. Both current and former smokers have the worst subjective and objective (dynamic peak systolic velocity at penile Duplex ultrasound) erectile parameters. This might be due to a cigarette-induced alteration of lipid profile (higher triglyceride and lower HDL cholesterol in current smokers than in non-smokers or past-smokers) or to a higher used of medications potentially interfering with sexual function.

#### Conclusions

Our report demonstrates that smoking have a strong negative impact in male sexual life, even if it associated at an apparently more sexual-favourable hormonal milieu.

### P652

#### Psycho-biological correlates of delayed ejaculation in male patients with sexual dysfunctions

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#### Introduction and objectives

Pathogenesis of delayed ejaculation (DE) is rather unknown, although the contribution of various psychological, marital, hormonal and neurological factors has been advocated.

#### Methods

In this study we systematically investigated the relative relevance of the aforementioned factors in a large sample (1632) of men, seeking medical help for sexual dysfunction. Delayed ejaculation was defined according to Kaplan criteria. In particular mild/moderate DE (MMDE) was diagnosed if ejaculation and climax were still possible, but only with great effort and after prolonged intercourse (mild DE) or possible only with autoerotism, although in the presence of the partner, but not during coitus (moderate DE). Anejaculation or severe DE (ASDE) was diagnosed if orgasm and ejaculation could not be obtained at all (anejaculation) or could be obtained but only with autoerotism conducted in the absence of the partner (severe DE).

#### Results

Mild and moderate DE (MMDE) generally recognized different risk factors than the most severe forms (anejaculation/severe DE; ASDE). ASDE was essentially coupled to the presence of neurological diseases or to the use of serotonergic drugs. Serotonergic drugs also significantly increase (by at least ten-fold) the risk for MMDE, which, however was also coupled to other relational (impaired partner's climax, patient's hypoactive sexual desire, HSD) or intra-psycho (stress at work) factors. At multiple regression analysis, some organic conditions (such as psychiatric disorders and hypogonadism) were also associated to MMDE. In particular, hypogonadism retained significance for DE even after adjustment for HSD (Adj. OR = 2.08[1.11–3.89];  $P < 0.05$ ), suggesting other effects of testosterone deficiency on the ejaculatory reflex, besides reduced libido.

#### Conclusions

In conclusion, the present study demonstrates that multiple psychobiological determinants are associated to DE, a still obscure condition which substantially impairs psychosexual equilibrium of the couple.

### P653

#### Psycho-biological correlates of free-floating anxiety symptoms in male patients with sexual dysfunctions

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#### Introduction and objectives

Anxiety has a relevant impact on everyday life, including sexual life, and therefore is considered the final common pathway by which social, psychological and biological stressors negatively affect sexual functioning. The aim of this study is to define the psycho-biological correlates of free-floating anxiety in a large sample of patients complaining erectile dysfunction (ED) based sexual problems.

#### Methods

We studied a consecutive series of 882 ED-patients using SIEDY, a 13 items structured interview, composed of three scales which identify and quantify organic, relational and intrapsychic domains. MHQ-A scoring from Middlesex Hospital Questionnaire (MHQ) was used as putative marker of free-floating anxiety symptoms (AS). Metabolic and hormonal parameters, nocturnal penile tumescence (NPT) test and penile doppler ultrasound (PDU) examination were also performed.

#### Results

MHQ-A score was significantly higher in patients complaining difficulties in maintaining erection and in those reporting premature ejaculation ( $6.5 \pm 3.3$  vs.  $5.8 \pm 3.3$  and  $6.6 \pm 3.3$  vs.  $6.1 \pm 3.3$  respectively; both  $P < 0.05$ ). Moreover, AS were significantly correlated to life stressors quantified by



SIEDY Scale 2 (relational component) and Scale 3 (intra-psychic component) scores, as dissatisfaction at work or within the family or couple relationships. Among physical, biochemical or instrumental parameters tested, only end-diastolic velocity at PDU was significantly ( $P < 0.05$ ) related to AS.

#### Conclusions

In patients with ED based sexual problems, AS are correlated to many relational and life stressors. Conversely, organic problems are not necessarily associated with MHQ-A score.

### P654

#### Histone deacetylase inhibitors exert estradiol-induced proliferation and hyperplasia formation in the mouse uterus

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It is suggested that estrogen hormones involve pathways controlling histone acetylation to brought about their effects in the uterus. However, it is not known how the level of histone acetylation affect estrogen-dependent processes in the uterus, especially proliferation and morphogenetic changes. Therefore, effects of histone deacetylase blockers, trichostatin A and sodium butyrate, on proliferative and morphogenetic reactions in the uterus under long-term estrogen treatment were examined. Ovariectomized mice were treated with estradiol dipropionate (4 microg per 100 g; s.c.; once a week) or vehicle and trichostatin A (0.008 mg per 100 g; s.c.; once a day) or sodium butyrate (1% in drinking water) or with no additional treatments for a month. In animals treated with estradiol and trichostatin A or sodium butyrate, uterine mass was increased, abnormal uterine glands and atypical endometrial hyperplasia were found more often. Both histone deacetylase inhibitors produced an increase in the numbers of mitotic and bromodeoxyuridine-labelled cells in luminal and glandular epithelia, in stromal and myometrial cells. Levels of estrogen receptors-alpha and progesterone receptors in uterine epithelia, stromal and myometrial cells were decreased in mice treated with estradiol with trichostatin A or sodium butyrate. Expression of beta-catenin in luminal and glandular epithelia was attenuated in mice treated with estradiol with trichostatin A or sodium butyrate. Both histone deacetylase inhibitors have similar unilateral effects, however the action of trichostatin A was more expressed than that of sodium butyrate. Thus, histone deacetylase inhibitors exert proliferative and morphogenetic effects of estradiol. Actions of trichostatin A and sodium butyrate are associated with changes in expression of estrogen receptors-alpha, progesterone receptors and beta-catenin in the uterus. This work was supported by grant from RFBR (03-04-48000).

### P655

#### Aetiologies of galactorrhea

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Galactorrhea (G) is a frequent symptom in endocrinology when breast are systematically examined. In literature there is few works about aetiologies of G. The aim of our work is to evaluate prevalence of G and to study its cause in 170 subjects with nipple discharge.

Our methodology is based on anamnestic history, clinical exam and a complete hormonal exploration. Women with normal cycle has a temperature curve, endometrial biopsy and progesterone evaluation. Patients who take medical treatment are excluded.

#### Results

In a personal consultation we have noticed 170 subjects with G among 686 patients who came for various symptoms = 27.7%. Among our population 163 are females and 7 are males. Mean age is  $28 \pm 8$  years in women and  $33 \pm 8$  years in males. For aetiologies 93 subjects from 170 (54%) are hyper (H) prolactinemic (PRL) and in 46% PRL is normal. In male cases PRL is always high (6 prolactinomas and 1 craniopharyngioma). In women G with normal PRL are more frequent. Among this last group luteal insufficiency represents 88%, primary hypothyroidism = 9.7% and pituitary tumors = 2.1% (Cushing's disease, craniopharyngioma). In female HPRL G are due to hypothalamus and/or pituitary abnormalities (prolactinoma, GH adenoma and empty sella) in 78%, primary hypothyroidism in 21% and luteal insufficiency in 2.9%.

#### Conclusion

Galactorrhea is very rare in males and when this abnormality exist, its aetiology is represented by HPRL. In female cases, G with normal PRL are more frequent and among those luteal deficit is the first cause, while HPRL G are often due to hypothalamus and/or pituitary disorders.

### P656

#### Standardisation and validation of a sensitive enzymeimmunoassay procedure to estimate 13,14-dihydro-15-keto-PGF<sub>2α</sub> (PGFM) in mithun (*Bos frontalis*) plasma

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Mithun (*Bos frontalis*), a free-range domesticated ungulate, reared primarily for beef and occasionally for milk, is mainly distributed in the South-East Asia. Much scientific reports on reproductive-endocrinology in this species are not available at present. The current study was designed to standardise and validate a simple, sensitive and direct enzymeimmunoassay (EIA) on microtitre plates using second antibody coating technique to determine 13,14-dihydro-15-keto PGF<sub>2α</sub> (PGFM) in unextracted mithun plasma. The assay was carried out directly in 20μl mithun plasma. PGFM standards prepared in hormone-free plasma were used in the assay. It was observed that the different plasma volumes (10, 20, 50 and 100 μl) did not influence the absolute binding sensitivity. However, a slight drop in OD<sub>450</sub> was observed with increasing plasma volume. The sensitivity of the assay was found 40 pg/ml plasma. The intra- and inter- assays coefficients of variation that determined using pooled plasma samples containing  $97.2 \pm 3.1$  and  $235.6 \pm 4.9$  pg/ml PGFM were found 5.9 and 8.3 and, 6.5 and 9.7 percent, respectively. Biological validation of the assay was carried out in plasma samples that collected from 10 mithun cows on different days of estrous cycle and during peri-estrus period. In estrous cycle, the plasma PGFM level ( $P < 0.01$ ) attained peak ( $481.0 \pm 43.4$  pg/ml) on day 4 prior to estrus and the lowest level ( $164.3 \pm 11.9$  pg/ml) was observed on estrus day. A pulsatile PGFM secretion pattern was observed prior to estrus when frequent samples were collected at 1 h intervals. A wide range of plasma PGFM concentration (93 to 612 pg/ml) were successfully detected in cyclic mithuns using the current EIA protocol. In conclusion, the present PGFM-EIA procedure has been found to be sufficiently sensitive and reliable to estimate different physiological levels of PGFM in mithun plasma.

### P657

#### "Siofor" efficacy in treatment of obesity and reproductive disorders

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The work presents results of examination of 60 obese fertile women (aged  $31.1 \pm 2.35$ ) with various reproductive disorders aiming at assessment of "Siofor" efficacy in restoration of endocrine and reproductive function.

The examination showed long-term ( $7.3 \pm 2.3$  years) menstrual cycle disorders in 35 women (58%), primary infertility with duration of  $6.5 \pm 3.2$  years being registered in 12 (20%), polycystic ovary syndrome in 13 (21.6%). All patients had obesity of various degrees; mean BMI being  $35.0 \pm 3.3$  kg/m<sup>2</sup> and waist/hip circumference ratio  $0.95 \pm 0.05$ . Concentrations of hormones were as follows:  $10.25 \pm 2.5$  MU/l of LH,  $6.69 \pm 1.2$  MU/l of FSH, 1.20 nmol/l of testosterone, 10.26 MU/l of PRL,  $633.2 \pm 34.6$  nmol/l of cortisol,  $43.7 \pm 0.24$  of progesterone and  $203.09 \pm 12.5$  of estradiol.

Results of glucose tolerance test show no confident difference of basal insulin ( $9.7 \pm 1.3$ ) and glucose ( $4.0 \pm 0.9$ ) from those in the control group ( $7.9 \pm 1.2$  and  $4.2 \pm 0.1$ , respectively). 2-hour glucose values in the examinees and control were confidently different, but there was no confident difference in final values ( $5.1 \pm 1.7$  and  $4.5 \pm 0.4$ ). Insulin in the examinees was significantly higher than in the control group ( $81.1 \pm 1.9$  vs  $11.6 \pm 0.9$ ). Mean body mass reduction by 10 kg was observed in 80% of the patients, BMI and WHI being decreased to  $30.2 \pm 0.9$  and 0.78, respectively. Menstrual cycle restoration was found in 36 (60%) women, in 18 (30%) of them ovulatory cycle being confirmed and 3 pregnancies being registered. In 20 (33%) patients testosterone reduction as well as confident decrease of LH and FSH levels ( $7.6 \pm 0.2$  and  $4.3 \pm 0.1$ , respectively) was found.

The study showed that "Siofor" contributes to body mass reduction, T level and LH/FSH ratio normalization resulting in menstrual cycle and ovulation restoration in some patients.

Thus "Siofor" could be used at the primary stage of therapy of obese patients with ovary dysfunction. It is expected to correct both on excessive body mass and reproductive system to allow avoiding of delaying hormone therapy.

#### P658

##### Endothelin-1 levels in men of reproductive age with idiopathic hypogonadism after treatment with tamoxifen or raloxifene

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An association between testicular function and vasoconstrictive peptide endothelin-1 levels, which is produced by Leydig and Sertoli cells, is observed. Endothelin-1 regulates blood flow in the testis and stimulates testosterone secretion.

The purpose of this study is (1) To estimate the influence of tamoxifene and raloxifene therapy on endothelin-1 levels in men of reproductive age with idiopathic hypogonadism. (2) To find an association between improvement of semen parameters and changes of peptide levels during therapy.

##### Materials and methods

Forty men (40) with idiopathic hypogonadism were studied. Group 1 consisted of twenty (20) men aged  $35 \pm 4.4$  yrs who received tamoxifen therapy (20 mg/day for three months). Group 2 consisted of twenty (20) men aged  $34.1 \pm 4.3$  yrs who received raloxifene therapy (60 mg/day for three months). Endothelin-1 levels were measured before and three months after initiation of therapy. All patients had a semen analysis at the same times.

##### Results

(1) Tamoxifene therapy improved morphology and movement of spermatozoa significantly whereas raloxifene therapy improved the number and morphology of spermatozoa. (2) Endothelin-1 levels didn't differ significantly between the two groups before therapy ( $58.6 \pm 27.4$  pg/ml vs  $46.7 \pm 25.4$  pg/ml respectively) (3) Tamoxifene increased levels of endothelin-1 but not significantly ( $58.6 \pm 27.9$  pg/ml vs  $76.3 \pm 26.2$  pg/ml). (4) Raloxifene did not alter endothelin-1 levels ( $46.7 \pm 25.4$  pg/ml vs  $46.9 \pm 28.2$  pg/ml). (5) After three months of therapy, endothelin-1 levels were higher in men in group 1 than the ones in group 2. (6) No association was found between endothelin-1 levels and semen parameters before and three months after therapy.

##### Conclusions

The present study shows that tamoxifene tends to increase endothelin-1 levels and this action is not observed after raloxifene therapy. Furthermore, small changes in endothelin-1 levels are not associated with improvement of semen parameters after therapy with either of the two medications (tamoxifene and raloxifene).

#### P659

##### Clinical, hormonal, neuroradiological characteristics and response to treatment in 29 macroprolactinomas

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Most macroprolactinomas are responsive to dopamine agonists drugs. Bromocriptine (BRC) has been available and extensively used in our country. The aims are to assess the outcome in 29 patients with macroprolactinomas who were treated with BRC.

##### Material and methods

A multicentric study of 29 patients with macroprolactinoma (19 female; 10 males). The mean age was 33 years. The pituitary mass was larger than 10 mm in diameter. There was no endocrinological evidence of mixed tumour or primary hypothyroidism.

##### Results

The primary presenting complaint included headaches and/or visual abnormalities which were present in 68% of female vs 90% in men, menstrual dysfunction (94%), impotence (57%), decreased libido (80%), gynaecomastia (40%). The mean duration of symptoms before diagnosis was  $43.6 \pm 33.6$  months in females and  $27.7 \pm 25$  months in men. The mean maximal tumor diameter was 20 mm and were larger in men ( $25 \pm 9$  mm) than in women ( $17 \pm 6$  mm). Extra sellar extension was observed in 68.5% in female vs 80% in male. The mean pre-treatment prolactin level was

1501 ng/ml (range: 70–20476 ng/ml) and correlated significantly with tumour size ( $P = 0.02$ ). Apoplexy was clinically evident in one patient and silent three others diagnosed on radiologic imaging.

The cumulative radiological response to BRC seemed to be associated to a longer duration of treatment. A tumor reduction greater than 50% or a shrinkage was seen in 38% after one year of treatment, rose to 43% after two years and to 67% after three years over. Empty sella was seen in 5 patients. The mean doses needed of BRC were correlated to basal prolactin and women received lower doses than men.

##### Conclusion

Bromocriptine was the main dopamine agonist used in our patients and was safe and effective. Doses varied with the mean prolactin level and tumor size.

#### P660

##### Prevalence of macroprolactinemia during pregnancy of women with prolactin-producing pituitary microadenomas

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During pregnancy of healthy women, a great increase of serum little prolactin occurs. It has been also reported that a significant amount of serum big big prolactin (termed macroprolactinemia) may be detected in about 4% of healthy pregnant women. Because the occurrence of macroprolactinemia during pregnancy of women with prolactin-producing pituitary microadenomas has not been extensively investigated, we determined both little and big big prolactin in serum of 97 women with prolactin-producing pituitary microadenomas who became pregnant after bromocriptine treatment. In all patients bromocriptine was discontinued during the first 12 weeks of pregnancy, and blood samples were obtained for prolactin measurements from the 6th until the 13th week of pregnancy (trimester I), from the 14th until the 27th week (trimester II) and from the 27th week until delivery (trimester III). Total prolactin (assayed directly) and little prolactin (measured following polyethylene glycol precipitation) were determined using an ECLIA assay (Elecsys 2010, Roche), and the samples were considered to contain significantly large amounts of macroprolactin when the proportion of little prolactin was less than 55% of total prolactin. Of the 97 women, 84 had negligible amount of macroprolactin in each of the three period of sampling (serum total and little prolactin in trimester I, mean  $\pm$  SD,  $88 \pm 55$  and  $80 \pm 54$  ng/ml, respectively; in trimester II,  $150 \pm 61$  and  $143 \pm 62$  ng/ml, respectively; in trimester III,  $196 \pm 76$  and  $186 \pm 73$  ng/ml, respectively). In contrast, a significantly large amount of macroprolactin (corresponding to >45% of total prolactin) was found in 13 women, in whom total prolactin (mean  $\pm$  SD in trimester I, II, and III,  $174 \pm 183$ ,  $281 \pm 148$  and  $368 \pm 61$  ng/ml, respectively), but not little prolactin levels (mean  $\pm$  SD in trimester I, II and III  $54 \pm 44$ ,  $100 \pm 52$  and  $146 \pm 46$  ng/ml, respectively) were significantly higher than the corresponding values in woman without significant macroprolactinemia. We conclude that a significant macroprolactinemia, associated with a significant increase of total prolactin, occurs in 12% of women with prolactin-producing microadenomas during pregnancy.

#### P661

##### Hormone replacement therapy in postmenopausis fails to normalize gonadotropins – does it matter?

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Level of pituitary trophic hormone dictates dose of hormone replacement (HRT) in endocrine gland hypofunction. We examined 28 women receiving HRT in postmenopausis aged 46–63 yrs ( $54 \pm 4.27$  yrs). In average patients started HRT two years after last spontaneous menstrual bleeding ( $28 \pm 3.76$  months, 1–132 months). 8 patients used transdermal estradiol, 10 peroral combination of estradiol, estriol and norethisterone and 10 patients were treated by fixed peroral combination of estradiol and norethisterone. Average duration of treatment was  $24 \pm 17.96$  (3–72) months. Hormones were determined by commercial kits. Follicle stimulating hormone (FSH) and luteinizing hormone (LH) values were  $39.42 \pm 22.74$  (4.3–83) U/l (Normal values: 2.4–9.3– follicular phase) and  $24.43 \pm 14.41$  (2–53) U/l (Normal values: 1.6–9.3) respectively. At the same time estradiol level was  $0.21 \pm 0.12$  (0.05–0.44) nmol/l (Normal

values for follicular phase:  $0.08-0.79$ ) and progesterone ranged from  $0.26-1.9$  ( $0.87 \pm 0.37$ ) nmol/l (Normal values for follicular phase:  $0.6-3.6$ ). FSH was normal in only one patient receiving fixed combination of estradiol and norethisterone, LH was normal in six patients. All patients were free of menopausal symptoms and side effects of HRT. Results show lack of normalization of gonadotropins despite normal values of estradiol. And yet this way of treatment is considered satisfactory in terms of achieving main targets of HRT in postmenopausis: relieving menopausal symptoms, prevention of osteoporosis, protection of cardiovascular system and eventually central nervous system. Authors wonder if different approach in dosing would improve any outcome of HRT.

## P662

### All that is hypogonadal in haemochromatosis is not due to iron deposition

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Hypogonadism is the most common non-diabetic endocrinopathy in patients with hereditary haemochromatosis (HH). A recent review suggests a prevalence of hypogonadism in males with haemochromatosis to be 6.4%.<sup>1</sup> We report three patients with HH in whom hypogonadism was not due to iron deposition.

Patient 1 was homozygous for C282Y and liver biopsy revealed grade III siderosis without cirrhosis. His testosterone was borderline low ( $6.7$  nmol/L), and FSH and LH were appropriately elevated. Karyotype analysis confirmed the clinical impression of Klinefelter's syndrome.

Patient 2 was homozygous for C282Y, with liver biopsy showing grade III siderosis and cirrhosis. He had a similar phenotype and hormonal profile to patient 1 and karyotyping again confirmed Klinefelter's.

Patient 3 was a compound heterozygote (C282Y/H63D). Ferritin was elevated ( $547$  ng/ml) and liver biopsy showed grade II siderosis without cirrhosis. FSH, LH and testosterone were low. The absence of cirrhosis and the modest elevation of ferritin raised doubt that HH was responsible for the hypogonadism.<sup>1</sup> On direct questioning, the patient admitted to chronic high dose use of androgenic anabolic steroids while representing Ireland in weight-lifting in the recent past.

In our large series of patients with HH, hypogonadism was due to causes other than iron deposition in 25% of cases.

These cases underscore the importance of careful evaluation of hypogonadism in HH.

1. McDermott JH & Walsh CH. Hypogonadism in Hereditary Haemochromatosis. *J Clin Endocrinol Metab* 2005; 90(4): 2451-2455.

## P663

### Nitric-oxide-cGMP and ATP-cAMP pathways in pregnant women with gestational diabetes mellitus

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Gestational diabetes mellitus (GDM) is a clinical condition which is ideal for evaluating short-term effects of impaired glucose metabolism, ruling out the possibility that the platelet abnormalities are a consequence of diabetic complications.

The aim of this study was to measure the velocity of platelet disaggregation and the activation of the platelet second messengers cGMP and cAMP by sodium nitroprusside (SNP) and ATP, respectively, *in vitro* in pregnant women with GDM.

Materials and methods

We compared 12 pregnant women with GDM ( $24.68 \pm 5.12$  years and gestational age (24-26 weeks)), and 10 healthy pregnant women ( $23.96 \pm 5.18$  years). There were no significant differences in fasting glycaemia ( $5.38 \pm 0.41$  mmol/l for GDM and  $4.61 \pm 0.17$  mmol/l for healthy pregnant women) or in H<sub>B</sub>A<sub>1c</sub> levels ( $6.78 \pm 0.21$  vs.  $5.83 \pm 0.61\%$ ). The study was performed in accordance with the principles of the Declaration of Helsinki as revised in 1996. Platelet disaggregation was determined by light transmission using an AP 2110 computerized analyzer of platelet aggregation (SOLAR, Belarus). Concentrations of cAMP and cGMP were determined radioimmunologically with a  $\gamma$ -counter (LKB\_Wallac, Finland). Addition of SNP ( $400 \mu\text{mol/l}$ ) or ATP ( $200 \mu\text{mol/l}$ ) to platelets preaggregated with ADP ( $1.5 \mu\text{mol/l}$ ) induces platelet disaggregation.

Results

The velocity of SNP-induced disaggregation at the pregnant women with GDM was higher than in control group in 1.8 times, however differences between groups of cGMP levels were inappreciable ( $1.5 \pm 0.1$  and  $1.6 \pm 0.1$  pmol per  $10^9$  platelets accordingly). There was no significant difference between ATP-induced disaggregation and cAMP levels in pregnant women with GDM and healthy pregnant women.

We conclude that, the NP-induced platelet disaggregation in investigation groups of the donors is not linked to cGMP-signalling systems, ATP-cAMP pathways is not activated in investigation group and under activity of ADP are formed less stable platelet aggregates, than in control group.

## P666

### The influence of estradiol on erythrocyte antioxidative enzyme system activity in pre- and postmenopausal women

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Free radicals are continuously produced during normal metabolism. They play important role in the pathogenesis of atherosclerosis, carcinogenesis, degenerative processes of the brain and skin, and some diseases connected with aging. Among several natural antioxidants, estrogens are important free-radical scavengers due to the hydroxyphenolic structure of their molecules. Some authors have suggested that estrogens may also influence the activity of the cellular antioxidative enzyme system, but these data remain controversial. The aim of our study was to investigate the effects of estradiol deficiency after menopause and the influence of estradiol therapy (ET) on cellular antioxidative enzyme system: erythrocyte dismutase (SOD), glutathione peroxidase (GSH-Px) and catalase (CAT) activity. Glutathione (GSH) and selenium (Se) concentrations were also estimated. Serum lipid peroxide (LPO) levels were measured as an indicator of free-radical production and cell membrane phospholipids peroxidation. Materials and methods: The study group consisted of 26 women with surgically induced menopause and climacteric symptoms. Forty premenopausal healthy volunteers served as controls (C group). The postmenopausal women were treated for 4 months and received estradiol transdermally in a dose of  $50 \mu\text{g}$  daily. The blood was collected for SOD, GSH-Px, CAT, GSH, Se and LPO estimation before and after therapy. The study protocol was approved by Ethical Committee of Medical University in Wroclaw. Results: LPO was higher in postmenopausal women and decreased after ET. GSH-Px and GSH were lower in the postmenopausal groups, but increased significantly after estrogen therapy. Se concentrations did not differ significantly among the groups. CAT activities were similar in all groups and decreased after ET. SOD activities in postmenopausal women were similar to those in C group and did not change significantly after ET.

Conclusions

Our findings indicate that oxidative stress increased after menopause and the administration of natural estrogens to postmenopausal women diminishes oxidative stress and increases antioxidative cell potency.

## P667

### The influence of the Cimicifuga Racemosa on the vegetovascular and psychoemotional disorders in women with climacteric syndrome

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Aim

To investigate the influence of Cimicifuga Racemosa in women with peri- and early postmenopause.

Materials and Methods

125 women were investigated. They created 2 groups. The group I - perimenopausal women, their age varied between 36-45 years ( $n = 85$ ); II - women with early postmenopause, their age varied between 46-55 years ( $n = 40$ ). They complained about hot flashes, skin irritating, excess hyperhidrosis, tachycardia, giddiness, pulsation in the head, lability of the pulse and arterial tension, oligoamenorrhea, the soreness of the breast. All this patients received Cimicifuga Racemosa - 2 tablets daily during 6 months.

Results

After the treatment with Cimicifuga Racemosa the significant improvement of the condition was observed. In both of the groups in 95 women the complaints were either reduced, or vanished. In the rest of the cases the arterial tension stayed elevated, what probably is related to the concomitant disease - arterial hypertension.



Conclusions

Cimicifuga Racemosa is a very effective medication in peri – and early postmenopausal women.

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**P668**

**Gonadal dysfunction in hypercortisolic men**

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Cushing's syndrome is rare in males. Hypercortisolic consequences on male gonadal function are unknown. The aim of our study is to research gonadal and sexual abnormalities in 17 male subjects with endogenous cortisol excess: 10 Cushing's diseases, 5 paraneoplastic Cushing syndrome, 1 malignant corticosteroidoma, 1 micronodular dysplasia. Their mean age is 30.2 years (18–50). Our work is based on anamnestic, clinical and hormonal results. Our population is compared to normal men without medical or endocrinological diseases. Nobody has taken medical treatment before or during biological exploration.

Our results are as follow: decreased libido and/or impotency is noticed in 7/15 subjects (46%), there is a gynecomastia in one case (5.8%) and testosterone of hypercortisolic subjects is significantly lower than testosterone of normal subjects (2.9 ng/ml versus 5.05,  $P < 0.01$ ). Mean prolactin (PRL) of our population is higher than PRL of control group (11 ng/ml versus 2.5,  $P < 0.005$ ). For gonadotrophins there is no difference between the two groups (FSH of hypercortisolic subjects = 4.2 mU/ml versus 3.3 and LH = 2.9 mU/ml versus 3.0,  $P > 0.05$ ).

**Conclusion**

Men with endogenous hypercortisolism have a decreased libido and/or impotency in 46%. Their testosterone is significantly lower than normal subjects and their prolactin is higher. Gonadotrophins of hypercortisolic group are similar to those of control group. The mechanism of this normogonadotrop hypogonadism will be discussed.

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**P669**

**Role of estrogen receptor  $\alpha$ - or  $\beta$ -isoforms in sex-specific brain organization**

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Male- or female-like neural control of gonadal function and reproductive behaviour is determined by sex-specific activation of estrogen receptors (ER) in the developing brain during a limited, critical period during ontogenesis. Exposure of the neonatal female brain to inappropriately high estrogen concentrations results in irreversible defeminization of neuroendocrine function which is manifested by disturbed cyclic ovarian activity and decreased female sexual receptivity in adulthood. Two major ER isoforms, alpha and beta, are present in neural circuits which govern ovarian cycle and sexual behavior. Using highly selective ERalpha or beta-agonists, this study provides evidence for distinct contribution of individual ER isoforms to the process of estrogen-dependent brain defeminization in the rat. Neonatal activation of the ERalpha results in impairment of cyclic ovarian activity and female sexual behavior in adulthood; these effects are associated with male-like alterations in the morphology of the anteroventral periventricular (AVPV) and sexually dimorphic nucleus of the preoptic area (SDN-POA) as well as refractoriness to estrogen-mediated induction of sexual receptivity. Exposure to an ERbeta-selective agonist abolished cyclic gonadal function and had a strong defeminizing effect on the morphometry of the hypothalamic gonadotrophin "surge generator" AVPV. However, neonatal ERbeta-activation failed to alter female sexual behaviour, responsiveness to estrogens and morphometric appearance of the behaviourally relevant SDN-POA. Thus, although co-present in several brain regions involved in the control of female reproductive function, ER isoforms convey different and, probably, not synergistic, chemical signals in the course of neonatal sex-specific brain organization.

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**P670**

**An immortalised cell line (SK11) derived from immature mouse testes express functionally active androgen and oestrogen receptors**

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Spermatogenesis is a complex process that requires the formation of junctional complexes between somatic Sertoli cells and germ cells and hormonal regulation of the Sertoli cells. Androgens and oestrogens are both synthesised in the testis. In adults Sertoli cells contain both androgen receptor (AR) and oestrogen receptor beta (ER $\beta$ ). In the present study we used a transformed Sc cell line (SK11) that was prepared from the testes of mice expressing the large T antigen. When grown at 34°C the cells are mitotically active but when transferred to 39°C they stop dividing.

SK11 cells expressed mRNAs for AR and ER $\beta$  as well as for other proteins found in Sc *in vivo* ( $\beta$ -tubulin,  $\beta$ -actin, aromatase, sulphated proteins 1 and 2). Transfer of cells from 34°C to 39°C resulted in a decrease in expression of proliferating cell nuclear antigen (PCNA) and an increase in expression of SGP-2 as well as a change in cell shape and re-organisation of the cytoskeleton. These changes parallel those seen in Sc as they undergo functional maturation during the first wave of spermatogenesis. Messenger RNA for the androgen-regulated Sc product Pem was expressed in the SK11 cells and was increased in cells after incubation at 39°C. Functional activity of AR and ER $\beta$  was investigated using transient transfections with plasmid reporter constructs containing either 3XERE or pem-ARE-promoters. Expression of the ERE was induced following incubation with E2 or 3 $\beta$ Adiol consistent with reports that 3 $\beta$ Adiol (a metabolite of DHT) can activate ER $\beta$ . Activation of the ERE-reporter did not occur following targeted knockdown of ER $\beta$ . Up-regulation of the ARE reporter was only induced in the presence of T or DHT but not with E2 or 3 $\beta$ Adiol.

In conclusion, SK11 cells seem to provide a useful model that can be used to complement studies using Sertoli cell selective gene ablation.

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**P671**

**Clinical and laboratory relationships in women with hirsutism as a primary symptom**

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Hirsutism may be the expression of hyperandrogenia of ovarian or adrenal origin, iatrogenic or idiopathic/familial. Regardless of the etiology, there is a negative impact of body hair excess on the self-image, the self-esteem and the sexual satisfaction of subjects. The present study aimed to evaluate 60 women, aged 26.3 years, with hirsutism as the primary symptom. At presentation, basal FSH, LH and testosterone levels, urinary 24-hours 17-ketosteroids and DHEA, and an abdominal ultrasound evaluation were performed. In subjects in whom abnormal results were obtained, the diagnostic evaluation continued. Records of clinical data showed that 75% of women were obese and in 53% of women other signs of hyperandrogenia were noticed. Amenorrhea was recorded in 62.5% of cases. Of the 60 patients, a tumor (ovarian, adrenal or pituitary) was found in 11 women (18.3%), 23 were diagnosed with polycystic ovary disease (38.3%) and 12 (20%) had laboratory data suggesting an adrenal enzymatic defect. Of the 60 patients, 10 (16.6%) women with hirsutism and clinical signs of metabolic syndrome had slightly increased basal testosterone levels but normal day 3 FSH, LH levels, FSH/LH ratio and ultrasound evaluation. Only 4 (6.6%) of women were diagnosed with idiopathic hirsutism. Mean levels of testosterone, 17-ketosteroids and urinary DHEA were above the upper range in the studied group; mean testosterone concentration was significantly higher in women suffering from virilizing syndrome as compared to women with hirsutism as the solely complaint ( $2.3 \pm 0.7$  ng/ml vs.  $1.7 \pm 0.5$  ng/ml,  $P < 0.01$ ) but no difference was seen with regard to urinary 17-ketosteroids levels ( $17.7 \pm 4.1$  mg/24 h vs.  $16.5 \pm 3.1$  mg/24 h,  $P > 0.05$ ) or urinary DHEA levels ( $1.9 \pm 0.8$  mg/24 h vs.  $1.9 \pm 0.6$  mg/24 h,  $P > 0.05$ ). In conclusion, in most cases hirsutism is the expression of a dysfunctional hormonal state. There is a significant correlation between testosterone levels and clinical characteristics.

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**P672**

**Bone mass density in 180 women with premature ovarian failure**

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Premature ovarian failure (POF) is characterized by amenorrhea, hypergonadotropism and hypoestrogenism in women under 40 years. Sex steroids play an important role in maintaining bone mass and cessation of ovarian function results in significant bone loss.

#### Objective

To evaluate bone mass density (BMD) and factors influencing it in women with POF. Design & methods: First group of 144 women with spontaneous POF (I),  $39.7 \pm 7.6$  y's old, BMI =  $29.4 \pm 2.1$  kg/m and second group of 36 women with bilateral adnexectomy (II),  $41 \pm 7.8$  y's, BMI =  $24.9 \pm 2.9$  kg/m. Risk factors (eating habits, smoking, alcohol intake, training) were tested. FSH, LH, prolactin, estradiol, progesterone and testosterone were detected by RIA. BMD was measured at the lumbar spine by DEXA. Statistics: Kruskal-Wallis ANOVA, Chi-square test, Spearman's correlation.

#### Results

No significant difference was found for amenorrhoeic period, menarche, W/H ratio. High correlation was found between physical training, non smoking, regular cycle, normal BMI and T score implying on the low risk for osteoporosis ( $P < 0.01$ ). The higher risk was found in the II group. According to amenorrhoeic period ( $< 5$  y's; 6–10;  $> 11$  y's) T scores in I vs. II group were:  $-1.6$  vs.  $-1.8$ ;  $-1.8$  vs.  $-2.1$ ;  $-1.9$  vs.  $-3.2$ .

#### Conclusions

Young women with POF need early education regarding on strategies to maintain their BMD and appropriate hormone replacement therapy in achieving better quality of life.

### P673

#### Oxidized low-density lipoprotein autoantibodies in patients with polycystic ovary syndrome

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Polycystic ovary syndrome (PCOS), affects approximately 5% to 10% all women. Insulin resistance is highly associated with this syndrome. Insulin resistance is associated with increased oxidation of low density lipoprotein (LDL) cholesterol. Oxidized LDL autoantibody levels are generally measured to evaluate the degree of LDL oxidation. In the present study, we aimed to measure serum titers of oxidized LDL autoantibodies in patients with PCOS.

Twenty-five patients with PCOS, age (mean  $27 \pm 3.5$ ) and BMI matched healthy women were studied. Serum concentrations of oxidized LDL autoantibodies were determined using an enzyme-linked immunosorbent assay from a commercially available test kit (Biomedica, Vienna, Austria) designed to directly determine human autoantibodies to Cu<sup>2+</sup> oxidized LDL in serum. Homeostasis model assessment (HOMA-IR) index was used for insulin resistance.

Mean HOMA-IR was higher in the PCOS group than in the control group (respectively,  $2.84 \pm 1.16$  and  $1.23 \pm 0.58$ ,  $P < 0.001$ ). Oxidized LDL autoantibodies were significantly different in the PCOS group ( $491 \pm 109$  mU/ml) than in the control group ( $268 \pm 86$  mU/ml) ( $P < 0.001$ ). In the PCOS group, oxidized LDL autoantibody was positively correlated with HOMA-IR ( $r = 0.24$ ,  $P < 0.005$ ).

This study suggests that increased oxidized LDL autoantibodies could contribute to the increased risk of cardiovascular disease in patients with PCOS.

### P674

#### Transforming growth factor beta 1 and decidualisation of the human endometrium

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#### Introduction

Decidualisation of the endometrial stromal cell (ESC) is pivotal for successful implantation and is initiated in response to increasing

progesterone levels. Transforming Growth Factor- $\beta$ 1 (TGF $\beta$ -1), a potent cytokine involved in many diverse cellular responses, is activated from the latent form in the mid-late secretory phase. Previous reports suggest that TGF $\beta$ -1 might oppose the action of progesterone, although no mechanism has been proposed. We have investigated whether TGF $\beta$ -1 may have an effect on progesterone receptor (PR) expression or function and inhibit decidualisation.

#### Methods

Quantitative real time PCR (TaqMan) and Promoter-Reporter studies were used to investigate the role of TGF $\beta$ -1 in mediating nuclear PR expression and the decidualisation markers, IGFBP-1 and Prolactin expression, within ESCs decidualised *in vitro*. ESCs were isolated from patients undergoing gynaecological procedures for benign indications. Institutional ethical approval and written informed consent were obtained.

#### Results

TGF $\beta$ -1 (10 ng/ml, 2h) upregulated nuclear PR expression in decidualised ESCs 2-fold ( $P < 0.001$ ) ( $n = 5$ ) however, at 12h, 24h and 36h this upregulation was not seen. At 72 hours the nuclear PR mRNA expression levels were significantly downregulated 2-fold ( $P < 0.05$ ) ( $n = 5$ ). Western blotting and immunocytochemistry validated protein expression. The Promoter-Reporter study demonstrated that TGF $\beta$ -1 does not interfere with the transactivation potential of PR, indicating that the TGF $\beta$ -1 effect on PR expression is due to a decrease in genomic PR. IGFBP-1 and Prolactin mRNA and protein levels are downregulated with addition of TGF $\beta$ -1 indicating that TGF $\beta$ -1 inhibits decidualisation.

#### Summary

We have shown that TGF $\beta$ -1 interacts with progesterone, via its receptor, and could inhibit the decidualisation process. These findings highlight the complexity of interactions controlling the hormonal responses of the endometrial stromal cell.

### P675

#### Evidence for synergy of SHBG and androgen receptor genes in PCOS phenotype

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Polycystic ovary syndrome (PCOS) is a common endocrinopathy with hyperandrogenemia to be its strongest genetically determined characteristic. Our aim was to investigate the potential synergy of two functional polymorphisms: the (TAAAA)n polymorphism of the sex hormone-binding globulin gene (*SHBG*) known to be associated with PCOS and influence serum SHBG levels (longer repeats were associated with lower SHBG levels) and the (CAG)n polymorphism of androgen receptor gene (*AR*) known to affect the transcriptional activity of *AR* (shorter repeats were associated with higher transcriptional activity).

#### Subjects and methods

We studied 180 women with PCOS and 168 healthy women of reproductive age. The body mass index (BMI) was recorded and the hormonal profile was determined on 3–5th day of menstrual cycle. DNA was extracted from peripheral blood leucocytes and the *SHBG*(TAAAA)n and *AR*(CAG)n polymorphisms were genotyped. All subjects gave their consent and the local Ethical Committee approval was obtained.

#### Results

Genotype analysis revealed 6 *SHBG*(TAAAA)n alleles with 6–11 repeats and 19 *AR*(CAG)n alleles with 6–32 repeats. Women with PCOS had a greater frequency of longer *SHBG* alleles ( $> 8$  repeats) than normal women ( $P = 0.001$ ) while there was no difference in the distribution of *AR* alleles between patients and controls. Among patients, those with both long *SHBG* ( $> 8$  repeats) and short *AR* ( $\leq 20$  repeats) genotypes had the lowest SHBG levels ( $P = 0.001$ ) and the highest DHEAS ( $P = 0.001$ ), total testosterone ( $P = 0.03$ ), FAI ( $P < 0.001$ ) and 17 hydroxyprogesterone levels ( $P = 0.03$ ) independently of BMI.

#### Conclusion

Both genes affecting androgen transportation and androgen action act synergistically in PCOS phenotype. Furthermore Individuals with both the *SHBG*(TAAAA)n variants associated with low SHBG levels and *AR*(CAG)n variants associated with increased transcriptional *AR* gene activity are likely to be exposed to excess androgen even during fetal life and this may "programme" their PCOS phenotype in later life.

**P676**

**Factor analysis identifies three independent factors within PCOS**

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**Background**

The polycystic ovary syndrome (PCOS), a condition defined by hyperandrogenism and ovulatory dysfunction, is the most common endocrinopathy in women of reproductive age. Women classified as having PCOS according to the most recent consensus statement are heterogeneous both clinically and biochemically, and are therefore likely to have differing aetiologies, long term sequelae and possible treatment options.

**Aim**

To utilise principal components analysis in a cohort of women with PCOS, aiming to define orthogonal factors associated with the syndrome that may be of use delineating subgroups within PCOS.

**Methods**

A retrospective chart review studying data from a clinic population of 261 women with PCOS, as defined by the 1990 NIH criteria, mean age 29 (± 8.8yrs). SPSS 11.0 was used for analysis.

**Results**

Three factors that explained 61% of variance were recognised. (See table). Factor one includes the components reflective of insulin resistance and obesity, factor two includes hypertension and dyslipidaemia, and factor three includes higher LH:FSH ratio and higher plasma testosterone. Factor analysis results in PCOS\*.

Factor 1	Factor 2	Factor 3
↑ HOMA-IR (0.64)	↑ Systolic BP (0.54)	↑ Testosterone (0.77)
↑ Waist circumference (0.80)	↑ LDL (0.71)	↑ LH:FSH ratio(0.63)
↑ Body mass index (0.79)	↑ Triglycerides(0.72)	
↓ HDL (-0.72)		
↓ SHBG (-0.74)		

\*(Factor loadings >0.5 are reported)

**Conclusion**

Three independent factors within a cohort of PCOS women have been identified using principal components analysis. The findings suggest different pathogenetic entities within this 'syndrome' that may have both prognostic and therapeutic implications for the long-term health outcomes for women with PCOS.

**P677**

**Impaired vascular function in hypogonadal males is unaltered during monthly treatment cycle with intramuscular testosterone esters**

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**Background**

Male hypogonadism is associated with an increased incidence of cardiovascular risk factors and approximately 25% of males suffering with coronary heart disease are hypogonadal. Few studies have assessed the impact of testosterone therapy on vascular function in hypogonadal individuals and none have investigated the weekly impact of gradually reducing testosterone concentrations on vasculature.

**Patients and methods**

10 hypogonadal subjects (6 with 1<sup>0</sup> hypogonadism, 4 with 2<sup>0</sup> hypogonadism) were studied on a weekly basis throughout a monthly treatment cycle with intra-muscular Sustanon 250. Each subject acted as their own control. Subjects with known cardiovascular risk factors, AF and hypopituitarism were excluded. Lipid profiles, glucose, insulin and haematocrit were measured in each subject in addition to sex hormone profiles. Arterial stiffness and central arterial compliance was analysed with pulse wave

analysis (Sphygmocor apparatus) using inhaled salbutamol and GTN as surrogate markers to assess endothelial dependent and independent vasodilatation respectively. Further assessment of endothelial function was determined using flow mediated dilatation in the brachial artery.

**Results**

FMD and the Augmentation Index were unaltered by the gradual reductions in testosterone concentrations (wk 1 35.98 ± 22.21 nmol/l vs wk 2 16.05 ± 8.65 nmol/l, wk 3 11.21 ± 6.14 nmol/l, wk 4 6.32 ± 2.91 nmol/l, P < 0.0005) throughout a monthly treatment cycle with Sustanon. Despite this, FMD in hypogonadal subjects was impaired (8.9 ± 3.0%), compared with previous studies on healthy eugonadal males.

**Conclusion**

Hypogonadal males have impaired vascular reactivity which is unaffected by weekly fluctuation in testosterone concentrations.

**P678**

**Changes in GnRH gene expression in the medial basal hypothalamus of rhesus macaques across the menstrual cycle**

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Rhesus macaques, like humans, express two molecular forms of gonadotropin-releasing hormone (GnRH-I and GnRH-II). Although the role of GnRH-I in the control of the reproductive axis is well-established, the physiological role of GnRH-II is less clear. Both forms of GnRH are highly expressed in the monkey hypothalamus and both forms are highly effective at stimulating LH and FSH release *in vivo*. However, estradiol appears to affect GnRH-I and GnRH-II gene expression differentially, causing a suppression of the former and a stimulation of the latter, raising the possibility that GnRH-I and GnRH-II may have different reproductive roles. To examine this hypothesis, we isolated RNA from the medial basal hypothalamus (MBH) of female rhesus macaques at three different stages of the menstrual cycle (N = 3/group): (1) the early follicular phase, when circulating estradiol and progesterone concentrations are low; (2) the late follicular phase, after the preovulatory estradiol peak while progesterone concentrations are still low; and (3) the mid-luteal phase, when circulating estradiol concentrations are low and progesterone concentrations are high. The samples were subjected to GeneChip microarray analysis (Affymetrix HG-U133A), and the data were analyzed using ANOVA and Newman-Keuls. Although expression of the GnRH-I gene was high in the MBH, it did not change across the menstrual cycle. In contrast, expression of GnRH-II gene in the MBH was low during the early follicular and mid-luteal phases but significantly (P < 0.05) elevated during the late follicular phase. The coincidence of elevated GnRH-II gene expression during the preovulatory rise in circulating estradiol supports the hypothesis that estradiol exerts a positive feedback effect on GnRH-II but not GnRH-I. Moreover, the data give credence to the view that GnRH-II may be the primary GnRH form responsible for triggering the preovulatory LH surge in primates.

**P679**

**Mechanisms of Wolffian duct differentiation; development of a model for studying androgen-driven stromal-epithelial interactions**

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After they are released from the testis, spermatozoa pass into the epididymis where they mature. The epididymis is a single, highly coiled duct that develops from a portion of the embryonic Wolffian duct (WD) under the control of testosterone. We have used a model system in which the androgen receptor (AR) antagonist flutamide is administered to pregnant rats to investigate the cellular mechanisms responsible for androgen-dependent WD differentiation. Time-mated pregnant rats were treated daily from day 15 of pregnancy (e15) with flutamide (50 or 100 mgkg<sup>-1</sup>) or vehicle alone (controls). WDs were recovered from fetuses on e19-e21. The appearance and luminal length of the ducts was recorded at isolation. WDs were immunostained for AR, for specific cell compartment markers (e.g. smooth muscle actin, cytokeratin,

laminin), for proliferation (histoneH3) and apoptosis (cleaved caspase 3). At e20 and e21 (but not at e19) there was a highly significant reduction in both the length, and degree of coiling, of WDs from flutamide-treated mothers compared to controls. This was associated with a significant decrease in proliferation of stromal and epithelial cells but no change in apoptosis. AR was expressed in control and treated WDs; immunostaining was more intense in stromal cell nuclei than in epithelial cells, consistent with the primary site of action of androgens in WD differentiation being the stromal cell. Changes have been identified in a number of structural proteins that may reflect stromal-epithelial interactions to lay down the basal lamina; laminin showed a treatment-related decrease while vimentin epithelial expression increased in WD from flutamide-treated mothers compared to controls.

In conclusion, we have confirmed that androgen action via AR is essential for normal coiling of the WD, that the primary site of androgen action appears to be the stromal cell and that maintenance of the basal lamina appears vital in WD differentiation.

## P680

### Neonatal, but not maternal, plasma lipid profiles are altered in pregnancy in women with polycystic ovarian syndrome (PCOS), compared with weight matched controls

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PCOS is a disorder of chronically abnormal ovarian function and hyperandrogenism, and associated sub-fertility. Insulin resistance is an integral feature of PCOS, particularly in obese women, and non-pregnant women with PCOS exhibit many of the features of the metabolic syndrome, including disturbed lipid metabolism. Treatments to improve conception rates include lifestyle intervention and insulin-sensitising therapy. When a woman with PCOS becomes pregnant she is at increased risk of adverse pregnancy outcome, but it is difficult to determine whether this is independent of obesity. The impact of PCOS on maternal and fetal lipid profiles during pregnancy is unknown. The aim of this study was to assess maternal third trimester and fetal cord blood levels of cholesterol, triglyceride (TG) and high density lipoprotein (HDL) in a BMI-matched case control study ( $n = 21$  per group) of PCOS pregnancy. Fetal samples were available from 10 BMI matched case control pairs. Maternal cholesterol [mean (SD) case vs control 6.46(1.37) vs 6.14(1.47) mmol/L,  $P = 0.48$ ], triglyceride [3.13(1.15) vs 2.80(0.92) mmol/L,  $P = 0.38$ ] and HDL [1.84(0.48) vs 1.73(0.37) mmol/L,  $P = 0.42$ ] did not differ between PCOS and control pregnancies. However, in fetal cord plasma TG was higher [0.75(0.12) vs 0.39(0.04) mmol/L,  $P = 0.017$ ] and HDL lower [0.58(0.13) vs 0.94(0.32) mmol/L,  $P = 0.007$ ] in the offspring of PCOS pregnancy. There was no difference in cord blood cholesterol levels. These data suggest that PCOS women who conceive have normalised lipoprotein metabolism that does not differ from healthy, BMI matched women during pregnancy. On the other hand, offspring of women with PCOS do have altered lipoprotein metabolism suggesting that they are sensitive to metabolic features of the intrauterine environment. PCOS mothers may influence offspring development by mechanisms including insulin sensitivity, genetic inheritance or intrauterine effects upon the embryo and foetus via maternal metabolism and placental transfer of nutrients.

## P681

### Kisspeptin-54 stimulates the hypothalamic-pituitary gonadal axis in human males

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Mutation of the GPR54 receptor is associated with a failure of reproductive function. The endogenous neuropeptide agonist for GPR54, kisspeptin, potently stimulates the hypothalamic-pituitary-gonadal (HPG) axis in rodents and primates.

#### Objective

The present study was designed to determine the effects of elevating circulating kisspeptin levels on LH, FSH and testosterone in male volunteers.

#### Method

Study (i) Volunteers were infused with a 90 minute infusion of kisspeptin-54 (0.125, 0.25, 0.5, 1, 2, 4, 8, 12, 18, 27 or 40 pmol/kg/min,  $n = 3$  per dose). Blood was sampled for LH. Study (ii) Volunteers ( $n = 6$ ) received a 90 minute intravenous infusion of kisspeptin-54 (4 pmol/kg/min) and a control infusion of saline (0.9%) at least 3 days apart in random order. Blood was sampled for kisspeptin, LH, FSH and testosterone measurements. This study was approved by the local Ethical Committee.

#### Results

(i) There was a dose-dependent increase in mean LH over time from an infusion rate of 0.25 pmol.kg<sup>-1</sup>.min<sup>-1</sup> up to 4 pmol/kg/min. The latter dose of kisspeptin-54 was chosen for study (ii) as it was the lowest infusion dose which maximally increased LH levels in male volunteers.

(ii) Kisspeptin-54 infusion significantly increased plasma LH, FSH and testosterone concentrations compared to saline infusion (mean 90 minute LH: kisspeptin 10.8 ± 1.5 vs. saline 4.2 ± 0.5U/l,  $P < 0.001$ ; mean 90 minute FSH: kisspeptin 3.9 ± 0.7 vs. saline 3.2 ± 0.6U/l,  $P < 0.001$ ; mean 180 minute testosterone: kisspeptin 24.9 ± 1.7 vs. saline 21.7 ± 2.2 nmol/l,  $P < 0.001$ ). The plasma half-life of kisspeptin-54 was calculated to be 27.6 ± 1.1 min. The mean metabolic clearance rate (MCR) was 3.2 ± 0.2 ml/kg/min and the volume of distribution (VD) was 128.9 ± 12.5 ml/kg.

#### Conclusion

Elevation of plasma concentrations of kisspeptin in human males significantly increases circulating LH, FSH and testosterone levels. Kisspeptin infusion provides a novel mechanism for HPG axis manipulation in disorders of the reproductive system.

## P682

### Effect of chronic tadalafil administration on penile hypoxia induced by cavernous neurotomy in the rat

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#### Objectives

Radical prostatectomy is an effective therapy for clinically localized prostate cancer. A significant number of men develops post-prostatectomy erectile dysfunction (PDED), due to surgery-related nervous damage. PDED is relatively refractory to PDE5i therapy. In a rat model of bilateral cavernous neurotomy we evaluated whether chronic tadalafil treatment (CTT) could ameliorate anatomical and functional damage to corpora cavernosa (CC).

#### Methods

Tadalafil (2 mg/Kg/daily) was added in drinking water in a subgroup of neurotomy rats (CTT). After 3 months, penile tissues were removed and hypoxia and muscular/fibrous ratio revealed using semi-quantitative immunohistochemistry with hypoxyprobe<sup>TM</sup> and Masson staining, respectively. Endothelin receptor B (ETB), PDE5, nNOS and eNOS expression and functional activity were also studied.

#### Results

Penile denervation induced massive hypoxia and a decreased muscular/fibrous ratio, which were completely restored by CTT. Functional studies indicated that hypoxic tissues were hypersensitive to the relaxant effect of the ETB agonist IRL-1620, due to the previously described hypoxia-induced over-expression of ETB (Granchi et al., Mol Hum Reprod 8:1053, 2002; Filippi et al., Mol Hum Reprod 9:765, 2003). CTT restored normal sensitivity to IRL-1620, and normalized ETB gene (real-time RT-PCR) and protein (Western) expression. Hypoxic CC were more sensitive to the relaxant effect of the NO-donor sodium nitroprusside (SNP), while they were unresponsive to acute tadalafil (100 nM) amplification of SNP effect. According to these findings, PDE5 mRNA and protein expression were reduced in neurotomy penile tissue. By restoring PDE5, CTT decreased SNP-induced relaxation and rescued sensitivity to acute tadalafil (100 nM). However, in hypoxic CC, CTT was unable to normalize other observed events, as acetylcholine hypo-responsiveness or decreased nNOS and eNOS, at both mRNA or protein levels.

#### Conclusion

CTT restores several (but not all) of the neurotomy-induced penile alterations, including PDE5 expression and *in vitro* responsiveness to PDE5 inhibitors, such as tadalafil.



**P683**

**Neuromedin U (NMU) and Neuromedin S (NMS) function as putative regulators of the gonadotrophic axis in the rat**

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Neuromedin U (NMU) is a brain-gut peptide originally isolated from porcine spinal cord, and later found in other species. NMU acts through two receptors named NMU1R (abundant in peripheral tissues) and NMU2R (apparently restricted to specific brain regions). Besides its potential implication in the control of stress responses, NMU is abundantly expressed in the ventromedial hypothalamic area and has been involved, as satiety factor, in the regulation of food intake. Very recently, a novel neuropeptide, structurally related to NMU, has been identified in rat brain, and termed neuromedin S (NMS). NMS shares its C-terminal region with NMU and acts through the same receptors. NMS has been reported as potent anorexigenic factor in the hypothalamus, and it may play a role in the regulation of circadian rhythms.

A wealth of data has now demonstrated that a large number of regulators of feeding behaviour (e.g., leptin, ghrelin and orexin) are also implicated in the control of the gonadotrophic axis, thereby contributing to the joint regulation of energy balance and reproduction. However, the implication of NMU and NMS in the regulation of gonadotropin secretion remains so far scarcely evaluated. In the present study, we analyzed the effects of NMU and NMS on LH secretion, as well as hypothalamic NMUR2 mRNA expression, in different experimental models. Intracerebral administration of NMU or NMS stimulated basal LH secretion in peripubertal male and female rats, as well as in cyclic females at diestrous (i.e. low levels of circulating LH). On the contrary, NMU failed to alter the stimulated levels of LH at the afternoon of proestrus (at 18:00), while it decreased the elevated LH levels in male rats after orchidectomy or KiSS-1 injection. In addition, expression analyses revealed that NMU2R mRNA significantly varied during postnatal sexual development and along the estrous cycle. In summary, our present data substantiate the potential role of NMU and NMS as novel regulators of gonadotropin secretion, and suggest the potential implication of these neuropeptides in the joint control of energy balance and reproductive function.

**P684**

**Circadian changes in melatonin secretion and its relationship to gonadotropins in premenopausal women**

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Through its circadian pattern of melatonin (MT) secretion, the pineal gland helps to keep the internal physiology of animals in synchrony with the day-night cycle and with the annually changing seasons.

Currently, however, the influence of MT on the human reproductive axis appears to be marginal and the available evidence supporting its physiological role is largely circumstantial; there is no clear consensus whether MT levels change during the menstrual cycle.

**Objective**

The present study evaluated the diurnal rhythm both of melatonin and gonadotropins in healthy women, in order to clarify the implications of circadian neuroendocrin axis.

**Methods**

Hormone measurements were performed in early follicular phase, ovulation and midluteal phase. aMT6s, a reliable index of MT secretion and gonadotropins, LH and FSH were assayed in urine samples sequentially collected for 30–36 h. The reproductive hormones were measured in blood samples prelevated at 8 a.m.

**Results**

As expected, a marked 24-h variation in MT secretion was found during menstrual cycle. Cosinor analysis of circadian profiles of aMT6s showed discrete changes among phases of menstrual cycle. Mean of amplitudes at ovulation showed a rise at limit significance (F:  $11.62 \pm 2.49$ ; O:  $18.08 \pm 5.63$ ; L:  $15.5 \pm 3.58$ ). Melatonin and gonadotropin secretion showed a positive correlation in early follicular phase and negative one in ovulation. Gonadotropins, LH and FSH, exhibited an evident diurnal rhythm only in ovulation.

**Conclusions**

The data suggest the hypothesis that melatonin induces chemical changes in secretion of reproductive hormones that respond to light/dark cycles for

keeping the balance of reproductive axis. Melatonin secretion positively correlated with gonadotropin secretion in early follicular phase could be explained by a synergic action with gonadotropins, necessary for normal follicular development. At ovulation, melatonin secretion negatively correlated with rising levels of gonadotropin secretion may exert a tonic negative feedback effect for the ovulatory stimulus.

**P685**

**Serum levels of PSA do not change in healthy pre-menopausal and in menopausal women, but are increased in subjects with polycystic ovary syndrome (PCOS)**

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**Background/aims**

Prostatic specific antigen (PSA) is the best prostatic tumor marker in man; Recently PSA has been detected in a variety of tissues and fluids in women and is considered a marker of hyperandrogenism. However no precise information are available about the physiology of PSA in females. The goal of this study was to assess serum concentrations of PSA in healthy pre-menopausal women (healthy pre-menopausal group) and menopausal women (menopause group) and compare them with affected by a specific kind of hyperandrogenism such as polycystic ovary syndrome (PCOS group).

**Methods**

PSA, androgens, LH, FSH, 17-b-Estradiol (E2), Progesterone (Pg), were assessed by measurement in 40 post-menopausal women and 35 fertile controls. Thirty-five women with PCOS were also included in the study.

**Results**

No significant difference in PSA concentrations could be demonstrated in different phases of the menstrual cycle in healthy pre-menopausal group and between subjects in pre- and post-menopausal groups. No correlations could be demonstrated between serum PSA levels and the following parameters: Age, BMI, LH, FSH, E2, T, DHEAS, and SHBG, both in pre- and post-menopausal women. In the PCOS group, all the subjects showed a significantly higher PSA levels (median = 14 pg/ml) as compared to pre-menopausal (median = 5 pg/ml) and menopausal (median = 5 pg/ml) groups, as well as increased androgen concentrations.

**Conclusions**

Only minor fluctuations of serum PSA concentrations are observed in the healthy state, while serum level is higher in PCOS. PSA can be considered a valid marker of female diseases such as pathological hyperandrogenism.

**P686**

**Expressions of KISS1 and GPR54 are differentially regulated by estradiol and GnRH in adult female rat pituitary**

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Expressions of *KISS-1* and *GPR54* have been demonstrated in a variety of tissues, including pituitary. Given their role in reproduction and in gonadotrophin secretion, we examined the regulation of their expression by steroids and GnRH in the rat pituitary using real-time PCR procedure.

After ovariectomy (OVX), pituitary *KISS-1* mRNA levels dramatically decrease whereas *GPR54* transcripts slightly increase compared with intact cycling rats in metestrus. These variations are reverted *in vivo* by E2 replacement. In addition, activation of ER $\alpha$  by the selective ligand PPT mimics this effect. By contrast, activation of ER $\beta$  by the selective ligand DPN does not stimulate *KISS1* expression while it suppresses the ovariectomy-induced increase of *GPR54* mRNAs.

E2 acts on the hypothalamus and the pituitary gland. Indeed, ovariectomy increases and estradiol replacement restrains, both GnRH pulse frequency and amplitude. To determine whether estradiol acts on the pituitary directly or indirectly via hypothalamic GnRH secretion, OVX rats were treated with a GnRH antagonist or GnRH antagonist plus estradiol. GnRH antagonist administration results in a slight decrease in both *KISS1* and *GPR54* mRNAs suggesting that GnRH stimulates both *KISS1* and *GPR54* expression. When E2 was added, *KISS1* transcripts increased while that of *GPR54* decreased, demonstrating that estradiol acted also at the pituitary level. However, the result observed for *KISS1* is conflicting with that observed after ovariectomy, a condition in which hypothalamic GnRH



secretion was increased. Taken together, these data suggest that results observed under GnRH antagonist are related on estrogen deprivation.

In conclusion, we clearly demonstrated that *KISS1* and its cognate receptor *GPR54* are expressed in the pituitary. Estrogens have a main role on the regulation of *KISS1* gene expression via ER $\alpha$  signalling pathway. *GPR54* expression is regulated by estradiol and GnRH in a manner which appears to parallel that of LH $\beta$ .

## P687

### Anti-Mullerian hormone (AMH) production by and amh type-II receptor (AMHRII) in normal human ovaries

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AMH, the fetal testicular sexual differentiative factor, is now implicated in adult ovarian function. Antral follicle immunohistochemistry demonstrated AMH protein and message, the staining peaking around 4 mm. Interestingly, AMH-knockout mice have increased FSH sensitivity. Our aim was to measure AMH in follicular fluid and cell-conditioned medium and AMHRII in normal ovaries from women undergoing TAH/BSO.

Follicles were dissected intact, follicular fluid aspirated and granulosa and theca cultured +/- gonadotrophins. Granulosa luteal cells (GLC) were harvested from women undergoing IVF. AMH in fluid and media was measured by ELISA (DSLabs). Characterisation of the assay for media use included: parallel dilution of samples with the standard curve, good recovery and no loss with repeated freeze/thaw cycles. The detection limit was 0.025 ng/ml. AMHRII expression was assessed by PCR of RNA extracted from granulosa, GLC and theca cells.

Follicular fluid AMH concentrations were mean (range) 4 (0.3–16) ng/ml ( $n = 18$ ). Levels declined exponentially with increasing follicle size, being undetectable in follicles >9 mm. AMH in granulosa-conditioned medium ranged from undetectable to 1.7 ng/ml ( $n = 17$ ) with levels again falling with follicle size: mean at 5 mm = 1.47, mean at 10 mm = 0.17 ng/ml and undetectable above 10 and in GLC. Levels in stroma and theca were below or at the detection limit. Incubation with FSH or LH (5 ng/ml) had no consistent effect. AMHRII was present in granulosa, GLC and theca.

In summary, the DSLabs AMH ELISA is suitable for use with ovarian cell culture medium. AMH is produced primarily by granulosa cells in the human ovary and there is a rapid decline in production as follicles approach 10 mm in diameter. These data add further weight to AMH having an important role in follicle selection. As all compartments of antral follicles express the receptor, further elucidation of the action of AMH in the ovary is now essential.

## P688

### Identification and functional study of a new FSH receptor gene mutation in women affected by polycystic ovary syndrome (PCOS)

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Polycystic ovary syndrome (PCOS) is one of the most common endocrine diseases in women, affecting up to 10% of women in reproductive age. The evidence of polycystic ovaries at ultrasonography has been recently considered as a further criteria to diagnose PCOS. In order to determine whether FSH receptor gene affects hormonal and/or metabolic parameters of PCOS women the presence of FSH receptor gene mutations was investigated. Forty women aged between 18 and 40 years with polycystic ovaries, body mass index (BMI) >25 kg/m<sup>2</sup>, testosterone >2.5 nmol/L, sex hormone binding globulin (SHBG) <30 nmol/L, oligoamenorrhea and/or hirsutism were included in this study. All of them had a normal development of secondary sexual characteristics and a normal karyotype (46,XX). Pelvic ultrasonography showed polycystic ovaries of abnormal size. After genomic DNA extraction from peripheral-blood lymphocytes of the women, exons 1–10 of the FSH receptor gene were amplified by PCR by specific primers, and directly sequenced. The genetic analysis showed a mutation of the FSHr in heterozygous state located in exon 10 of the receptor (T411D) in one patient. The parents had only wild-type sequence. The T411D mutation was

not present in 30 normal subjects. The functional study of the identified FSHr mutation was performed. The mutated FSHr was obtained by site-directed mutagenesis. COS-7 cells transfected with the wild type FSHr (500 ngr/mL) and the mutated receptor (500 ngr/ml) were used to determine cAMP production after stimulation with increasing concentrations of hrFSH (1–5000 mU/mL) or hrCG (0.1–300.000 mU/mL). The T411D FSHr mutant did not show a different cAMP production in response to hrFSH stimulation with respect to the wtFSH. Very high concentrations of hCG were not able to stimulate cAMP production in COS-7 cells transfected with the mutated receptor.

#### Conclusion

We identified a FSHr mutation in a patient with PCOS. Transfection studies in eukaryotic cells did not show any modification of the functional properties of the mutated receptor with respect to the wild type.

## P689

### Plasma kisspeptin is a novel tumour marker in patients with gestational trophoblastic neoplasia

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Kisspeptin is a 54 amino acid peptide, encoded by the anti-metastasis gene *KiSS-1*, that activates the G-protein-coupled receptor, GPR54. The kisspeptin/GPR54 system is critical to normal reproductive development. *KiSS-1* gene expression is increased in the human placenta in normal and molar pregnancies. Circulating kisspeptin is dramatically increased in normal pregnancy but levels in gestational trophoblastic neoplasia (GTN) have not previously been reported. The objective of this study was to determine if plasma kisspeptin levels are altered in patients with malignant GTN. Method: Thirty nine blood samples were taken from 11 patients with malignant GTN at presentation, during and following chemotherapy. A single blood sample was taken from normal female volunteers ( $n = 11$ ). Plasma kisspeptin immunoreactivity (-IR), human chorionic gonadotrophin (hCG), progesterone and oestradiol concentrations were measured. Reverse-phase FPLC chromatography was used to further analyze kisspeptin-IR extracted from plasma by Sep-Pak cartridge. This study was approved by the local Ethical Committee.

#### Results

Plasma kisspeptin-IR in healthy females was <2 pmol/L. Plasma kisspeptin-IR and hCG concentrations in patients with malignant GTN were elevated at presentation and fell during and following treatment with chemotherapy in each patient (mean plasma kisspeptin-IR: pre-chemotherapy 1363  $\pm$  1076 pmol/L vs. post-chemotherapy <2 pmol/L,  $P < 0.0001$ . Mean plasma hCG: pre-chemotherapy 227191  $\pm$  152354 U/L vs. post-chemotherapy 2U/L,  $P < 0.0001$ ). Plasma kisspeptin-IR strongly positively correlated with plasma hCG levels ( $r^2 = 0.99$ ,  $P < 0.0001$ ). Plasma kisspeptin-IR showed significant positive correlations with circulating levels of progesterone ( $r^2 = 0.92$ ,  $P < 0.0001$ ) and oestradiol ( $r^2 = 0.70$ ,  $P < 0.0001$ ) as did hCG ( $r^2 = 0.89$ ,  $P < 0.0001$  for progesterone and  $r^2 = 0.64$ ,  $P < 0.0001$  for oestradiol). In each plasma extract the kisspeptin-IR eluted in a single peak corresponding to the elution position of synthetic kisspeptin-54.

#### Conclusion

Our results suggest that measurement of plasma kisspeptin-IR may be a novel tumor marker in patients with malignant GTN.

## P690

### Chronic subcutaneous administration of kisspeptin-54 causes testicular degeneration in adult male rats

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The kisspeptins are *KiSS-1* gene-derived peptides which signal through the G protein coupled receptor 54 (GPR54), and have recently been shown to be critical regulators of reproduction. Acute intracerebroventricular (ICV) or peripheral administration of kisspeptin to rodents and primates, and peripheral administration to humans, stimulates the hypothalamic-pituitary gonadal (HPG) axis. This effect is thought to be mediated via the hypothalamic gonadotrophin-releasing hormone (GnRH) system. Chronic

administration of GnRH agonists paradoxically suppresses the HPG axis following an initial agonistic stimulation. We investigated the effects of chronic kisspeptin administration in adult male rats. Initially we compared the effects of acute subcutaneous administration of equimolar doses of kisspeptin-10, -14 and -54 on the HPG axis. Kisspeptin-54 produced the greatest increase in plasma luteinizing hormone (LH) and total testosterone at 60 mins post injection and was consequently used in the subsequent chronic administration experiment. Kisspeptin-54 at 50 nmol per day was administered subcutaneously to adult male rats using Alzet<sup>®</sup> osmotic minipumps. Chronic subcutaneous administration of 50 nmol kisspeptin-54 per day for 13 days significantly decreased testicular weight. Histological examination showed degeneration of the seminiferous tubules, with varying degrees of maturation arrest, sloughing and death of germ cells, focally with complete loss of germ cells and degeneration of residual Sertoli cells. There were no measurable differences in Leydig cell morphology. The testicular degeneration was associated with a significant decrease in the circulating levels of the testes-derived hormone, inhibin B. Free and total testosterone were also lower in the kisspeptin-54 treated group, though this change did not reach statistical significance. Chronic administration of GnRH agonists can produce similar testicular effects. These findings indicate that kisspeptin may provide a novel tool for the manipulation of the HPG axis and spermatogenesis.

### P691

#### Oxidative stress, endothelial function, and arterial stiffness in young patients with polycystic ovary syndrome

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Polycystic Ovary Syndrome (PCOS) is currently believed a common endocrine-plurimetabolic disorder often associated to insulin resistance, diabetes mellitus, hypertension, hyperandrogenism, dyslipidemia and obesity, pathological conditions predisposing to high cardiovascular risk. Oxidative stress and endothelial dysfunction could represent early mechanisms of the cardiovascular risk in patients with PCOS. In order to confirm this hypothesis, 25 young (23.8 ± 1.3 yr), over-weight/obese (31.5 ± 1.5 Kg/m<sup>2</sup>) PCOS women and 16 healthy age- and body mass index (BMI)-matched controls were enrolled. Blood pressure, oxidative stress markers (Ferric Reducing Ability of Plasma, FRAP; Lipoperoxides, LOOH; Malonaldehyde, MDA), endothelium dependent (post-ischemic flow mediated dilation, FMD) and independent vasodilation (response to sublingual nitroglycerine, NTG) in brachial artery and peripheral vascular stiffness (Augmentation Index, AIX, and pulse wave velocity, PWV), were investigated in both groups. Results obtained in PCOS women did not significantly differ from those obtained in controls concerning blood pressure (122.4 ± 2.1/80.1 ± 1.6 vs 123.1 ± 4.7/84.4 ± 4.0 mmHg), oxidative stress markers (FRAP: 736.5 ± 31.1 vs 639.6 ± 32.0 μmol/l; LOOH: 3.7 ± 0.37 vs 3.2 ± 0.55 μmol/l; MDA: 2.56 ± 0.5 vs 2.0 ± 0.24 μmol/l), endothelial function (FMD: 8.5 ± 0.7 vs 8.95 ± 0.76%; NTG: 11.7 ± 0.98 vs 13.17 ± 1.21%) and peripheral vascular stiffness (AIX: 6.72 ± 1.71 vs 8.21 ± 3.02%; PWV: 5.61 ± 0.13 vs 6.04 ± 19%). Our results indicate that young and over-weight/obese patients with PCOS show, despite an adverse clinical assessment, a haemodynamic picture, oxidative stress profile, conduit vascular reactivity and arterial stiffness comparable to those of age- and BMI- matched controls, suggesting that these patients are not exposed to an early and higher cardiovascular risk, at least in young age.

### P692

#### Aldosterone and renin plasma levels in young patients with polycystic ovary syndrome

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Polycystic Ovary Syndrome (PCOS) is the most common endocrine disorder in women of reproductive age often associated with insulin resistance, diabetes mellitus, hyperandrogenism, dyslipidemia, obesity and hypertension, pathological conditions predisposing to high cardiovascular risk. Insulin resistant subjects may have higher plasma renin activity and it is well known

that aldosterone plays a well-recognized role in the pathophysiology of hypertension and in the development of cardiovascular diseases. In order to evaluate this hypothesis, 25 young (23.8 ± 1.3 yr), over-weight/obese (31.5 ± 1.5 Kg/m<sup>2</sup>) PCOS women and 16 healthy age- and body mass index (BMI)-matched controls were enrolled. Blood pressure, renin-angiotensin-system parameters (Plasma Renin Activity, PRA and plasma Aldosterone) and androgens profile were investigated in both groups after a seven days hyposodical-normocaloric diet. Insulin, glucose, and lipid profile were also evaluated and the Homeostasis Model Assessment of insulin Resistance Index (HOMA-IR) was calculated. Results obtained in PCOS women did not significantly differ from those obtained in controls concerning blood pressure (122.4 ± 2.1/80.1 ± 1.6 vs 123.1 ± 4.7/84.4 ± 4.0 mmHg), circulating renin-angiotensin-system (PRA: 3.7 ± 0.5 vs 3.0 ± 0.6 ng/ml/h; Plasma Aldosterone: 21.1 ± 2.5 vs 27.9 ± 5.3 ng/dl) and PRA/Aldosterone rate was 6.85 (nv < 70). Our results indicate that young and over-weight/obese patients with PCOS, despite an adverse clinical assessment, show similar concentrations in renin and aldosterone plasma levels comparable to those of age- and BMI- matched controls, non positive correlations were found between HOMA, BMI and androgens plasma levels and renin, aldosterone and renin-aldosterone rate. These data suggest that these patients are not exposed to an early and higher cardiovascular risk, at least in young age.

### P693

#### Association between polymorphisms within the SUR1/Kir6.2 gene region and polycystic ovary syndrome: a case-control study

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Hyperinsulinaemia has an important role in the aetiology of Polycystic Ovary Syndrome (PCOS). Logical candidate genes for the development of PCOS therefore include those genes that influence β-cell insulin secretion such as *ABCC8* and *KCNJ11* on human chromosome 11. These two genes encode the SUR1 and kir6.2 components of the β-cell KATP channel respectively. To our knowledge, these genes have not been studied in women with PCOS previously.

The objective of these genetic analyses was to establish whether alleles of 6 tag Single Nucleotide Polymorphisms (SNPs) occurring within haplotype block 5 of the SUR1/Kir6.2 gene region are associated with PCOS susceptibility. These tag SNPs are located within the genes *ABCC8*, *KCNJ11* and *NUCB2*. (The tag SNP, rs5219 responsible for the E23K polymorphism within *KCNJ11* is currently being genotyped). A case-control association analysis was performed on a UK population comprising 374 cases and 540 control subjects (all of British/Irish origin). The main outcome measures were genotype frequencies between cases and controls. The Kruskal-Wallis  $\chi^2$  method was used to test for genotypic associations. All SNPs were in Hardy-Weinberg equilibrium. There were no significant differences in genotype distributions between the case and control groups, a significant *P*-value being defined as less than 0.05 (*ABCC8* rs2074310, *P* = 0.33; *ABCC8* rs2067043, *P* = 0.09; *NUCB2* rs1073443, *P* = 0.87; *KCNJ11* rs1800467, *P* = 0.86; rs1557764, *P* = 0.05; rs2354867, *P* = 0.06). The distribution of haplotypes in the cases was nominally significantly different from that in the controls (*P* = 0.015). The main haplotype driving this result was CTGCC which was reduced in frequency in the cases (0.232 versus 0.284 in controls). The proportion of haplotypes captured using the 6 tag SNPs was 96%. These results suggest that polymorphisms within the 6 tag SNPs located within haplotype block 5 of the SUR1/Kir6.2 gene region are not strongly associated with PCOS.

### P694

#### Effect of family history of type 2 diabetes on metabolic status of women with polycystic ovary syndrome

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#### Background and aims

Polycystic ovary syndrome (PCOS) and the metabolic syndrome (MS) have many features in common and may share the same pathogenesis. We

examined the prevalence of the MS according to the ATP III criteria and the effect of family history for DM 2 on metabolic profile in young women with PCOS.

#### Subjects and methods

We studied 75 women with PCOS aged ( $23.9 \pm 5.4$ ) and 75 healthy age-matched women. We measured BMI, WHR and blood pressure (BP). After an overnight fast and hormone, lipid and glucose blood levels were determined. Homeostasis model assessment score of insulin sensitivity (HOMA-IR) and free androgen index (FAI) were calculated. Women with impaired glucose tolerance or DM 2 were excluded from the study. If one of the parents suffered from DM 2 this was considered a positive family history (FHP).

#### Results

Subjects and control women were similar in age, BMI and smoking habits. However, PCOS subjects had significantly higher WHR ( $0.79 \pm 0.07$  vs  $0.75 \pm 0.04$ ,  $P = 0.001$ ), HOMA-IR ( $3.2$  vs  $1.9$ ,  $P < 0.001$ ) and FAI ( $10.5$  vs  $2.8$ ,  $P < 0.001$ ). Additionally, patients with PCOS had higher Total ( $187 \pm 35$  vs  $178 \pm 28$ ,  $P = 0.02$ ) and LDL-cholesterol ( $118 \pm 35$  vs  $103 \pm 24$ ,  $P = 0.01$ ) and lower HDL-cholesterol levels ( $49 \pm 14$  vs  $61 \pm 13$ ,  $P < 0.001$ ) compared to control women. The prevalence of MS and FHP by  $\chi^2$  test was higher in PCOS women than controls ( $21.3\%$  vs  $5.3\%$ ,  $P = 0.004$ ) and ( $32\%$  vs  $12\%$ ,  $P = 0.02$ ) respectively.

Multiple regression analysis showed that apart from age ( $\beta = 0.28$ ,  $P < 0.001$ ), MS was also positively associated with HOMA-IR ( $\beta = 0.66$ ,  $P < 0.001$ ) and FAI ( $\beta = 0.43$ ,  $P < 0.001$ ). Logistic regression analysis revealed that a FHP was associated with an increased risk for MS (OR = 2.41,  $P < 0.01$ ).

#### Conclusions

MS and a FHP of DM 2 are present with significantly higher frequency in young women with PCOS. FHP has a substantial impact on the metabolic profile of these women and can be used as a predicting factor for the MS.

### P695

#### A review of cimetidine (tagamet) effects as a reproductive toxicant in male rats prostate and seminal vesicle

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Cimetidine, an H2 receptor antagonist, has been shown to be clinically valuable in the treatment of peptic ulcers. Recently, the effect of cimetidine on the reproductive system has been studied in detail and there are some reports showing the untoward effects of cimetidine on this system. In this paper was undertaken to evaluate the effect of cimetidine on serum testosterone, testes, prostate, seminal vesicle and vas deferens. Apart from the above objective, the mechanism through which cimetidine can affect sperm motility and count was also sought. Oral repeated dose studies in rats at dosage levels of 150, 378 and 950 mg/kg cimetidine for periods up to 12 months have been reported. Few adverse effects were noted at all dose levels and no significant differences between the groups were observed in body weight, food consumption, blood chemistry, urinalysis. The livers of the 950 mg/kg dose group were heavier than those of controls. This was attributed to increased metabolic work load. After 12 months dosing there was a reduction in the size of prostates of the rats in all dosed groups and a reduction in the size of testes and seminal vesicles of the top dose group. No histopathological abnormalities were attributable to cimetidine treatment.

### P696

#### A review of male rat genital system morphology followed cimetidine injection

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The development of genital organs of rats chronically treated with cimetidine showed that the drug may present anti-androgenic activity. Rats were treated i.p. with cimetidine at a dose of 50 mg/kg body weight for 59 days. Accessory sex organ weights, were significantly reduced in the high dose treated groups. A high degree of variability characterized testis histology, with most tubules appearing normal and some tubules (15–17%) partially lacking or devoid of germ cells. Morphometry showed that although seminiferous tubule volume was not significantly changed, the volume of peritubular tissue was reduced in the high dose group. There was extensive duplication of the basal lamina,

lamina densa in both apparently normal spermatogenic tubules and severely damaged tubules. Apoptotic peritubular myoid cells were also found. Peritubular cells are lost from the testis, it is suggested that the primary event in cimetidine-related damage is targeted to testicular smooth muscle cells. Since no change in serum testosterone levels was verified in cimetidine-treated rats, the authors could not exclude the possibility that besides an antiandrogenic effect, other biochemical factors necessary for normal spermatogenesis could be involved in the testicular alterations. This is the first *in vivo*-administered toxicant to be described that targets myoid cells, resulting in abnormal spermatogenesis.

### P697

#### A review of cimetidine (tagamet) effects as a reproductive toxicant in male rats leydig cells

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Cimetidine is a selective antagonist of histamine at gastric H<sub>2</sub>-receptor sites. A 24 month oral cimetidine toxicity and carcinogenicity study in rats at dose levels of 150, 378 and 950 mg/kg was performed. Cimetidine appeared to produce changes involving the liver and the male reproductive organs. In this paper non-tumour Leydig cell morphology was normal in cimetidine treated groups. It was concluded that the higher statistically significant incidence (apparent only in aged rats) of benign Leydig cell tumours seen in cimetidine treated groups compared to controls probably represented a chance occurrence and did not imply a tumorigenic risk for man.

### P698

#### Functional restoration of intact rabbit ovary after cryopreservation and transplantation

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#### Objective

Ovarian cryopreservation is an emerging technique that may advance breeding of endangered species and preservation of reproductive capacity in young women cancer patients who will undergo chemo/radiotherapy destruction of ovarian tissue.

#### Materials and methods

A total of twelve mature female rabbits were used in experiment. Rabbits were anesthetized and then subjected to laparotomy. Bilateral oophorectomy was used to save one ovary as fresh control and the other as experiment in each rabbit. The ovary to be experimented was isolated with retained ovarian vessels 2.5 cm from the ovarian hilum and embedded in a wedge of mesenteric fat after dissection of soft tissue and ligation of peripheral vascular connections. The ovarian artery was cannulated and perfused with cryoprotectant. The freezing protocol was based on a slow freezing rate to store intact ovary at  $-196^{\circ}\text{C}$  and rapid thawing rate by perfusion cryoprotectant (CPA) of 1.5M dimethylsulfoxide (DMSO) and reversal concentration gradient respectively. Each rabbit was grafted one intact cryopreserved ovary in groin area by vascular anastomosis. Ovarian function was evaluated by vaginal cytology, hormone assays and ovarian biopsy until 6 months later.

#### Results

Fully 83.3% of rabbits had ovarian function as shortly as one week after transplantation. Mean survival rate of primordial follicles is  $74.9 \pm 4.5\%$  after whole ovarian cryopreservation and 6 month post-transplantation. Cases of failure to regain ovarian function showed cracking of mesenteric fat after ovarian freezing, in contrast to functional ovaries, which had intact mesenteric fat. Cracking of mesenteric fat after the freezing process seems to affect the architecture of the graft complex and thus may be used to predict adverse freezing outcome.

#### Conclusions

By microvascular manipulation and anastomosis, cryopreservation of an intact ovary followed by transplantation may overcome revascularized ischemia and last reasonable graft longevity, which supports the promising role of whole ovarian cryopreservation.



**P699**

**Effects of metformin therapy in lean subjects with PCOS on insulin resistance indices**

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**Objective**

Polycystic ovary syndrome (PCOS) is closely associated with insulin resistance (IR) and about 50–70% of women with PCOS have significantly increased IR. Our study was performed to determine the effects of metformin on IR indices with lean PCOS patients.

**Methods**

Our study was a single center, randomized prospective study, approved by the local ethical committee. 53 patients with BMI <25 kg/m<sup>2</sup> who had chronic anovulation and hyperandrogenism without any other specific causes of adrenal or pituitary disease and also met the diagnostic criteria for PCOS of the NIH Consensus, were considered to be eligible for the study. Patients were treated with 850 mg metformin twice daily for 24 weeks.

**Results**

Various indices of insulin sensitivity were used. Fasting insulin, Raynaud index, HOMA-IR and FIRI decreased significantly after treatment with metformin ( $P < 0.05$  for all). Fasting Belfiore index, QUICKY index, ISI HOMA and FIRI<sup>-1</sup> increased significantly at the end of 24 weeks of treatment period ( $P < 0.05$  for all).

**Conclusion**

In conclusion, even in lean patients with PCOS, metformin treatment provided a significant improvement in IR. Therefore metformin should be considered an integral part of therapy also in lean PCOS patients.

**P700**

**Comparison of various insulin sensitivity indices in idiopathic hirsutism**  
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**Objective**

Hirsutism, is characterized by excessive growth of terminal hair in a male pattern. Idiopathic hirsutism (IH) is a common cause of hirsutism. Since there are few data demonstrating IH is associated with insulin resistance we tried to assess various insulin sensitivity indices in lean IH and compare with healthy subjects.

**Methods**

A cross-sectional study was performed in 71 lean (BMI between 20 and 25 kg/m<sup>2</sup>) women (17–39 years old), 31 with IH and 40 healthy individuals. Blood glucose, insulin, HOMA-IR, hepatic insulin sensitivity (ISI<sub>HOMA</sub>), Quicky index, reciprocal fasting insulin resistance index, fasting Belfiore index, and fasting glucose/insulin ration (GIR) were estimated using a single fasting sample of glucose and insulin levels. Raynaud index calculated using the mathematical estimation in a single fasting sample of insulin levels were determined and compared in two groups.

**Results**

Fasting insulin, Raynaud index, HOMA-IR and FIRI results were higher in IH group than in controls ( $P < 0.01$ , for all). Fasting Belfiore index, QUICKY index, ISI HOMA and FIRI<sup>-1</sup> results were lower in IH group than in controls ( $P < 0.01$ , for all).

**Conclusion**

Our study showed that IH patients were more insulin resistant than healthy subjects. We propose that insulin sensitivity indices are useful methods for measuring insulin resistance in IH.

**P701**

**Metformin therapy decreases hyperandrogenism and hyperinsulinaemia in women with polycystic ovary syndrome**

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Insulin resistance has been suggested to have a pathogenic role in polycystic ovary syndrome (PCOS). The use of insulin sensitising drugs might therefore have a therapeutic role in its management.

We studied 20 patients (mean age 32.6 years) with PCOS (diagnosed by the Rotterdam Consensus Workshop group criteria, 2003) before and after treatment with metformin (1.5–3 grams daily) for 6 to 12 months.

Clinical features studied were menstrual pattern, body mass index (BMI) and hirsutism by means of Ferriman & Gallwey (F&G) scores.

Biochemical parameters included fasting glucose, insulin, lipid profile, total testosterone, FSH, LH, SHBG, DHEAS, androstenedione, basal and ACTH stimulated 17-hydroxyprogesterone levels. Free and bioavailable testosterone levels were calculated using a software programme available from [www.issam.ch/freetesto.htm](http://www.issam.ch/freetesto.htm).

Insulin resistance and sensitivity% were determined using the homeostatic model assessment (HOMA 2) software programme available from [www.dtu.ox.ac.uk](http://www.dtu.ox.ac.uk).

Menstrual cycles became regular in 15 patients (75%). F&G significantly improved ( $P = 0.009$ ). There was no significant change in BMI. Significant reductions were recorded in fasting glucose, insulin, total testosterone, LH, free testosterone, bioavailable testosterone and insulin resistance (all  $P < 0.05$ ). Insulin sensitivity % also significantly improved ( $P < 0.05$ ). There were no significant changes in lipid profiles, FSH, SHBG, androstenedione, DHEAS and 17 hydroxyprogesterone levels. There was a significant positive correlation between the fall in insulin levels and basal BMI, glucose and insulin levels ( $r = 0.79, 0.77, \text{ and } 0.91$  respectively).

This study suggests a useful role for metformin in the management of PCOS. Improvement in clinical features and hyperandrogenism paralleled improvements in insulin sensitivity.

**P702**

**A unique subgroup of patients with polycystic ovary syndrome identified by clinical and biochemical features**

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Hyperandrogenism of adrenal origin evidenced by elevated dehydroepiandrosterone sulphate (DHEAS) levels has been reported in 20% to 30% of patients with polycystic ovary syndrome.

We studied 50 patients with PCOS (defined by the Rotterdam Consensus Workshop group criteria, 2003). Adrenal hyperandrogenaemia was defined as a DHEAS level > 10.5 micromoles/litre was found in 10 patients (20%). Clinical features studied were body mass index (BMI) and hirsutism by means of Ferriman and Gallwey (F&G) scores. Biochemical parameters included fasting glucose, insulin, total testosterone, FSH, LH, SHBG, DHEAS, androstenedione, basal and ACTH stimulated 17 hydroxyprogesterone levels. Free and bioavailable testosterone levels were calculated using a software programme available from [www.issam.ch/freetesto.htm](http://www.issam.ch/freetesto.htm). Insulin sensitivity% and insulin resistance were determined using the homeostatic model assessment (HOMA 2) software programme available from [www.dtu.ox.ac.uk](http://www.dtu.ox.ac.uk).

Significant differences were found between patients with adrenal hyperandrogenaemia (AH) and normal adrenal androgens (NA). BMI  $30.80 \pm 7.12/35.60 \pm 9.15$  kg/m<sup>2</sup> ( $P 0.078$ ), androstenedione  $19.76 \pm 4.55/14.18 \pm 4.61$  nmol/l ( $P 0.001$ ), insulin  $65.60 \pm 64.47/129.55 \pm 129.63$  pmol/l ( $P 0.048$ ), insulin resistance  $1.2 \pm 1.16/2.26 \pm 0.9$  ( $P 0.046$ ) and insulin sensitivity  $131.82 \pm 77.97/84.93 \pm 71.81\%$  ( $P 0.047$ ) for AH/NA respectively. All data expressed as mean + standard deviation. No significant differences were found in F&G score, fasting glucose, total testosterone, FSH, LH, SHBG and 17 hydroxyprogesterone levels. Negative correlations were found between DHEAS and fasting insulin levels ( $r = -0.04, P < 0.05$ ) and DHEAS and BMI ( $r = -0.33, P < 0.05$ ).

These results suggest the existence of a unique subpopulation of patients with PCOS identified by low BMI, absence of insulin resistance and adrenal hyperandrogenaemia. The similar basal and stimulated 17-hydroxyprogesterone levels indicate a difference in downstream adrenal androgen generation.

**P703**

**Obstetric hazards of maternal obesity**

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**Introduction and objective**

Prevalence of obesity is increasing in fertile women. We aimed to investigate its relation with pregnancy outcome.

**Methods**

A retrospective analysis of 37 obese pregnant women (O group) was undertaken in the Endocrinology/ Obstetric Unit, from 1998 to 2004. Control group included 33 pregnant women (C group) with normal body mass index (BMI).

**Results**

Our O and C groups were age matched (O:  $27.9 \pm 4.35$ ; C:  $28.6 \pm 5.36$  years; ns), average BMI of 38.38 (O group) and 22.5 (C group,  $P < 0.0001$ ). Weight gain during pregnancy was significantly more pronounced in the C group (11.82) – O group (7.89,  $P < 0.01$ ). Obstetric history revealed an incidence of 24.3% of spontaneous abortions in obese patients – 6% in the control group. Complications during pregnancy, women in the O group had higher incidence of GDM (16.2%) than in the C group (3%,  $P = 0.066$ ). Furthermore, 27% developed hypertension – C group (3%,  $P < 0.005$ ). There was a case of repeated urinary infection in the O group.

Delivery occurred at 39.03 weeks in the O group; 38.55 in the C group; 27% of caesarean section in the former group as compared to 12.12% in the later. Birth weight was significantly higher in the O group (3359g vs 3096g,  $P < 0.01$ ). Congenital malformations occurred in 13.5% (5) in the O group compared to 3% (1) in the C group. There wasn't any foetal death.

A significant correlation was found between increased BMI, hypertension ( $P = 0.009$ , Man-Whitney test) and GDM ( $P = 0.012$ ). No significant correlation between type of delivery and BMI.

**Conclusions**

Obesity is associated with a higher incidence of maternal and foetal morbidity, diabetes. This implies that obese women require information regarding potential hazards and advice concerning pregnancy planning.

**P704****Cardiopulmonary impairment in young women with polycystic ovary syndrome**

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The polycystic ovary syndrome (PCOS) is not only the first cause of female infertility but also a complex, endocrine, multifaceted disease with important health implications. PCOS is often associated to the metabolic syndrome, and insulin resistance represents the main characteristics for both these syndromes. The metabolic disturbances characteristic of insulin-resistant states have been linked to the role of mitochondrial function and in particular to the maximal oxygen consumption ( $VO_{2max}$ ).

The aim of this study was to assess functional capacity in PCOS. Thirty PCOS women age- and body mass index-matched with 30 healthy women were enrolled. In each subject the assessment of functional capacity by cardiopulmonary exercise testing was performed, evaluating:  $VO_{2max}$ ,  $VO_{2AT}$  (oxygen consumption at anaerobic threshold),  $Watt_{max}$  (maximal workload at peak exercise). Biochemical and hormonal patterns were evaluated; insulin, glucose, and lipids levels were measured and the Homeostasis Model Assessment of insulin resistance (HOMA-IR) index was also calculated.

$VO_{2max}$  ( $17.1 \pm 2.9$  vs.  $26.8 \pm 3.5$  ml/Kg/min,  $P < 0.001$ ),  $VO_{2AT}$  ( $13.7 \pm 3.2$  vs.  $21.4 \pm 3.6$  ml/Kg/min,  $P < 0.001$ ) and  $Watt_{max}$  ( $101.3 \pm 25.2$  vs.  $135 \pm 22.6$  W,  $P < 0.001$ ) resulted significantly reduced in PCOS compared to healthy women. HOMA-IR was significantly increased in PCOS compared to controls ( $4.6 \pm 1.8$  vs.  $1.3 \pm 0.5$ ,  $P < 0.001$ ). There was a significant linear correlation between  $VO_{2max}$  and HOMA-IR in PCOS ( $r = 0.70$ ,  $P < 0.001$ ).

In conclusion our data firstly demonstrate a cardiopulmonary impairment with a reduced functional capacity in young PCOS women probably related to insulin resistance. Functional capacity could be a powerful further marker to assess the cardiovascular risk in PCOS.

**P705****Metformin decreases CRP level and cardiovascular risk in PCOS women**

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Hyperinsulinaemia and insulin resistance play a key role in the development of the PCOS. Amenorrhoea, polycystic ovaries, hirsutism, high fasting insulin and high testosterone concentrations characterize polycystic ovary syndrome (PCOS). Hyperinsulinaemia is associated with serum C-reactive protein (CRP) levels. Elevated CRP in association with hyperinsulinemia is a significant risk factor for cardiovascular diseases.

We aimed to evaluate the effect of metformin on serum CRP levels in PCOS women. Thirty women with PCOS [BMI =  $27.8 \pm 1.9$  kg/m<sup>2</sup>, aged 18–33 yr] were studied. Patients received metformin orally the dose of 850 mg/d. The patients were carefully interviewed, clinically examined, and laboratory tested to eliminate conditions probable to provoke an inflammatory response which was an exclusion criterion. At all patients we determined level of CRP, fasting insulin, blood glucosae, C-peptid, lipids, testosterone, FSH, LH, E2, T3, T4, TSH, PRL, cortisol, ACTH, GH. Serum CRP levels were measured with immunometric assay, level of insulin were measured with RIA before and after metformin treatment. Student's T-test and percentile was reformed in statistical analysis.

Mean serum C-RP levels significantly decreased after metformin treatment ( $6.98 \pm 1.92$  vs.  $1.98 \pm 0.68$  mg/l;  $P < 0.05$ ). Level of insulin reduced for 38% after metformin treatment ( $242 \pm 71$  v.s.  $151 \pm 38$  pmol/l). Mean total testosterone level decreased with metformin treatment ( $3.52 \pm 0.89$ ,  $1.66 \pm 0.47$ ,  $P < 0.05$ ). Total cholesterol and low-density lipoprotein cholesterol levels decreased as well.

Level of CRP significantly correlated to the level of fasting insulin ( $r = 0.57$ ).

PCOS women have insulin resistance and high CRP level. In sum, metformin decreased level of CRP and insulin and decreased risk for cardiovascular disease. Metformin therapy improved hirsutism and menstrual cycles, as well.

**P706****Functional characterization of the follicle-stimulating hormone receptor core promoter: applying a comparative approach among primates**

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Follicle-stimulating hormone (FSH) is essential for primate reproduction, acting via the FSH-receptor (FSHR) which is expressed in Sertoli and granulosa cells only. Despite its highly specific expression pattern, knowledge of the FSHR promoter and transcriptional regulation is still very limited. To gain insights into the regulatory elements controlling FSHR expression we characterized the core promoter activity of all important primate lineages including human. We isolated DNA fragments covering nucleotides -1 to -257 relative to the translational start site of the human, chimpanzee (*Pan troglodytes*), bonobo (*Pan paniscus*), cynomolgus monkey (*Macaca fascicularis*), marmoset (*Callithrix jacchus*) and lemur (*Microcebus murinus*) FSHR gene. The DNAs were cloned into the pGL3 vector to drive the expression of the luciferase reporter in transiently transfected COS7 and SK11 (mouse Sertoli) cell lines. Finally relative luciferase activity (RLA) was determined. Promoter activities varied significantly between species. Compared to the human FSHR promoter the chimpanzee displayed a 3.7-fold higher RLA, while for the bonobo only a 0.5 RLA was detected. The other primates displayed promoter activities similar to the human. Comparison of the human, chimpanzee and bonobo nucleotide sequences revealed only very few mismatches. Subsequent *in-vitro* mutagenesis of the human FSHR core promoter introducing one selected chimpanzee-specific alteration caused a significant 5-fold increase in RLA. Introducing the human nucleotide into the chimpanzee promoter decreased promoter activity to the bonobo wildtype level. Sequence analysis identified a binding site for an ETS transcription factor to be involved, hitherto unknown for the FSHR promoter. EMSA and western blot analysis will precisely identify transcription factor(s) involved. Although FSHR promoters show very high degrees of sequences homology among primates, single nucleotide changes may have significant impact on FSHR promoter activities. Thus comparative functional studies using closely related species could yield important insights on different regulatory promoter elements within the same gene.

**P707**

**Testosterone restores diabetes-induced erectile dysfunction and sildenafil responsiveness in two distinct animal models of chemical diabetes**

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**Objectives**

Hypogonadism is often associated with diabetes mellitus and both conditions represent major risk factors for erectile dysfunction (ED). The aim of this study was to evaluate the effect of diabetes-induced hypogonadism on ED using two distinct animal models: alloxan-rabbits and streptozotocin (STZ)-rats.

**Methods**

A subgroup of diabetic animals was Testosterone (T) replaced. After 8 weeks from induction of diabetes, erectile function was evaluated by *in vitro* contractility studies (rabbit) and *in vivo* cavernous nerve electro-stimulation (ES, rat).

**Results**

In both models of chemical diabetes an overt hypogonadism was observed, as derived by the reduced T plasma concentrations and by atrophy of the androgen-dependent accessory glands (prostate and seminal vesicles). In diabetic animals, T substitution completely reverted hypogonadism and diabetes-induced penile hyposensitivity to *in vitro* (acetylcholine, rabbit) or *in vivo* (ES, rat) relaxant stimuli. Moreover, T replacement was able to restore nitric oxide synthase (nNOS) expression, which was reduced ( $P < 0.05$ ) in STZ-rats. In diabetic animals, T substitution reinstated sildenafil-induced enhancement of both *in vitro* nitric oxide-donor (NCX 4040) relaxant effect (rabbit) and *in vivo* ES-induced erection (rat). The abolishment of the sildenafil effect on erectile response in diabetic animals might be due to the hypogonadism-induced PDE5 deficiency. Accordingly, PDE5 resulted reduced in diabetic STZ-rats ( $P < 0.05$ ) and normalized by T. In STZ-rats, intracavernous injection of sodium nitroprusside (SNP) induced a more sustained erection than in control rats, which was no further enhanced by sildenafil. T substitution normalized both hyper-responsiveness to SNP and sildenafil efficacy along with PDE5 expression.

**Conclusion**

In conclusion, in two experimental models of diabetes T deficiency underlies biochemical alterations leading to ED. Normalizing T in diabetes restores nNOS and PDE5, and reinstates either sensitivity to relaxant stimuli and responsiveness to sildenafil.

**P708**

**Androgenic status is influenced by AR polymorphism (CAG repeats number)**

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Exon 1 of AR gene contains a variable number of CAG triplets, which encode a polyglutamine stretch of variable length in the N-terminal domain of the receptor. Experimental evidence has accumulated in demonstrating that the length of this stretch influences AR transcriptional activity and therefore modulates target organs responsiveness to androgens. Aim of our study was to evaluate CAG repeats length [(CAG)<sub>n</sub>] in various conditions hypothetically influenced by AR function. 35 infertile, 32 hypoandrogenized (hypo) patients (absent beard and body hair, reduced epididymal and prostate size, recurvatum, etc.) and 91 normal controls (all fathers, normal muscle and bone structure, normal body hair) were analysed. Y-microdeletions and other congenital abnormalities were excluded in infertile patients. DNA was extracted from blood and amplified by PCR using the primers flanking the CAG repeat in the AR gene. PCR products were electrophoresed on the autosequencer to determine the exact (CAG)<sub>n</sub>.

The normal Caucasian population (subject from Central Italy), is super imposable to other European population for (CAG)<sub>n</sub> distribution. Hypoandrogenetic status is characterized by an increased number of CAG repeats; conversely, infertile patients are not statistically different from the control group (see Table 1).

	Infertile	Hypo	Controls
Mean	23.2	25.8	21.5
SD	2.1	3.0	1.7
90 <sup>th</sup> perc	24.0	29.5	23.4

T levels do not correlate to (CAG)<sub>n</sub>. Prostate size is significantly different between hypo and controls ( $P < 0.003$ ).

In conclusion, given the same amount of circulating testosterone (as in hypoandrogenized and control group), the final net androgenic phenotypical effect is due to AR polymorphism.

**P709**

**Role of D327N sex-hormone binding globulin gene polymorphism in the pathogenesis of polycystic ovary syndrome**

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SHBG (sex hormone-binding globulin) is a transport protein specific for dihydrotestosterone, testosterone and estradiol. The missense mutation in exon 8 (GAC → AAC) causing the amino acid exchange D327N correlates accordingly to literature data with higher SHBG levels.

We studied the possible association of this polymorphism with polycystic ovary syndrome (PCOS) and its influence on anthropometric and biochemical parameters in 247 PCOS patients in comparison to 109 healthy control women.

The D327N polymorphism (wild-type a variant allele) was detected using PCR-RFLP method (restriction enzyme Bbs-I). Statistical evaluation:  $\chi^2$  test, ANOVA (Statgraphics Plus v.5.1, USA). There was no significant difference in genotype distribution between PCOS and controls ( $\chi^2 = 0.30$ ,  $P = 0.58$ ). Biochemical parameters were evaluated in women without any medication i.e. in 73 control women and in 247 PCOS. We did not find any association of the variant allele with plasma SHBG level. SHBG was associated only with body mass index ( $P < 0.0004$ ). The variant allele carriers had significantly lower levels of testosterone ( $P < 0.05$ ) and 17-hydroxyprogesterone ( $P < 0.05$ ), but the significance was achieved only in the age group above 30 years.

**Conclusion:** The influence of adiposity on SHBG level was probably stronger than the contribution of D327N polymorphism. However, this polymorphism could influence the androgen levels.

Study was approved by local Ethical Committee, supported by grant GA CR 301/04/1085 and IGA MH CR NR/7809-5.

**P710**

**Endocrine evaluation of a large cohort of male cancer survivors compared with healthy controls**

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**Background**

Men who receive gonadotoxic treatment for cancer may be at risk of hypogonadism. The extent of this risk is unknown. Severe hypogonadism is associated with fatigue, sexual dysfunction, reduced muscle mass and obesity. Diagnosing mild or moderate hypogonadism is difficult and requires evaluation.

**Aim**

The aim of this study was to identify hypogonadism in young male survivors of cancer.

**Subjects and methods**

The study was approved by the local ethics committee. 176 male cancer survivors (mean age 37.3 y SD 5.75) who previously received gonadotoxic therapy and were more than 2 years since end of treatment, and 213 controls (mean age 36.3y SD 6.0) with no previous history of cancer were recruited. Height (m), weight (kg), body mass index (BMI, kg/m<sup>2</sup>) and several biochemical markers were measured including total testosterone (tT), oestradiol (E<sub>2</sub>), LH, FSH, SHBG, T<sub>4</sub>, TSH, IGF-1. Free androgen index (FAI) and free oestrogen index (FEI) were also calculated. Comparisons were made by independent t-test using SPSS v11.

**Results**

There were no significant differences between survivors and controls for age, height,  $T_4$ ,  $tT$ ,  $E_2$ , FAI, FEI, SHBG and IGF-1. Cancer survivors were significantly heavier (88.9 vs 85.5 kg,  $P = 0.011$ ), with a greater BMI (27.6 vs 26.5,  $P = 0.005$ ). TSH was significantly higher (2.4 vs 1.9,  $P < 0.001$ ) suggesting hypothyroidism. FSH and LH were significantly higher (FSH 12.9 vs 3.9,  $P < 0.001$ , LH 7.2 vs 4.8,  $P < 0.001$ ).

**Conclusion**

Cancer survivors may have subclinical hypogonadism and further analysis of fatigue, sexual function and detailed body composition is required.

**P711****The use of prolonged human chorionic gonadotrophin stimulation testing in the evaluation of undescended testes**

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**Introduction**

The hCG stimulation test is a useful indicator of functional testicular tissue and may also have a place in treating undescended testes (UDT). However, its utility is limited by the inconsistency in the regimens used. We have looked at the clinical and biochemical response in a group of children with UDT who had a prolonged hCG stimulation as part of their clinical management.

**Methods**

The retrospective review in 17 cases with a median EMS (10th, 90th centiles) of 9 (5.6, 10.5) included analysis of age, pre and post hCG - serum concentrations of testosterone (T), DHT and androstenedione (A). These 17 cases included one child who was later discovered to be 46XX and another who was anorchic. These were both excluded when assessing clinical outcome by palpation of testes and the need for orchidopexy.

**Results**

Amongst the 17 cases, all except one had serum testosterone measurements on D1, 4 and 21, as per the protocol. 9, 15 and 16 patients had a pre and a post DHT, A and SHBG, respectively. Testicular descent occurred in 8/26 (32%) undescended testes. As a result, immediate surgery was deemed unnecessary in 7/15 (47%) cases. Three patterns of changes in serum T were noticed; in 4/17 (24%), the expected rise in T was by D4, in 6/17 (35%) the rise was by 21 and not D4 and in the remainder, serum T stayed low. The median D1 T:A rose from 0.4 (0.2,1.6) to 1.9 (0.3,4.8) at D4 ( $P < 0.01$ ). There was no further rise in the ratio by D21. The median DHT:T at D1 and D4 remained unchanged at 0.26 (0.06,0.4) and 0.26(0.2,0.66), respectively.

**Conclusion**

Prolonged hCG stimulation not only helps to establish the presence of functioning testicular tissue and determine abnormalities in testosterone biosynthesis, but it may also reduce the need for orchidopexy.

**P712****Stage-specific mRNA expression of androgen receptor correlated with FSH receptor in individual pre-antral follicles isolated from human ovary**

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Recent evidence indicates that the increase in follicle numbers seen in PCOS occurs early in folliculogenesis, and that androgens are implicated in this. In primates and sheep, androgen excess *in-utero* results in ovarian changes similar to those in PCOS. We have recently shown using a novel *in ovo* model, that testosterone added to implanted human tissue increased primary follicles compared to untreated tissue (Qureshi *et al.*, 2005). This is similar to the published results of follicle counts in polycystic ovaries (Webber *et al.*, 2003, Maciel 2004). It is important therefore to determine the follicle stage at which androgen receptors (AR) are first expressed. The problem has been the sensitivity of the techniques available for use with the limited amount of material.

The aim of this study therefore was to determine the origin of AR mRNA expression in individual, well characterised pre-antral follicles isolated from human, ovarian cortex using nested RT-PCR and correlate this to the appearance of mRNA for follicle stimulating hormone receptor (FSHR) in the same follicles. Ovarian tissue was obtained with informed consent from women undergoing TAH/BSO ( $n = 3$ ) and cortical biopsies from women undergoing elective Caesarean sections ( $n = 4$ ). Follicles were manually

isolated from tissue following brief enzymatic digestion, staged under light microscope and photographed. Individual follicles were lysed, snap-frozen and stored at  $-80^{\circ}\text{C}$ , prior to reverse transcription. To confirm the presence of cDNA, nested PCR for beta-actin was run on each follicle. 61/67 follicles examined were positive for beta-actin, and of these 15 were positive for AR and 4 for FSHr. The AR positive follicles were: 0 primordials, 3 transitionals (primordial-primary), 6 primaries, 3 transitionals (primary-secondary), 2 secondaries and 1 pre-antral. The FSHr positive follicles were: 3 primaries and 1 pre-antral. We have shown that early follicles acquire AR prior to FSHr, demonstrating that androgens could be exerting their effects from initiation of follicle growth onwards. This adds further weight to the hypothesis that androgens cause disordered early folliculogenesis in PCO.

**P713****Hyperprolactinaemia due to big prolactin**

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Prolactin is present in serum in a variety of forms defined by different molecular masses. Macroprolactin is the best-known variant, a high molecular mass (150–170 kDa) form of prolactin usually representing a prolactin-IgG complex which reacts in immunoassays causing apparent hyperprolactinaemia. Big-prolactin is a smaller high molecular mass (50–60 kDa) form of prolactin found more commonly than macroprolactin; however, the origin and significance of big-prolactin are poorly understood. Screening for larger molecular mass forms of prolactin is performed by measurement of serum prolactin after polyethylene glycol (PEG)-precipitation; low recovery (<40%) indicates the presence of macroprolactin, and intermediate recovery (40–60%) may require gel filtration chromatography (GFC) for clarification. We report the results of Sephacryl S-100 high resolution GFC to study the contribution of big-prolactin to total serum prolactin in 17 consecutive hyperprolactinaemic serum samples (total prolactin 724–7743 mU/L) with intermediate recovery after PEG-precipitation. Big-prolactin was the predominant high molecular mass prolactin form in 15 (79%) cases, representing 17–49% of total serum prolactin (56–91% of the high molecular mass forms). Chromatography of the precipitate after re-dissolution confirmed that PEG precipitates both macroprolactin and big-prolactin. Chromatography after adsorption of serum with protein G-Sepharose showed that only part of the big-prolactin peak was adsorbed, indicating an IgG component. Re-assay of big-prolactin chromatography fractions in seven commercial immunoassays confirmed that big-prolactin reacts in all prolactin assays widely used in the United Kingdom.

We conclude that, in cases of intermediate prolactin recovery after PEG-precipitation, big-prolactin is a substantial component of total serum prolactin and contributes to the hyperprolactinaemia seen in these cases. The molecular nature of big-prolactin remains unclear, but our data suggest that an IgG component is involved. Further work is required to define the biological activity of big-prolactin and thus to guide clinical interpretation of the results in these cases.

**P714****Efficacy of Testogel in the treatment of hypogonadism in routine clinical practice**

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**Introduction**

Testogel is now established treatment for male hypogonadism. The aim of TRT is to resolve hypogonadal symptoms and achieve physiological levels of testosterone.

**Objective**

To determine the efficacy of Testogel 50 mg/day, in achieving symptomatic benefit and physiological testosterone level in 100 male patients.

**Method**

Retrospective review of 100 case notes from the andrology clinic. Men included were between 16 and 80 years. A database was established recording their demographic data, treatment regimes, serum testosterone [TT], calculated bioavailable testosterone, SHBG, FSH, LH, PSA, Hb, and Hct were at baseline, 3, 6, 9, and 12 months after treatment with Testogel.



## Results

Fifty-nine percent were aged 51–70 years. 39% had secondary, 36% primary and 25% mixed hypogonadism. 45% were started on Testogel de novo, and 55% were switched over from other treatments. In de novo patients mean TT was 16.3 nmol/l, 18.3 nmol/l, then 19.7 nmol/l at 3, 6, and 12 months respectively. For those switched to Testogel, mean TT was 13.1 nmol/l, 13.0 nmol/l, and 15.6 nmol/l for the same intervals. Collectively the mean TT levels demonstrated as expected a statistically significant rise with  $P = 0.038$  using a paired t test. On Testogel serum TT failed to rise to physiological levels [11–30 nmol/l] in 19% at 3 months, 17% at 6 months and in 13% at 12 months despite dose adjustment. Supraphysiological levels [ $> 30$  nmol/l] of testosterone were reached in 4% after 3 months treatment 5% after 6 months and 6% after 12 months. During this period of treatment 7% changed to other modes of testosterone therapy. 6% are unhappy on testogel and planning to change. One patient was diagnosed with prostate cancer and his treatment stopped.

## Discussion

Testogel achieves physiological TT in majority of patients on TRT and was associated with symptomatic improvement. Not all patients achieved consistent levels of testosterone within the physiological range, which potentially could be due to compliance, area of application, or difference in dermal absorption.

## P715

### Which clinical features of polycystic ovary syndrome are improved with metformin therapy? A retrospective study

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#### Background

Metformin is increasingly used in the management of polycystic ovary syndrome (PCOS) but there remain differences in opinion as to its precise role in terms of clinical benefit. We retrospectively reviewed the effects of metformin on menstrual regularity, fertility, weight and hirsutism in 244 patients with PCOS treated routinely with metformin in our clinics.

#### Results

Patients were of median age 27 years (range 12–47) with median BMI of 35.1 kg/m<sup>2</sup> (range 20.5–61.4). BMI was  $< 25$  in 6%, 25–30 in 15%, 30–40 in 51% and  $> 40$  in 28%. Menstruation was restored in 52% of patients presenting with amenorrhoea (17/33) and in 60% of patients with oligomenorrhoea (102/170). Pregnancy was achieved in 39% of patients with a recorded diagnosis of primary or secondary infertility (28/72). Four of these experienced miscarriage. In patients who had taken metformin for at least 6 months, 49% (87/177) lost greater than 2 kg in weight. Clinically significant improvement in hirsutism was documented in only 14% (17/123). Type 2 diabetes had been diagnosed in 15 patients before or at the time of starting metformin, but was subsequently diagnosed in a further 5 patients.

#### Conclusion

Our findings from routine clinical practice support the efficacy of metformin in the treatment of menstrual disturbance and infertility in women with PCOS. We found weight reduction in a significant minority but limited efficacy in the treatment of hirsutism. As expected, Type 2 Diabetes is relatively common in this population, and a systematic approach to documentation of glucose tolerance is appropriate. Since this is a retrospective, non-randomised study we cannot assess the extent to which dietary, exercise or lifestyle improvements contributed to the response to metformin. Further prospective clinical studies are required to clearly define the role of metformin in clinical practice in PCOS.

## P716

### Comparison of Insulin Resistance levels in pre and post-menopausal women, women with polycystic ovarian syndrome and postmenopausal women with Type 2 Diabetes

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#### Objectives

There is strong epidemiological evidence that insulin resistance confers a significantly increased risk of cardiovascular disease independent of other cardiovascular risk factors. The aim of the study is to compare the insulin

resistance levels in metabolic syndrome of polycystic ovarian syndrome (PCOS) with that of type 2 diabetes (T2DM).

#### Methods

The biological variation of IR was assessed by measuring IR at four day intervals on 10 consecutive occasions in 12 overweight patients with PCOS (median age, 28 yrs, range, 18–31), 12 postmenopausal Caucasian subjects with T2DM (median age 62 yrs, range 50–73), 11 healthy women with normal menstrual periods (median age 30 yrs, range 19–33) and 11 healthy, weight matched, postmenopausal women (median age 56 yrs, range 48–70). Insulin resistance was derived using the Homeostasis Model Assessment method (HOMA-IR) from fasting measures of serum insulin and plasma glucose. All subjects gave their informed written consent before entering the study and local ethical committee approval had been obtained.

#### Results

The HOMA-IR in women with PCOS was  $5.85 \pm 5.3$ , in postmenopausal T2DM was  $4.33 \pm 2.3$ , in pre-menopausal women was  $1.67 \pm 0.63$  and in postmenopausal women was  $2.11 \pm 0.79$ . HOMA-IR did not differ significantly between women with PCOS as compared to postmenopausal women with T2DM, and were much higher than those seen in normal pre and postmenopausal women.

#### Conclusion

As insulin resistance is an independent risk factor for cardiovascular disease, this implies that PCOS is associated with an increased risk of cardiovascular disease, similar to those with T2DM.

## P717

### 11 $\beta$ -hydroxysteroid dehydrogenase (11 $\beta$ HSD) activities in porcine granulosa cells from ovarian follicles and cysts

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In the ovary, glucocorticoids are metabolised by 11 $\beta$ HSD enzymes. The bi-directional 11 $\beta$ HSD1 enzyme usually acts as an NADPH-dependent 11-ketoreductase to regenerate cortisol (F) from circulating cortisone (E), while 11 $\beta$ HSD2 inactivates F using NAD<sup>+</sup> as cofactor. Having isolated endogenous inhibitors of 11 $\beta$ HSD1 from porcine follicular fluid (pFF) and ovarian cysts, the aims of this study were to establish whether 11 $\beta$ HSD enzymes interconvert F and E in porcine granulosa cells (GCs) and, if so, whether this changes during follicle growth.

Porcine GCs were isolated from small, medium and large antral follicles (2–4 mm, 4–8 mm and  $> 8$  mm in diameter, respectively), and from spontaneous ovarian cysts (25–40 mm diameter) ( $n = 5$  in each group). 11 $\beta$ HSD activities were measured over 4 h at 37.5°C in primary cell cultures and in GC homogenates using radiometric conversion assays. Intact GCs were incubated with either <sup>3</sup>H–F or <sup>3</sup>H–E (100 nM) in serum-free medium supplemented with 10 ng/ml insulin, 10 ng/ml IGF-1, 5  $\mu$ g/ml transferrin, 0.04 ng/ml sodium selenite, 100 nM androstenedione and 1 ng/ml FSH. Cell homogenates were incubated either with 100 nM <sup>3</sup>H–F plus 4 mM NADP<sup>+</sup>/NAD<sup>+</sup>, or 100 nM <sup>3</sup>H–E plus 4 mM NADPH  $\pm$  10 mM glucose-6-phosphate.

In intact GCs, net oxidation of F increased with follicle diameter (from  $0.8 \pm 0.3$  pmol in GCs from small follicles to  $2.1 \pm 0.5$  pmol in dominant follicles) but was significantly decreased in ovarian cysts ( $0.7 \pm 0.1$  pmol;  $P < 0.05$  versus large antral GCs). In GC homogenates, addition of both NADP<sup>+</sup> and NAD<sup>+</sup> increased F metabolism, and both activities increased with follicle diameter. There was no significant metabolism of <sup>3</sup>H–E either in intact cells or GC homogenates, irrespective of follicle size or added cofactors. We conclude that porcine GCs can inactivate F using both NADP<sup>+</sup>-dependent 11 $\beta$ HSD1 and NAD<sup>+</sup>-dependent 11 $\beta$ HSD2 enzymes, but that 11 $\beta$ HSD1 appears to lack any 11-ketosteroid reductase activity in these ovarian cells. Furthermore, rates of F oxidation are lowest in rapidly growing small antral follicles and in ovarian cysts.

This work was supported by a BBSRC-Sygen CASE studentship.

## P718

### Secretion of adiponectin by human placenta: differential modulation by cytokines

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Pregnancy, a state of insulin resistance (IR), is associated with elevated levels of cytokines and profound alterations in metabolism, directed towards supplying adequate nutrition for the fetus. Adiponectin, an adipokine with anti-inflammatory and insulin sensitising properties, plays an important role in energy homeostasis. Interestingly, in gestational diabetes mellitus (GDM), a state of greater insulin resistance, serum adiponectin levels are lower than normal subjects. We hypothesised that the human placenta is a source of adiponectin, and we investigated its expression, secretion, and regulation. The study was approved by the Local Research Ethics Committee and all patients involved gave their informed consent.

Adiponectin gene is present in the human term placenta, with expression primarily in the syncytiotrophoblast. Radioimmuno-analysis of conditioned media from explant experiments revealed that the placenta can secrete adiponectin *in vitro*. Addition of conditioned media to HEK-293 cells transfected with adiponectin receptor-1 altered the phosphorylation status of ERK<sub>1/2</sub> and p38 MAPK, an effect abolished after preabsorption with adiponectin antibody.

Cytokines including TNF- $\alpha$ , IFN- $\gamma$ , IL-6 and leptin, differentially modulated placental adiponectin receptors as well as adiponectin gene expression and secretion. Interestingly, in GDM placentae, there was a significant downregulation of adiponectin and adipOR2, but not adipOR1 mRNA.

Our results indicate the novel production and secretion of adiponectin from the human placenta, differential regulation by cytokines and altered expression in GDM. Collectively, our data suggest that adiponectin may play a role in adapting energy metabolism at the materno-fetal interface.

## P719

### Functionality of largest follicles is affected in gonadotrophin-stimulated cycles

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Nowadays, though highly improved, the efficiency of human assisted reproduction protocols remains affected by a decrease in embryo yields after IVF. The aim of the current study was to evaluate a possible relationship between poor embryos and the functionality of follicles stimulated to grow with exogenous gonadotrophins, using a monovular species (sheep) as a model. In ewes, follicles can ovulate from  $\geq 6$  mm in unstimulated cycles, but from  $\geq 4$  mm in FSH treatments. Fourteen Manchega ewes were superovulated with a commonly used protocol, consisting of 8 step-down doses of oFSH (OVAGEN<sup>TM</sup>, ICP, New Zealand), twice daily during the last days of an intravaginal progestagen treatment. At oestrus detection, all preovulatory-sized follicles ( $\geq 4$  mm,  $n = 230$ ) were dissected and both follicular fluid and oocytes were individually obtained. Follicular function was determined by measuring intrafollicular concentrations of oestradiol (E<sub>1F</sub>), androstenedione (A<sub>1F</sub>) and inhibin A (I<sub>1F</sub>) in individual follicles, as markers for follicular health. Oocytes were morphologically evaluated at recovery and thereafter *in vitro* matured for 24 h in individual wells. Results showed that intrafollicular hormone concentrations were related to follicle size, but surprisingly were higher in smaller follicles. E<sub>1F</sub> was higher in follicles with 4 mm in diameter in comparison with follicles with 5 and 6 mm, ( $3.4 \pm 1.2$  ng/ml vs  $1.2 \pm 0.3$  ng/ml,  $P < 0.01$ , and  $0.8 \pm 0.1$  ng/ml,  $P < 0.0005$ , respectively). The ratio E<sub>1F</sub>: A<sub>1F</sub> was also higher in 4 mm follicles than in 5 mm follicles ( $0.2 \pm 0.0$  vs  $0.08 \pm 0.0$ ;  $P < 0.0005$ ). I<sub>1F</sub> concentrations were similar in 4 and 5 mm follicles ( $13.6 \pm 1.6$  and  $14.0 \pm 1.1$   $\mu$ g/ml, respectively) but significantly lower ( $8.5 \pm 0.5$   $\mu$ g/ml,  $P > 0.0005$ ) in 6 mm follicles. No relationship was found between follicle diameter and the ability of oocytes to resume meiosis. Nevertheless, the reduced hormone secretion of the largest follicles, indicates follicular malfunction and could be related to failures in the later embryo development.

## P720

### Screening for gene SRY by FISH in patients with Turner Syndrome

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Turner's Syndrome is one of the most common chromosomal abnormalities found in 1 in 2500 live female births. Y chromosome material is detected in up to 6% of patients by karyotype, but with new technologies (DNA analysis), Y chromosome sequences have been reported in 60% of patients. The presence of Y material has been associated with virilization and with the development of gonadal neoplasia.

#### Aim

Determine the frequency of Y chromosome sequences in patients with Turner's Syndrome, using FISH and the clinical implications.

#### Material and methods

We studied 21 patients with Turner's Syndrome, confirmed by karyotype and metaphase FISH analysis had been performed to identify SRY gene.

#### Results

Eight patients (38%) had a 45,X karyotype and the others had mosaicism or a normal X chromosome. Two patients (9,5%) were positive for SRY FISH-probe tested. One patient underwent gonadectomy but was negative for gonadoblastoma. The pelvic ultrasound of the other patient was normal and was not performed yet a gonadectomy because of the small number of cells positive for SRY gene (0,5%) and she is still waiting for DNA analysis.

#### Conclusions

The presence of a Y chromosome has been associated with gonadal neoplasia but we didn't find any case. Even though gonadoblastomas are benign tumors, it has been reported that 50% will progress into invasive dysgerminomas and prophylactic gonadectomy is recommended for Turner's patients with a Y chromosome material.

## P721

### Serum Anti-Mullerian hormone (AMH) concentrations in infants and children

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#### Introduction

AMH is produced by the sertoli cells in the testes and is responsible for the regression of the Mullerian structures in the male fetus. It is also produced by the granulosa cells of the ovaries in females to a lesser degree. Serum AMH measurements may be important for detecting testicular tissue and monitoring ovarian activity. The objective of this study was to establish a cross-sectional reference range for AMH in UK children.

#### Method

Serum AMH (pmol/l) was measured by enzyme immunoassay (Immuno-tech) on spare serum left over from samples collected from 281 children (m,152) for routine clinical indications. Diagnoses were noted and those with reported genital abnormalities were excluded from the reference range data.

#### Results

The results showed that AMH concentration in males increased and peaked during the second half of first year. Subsequently they fell gradually until the age of early puberty following which there was a more precipitous fall. The table below shows the AMH concentration (pmol/L) for males of different age ranges (years) and the AMH concentration for the total female study population.

	0-1 yr	1-4 yr	5-8 yr	9-12 yr	13-16 yr	Females 0-16 yr
<i>n</i>	31	27	32	34	28	129
Med AMH	603	535	598	240	53	6.4
10th centile	199	230	202	78	30	0.3
90th centile	1017	1285	851	781	426	30

In females, AMH remained low from birth until age of 16 with no obvious peak. In one 10 month old boy with Persistent Mullerian Duct Syndrome, AMH was undetectable. In another boy with bilateral cryptorchidism and poor testosterone response to HCG, AMH levels were low for age.

#### Conclusion

Our study establishes, for the first time, an age and gender related reference range for AMH infants and children in the UK and confirms the clinical utility of serum AMH measurement in evaluating disorders of sex development.

## P722

### Prenatal diagnosis of P450 oxidoreductase deficiency

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P450 Oxidoreductase Deficiency (ORD) is a novel form of congenital adrenal hyperplasia with biochemical evidence of combined 21-hydroxylase and 17 $\alpha$ -hydroxylase deficiency. Mutations in P450 oxidoreductase (POR), a crucial electron donor to microsomal P450 enzymes including CYP17 and CYP21, have recently been identified as the cause of disease. Clinical features in ORD include ambiguous genitalia, glucocorticoid deficiency and craniofacial malformations resembling the Antley-Bixler phenotype. Here we have performed prenatal diagnosis in two pregnancies affected by ORD, utilizing GC/MS analysis of maternal urinary steroid excretion and direct sequencing of the POR gene in DNA from cultured amniocytes. Case 1 was the second pregnancy of non-consanguineous parents with a first child (46, XX) affected by ORD caused by a homozygous A287P mutation and manifest with craniofacial malformations and virilised genitalia. The maternal urine contained excessive amounts of androgen and pregnenolone metabolites, indicative of ORD. This was confirmed by direct sequencing revealing a homozygous A287P mutation also in this second child (46,XY), which will therefore require special perinatal care. Case 2 is the first pregnancy to healthy, non-consanguineous parents. A routine ultrasound scan at 16 weeks revealed an abnormally shaped skull and bowed femora. Follow-up scans also showed underdeveloped external genitalia in the foetus (46, XY). In view of the malformations the parents decided for termination of pregnancy and autopsy showed coronal synostosis, midface hypoplasia, radiohumeral synostosis, a small penis and an absent scrotum. Sequencing analysis commenced after termination of pregnancy revealed a paternally inherited heterozygous A287P mutation, the maternal mutation has yet to be found. Again, urine analysis was suggestive of ORD. Prenatal diagnosis is demanding with regard to its ethical implications and important immediate clinical consequences but may become a valuable tool in families affected by ORD, benefiting the optimum perinatal care based on imminent advances in genotype-phenotype analysis.

## P723

### Isolated progesterone secretion by an ovarian Leydig cell tumour: I, hormonal and immunohistochemical characterization II, effects on the gonadotrope axis

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A 20 yr old woman was referred for primary amenorrhea. At examination BMI was 19 and displayed a pubertal development at S4P4. Hormonal evaluation showed normal prolactin, low estradiol (18 pg/ml) and gonadotropins (LH = 1.5IU; FSH = 1.9 IU/L). Testosterone was normal (0.25 ng/ml) but curiously plasma progesterone (P) was increased (from 3.9 to 5 ng/ml). Initial ovarian sonography and adrenal CT scan didn't show any abnormal mass. ACTH stimulation tests showed normal responses excluding 21.17 or 11 hydroxylase deficiencies. During these tests circulating P didn't increase and was not suppressed by dexamethasone. Neither GnRH agonist (Dtrp-6, 3 mg IM) stimulated (flare up) nor blunted (desensitization) gonadotropins increased or decreased P levels. Very high P in the right ovarian vein effluent (107 vs 4.9 in the left effluent (ng/ml)) indicate a right ovarian source of P but ovarian sonography remained normal. The LH secretion profile studied during follow up showed 2 LH pulses in 4 hours, a frequency similar to those observed in the luteal phase of normal women. Pulsatile GnRH administration for 21 days (20  $\mu$ g/90 minutes, sc) failed to increase low E2 levels and to induce the recruitment of a dominant follicle. A 30mm right ovarian tumour was discovered by ultrasound after 18 month follow up and removed thereafter. Surgical tumour resection was followed by decrease in P levels and appearance of regular menses. Immunohistochemical analysis of the tumour showed expression of P450 scc, 3 $\beta$ -HSD but no expression of P450c17. To our knowledge, this is the first case of isolated progesterone secretion by an ovarian Leydig cell tumour. This model provide a unique opportunity to

evaluate the consequence of a permanent P secretion on the gonatropo axis. Our results indicate that P can exert an inhibitory effect on gonadotropin secretion at both the hypothalamic and pituitary levels.

## P724

### Serum inhibin levels in patients with polycystic ovary syndrome

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The possible role of inhibin in the etiology of polycystic ovary syndrome (PCOS) is controversial. This study was designed to investigate levels of serum inhibin in women with PCOS.

In a case-control setting 41 women with PCOS and 44 women with normal cycles (control group) aged 15-40 year-old were evaluated. Mean age of cases and controls was  $23.6 \pm 5.3$  and  $23.1 \pm 3.9$  years, respectively. Mean body mass index (BMI) in cases and controls were  $25.07 \pm 5.45$  and  $21.3 \pm 3.246$  Kg/m<sup>2</sup>, respectively. There was no statistically significant difference in mean age between the two groups ( $P > 0.05$ ) but mean BMI was significantly different between the two groups ( $P < 0.001$ ). Mean serum levels of inhibin in cases and controls were  $1.62 \pm 1.23$  and  $2.26 \pm 2.26$  U/ml, respectively which was not significantly different between the two groups ( $P: 0.16$ ).

We concluded that basal inhibin levels cannot be used for routine screening in women with PCOS.

## P725

### Outcomes of pregnancy and glycaemic control in patients with type 1 diabetes

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#### Background

It is generally believed that glycemic targets in diabetic pregnancies should mimic those found in normal pregnancies.

#### The aim

of the present work was to reveal how glycemia indices in pregnant patients with type 1 diabetes mellitus (T1DM), their infants' birth weight (IBW).

#### Methods

Totally, 128 pregnant women with T1DM were enrolled in the study (mean age -  $26 \pm 7$  yrs; diabetes duration -  $10.2 \pm 5.4$  yrs). Patients with nephropathy were excluded. Strict metabolic control was maintained and fetal surveillance was performed throughout the pregnancy. Data obtained for home-blood glucose monitoring (five-point profiles), postprandial (PG), fasting (FG), mean blood glucose (MG) were enrolled in this work. Healthy infants were born to diabetic mothers at 38-40 week of gestation. According to the IBW, the patients were divided into 2 groups (GR): GR.1,  $n = 97$ ,  $IBW < 4000$  g; Gr. 2,  $n = 31$ ,  $IBW > 4000$  g.

#### Results

HbA1c, PG, FG and MG levels were statistically higher in GR.2, than in Gr.1: HbA1c ( $7.6 \pm 1.38\%$  vs  $5.6 \pm 1.71\%$ ,  $P < 0.001$ ), PG ( $168.1 \pm 17.1$  vs  $110.4 \pm 14.7$ ,  $P = 0.000$ ), FG ( $136.5 \pm 15.8$  vs  $102.5 \pm 13.4$ ,  $P = 0.000$ ), MG ( $155.2 \pm 12.17$ ; vs  $104.7 \pm 12.1$ ,  $P = 0.000$ ). Pre-pregnancy BMI (kg/m<sup>2</sup>) indices were higher in GR.2, than in Gr.1 ( $27.2 \pm 0.58$  vs  $22.5 \pm 0.62$ ,  $P < 0.001$ ).

Strong correlation was observed between IBW and PG ( $r = 0.882$ ;  $P = 0.000$ ), IBW and HbA1c ( $r = 0.794$ ;  $P = 0.04$ ), IBW and MG ( $r = 0.703$ ;  $P = 0.012$ ) for Gr.1, and between IBW and PG ( $r = 0.814$ ;  $P = 0.000$ ), IBW and pre-pregnancy BMI ( $r = 0.866$ ;  $P = 0.001$ ) for GR.2.

#### Summary

IBW strongly correlated with PG, MG and HbA1c in the pregnant patients with T1DM. Pre-pregnancy BMI  $> 26$  kg/m<sup>2</sup> and postprandial glycemia levels of  $> 160$  mg/dl may be the predictors of fetal macrosomia.

## P726

### Characteristic changes of skin and it's accessories in relation to diabetic peripheral neuropathy in patients with type 2 diabetes mellitus

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**Background**

The different changes are seen on skin and its accessories in patients (pts) with Type 2 Diabetes (T2D); some of them represent the prognostic indicator of complicated diabetes and some directly contribute in development of them. The aim of our study was to examine the prevalence of characteristic changes of skin and its accessories in T2D considering the grade of severity of Diabetic Peripheral Neuropathy (DPN).

**Materials and methods**

The objective data on foot examination of 195 pts with T2D were analyzed. (Age 38-78 yrs., DD  $14.5 \pm 9$  yrs., male/female 1.5/1). Pts were divided into 4 groups according to the grade of DPN; Gr.1: without DPN; Gr.2: sub clinical DPN; Gr.3: clinical DPN; Gr.4: disabling DPN (classification suggested by Boulton, 1998); the following types of skin humidity were described: normal/smooth skin; dry/flake skin and very dry/rough skin with fissures and keratoses.

**Results**

Skin humidity, which is the common manifestation of diabetic autonomic injury was related to the severity of DPN, particularly, DPN 2-4 times raises the possibility of dryness of the skin on the foot; the percentile of pts with very dry skin according to the groups were 11%/17%/38%/43%; the risk of callus development was significantly high in case of clinical (63%) and disabling (91%) DPN, the possibility of neuropathy ulcer was also high in Gr.4 - 2 out of 7 pts. The high incidence of onychomycosis was noted, especially in Gr.3 (37.4%) and Gr.4 (57.1%). The incidence of Yellow nails syndrome was very high - 39.4% of all cases in all groups. the Melin's shin spots were found in fourth of the patients, mainly in men and it was not related to the severity of DPN; a few cases of rubeosis plantarum and bulbosis diabeticorum were revealed; the cases of necrobiosis were not noted.

**Conclusion**

The prevalence of the characteristic changes of skin and its accessories in Type 2 Diabetes Mellitus is sufficient high; The incidence of dryness of the skin, callosities and onychomycosis is depended on the severity of DPN, and may have a harmful impact on the course of the foot problems and its outcome; by using of their early detection and treatment many problems concerning to diabetic foot may be avoided.

**Steroids****P727****High risk of adrenal insufficiency after single intra- or peri-articular steroid administration in young healthy sportsmen**

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**Objectives**

To determine if a single intra- or peri-articular injection of corticosteroid for post-traumatic or microtraumatic skeleton injuries in healthy young subjects would induce a suppression of hypothalamo-pituitary-adrenal axis activity and reactivity.

**Methods**

10 young healthy male sportsmen ( $28.8 \pm 2.5$  years) received a single intra- or peri-articular injection of either cortivazol or betamethasone for post-traumatic or microtraumatic skeleton injuries. Morning cortisol levels were measured on 4 occasions: the first day immediately before steroid injection (D0) & 2d (D2), 7d (D7) and 14d (D14) later. On D2, a ACTH test (1 µg) was performed.

**Results**

On D2, adrenal insufficiency (cortisol levels < 100 nmol/l and/or blunted peak cortisol after stimulation with ACTH 1 µg) occurred in 9 out of 10 subjects.

On D7, cortisol levels were still decreased in all subjects ( $48.2 \pm 7.3\%$  of D0 levels). Only one subject displayed a normal adrenal function (cortisol level > 500 nmol/l).

On D14, cortisol levels remained significantly decreased compared to pre-injection levels ( $P = 0.02$ ) and averaged  $77.3 \pm 8.3\%$  of D0 levels. Only 3 participants out of 10 displayed a normal adrenal function. The magnitude of the adrenal suppression was correlated with the injected dose.

**Conclusions**

Some sportsmen are exposed to a high risk of traumatism which can potentially lead to an acute adrenal crisis should the HPA axis be non reactive. Thus, it is important to provide them some advice about the clinical signs that should lead to consult a physician after an intra or peri-articular corticosteroid injection.

**P728****Capillary blood, an alternative to saliva for the diagnosis of Cushing's syndrome?**

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**Introduction**

As an alternative to cortisol assays in the serum or saliva to diagnose hypercorticism we evaluated the ability of a commercial cortisol assay to assess capillary blood cortisol concentrations using routine procedures.

**Methods**

Capillary blood was obtained using a device used by diabetics to routinely assess capillary glucose levels and deposited on Schleicher-Scheull 903 blotter paper (routinely used for neonatal diagnosis of hypothyroidism, CAD. .). In separate experiments, blood was sampled from veins into EDTA-containing tubes to allow precise volume distribution on the blotter paper (15 µL). Discs were punched from blotter paper (6.5 mm Ø). Two solid phase cortisol RIA (Gamma-Coat Diasorin) were performed: method A routine assay in serum, or method B modified procedure to assay in saliva. Results

There was a significant relationship between serum cortisol and capillary blood cortisol whatever the method used:  $r^2$  0.83 & 0.87 (A & B methods respectively). A multivariate analysis did not show any influence of hematocrite.

Intra- and inter-assay coefficients of variation were: 13 & 5 and 13 & 11%, (A & B methods respectively).

Detection threshold (intra-assay CV <20%) were 40 & 2.7 nmol/l corresponding to 40 & 80 nmol/l serum cortisol levels (A & B methods respectively).

**Conclusion**

Capillary blood cortisol assessment is reliable enough to initially investigate diurnal rhythm or minute dexamethasone test in patients in which venous sampling is notoriously difficult. Advantages of method A are the low detection threshold and the procedure identical to serum assay allowing simultaneous management of serum and capillary samples; advantages of method B is to allow duplicate assay.

**P729****Gonadal dysfunction in male hypercortisolic subjects**

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Cushing's syndrome is rare in males. Hypercortisolic consequences on male gonadal function are unknown. The aim of our study is to research gonadal and sexual abnormalities in 17 male subjects with endogenous cortisol excess: 10 Cushing's diseases, 5 paraneoplastic Cushing syndrome, 1 malignant corticosurrenaloma, 1 micronodular dysplasia. Their mean age = 30.2 years (18-50). Our work is based an anamnestic, clinical and hormonal results. Our population is compared to group of normal men without medical or endocrinological diseases. No body has taken medical treatment before or during biological exploration.

Our results are as follow: decreased libido and/or impotency is noticed in 7/15 subjects (46%), there is a gynecomastia in one case (5.8%) and testosterone of hypercortisolic subjects is significantly lower than testosterone of normal subjects (2.9 ng/ml versus 5.05,  $P < 0.01$ ). Mean prolactine (PRL) of our population is higher than PRL of control group (11 ng/ml versus 2.5,  $P < 0.005$ ). For gonadotrophines there is no difference between the two groups (FSH of hypercortisolic subjects = 4.2 mU/ml versus 3.3 and LH = 2.9 mU/ml versus 3.0,  $P > 0.05$ ).

**Conclusion**

Men with endogenous hypercortisolism have a decreased libido and/or impotency in 46%. Their testosterone is significantly lower than normal subjects and their prolactine is higher. Gonadotrophines of hypercortisolic group are similar to those of control group. The mechanism of this normogonadotrop hypogonadism will be discussed.

**P730****A ras recruitment screen for glucocorticoid receptor-interacting proteins using the entire receptor as bait**

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Glucocorticoid hormones play a key role in glucose, fat and protein metabolism and dampen immune and inflammatory reactions. Synthetic equivalents are widely used as therapeutic drugs. Upon hormone binding, the glucocorticoid receptor (GR) detaches from a cytoplasmic multiprotein complex, translocates to the nucleus and regulates the transcription of target genes either directly or indirectly through interference with other transcription regulators.

While many GR interaction partners have been identified using transcription-based assays, less effort has been invested in unbiased screening for protein-protein interactions.

One classical tool to detect protein-protein interactions, the yeast two-hybrid system, is inherently ill-equipped to handle transcription factors, as it relies on the reconstitution of a transcription factor activity by the sought interaction.

To overcome these limitations, we used an alternative yeast two hybrid system developed by A. Aronheim, the so called "reverse ras recruitment system". Here, successful interaction between bait and prey reconstitutes defective Ras/Adenylate cyclase signaling in the yeast mutant *cdc25-2*. Using the entire human GR as bait, we are screening a HeLa cDNA library. Positive candidates identified in this screen are expressed as FLAG-tagged fusion proteins and validated in mammalian cells by coimmunoprecipitation experiments. Potential colocalization of receptor and interaction partner is monitored by fluorescence microscopy and live cell imaging by expressing preys as YFP- and the receptor as CFP-tagged fusion proteins. So far, screening of 200000 clones yielded 40 (0.02%) primary interaction candidates. The isolation of proteins that have previously been described to make contact with the GR, such as Bip, proves the usefulness of this approach. Several novel interaction partners were identified, some of which are currently more closely investigated.

Support: FWF (P16013).

### P731

#### Hormones of cortex of adrenal glands in patients after cardiac arrest in early postresuscitation period

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#### Aim

To assess hormonal function of cortex of adrenal glands in patients (pts) after cardiac arrest (CA) and its role for survival.

#### Material

CA-pts- 52 pts after CA in the age  $62 \pm 13$  years. 26 pts died after CA (CA-D-pts), 26 pts survived and were discharged from hospital (CA-S-pts). H-pts- 22 pts in the age  $60 \pm 11$  years with stable coronary artery disease.

#### Methods

In CA-pts concentrations (conc) of adrenocorticotropin (ACTH), cortisol, aldosterone nad dehydroepiandrosterone sulfate were measured in venous blood samples taken just after CA and in two consecutive days at 8.00 am. Conc of this hormones in H-pts were measured once, at 8.00 am. A comparison of conc of the hormones between CA-D-pts and CA-S-pts was performed.

#### Results

Significantly higher conc of ACTH and cortisol was found in CA-pts than in H-pts in each day after CA. Conc of aldosterone was higher in CA-pts only just after CA. No difference in conc of dehydroepiandrosterone sulfate between these groups was found.

Compared to CA-S-pts, in CA-D-pts conc of ACTH was lower just after CA ( $5.2 \pm 10.8$  vs  $7.9 \pm 7.7$  nmol/l,  $P < 0.037$ ). Conc of cortisol was higher in CA-D-pts than in CA-S-pts just after CA ( $3733 \pm 5869$  vs  $1635 \pm 1484$  nmol/l,  $P < 0.1$ ) and in the first ( $2084 \pm 2119$  vs  $1084 \pm 722$  nmol/l,  $P < 0.034$ ) and the second day ( $1325 \pm 1083$  vs  $711 \pm 401$  nmol/l,  $P < 0.03$ ) after CA.

#### Conclusions

(1) ACTH- cortisol response plays an important role for survival in pts after CA; (2) Significantly higher conc of cortisol in CA-D-pts, in comparison to CA-S-pts, suggests extremal involvement of this hormone for the attempt of survival after CA and may indicate pts in severe state after CA need substitutional administration of cortisol; (3) Incomparable elevated conc of ACTH to high conc of cortisol is a marker of bad prognosis after in pts after CA.

### P732

#### Clinical presentation of a patient with novel mutation in CYP17 gene

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The aim of this publication is to address an attention to possible misdiagnosis in patients with 17-alpha-hydroxylase deficiency.

A 24 year old woman was admitted to our Centre with diagnosis of aldosteroma (aldosterone – 486 pmol/l (22–478), hypertension, hypokaliemia, adrenal mass (d – 2.2 sm) defined by ultra sound). At the age of 15 years the patient was diagnosed with testicular feminisation as the cause of absent puberty (karyotype – XY, elevated LH and FSH), a year later gonadectomy was performed, since then the woman was on oestrogen/gestogen therapy. There were 2 episodes of severe hypokaliemia at ages 20 and 24 misdiagnosed as myocarditis and myasthenia, respectively. Later investigation did not confirm these diagnoses.

At the presentation the patient had eunuchoid stature, muscle weakness, blood pressure 140/100 mm Hg on antihypertensive therapy (lisinopril 10 mg/day, spironolactone 150 mg/day), which along with lab data (K – 3.31 mmol/l, ACTH – 149 pg/ml, cortisol in blood – 44.6 nmol/l, free cortisol in 24 hour urea – <36 nmol/l, testosterone – 0.3 pmol/l) and data of MRI (adrenal hyperplasia) helped us to suspect the diagnosis of 17-alpha-hydroxylase deficiency. The subsequent genetic analysis revealed compound heterozygosity for 2 mutations: deletion of 2 nucleotides CT in codon S117 (S117delCT) and substitution Arginin to Histidin in codon 440 (R440H) from which S117delCT mutation is novel.

After initiation of prednisolone at dose of 5 mg/day muscle strength dramatically improved, blood pressure remained at the level 110/70 mm Hg without any therapy, K level normalized.

### P733

#### Hypothalamic-pituitary-adrenal axis in diabetes: role of autonomic unbalance

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In order to evaluate hypothalamic-pituitary-adrenal activity in diabetic patients in relation to neuroautonomic balance, we studied 50 consecutive hospitalized T2D patients (22F, 28M) without symptoms of neuropathy or hypercortisolism. We measured: morning, midnight and post-dexamethasone suppression cortisol (F8, F24 and F-Dex respectively) 24-hours urinary free cortisol (UFC), morning ACTH, systolic and diastolic blood pressure levels (SBP, DBP) and performed deep-breathing (DB), lying-to-standing (LS) and postural hypotension (PH) tests. Patients were subdivided according to the presence of parasympathetic (Group A,  $n = 11$ ), sympathetic (Group B,  $n = 11$ ), para- and sympathetic (Group C,  $n = 9$ ) dysfunction or the absence of autonomic failure (Group D,  $n = 15$ ). Age (whole group  $58.2 \pm 9.3$  yrs), BMI (whole group  $30.3 \pm 4.9$  kg/m<sup>2</sup>) and glycated haemoglobin (HbA<sub>1c</sub>, whole group  $10.3 \pm 2.2\%$ ) were comparable among the 4 groups.

HPA activity was significantly increased in Group A compared to Group D (UFC  $48.6 \pm 21.4$  vs  $21.6 \pm 9.8$  µg/24 h,  $P < 0.0001$ ; ACTH  $27.0 \pm 8.6$  vs  $15.7 \pm 5.7$  pg/dL,  $P < 0.01$ ; F8  $20.4 \pm 4.5$  vs  $13.6 \pm 3.8$  µg/dL,  $P < 0.05$ ; F-Dex  $1.2 \pm 0.4$  vs  $0.8 \pm 0.6$  µg/dL,  $P < 0.05$ , respectively) and Group B (UFC  $26.3 \pm 11.0$  µg/24 h,  $P < 0.0001$ ; ACTH  $19.9 \pm 8.0$  pg/dL,  $P < 0.05$ ). SBP and DBP were significantly increased in Group A compared to Group D (SBP  $136.4 \pm 16.8$  vs  $120.7 \pm 12.9$  mmHg,  $P < 0.01$  and DBP  $81.0 \pm 7.7$  vs  $74.0 \pm 6.1$  mmHg,  $P < 0.05$  respectively) and Group B (SBP  $123.3 \pm 14.6$ ,  $P < 0.05$ ; DBP  $75.5 \pm 7.2$ ,  $P < 0.05$ ).

Values of DB and LS tests resulted to be correlated to UFC ( $R = -0.39$ ,  $P = 0.006$ ;  $R = -0.46$ ,  $P = 0.009$ ), SBP ( $R = -0.32$ ,  $P = 0.025$ ;  $R = -0.30$ ,  $P = 0.04$ ) and DBP ( $R = -0.28$ ,  $P = 0.046$ ;  $R = -0.32$ ,  $P = 0.025$ ). Multivariate linear regression analysis showed that UFC levels were significantly associated with parasympathetic neuronal dysfunction as reflected by LS test ( $\beta = -0.44$ ,  $P = 0.003$ ) after adjusting for BMI, HbA<sub>1c</sub> and disease duration. Asymptomatic diabetic autonomic unbalance is associated to increased activity of HPA axis and blood pressure levels, related to the degree of the neuronal dysfunction.



## P734

**Establishing circadian rhythm profiles for salivary testosterone in women: evidence of decline during ageing**EAS Al-Dujaili<sup>1</sup> & MA Sharp<sup>2</sup><sup>1</sup>Queen Margaret University College, Edinburgh, United Kingdom;<sup>2</sup>University of Edinburgh, Edinburgh, United Kingdom.

Androgens are increasingly known to play an important role in female health, development and well being. Alongside a controversial increase in androgen therapy there is a realisation that what actually constitutes androgen insufficiency is poorly understood. The aim of this study was to establish circadian profiles for salivary testosterone in healthy women from the age of 19 through 69 year old at 3 points during the menstrual cycle. Females were subdivided into these groups: 19–29, 30–39, 40–49 (peri- and post menopausal), 50–59 and 60–69 years were investigated. The study was approved by the University College Ethical Committee and all subjects provided 8 saliva samples per day on the 4th, 14th and 21st day of their cycle. Post-menopausal women collected saliva samples on the 4th, 14th and 21st day of the calendar month. The women were not on any hormonal medication such as the contraceptive pill and HRT; nor did they suffer from any major illness. Testosterone levels were estimated using our in-house highly sensitive and specific ELISA method. The results indicated that female salivary testosterone concentration showed a circadian rhythm similar to that found in males, though at much lower levels. Perhaps more importantly, throughout the course of the day testosterone levels were highly variable with episodic fluctuations of individual data points exceeding the 09.00 hours levels by up to 90% on some occasions, indicating a role for the hormone in reproduction and sexual health. There was marked variation in testosterone concentration between day 4, 14 and 21 of the cycle, though not statistically significant except for the age group of 30–39 year ( $P < 0.02$ , one-way repeated measure ANOVA). However, a significant decline in female salivary testosterone levels after the age of 39 years was evident. Day 4 of the cycle always exhibited near absence of salivary testosterone circadian rhythm. In contrast, day 21 testosterone values showed the best clear indication of the circadian rhythm profile compared to day 4 of the cycle. In conclusion, our study has established circadian profiles for female salivary testosterone and indicated that the testosterone levels were seriously low in some post-menopausal women and those above the age of 50 years that may justify some intervention to improve the health and well being of women. It is nevertheless an important hormone for.

## P735

**Prevalence of classical forms of 21-hydroxylase deficiency in Western Siberia according to the results of neonatal screening of congenital adrenal hyperplasia**LA Suplotova, EB Hramova, OB Makarova, NU Yuzakova & TV Barcova  
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## The purpose

To study prevalence of classical forms of 21-hydroxylase deficiency according to the results of neonatal screening on a congenital adrenal hyperplasia (CAH) in Tyumen, Russia.

The analysis of neonatal screening on CAH results in Tyumen for the period of 2003–2004. For the period under research 20011 newborn have been tested on the 17-OHP level. Measurement of 17-hydroxyprogesterone in blood samples was done in the central laboratory by the ELISA method using sets "Delfia Neonatal 17-OHP". The maximum normal quantity of 21-hydroxylase is 17 - OHP 60 nmol/L.

## Results

In the first test among 20011 newborn Tyumens there were 124 persons with 17-OHP level above 60 nmol/L, that makes 0.61%. According to the results of repeated test for 17-OHP from these 124 persons 2 male children with 21-hydroxylase deficiency have been revealed. Thus, frequency of classical forms of CAH in the Tyumen population is determined as 1:10005 newborns. The rest of newborn with 17-OHP level above normal – 122 persons (98.3%) had transitional character of 17-OHP level increase.

Sensitivity of the research method is determined as 100%, i.e. children with CAH in 100% had rising 17-OHP level. Specificity has made 99%, efficiency of the used method – 99.4%.

In order to study influence of gestational age and body weight of newborn on the level of neonatal 17-OHP, all the tested newborn children have been divided in 2 groups. In the first one the average weight at birth was of 2139 ± 345 g. and in the second one of

3448 ± 504 g. ( $P = 0.000$ ), the average gestation term was of 33 ± 1.4 week and 38.8 ± 0.9 weeks respectively ( $P = 0.000$ ). The 17-OHP level obviously differed in these 2 groups  $P = 0.015$ . The correlation analysis also revealed weak negative interrelation between these characteristics  $r = -0.18$ ,  $P = 0.007$ . Thus, the research shows the dependence of the level of neonatal 17-OHP on body weight and gestation age of newborn children.

## P736

**The New Zealand White Albino Rabbit is a suitable model for the evaluation of 11 $\beta$ -hydroxysteroid dehydrogenase type 1 activity in ocular tissues**CU Onyimba<sup>2</sup>, P Khosla<sup>2</sup>, SV Hughes<sup>2</sup>, PI Murray<sup>1</sup>, PM Stewart<sup>2</sup>, EA Walker<sup>2</sup> & S Rauz<sup>1</sup><sup>1</sup>Academic Unit of Ophthalmology, Division of Immunity and Infection, University of Birmingham, Birmingham, United Kingdom; <sup>2</sup>Department of Endocrinology, Division of Medical Sciences, University of Birmingham, Birmingham, United Kingdom.

Ion and fluid transport mechanisms within the eye are important for several key physiological processes including the maintenance of corneal transparency and the regulation of intraocular pressure (IOP). The mechanism involved in epithelial sodium transport in the eye is regulated by corticosteroids and at a pre-receptor level, by 11 $\beta$ -hydroxysteroid dehydrogenase (11 $\beta$ -HSD) activity. Recent studies localised type 1 (11 $\beta$ -HSD1), an oxo-reductase that activates cortisol from cortisone, to the non-pigmented layer (NPE) of the human ocular ciliary body (CB) epithelium and corneal epithelium (CE). Its potential role in aqueous humour (AH) production provides a novel target for AH suppression and treatment of IOP. Animal models are essential for further evaluation of 11 $\beta$ -HSD1 activity within the eye and the New Zealand White Albino rabbit (NZWAR) has been widely used to study IOP and a variety of ocular diseases. In-house generated primary antibodies to human 11 $\beta$ -HSD1 were used to study 11 $\beta$ -HSD1 expression within NZWAR eye sections and cells. Specific enzyme assays were also performed to assess 11 $\beta$ -HSD1 activity in this animal model.

Immunohistochemical studies showed that, as in human tissue, 11 $\beta$ -HSD1 localised to the NPE of the CB and the CE. Additionally, specific activity assays indicated predominant oxo-reductase activity compared to dehydrogenase activity in CB tissue (median, 44.4 ± 16% conversion vs 18.6 ± 12% conversion;  $P = 0.003$ ,  $n = 12$ ) and primary CE cells (oxo-reductase; median, 3.0 ± 1.9 pmol/mg/h, vs dehydrogenase, 0.5 ± 0.8 pmol/mg/h;  $P = 0.006$ ,  $n = 12$ ). Further confirmation of 11 $\beta$ -HSD1 expression was obtained by Western blot analysis of CB tissue and immunocytochemistry of primary cultured CE cells.

The results obtained indicate the NZWAR is a suitable *in-vivo* model for further investigation of 11 $\beta$ -HSD1 activity in the regulation of IOP and corneal physiology. This will help establish the NZWAR as a suitable model for the evaluation of specific topical 11 $\beta$ -HSD1 inhibitors for the treatment of raised intraocular pressure-related disorders.

## P737

**Androgen status in women with hypogonadotropic hypogonadism**

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Androgens play an important part in follicular development, sexual behavior, maintenance of skeletal homeostasis, normal affect and cognitive function. The negative influence of androgen deficiency is widely discussed in postmenopausal women. However, there is a cohort of young women with gonadal steroid deficit caused by the disorders of central regulation. It is well known that these patients are in lack of estrogens however their androgen status is not detailed.

We examined 25 women with hypogonadotropic hypogonadism (HH – group 1), age from 18 to 45 (mean 29 years 1 months), mean duration of HH 5 years 3 months, 12 of these patients had isolated HH (subgroup 1a), 13 women had HH associated with the other types of hypophyseal deficiency (subgroup 1b). Ten healthy women were included in control group (group 2). Concentrations of serum testosterone, SHBG and free testosterone were measured in all patients.

The mean concentrations of testosterone were 0.43 ± 0.12 ng/ml in group 1 and 1.02 ± 0.15 ng/ml in group 2 ( $P = 0.019$ ). The mean levels of SHBG were 161.75 ± 27.57 pmol/l and 82.35 ± 19.55 pmol/l respectively

( $P = 0.111$ ), whereas mean free testosterone concentrations were  $0.02 \pm 0.005$  pmol/dl in group 1 and  $0.14 \pm 0.028$  pmol/dl in group 2 ( $P < 0.0001$ ). Though the concentrations of total and free testosterone in subgroup 1a and in subgroup 1b were low there was the significant difference between them: mean levels of total testosterone  $0.78 \pm 0.09$  ng/ml and  $0.09 \pm 0.01$  ng/ml respectively  $P < 0.0001$ , mean levels of free testosterone  $0.04 \pm 0.01$  pmol/dl and  $0.01 \pm 0.001$  pmol/dl respectively  $P = 0.005$ . Mean SHBG concentrations were significantly higher in subgroup 1a compared to 1b:  $224.0 \pm 30.56$  pmol/l and  $99.50 \pm 23.43$  pmol/l,  $P = 0.012$ .

Thus the patients with hypogonadotropic hypogonadism have lack of androgens (particularly free testosterone) compared to healthy women. This deficiency is significantly more evident in patients with combined hypophyseal deficiency. These patients are on the list for the androgen replacement therapy.

### P738

#### Long-term glucocorticoid repression of pro-opiomelanocortin (*POMC*) expression

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#### Background

A functioning Hypothalamo-pituitary-adrenal (HPA) axis is essential for life. Prolonged high circulating levels of glucocorticoids inhibit pituitary Pro-opiomelanocortin (*POMC*) gene expression. Repression continues even in the absence of continued glucocorticoid exposure. To date there are no molecular explanations for this observation.

#### Hypothesis

Long-term exposure to glucocorticoids causes *de-novo* DNA methylation of the pituitary *POMC* promoter, or of the promoters of transcription factors that regulate *POMC* (*Ptx1*, *NeuroD1*, *Tpit* and *Nur77*), thereby imparting an inhibitory imprint and decreasing *POMC* expression even in the absence of ligand.

#### Methods

AtT-20 cells were cultured in standard growth media alone, or media supplemented with dexamethasone ( $10^{-7}$  M) for between 72 hours to 16 weeks. DNA and RNA were extracted by standard means. Real time quantitative PCR (qPCR) and standard RT-PCR was performed to establish expression of *POMC*, *Ptx1*, *NeuroD1*, *Tpit* and *Nur77*. Methylation patterns were assessed by bisulphite sequencing.

#### Results

At 4 weeks *POMC* and *NeuroD1* expression were absent in treated cells and normal in untreated cells, whilst dexamethasone treatment did not affect expression of *Tpit* and *Nur77* or *Ptx1*. The *POMC* promoter was, however, unmethylated in both treated and untreated cells.

#### Conclusions

*De novo* DNA methylation of the *POMC* promoter does not appear to be caused by long-term glucocorticoid exposure, at least for the doses and times studied. Prolonged glucocorticoid exposure inhibits expression of only one of the known transcription factors that stimulate *POMC* expression. Whether *NeuroD1* is methylated in this context remains to be elucidated.

### P739

#### A novel CYP11B2 gene mutation in an Asian family with aldosterone synthase deficiency

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Three siblings of Pakistani origin presented shortly after birth with failure to thrive and hyperkalemia and were found to have isolated hyperreninaemic hypoaldosteronism. They were all well controlled on fludrocortisone therapy during childhood and adolescence. When reassessed in adult life off fludrocortisone treatment, hyperreninaemic hypoaldosteronism was confirmed in all subjects, but with significant hyperkalemia in only one case. None of the subjects developed orthostatic hypotension or salt craving. Profiling of urinary steroid metabolites showed a biochemical pattern (elevated Tetrahydrocorticosterone/18-OH tetrahydro-11-dehydrocorticosterone

(THB/18-OH THA) ratio but normal 18-OH tetrahydro-11-dehydrocorticosterone/tetrahydroaldosterone (18-OH-THA/THaldo) ratio consistent with partial type 1 aldosterone synthase deficiency (ASD1). The CYP11B2 gene was sequenced and affected subjects were homozygous for a single nucleotide substitution (C925T) in exon 5, corresponding to a Serine to Proline mutation (S308P) in the predicted protein sequence. Two unaffected siblings and both parents were heterozygous for the mutation. Structural modelling indicates that the S308P mutation, located within the I alpha helix, is close to the haem binding and active site of the enzyme and therefore likely to be deleterious. We have identified the first CYP11B2 gene defect in patients of Asian origin in association with an ASD1 phenotype. Functional characterisation of the S308P mutant will determine the extent of loss of enzyme activity and its relationship to defective aldosterone biosynthesis *in vivo*.

### P740

#### Long-term effect of flutamide, metformin and their combination in dieting obese women with polycystic ovary syndrome

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The treatment of PCOS remains an unresolved issue due to the plethora of clinical manifestations of the syndrome. Aim of this long-term, prospective, placebo-controlled study was to define the effects of metformin, flutamide and their combination in dieting obese women with PCOS. Eighty obese women with PCOS (18–45 years) were included in the study. Hypocaloric diet was added to placebo, or metformin (1700 mg day), or flutamide (500 mg day), or metformin + flutamide for 12 months (20 subjects in each group). All patients underwent clinical, hormonal and metabolic assessments and a CT measurement of fat distribution at baseline, 6 months and 12 months. Respect to placebo, drugs had no further effects in decreasing body weight and waist circumference at any time. Flutamide had an additive effect in decreasing visceral fat after both 6 ( $P = 0.044$ ) and 12 months ( $P = 0.033$ ) and a progressive effect in reducing androgen levels and hirsutism score ( $P < 0.001$  after 6 and 12 months;  $P = 0.019$  12 months vs. 6 months). An additive effect of metformin in improving menses abnormalities became evident after 6 months ( $P = 0.039$ ) and further increased after 12 months ( $P < 0.001$ ;  $P = 0.013$  12 months vs. 6 months), when even flutamide effectively improved menses ( $P = 0.008$ ). After 12 months, metformin favoured a significant decrease of glucose-stimulated insulin levels ( $P = 0.014$ ), whereas flutamide further improved insulin sensitivity index ( $P < 0.001$ ) and LDL cholesterol levels ( $P = 0.003$ ). The combination of metformin and flutamide maintained the effect of each drug without any synergistic effect. These findings add relevance to the usefulness of insulin sensitizers and antiandrogens in the long-term treatment of dieting obese PCOS women and definitively provide a rationale for targeting different therapeutical options according to the required outcomes.

### P741

#### Can the 250mcg synacthen test be used to screen for primary hyperaldosteronism?

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Primary hyperaldosteronism secondary to bilateral adrenal hyperplasia (BAH) bears striking similarities pathophysiologically to essential hypertension. During stimulation studies to elucidate possible aberrant aldosterone responses in BAH we measured the aldosterone response to 250 mcg iv synacthen after 30 and 60 minutes in 7 patients with BAH and in 20 healthy controls. Patients had diuretics withheld for at least 4 weeks and betablockers and calcium channel blockers withheld for at least 2 weeks. Subjects were in the supine position for at least 30 minutes. For controls the serum aldosterone level (mean  $\pm$  standard error) was  $189$  pmol/l  $\pm$  21 at baseline,  $581$  pmol/l  $\pm$  48 at 30 minutes and  $565$  pmol/l  $\pm$  47 at 60 minutes. In the patient group, the serum aldosterone levels were  $722$  pmol/l  $\pm$  266 at baseline,  $2777$  pmol/l  $\pm$  633 at 30 minutes and  $2921$  pmol/l  $\pm$  790 at 60 minutes. Using a cutoff aldosterone value of 1200 pmol/l at 30 minutes

the synacthen test has a sensitivity of 85.7% and specificity of 100% for the diagnosis of BAH. At 60 minutes, using a cutoff value of 750 pmol/l, the sensitivity was 100% and specificity was 95%.

These unexpected results are promising for the possible use of the synacthen test in the screening for hyperaldosteronism if similar findings are confirmed in adenoma patients and if further studies in patients with essential hypertension show similar clear distinction from patients with hyperaldosteronism as have been seen with controls.

## P742

### The functional consequences of local glucocorticoid metabolism in synovial fibroblasts

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Tissue inflammation is usually transient but in diseases such as rheumatoid arthritis (RA) inflammation persists. It remains unclear why inflammation persists in some tissues and not in others. Recent studies have shown that stromal cells such as fibroblasts play a pivotal role in determining this persistence. We have hypothesized that glucocorticoid (GC) activation via the enzyme 11 beta-hydroxysteroid dehydrogenase type 1 (11 beta-HSD1) within fibroblasts plays a key role in modulating inflammatory responses. 11 beta-HSD1 converts inactive GCs (cortisone/prednisone) to their active counterparts (cortisol/prednisolone). 11 beta-HSD1 expression varies between fibroblasts from different tissues with activity and expression higher in synovial fibroblasts than fibroblasts from bone marrow or skin. We have now tested the functional consequences of these expression differences on fibroblast production of IL-6. IL-6 mRNA expression and protein levels were measured in primary fibroblasts isolated from dermis (DM), bone marrow (BM), and synovium (SY) in 3 subjects with RA. Cells were cultured in the presence or absence of GC and gene expression assessed by quantitative RT-PCR. IL-6 generation was measured by ELISA. Treatment with 100 nM cortisol decreased IL-6 mRNA expression in all fibroblasts (7-fold decrease with SY, 3.5-fold BM, 4.5-fold DM; all  $P < 0.01$ ). By contrast 100 nM cortisone decreased expression only in SY fibroblasts (5-fold) with no change in BM or DM fibroblasts. IL-6 ELISA assays displayed an identical pattern of expression with suppression by cortisol in all cells but cortisone only suppressing IL-6 generation in SY fibroblasts. The effects of cortisone, but not cortisol, were prevented by the 11 beta-HSD1 inhibitor glycyrrhetic acid. Local GC activation can decrease expression of IL-6, which in turn is likely to impact on leukocyte behaviour within the stromal environment. GC metabolism in synovial fibroblasts may be key to understanding the development of persistent inflammation in RA.

## P743

### Challenges in paediatric Cushing's disease

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#### Introduction

Paediatric Cushing's disease is rare and up to 50% have incomplete cure. <sup>1</sup>Besides hypopituitarism growth failure is particularly common and maybe due to GH deficiency, target tissue resistance. <sup>2</sup> or accelerated skeletal maturation. <sup>3</sup> Our patient is an 18-year-old obese male who first presented at the age of 16 with delayed puberty and short stature.

#### Results

His 24 hr urinary cortisol was 1472 nmol/l, ACTH 56 nmol/l, low & high dose dexamethasone suppression test reduced the early morning cortisol from 1023 to 288 and from 575 to 25 respectively and the rest of his hormone profile was normal. His fasting blood sugar was 10.9, HbA1c 12% and bone age was 16 years. Visual fields were normal and MRI showed a right-sided pituitary macro adenoma and normal adrenals. Oral hypoglycaemics were started and he underwent a trans-sphenoidal hypophysectomy with an uneventful recovery and pathology showed an ACTH – expressing pituitary adenoma. Pituitary testing showed (Table 1):

Table 1

	0 min	30	60	120	150	180
Testosterone nmol/l	3.1					
GH mU/L post GHRH & arginine	6.87	7.16	9.85	3.46	1.75	0.94
IGF 1 nmol/L	32					
Cortisol nmol/L	41					
24 hr urinary cortisol nmol/L	312					
ACTH	< 10					
TSH	0.62					
f T4	11.8					

#### Conclusions

Paediatric Cushing's can be missed for years and the overall prognosis is not as good as adult Cushing's. Management is challenging where the feasibility and timing of treatment can be very difficult. Post operative dynamic pituitary testing can sometimes be essential to unmask underlying hormone deficiencies.

1. Leinung et al. JCEM (1995); 80: 2475–2479.
2. Magiakou MA et al. NEJM (1994); 331: 629–636.
3. Hayes AB et al. Pediatrics (1966); 37: 19–25.

## P744

### Endogenous corticosteroid synthesis in subjects after bilateral adrenalectomy

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Corticosteroids can be synthesised in tissues other than the adrenal cortex but the contribution of this extra-adrenal corticosteroidogenesis to circulating levels in man is not known. We studied this in a group 10 subjects taking chronic glucocorticoid replacement following bilateral adrenalectomy. In phase 1, they were maintained on cortisol alone (30 mg/day). In phase 2, cortisol was replaced by dexamethasone (2 mg/day) and in phase 3, they received both cortisol and dexamethasone. Each phase lasted 3 days. A 24 h urine collection was made on the last day of each phase for analysis of steroid metabolite excretion by GCMS. Local ethics committee approval was obtained. Cortisol metabolite excretion rate (THE + THF + aTHF) fell from 9169 nmol/24 hr in phase 1 to 22 nmol/24 hr in phase 2 rising to 6843 nmol/24 hr in phase 3. Tetrahydroaldosterone excretion was low but detectable and did not alter significantly between phases (26.5, 23.5 and 28.5 nmol/24 hr respectively;  $P = 0.474$ ). In contrast, 18-hydroxycortisol (18-OHF) excretion was higher than in normal subjects in phases 1 and 3 (252.5 & 212 nmol/24/hr), falling substantially in phase 2 (12 nmol/24 hr).

Easily detectable amounts of aldosterone are synthesised in subjects who have undergone bilateral adrenalectomy. We conclude that this occurs at extra-adrenal sites, or in residual adrenal cortex tissue in an ACTH-independent manner. Synthesis of 18-OHF is entirely dependent on exogenous cortisol which must be 18-hydroxylated by 11 $\beta$ -hydroxylase or aldosterone synthase again either in residual or extra-adrenal tissue. This agrees with our previous report in normal subjects 1. The high levels of 18-OHF in phase 1 may indicate some post-adrenalectomy induction of these enzymes. Finally, the low, but detectable, excretion of cortisol metabolites in phase 2 also represents endogenous synthesis. Further studies of corticosteroid production within adrenalectomized subjects, particularly looking for evidence of adrenal re-growth or residual adrenal tissue, are clearly justified.

1. Freel EM et al. Studies on the origin of circulating 18-hydroxycortisol and 18-oxocortisol in normal subjects. JCEM 2004; 89: 4628–4633.



**P745**

**Tissue specific regulation of insulin signalling: a mechanism of glucocorticoid induced obesity?**

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The pathological effects of glucocorticoids (GC) are exemplified by patients with Cushing's syndrome who develop central obesity, insulin resistance and in some cases, type 2 diabetes mellitus. It is generally accepted that GC cause insulin resistance, however, both insulin and GC increase adipocyte differentiation. The question therefore arises as to how GC stimulate adipocyte differentiation whilst apparently making adipocytes insulin resistant. We have hypothesized that GC cause tissue specific changes in insulin sensitivity, enhancing insulin signalling in human adipose tissue in contrast to liver, and that this may represent a novel mechanism of GC induced obesity.

Human subcutaneous adipocytes (Chub-S7 cells) were grown to confluence and differentiated in chemically defined media and treated with or without GC (dexamethasone and/or cortisol). Insulin stimulated PKB/akt phosphorylation was determined by western blotting and glucose transport measured by tritiated glucose uptake. Tissue specificity was examined using the human liver cell line, HepG2 cells.

In differentiated human adipocytes, GC induced a dose (control  $1 \pm 0$  vs. 50 nM  $1.2 \pm 0.08$  vs. 250 nM  $2.25 \pm 0.24$  vs. 1000 nM  $3.4 \pm 0.17$ ,  $P < 0.001$ ) and time (control  $1 \pm 0$  vs. 6 hr  $4.11 \pm 1.7$  vs. 24 hr  $9.36 \pm 4.15$ ,  $P < 0.05$ ) dependent increase in insulin stimulated PKB/akt phosphorylation without alteration in total protein (western blotting) or mRNA (real-time PCR) expression. This effect was blocked by RU486 (GC receptor antagonist), and by wortmannin (PI3Kinase inhibitor). Furthermore, GC increased insulin stimulated glucose transport into differentiated human adipocytes in a PI3 kinase dependent manner (control  $1.8 \pm 0.2$  vs GC  $2.7 \pm 0.3$ ,  $P < 0.05$ ). In contrast, GC failed to regulate insulin stimulated PKB/akt phosphorylation in HepG2 cells.

We have demonstrated tissue specific regulation of insulin signalling by GC. In adipose tissue, GC enhances insulin signalling and we postulate that this represents a mechanism to increase adipocyte differentiation thereby contributing to GC induced obesity. Tissue specific regulation of GC action may therefore represent a novel therapeutic strategy.

**P746**

**BXL-628, a vitamin D analog, decreases RhoA/ROK signalling in rat and human bladder**

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BXL-628 is a non-hypercalcemic calcitriol analog successfully tested in a phase IIa trial for benign prostate hyperplasia therapy. Because part of low urinary tract symptoms (LUTS) are generated by overactive bladder (OAB) and bladder expresses the calcitriol receptor (VDR), we investigated the BXL-628 effects on bladder contractility and RhoA/ROK signalling activated in OAB, by *in vivo* (2-weeks; 30 mcg/Kg, Sprague-Dawley, SD, and spontaneously hypertensive rats, SHR) and *in vitro* (human bladder stromal cells, hBC) experiments. In SD bladder, BXL-628 did not affect maximal responsiveness to carbachol but increased the lag time to reach it, and reduced the maximal relaxant effect of the ROK inhibitor Y-27632. In SHR bladders, that over-express RhoA/ROK pathway and develop OAB, the Y-27632 effect was higher than in normotensive control rats Wistar-Kyoto (WKY). BXL-628 normalized SHR bladder sensitivity to Y-27632 up to WKY, while did not significantly affect RhoA/ROK expression (qRT-PCR) in rat bladder and in hBC. In immunokinase assay BXL-628 significantly decreased ROK activity, by reducing Y-27632 effect, of hBC extracts immunoprecipitated with anti-ROK. In SHR bladders ROK activity was higher than in WKY and significantly reduced by BXL-628. We therefore investigated whether BXL-628 impaired RhoA activation and its membrane translocation in hBC. Confocal microscopy, using pancadherin (a membrane marker) and RhoA immuno-labelling, revealed a reduction of RhoA membrane expression, which indicates its activation state, in BXL-628 treated hBC. Accordingly, BXL-628 significantly reduced the activated, rotekin-bound, RhoA fraction in hBC (Western blot). Because RhoA is

involved in cell motility, we tested the effect of BXL-628 on hBC migration. BXL-628 even at 0.01 pM inhibited cell migration to the same extent as other inhibitors of RhoA (simvastatin, C3 exoenzyme). Calcitriol was effective at higher concentrations (1nM). In conclusion, in human and rat bladders, BXL-628 inhibits RhoA/ROK signalling, suggesting a novel therapeutic opportunity for OAB and LUTS treatment.

**P747**

**Evaluation of aldosterone to renin ratio in patients with adrenal incidentalomas and/or hypertension**

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Primary aldosteronism is diagnosed with increasing frequency, especially between patients with hypertension. Thus, a reliable screening test is necessary to avoid costly and demanding diagnostic procedures to this population. The aim of the present study is the evaluation of aldosterone to renine ratio (A/R) in the diagnostic algorithm of patients with hypertension with or without adrenal insidentaloma.

A total of 115 subjects were studied: 13 patients with surgically proven hyperaldosteronism (Group I: age  $50.5 \pm 1.4$  yrs), 23 patients with adrenal incidentaloma (Group II: age  $60.3 \pm 1.8$  yrs, 11 hypertensive and 12 normotensive) and 79 subjects without known endocrinopathy (Group III: age  $48.7 \pm 1.3$  yrs, 27 hypertensive and 52 normotensive).

A ROC analysis was performed to define the A/R value that best discriminates patients with hyperaldosteronism. This analysis discloses that a A/R of 25 has a 100% sensitivity and A/R of 60 has a 100% specificity for primary hyperaldosteronism. A/R  $> 25$  was found in 1/11 hypertensive and in 2/12 non hypertensive patients of group II and in 4/27 hypertensive and in 2/52 non hypertensive subjects of group III. Patients of group II demonstrated significantly higher A/R than patients of group III ( $15.6 \pm 2$  pg/ml and  $9.88 \pm 0.9$  pg/ml respectively  $P = 0.002$ ) and lower renin levels ( $16.68 \pm 3.2$   $\mu$ U/ml and  $33.3 \pm 5.0$   $\mu$ U/ml respectively,  $P = 0.001$ ). No difference in A/R between hypertensive and normotensive individuals of groups II and III was found. Furthermore, patients of group II with subtle glucocorticoid hypersecretion demonstrated lower A/R compared to patients with normal cortisol secretion ( $11.7 \pm 2$  vs  $19.4 \pm 2.9$ ,  $P = 0.04$ ).

Our results demonstrate that A/R is a reliable screening marker for the general population and for the patients with adrenal incidentalomas in order to exclude primary hyperaldosteronism. Furthermore, subtle aldosterone hypersecretion, as indicated by increased A/R, in patients with adrenal insidentalomas is not associated with the presence of hypertension or subtle glucocorticoid hypersecretion.

**P748**

**Prenatal diagnosis of the "low-oestriol" disorders Smith-Lemli-Opitz syndrome (SLOS), oxidoreductase deficiency (ORD) and sulfatase deficiency (STSD)**

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**Introduction**

Unconjugated oestriol ( $uE_3$ ) is often measured in maternal serum during second trimester prenatal screening. While oestriol itself has little physiological importance, low values can indicate the presence of a disorder in fetoplacental synthesis with potential health implications. Fetal steroids pass through the maternal circulation and into the urine so analysis of maternal fluids can be used for diagnosing causes of low oestriol.

**Methods**

Samples of urine and serum were obtained from  $> 1,000$  pregnant women who exhibited low  $uE_3$  ( $< 0.3$  MoM). They were primarily from a multicenter study funded to investigate the feasibility of screening for SLOS. After solid phase extraction (SPE) of 1 ml samples, conjugated steroids were hydrolyzed, the free compounds extracted by SPE, derivatized and analyzed by GC/MS. Local ethical committee permissions were obtained for these studies.



**Results**

SLOS diagnosis was by measuring 7- and 8-dehydro-pregnenetriol and dehydro-oestriol. The key parameter for STS was excessive excretion of 16-hydroxyDHEA sulfate and ORD could be diagnosed by quantifying a pregnenolone metabolite epiallo-pregnanediol. Different quantitative ratios between known steroids ("precursor metabolite"/"product metabolite") have been tested for delineating affected and non-affected pregnancies. Cut-off values for these ratios in urine and serum were established in order to optimize the sensitivity and specificity of the method. The assay false positive rate for SLOS was 0%, and for STS was about 3.2%. The false negative rate for both SLOS and STS was 0%. Through quantifying these selected ratios 5 samples positive for SLOS, and 182 positive for STS were detected. Confirmatory diagnostic data were available through independent laboratory testing and/or postnatal clinical examination for all SLOS cases and 50% of STS cases. Three cases of ORD were found, independent of the primary multi-center program.

**Conclusions**

For SLOS, STS and ORD non-invasive second trimester diagnosis is readily attainable through GC/MS analysis.

**P749****Hydrocortisone replacement therapy- the controversy continues...**

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Although hydrocortisone is the standard replacement therapy for primary or secondary adrenal insufficiency (AI), there are limited data on dosage requirements and the value of salivary monitoring in this patient group. We assessed inter-individual variability in cortisol metabolism and relationships between plasma and salivary cortisol profiles in 27 patients. Ten (3 male) patients had primary AI and 17 (8 male) secondary AI. Salivary and plasma cortisol levels were measured at regular intervals following intravenous (IV, 20 mg bolus) and oral (10–20 mg) hydrocortisone. Local Ethics Committee approval was obtained. Cortisol levels were measured in saliva by radioimmunoassay (<sup>125</sup>I-cortisol) and in plasma by HPLC following solvent extraction.

Wide variability in plasma cortisol profiles was observed after IV administration (C<sub>max</sub> range 715–8313 nmol/L; AUC 1367–11658 nmol.h/L). Cortisol clearance was calculated as an indirect measure of inter-individual variability. This had a mean value (SD) of 20.5 (9.1) L/h and was influenced by weight (mean (SD) 0.273 (0.107) L/h/kg), with no additional effect of age, height, body surface area or sex. After oral administration, C<sub>max</sub> ranged from 422–1554 nmol/L, AUC 1160–4511 nmol.h/L and oral clearance had a mean (SD) of 0.245 (0.082) L/h/kg.

There was no clear relationship between paired saliva and plasma cortisol concentrations after IV or oral dosing. There was a significant correlation between plasma and salivary AUC<sub>2–8h</sub> after IV administration ( $r = 0.87$ ,  $p = 0.00$ ) but differences between predicted and measured plasma AUCs ranged from 3–90% (median 18%). There was a poor correlation between plasma and saliva AUC<sub>2–6h</sub> after oral administration ( $r = 0.4$ ,  $p = 0.08$ ). Wide inter-individual variability was identified in the handling of hydrocortisone in patients with AI making it difficult to recommend a standard 'dose' of hydrocortisone. Poor correlation between limited sample salivary and plasma AUCs suggests that salivary monitoring is unlikely to be a useful determinant of dosage requirements in patients with AI.

**P750****Dietary polyunsaturated fatty acid supplementation *in vivo* modulates ovine adrenal steroidogenesis *in vitro***

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Increased dietary intake of polyunsaturated fatty acids (PUFAs) has been reported to be associated with a decreased incidence of cardiovascular disease. Increased dietary intake of polyunsaturated fatty acids (PUFAs) has

been reported to be associated with a decreased incidence of cardiovascular disease. We have recently reported that ovarian steroid synthesis in ewes fed a diet high in n-3 PUFAs is significantly compromised.<sup>1</sup> Hence in this study we have assessed the impact of a diet high in n-3 PUFAs on adrenal steroid synthesis. Two groups of Welsh Mountain ewes were fed either a control diet or a diet supplemented with n-3 PUFAs (linseed) for six weeks. Ewes were then culled and adrenal glands removed and tissue either stored at –80°C for the analysis of protein expression or used for isolation of adrenal cells, which were cultured under basal conditions for 24 h in serum-supplemented media. Spent media was then removed and cells re-incubated in the presence or absence of 10<sup>–9</sup>M ACTH for a further 24 h after which spent media was stored at –20°C prior to analysis for cortisol by RIA. The cortisol response to ACTH in PUFA-fed ewes was significantly attenuated [(control: 4756.5 ± 683.9 vs n-3: 687.6 ± 57.3) ng/5 × 10<sup>4</sup> cells/24 h;  $n = 8$  per group  $P < 0.001$ ]. When adrenal tissue was assessed for protein expression, steroidogenic acute regulatory protein (StAR) and steroidogenic factor -1 (SF-1) were found to be inhibited compared to the control ( $P < 0.05$ ) whereas expression of steroidogenic enzymes, cholesterol side chain cleavage and 3(-hydroxysteroid dehydrogenase were unchanged. This data suggests that n-3 PUFAs modulate steroidogenesis through altering the expression of proteins involved in cholesterol transport and/or metabolism. 1. Chin *et al* (2005) Dietary Polyunsaturated Fatty Acids Inhibit Ovarian Progesterone Secretion through Changes in Ovarian StAR Protein Expression in the Ewe. 87th Annual meeting of the Endocrine society, P3-254.

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**P751****Human endothelial cells (HUVECs) potentiate aldosterone secretion from human adrenocortical cells through a PKA independent pathway**

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The mineralocorticoid hormone aldosterone is secreted by the adrenal cortex. It plays a major role in maintaining water and electrolyte balance and hence blood pressure homeostasis by the kidneys. The aldosterone synthesizing zona glomerulosa of the adrenal cortex is a highly vascularized region. This allows a complex and articulated interaction between the steroidogenic cells and the vascular endothelial cells regulating the hormonal output.

In this study we show that coculture of human adrenocortical cells (NCI-H295R) with freshly isolated human umbilical vein endothelial cells (HUVECs) over 24 h increased the aldosterone production in NCI-H295R cells. This stimulation reached 70 to 80% of angiotensin II (100 nM) or forskolin (20 μM) – induced stimulation of aldosterone secretion. A similar effect was observed with 24 h HUVEC-conditioned media (HCM). HCM-induced stimulation of aldosterone production was accompanied by a 30–40% increased phosphorylation of cyclic-AMP response element (CREB). The increase in aldosterone secretion was not affected by either the Protein kinase A (PKA) inhibitor H89 (10 μM), or by pertussis toxin (200 ng/ml), interacting with Gi proteins. Further studies with inhibitors for known aldosterone secretagogues from endothelial cells like nitric oxide, endothelin-1 or cyclo-oxygenase products with their respective inhibitors L-NAME, BQ-123, BQ-788, the endothelin receptor 1A and 1B inhibitors respectively, and indomethacin did not alter the increase mediated by HCM. These data show that the endothelial cells (a) stimulate aldosterone secretion from adrenocortical cells. (b) stimulation involves the phosphorylation of CREB. (c) the aldosterone secretion is independent of the classical cAMP dependent protein kinase A. (d) indicate the stimulating factor in HCM to be different from the known aldosterone stimulators endothelin-1, nitric oxide or cyclo-oxygenase products. Therefore, endothelial cells might play a hitherto unknown paracrine role in the regulation of steroid production and aldosterone release.

**P752****Alterations in Scavenger Receptor B1 and steroidogenic enzymes within the adrenal gland in response to dietary bile acids**

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Bile acids inhibit glucocorticoid and mineralocorticoid metabolism. However bile acids may also influence hormone action by modulating steroidogenesis in the adrenal gland. In rodents adrenal cholesterol supply for steroidogenesis is derived from circulating HDL through the actions of scavenger receptor SR-B1. Since hepatic SR-B1 expression is known to be regulated by bile acids, we have investigated whether adrenal SR-B1 is similarly affected and, if so, whether adrenal lipid accumulation and key steroidogenic enzymes also change.

Male Wistar rats ( $n = 8$ ) were fed fat-free diet (Control) or fat-free diet supplemented with chenodeoxycholic acid (CDCA; 1% w/w) for 4 weeks achieving a 3-fold increase in plasma bile acids. Plasma corticosterone, aldosterone and renin were measured by RIA and ACTH by ELISA. Abundance of adrenal SR-B1, 11 $\beta$ -hydroxylase (Cyp11 B1) and aldosterone synthase (Cyp11 B2) mRNA were quantified by real-time PCR. Adrenals were stained for lipids and morphology using Oil Red O and H&E respectively. Data are treatment vs control; mean  $\pm$  SEM; \* =  $P < 0.05$ .

SR-B1 mRNA abundance was elevated by 80%\* following CDCA treatment. Adrenal gland mass was not affected but histological changes were observed principally within the cortex. Zona glomerulosa cells in glands from treated rats were more numerous but were generally smaller ( $0.18 \pm 0.007$  vs  $0.21 \pm 0.008 \mu\text{m}^2$ \*) and contained larger lipid droplets. These changes were accompanied by increased CYP11B2 expression ( $15.8 \pm 2.4 \times 10^6$  vs  $8.2 \pm 1.5 \times 10^6$  Copies/ $\mu\text{gRNA}$ ), higher circulating aldosterone ( $3501 \pm 218$  vs  $2206 \pm 137$  pg/ml) levels and enhanced renin activity ( $24.14 \pm 3.93$  vs  $15.23 \pm 1.52$  ng Ang I/ml/hr). Conversely CYP11B1 expression was decreased ( $8.1 \pm 1.6 \times 10^6$  vs  $44.04 \pm 4.3 \times 10^6$  Copies/ $\mu\text{gRNA}$ ) with reduced circulating corticosterone ( $52.6 \pm 7.4$  vs  $84.9 \pm 10.3$  nM) and no change in ACTH ( $2.12 \pm 0.4$  vs  $1.40 \pm 0.6$  pg/ml) or in the size of corticosterone-synthesizing zona fasciculata/reticularis cells.

Thus, elevated circulating bile acids, as occurs in jaundice or obesity can have a profound impact on the hypothalamic-pituitary-adrenal and renin-angiotensin-aldosterone axes. These effects are mediated in part by inhibitory effects on hepatic steroid metabolism but also by stimulatory effects on adrenal cholesterol supply for steroidogenesis via increased expression of adrenal SR-B1.

### P753

#### Salivary cortisol as an alternative to serum cortisol in 1ug ACTH test

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One microgram ACTH (adrenocorticotropin) test is considered to have higher sensitivity for early diagnosis of subclinical hypoadrenalism. In the test, the response of total serum cortisol concentration after ACTH application is evaluated. The concentration of total cortisol in serum is influenced by various diseases and drugs (containing oestrogens) that affect the concentration of cortisol binding proteins and subsequently measured cortisol levels. By routine laboratory methods, it is difficult to measure free fraction of serum cortisol. It may be overcome by determination of concentration of salivary cortisol, which is known to reflect perfectly the free hormone levels and does not depend on the intensity of saliva production.

Thirteen healthy women aged from 22 to 40 years without oestrogen therapy were investigated in follicular phase of the menstrual cycle. The response of cortisol concentration in saliva and serum was determined before, and 30 and 60 minutes after administration of 1  $\mu\text{g}$  of ACTH (Synacthen 250  $\mu\text{g}$  in 250 ml of 0.9% NaCl, 1 ml i.m.). In all women who underwent the test, the cortisol levels exceeded 500 nmol/l in serum and 20 nmol/l in saliva 30 minutes after ACTH application.

Statistically significant increase in both parameters in the 30th minute of the test was observed. Both levels correlated well with a coefficient  $r = 0.631$  and  $p$  value  $< 0.0206$ . Comparison of both test proved that salivary cortisol determination can serve as an adequate alternative to the serum cortisol in 1  $\mu\text{g}$  ACTH test. This non-invasive sample collection and estimation of salivary cortisol reflecting its free fraction is especially advantageous for examination of adrenal function in women using oral contraceptives.

### P754

#### Novel mutations in the ACTH receptor gene as a cause of familial glucocorticoid deficiency

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Familial glucocorticoid deficiency (FGD) is a rare autosomal recessive disease resulting from adrenal unresponsiveness to ACTH. Patients present in early childhood with hyperpigmentation, hypoglycaemic episodes and seizures secondary to glucocorticoid deficiency. If left untreated this condition is fatal. Mineralocorticoid production is normal. Mutations in the ACTH receptor have been well described and account for approximately 25% of cases. We describe 3 additional novel mutations in the ACTH receptor. Patient 1 presented in the neonatal period with hyperpigmentation associated with very low cortisol and grossly elevated ACTH levels. Mutation analysis revealed compound heterozygous mutation S74I/H170L (serine 74  $\rightarrow$  isoleucine mutation on one allele and a histidine 170  $\rightarrow$  leucine mutation on the other). S74I homozygous mutations are well described and associated with Scottish ancestry. The S74I mutation effectively disables the receptor. The H170L mutation is novel, lying in the 2nd extracellular loop and hence may interfere with ligand binding. Patient 2 presented early in life with isolated glucocorticoid deficiency and carries a homozygous mutation resulting in a substitution of leucine  $\rightarrow$  proline at position 55 in the region of the 1st intracellular loop. Patient 3 presented with hypoglycaemia and seizures, has a homozygous nonsense mutation resulting in a truncated protein at position 180 of the ACTH receptor (S180X). The protein lacks the 6th and 7th transmembrane domains, 3rd intracellular loop, 3rd extracellular loop and the C terminal tail. In keeping with this mutation, patient 3 has a severe phenotype requiring high replacement doses of hydrocortisone and despite this remains darkly pigmented with very high ACTH levels. There remains scope for further functional characterisation of ACTH receptor mutations which may enhance our knowledge of ACTH receptor action.

### P755

#### Opposite effects of DHEA and DHEAS on chromaffin cells proliferation

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Dehydroepiandrosterone (DHEA) and its sulphate ester DHEAS are neurosteroids with potential effects on neurogenesis, neuronal survival and neuronal stem cells proliferation. DHEA is produced by the inner adrenocortical zone, which is in direct contact to the adrenomedullary chromaffin. Unlike the closely related sympathetic neurons, chromaffin cells are able to proliferate throughout the life span. The aim of the present study was to examine *in vitro* the effect of DHEA and DHEAS on bovine chromaffin cells proliferation induced by various growth factors and cytokines.

Graded concentrations of leukemia inhibitory factor (LIF) induced proliferation of chromaffin cells from young animals whereas epidermal growth factor (EGF) had no effect. On the contrary, EGF increased the cell proliferation in cells from adult animals, whereas LIF was inactive. In both cases, DHEA, as well as dexamethasone, decreased the proliferative effect induced by the growth factors. Neither DHEA nor dexamethasone did affect cell death. Surprisingly, DHEAS potentiated, in a dose-dependent-manner, the effect of growth factors on proliferation in cells from adult but not from young animals.

These data show that the sensitivity of bovine chromaffin cells to different growth factors is age-dependent. In addition, we show that the adrenal androgen DHEA and its sulphated form DHEAS can exert opposite functions in the control of chromaffin cell growth. Furthermore, DHEA and DHEAS probably interact with growth factor-induced proliferation and therefore exert a role in the control of adrenomedullary tissue formation. In summary, a differential regulation of adrenomedullary development with age-dependent differences in the sensitivity of these cells to growth factors and adrenal androgens is suggested.

## P756

**The glucocorticoid receptor 1a promoter in CD4<sup>+</sup>CD8<sup>+</sup> thymocytes is rapidly upregulated following dexamethasone treatment *in vitro***J Nixon<sup>1</sup>, J Sheridan<sup>2</sup>, C Blackburn<sup>2</sup> & K Chapman<sup>1</sup><sup>1</sup>Queens Medical Research Institute University of Edinburgh, Edinburgh, United Kingdom; <sup>2</sup>Institute For Stem Cell Research University of Edinburgh, Edinburgh, United Kingdom.

Glucocorticoid Receptor (GR) levels are increased in response to glucocorticoids (GC) in T-lymphoma cell lines susceptible to GC-induced cell death (GICD). The GR gene is transcribed from 5 promoters generating alternate first exons, 1A-1E, with 1A expression restricted to immune tissue and the cortex of the brain. Expression of the 1A-containing variant correlates with susceptibility to GICD in mouse thymocytes. Here we describe the time course of GC induction of total GR mRNA as well as 1A and 1B in mouse thymocytes.

Total mouse thymocytes were treated with 10<sup>-6</sup>M dexamethasone (dex) for up to 6h. RNA was extracted and Real-Time PCR assays for total GR mRNA and the 1A and 1B GR mRNA variants performed on cDNA. Total GR mRNA and 1A mRNA levels were increased 3-fold in thymocytes treated for 2h with dex, compared to untreated samples. Following 4h dex treatment, total GR mRNA levels in thymocytes were 1.5-fold higher than in untreated cells and remained at this level after 6h, whereas 1A-containing mRNA was elevated 2.8-fold above control levels after 4h dex treatment, falling to 2-fold above control after 6h. To ascertain which population of thymocytes show this change in GR mRNA levels, CD4<sup>+</sup>CD8<sup>+</sup> double positive (DP) and CD4<sup>-</sup>CD8<sup>-</sup> double negative (DN) thymocytes were FACS sorted and treated with dex over 6h. DP cells showed an identical increase in total GR mRNA and 1A at 2h and 6h to total thymocytes, consistent with the majority of the thymocyte population being DP cells. Levels of 1B-containing GR mRNA were elevated 2-fold above levels in control cells by 2h dex treatment, falling to control levels after 6h. In contrast to DP cells, preliminary data from DN cells suggested that 2h dex treatment caused a 1.5-fold increase in total GR mRNA, returning to control levels by 6h. No change was detected in levels of 1A-containing GR mRNA in DN cells following either 2h or 6h dex treatment. These data demonstrate a rapid and transient increase of GR mRNA in DP thymocytes, which correlates with DP cells being the population most sensitive to GICD.

The decline in total GR mRNA levels between 2h and 6h of dex treatment contrasts to the sustained increase seen in a human leukemic T-cell line and may be due to rapid induction of apoptosis in primary DP thymocytes with the highest levels of GR mRNA; this is currently under investigation.

## P757

**11beta-hydroxysteroid dehydrogenase is an early and essential marker of human adipogenesis**IJ Bujalska<sup>1</sup>, LL Gathercole<sup>1</sup>, JW Tomlinson<sup>1</sup>, C Darimont<sup>2</sup> & PM Stewart<sup>1</sup><sup>1</sup>University of Birmingham, Birmingham, United Kingdom; <sup>2</sup>Nestlé Research Centre, Lausanne, Switzerland.

The prevalence of obesity and its association with many health complications have evoked a high interest in adipose tissue metabolism. In man, glucocorticoid (GC) excess increases fat mass and the risk of developing Metabolic Syndrome. The enzyme responsible for modifying intracellular GC concentrations in adipose tissue is 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1). The aim of this study was to characterise the novel human preadipocyte cell line (Chub-S7) and explore the role of 11β-HSD1 in adipocyte differentiation. Chub-S7 cells were differentiated for up to 17 days in serum-free DMEM/F12 with insulin, PPARγ2 agonist and cortisol (F) and gene expression determined by real-time PCR. To study the effect of 11β-HSD1 on preadipocyte differentiation, cells were cultured in differentiation media containing 500 nM cortisone (E) (inactive GC) together with 1 μM glycyrrhetic acid (GE). Adipogenesis was studied by measuring expression levels of differentiation markers including fatty acid binding protein 4 (FABP4) and glycerol 3 phosphate dehydrogenase (G3PDH) by real-time PCR. Proliferating Chub-S7 cells did not express 11β-HSD1, FABP4 or G3PDH. From day 1 of differentiation, a linear increase in 11β-HSD1 mRNA was observed (29.3-fold change on day 1 and 290.6 on day 9 vs day 0, *P* < 0.001). mRNA expression paralleled oxo-reductase activity, E to F (0 day 1, 3.0 day 4 and 12.9 day 9, pmol/mg/h, *P* < 0.001). FABP4 and G3PDH mRNA expression significantly increased from day 6 onwards. Co-differentiation with E increased FABP4 (637.8-fold on day 17 vs control, *P* < 0.0001) and G3PDH mRNA expression (331.8-fold on day 17 vs control, *P* < 0.0001). Co-incubation with GE significantly reduced the cortisone induced increases in adipogenic markers. We

conclude that the increase in 11β-HSD1 mRNA expression and activity is essential for the early stages of adipogenesis. Specific 11β-HSD1 inhibitors may represent a novel approach for the treatment of obesity.

## P758

**Mechanisms of human prolactin regulation by oestrogen**AD Adamson<sup>1</sup>, S Friedrichsen<sup>1</sup>, S Semprini<sup>3</sup>, M Wilding<sup>1</sup>, CV Harper<sup>2</sup>, MRH White<sup>2</sup> & JRE Davis<sup>1</sup><sup>1</sup>Endocrine Sciences Research Group, University of Manchester, Manchester, United Kingdom; <sup>2</sup>Centre for Cell Imaging, School of Biological Sciences, University of Liverpool, Liverpool, United Kingdom; <sup>3</sup>Molecular Physiology Group, University of Edinburgh Medical School, Edinburgh, United Kingdom.

Oestrogens are capable of inducing rat prolactin gene transcription through liganded Oestrogen Receptor (ER) binding a degenerate oestrogen response element (ERE) sequence on the promoter 1440bp upstream of the transcription start site. ER binding is thought to interact with DNA bound Pit-1 leading to chromatin looping bringing enhancer elements into proximity with the transcription start site. This ERE sequence, TGTCActaTGTCa, differs from the consensus GGTCAnnnTGACC by two nucleotides, one in each half site. Oestrogens have also been implicated in regulation of prolactin transcription in humans, however the mechanisms are not understood.

We have previously shown a small but consistent activation of the human prolactin promoter at 24 hours in GH3 derived D44 cells, which drive luciferase expression from a 5000bp human prolactin promoter. Several putative EREs exist within this fragment. Deletion constructs of the promoter localised the oestrogen responsive sequence to be positioned within -828/-1779. A likely ERE sequence, TGTCActaTGTCc, is located at -1207/-1220bp, adjacent to a Pit-1 site. This sequence differs from the consensus ERE sequence by two nucleotides (and by three nucleotides compared to the rat prolactin ERE). Mutagenesis of this site abolished oestrogen induced promoter activation. Electromobility shift assays showed that this sequence bound ER but at a greatly reduced affinity compared to a consensus ERE sequence. Thus a degenerate, non-consensus ERE located adjacent to a Pit-1 binding site can regulate human prolactin gene transcription in a pituitary cell line. ChIP assays are presently being used to assess the chromatin modifying potential of ER at this site.

## P759

**Dimerisation of the melanocortin-2 receptor accessory protein MRAP**MI Almiro do Vale<sup>1</sup>, M Egerová<sup>2</sup>, MR Elphick<sup>2</sup> & AJ Clark<sup>1</sup><sup>1</sup>Department of Endocrinology, William Harvey Research Institute, Barts & The London, Queen Mary, University of London, London, United Kingdom; <sup>2</sup>School of Biological Sciences, Queen Mary, University of London, London, United Kingdom.

The melanocortin-2 receptor accessory protein (MRAP) is a type I integral membrane protein that is required for cell surface expression of the melanocortin 2 receptor (MC2R). Mutations in the N-terminal region of this protein are associated with familial glucocorticoid deficiency type II (OMIM#607398).

Here we have investigated the expression and biochemical properties of MRAP using polyclonal antibodies to a conserved peptide sequence in the N-terminal region of MRAP. Western blot analysis of lysates and membrane preparations from Y1 mouse adrenocortical cells subjected to SDS-PAGE under reducing conditions revealed two proteins with molecular masses of ~16 kDa and ~32 kDa (predicted mass for mouse MRAP is 14.1 kDa). Post-translational modification at two potential N-glycosylation sites was investigated by treatment with N-Glycosidase F but the molecular masses of the two proteins were unaffected. Western blot analysis of rat organs/tissues also revealed a ~32 kDa band in adrenal glands and in ovarian tissue, indicating that MRAP may exist *in vivo* as a dimeric protein. To test this hypothesis SK-N-SH cells transiently transfected with MRAP labelled with a Flag epitope tag at the C-terminus were analysed in co-immunoprecipitation experiments using Flag- and MRAP- antibodies. Both Flag- and MRAP- antibodies immunoprecipitated a protein with a mass of ~20 kDa, consistent with the expected mass for MRAP-flag monomer. However, Flag-antibodies also immunoprecipitated a protein of ~40 kDa, consistent with dimeric MRAP-flag. These data suggest that the N-terminal antigen targeted by the MRAP antibodies may be inaccessible when dimeric MRAP is in solution.

Collectively our data indicate that MRAP exists as a dimer *in vivo* and that dimerisation may occur in the N-terminal region of the protein. These



findings are consistent with other G-protein-coupled receptor interacting proteins such as RAMPs (receptor activity modifying proteins), which also exist as dimeric proteins.

#### P760

##### Expression of oestrogen receptor related proteins beta occurs in multiple cell types within human endometrium during the normal menstrual cycle

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Oestrogens are essential regulators of fertility in women. They induce changes in uterine cell function by binding to oestrogen receptors (ERs) two of which (ER $\alpha$  and ER $\beta$ ) have been identified. ER $\alpha$  and ER $\beta$  share significant sequence homology with three other steroid receptor superfamily members that are known as oestrogen related receptors alpha (ERR $\alpha$ ), beta (ERR $\beta$ ) and gamma (ERR $\gamma$ ). ERRs bind as monomers to the sequence TNAAGGTCA and as dimers to the consensus oestrogen response element (ERE).

We have previously mapped the pattern of expression of ER $\alpha$ , ER $\beta$  and an ER $\beta$  variant (ER $\beta$ 2) within the endometrium and demonstrated cell-specific patterns of expression. In the present study we have investigated whether ERR $\alpha$  and  $\beta$  are expressed in the human endometrium as the first step in determining what impact they might have on gene expression. Full thickness endometrial biopsies were obtained from women with regular menstrual cycles. ERR $\alpha$  and  $\beta$  were immunolocalised with antibodies obtained from Abcam. Immunopositive cell nuclei were detected at all stages of the normal menstrual cycle; ERR $\beta$  immunostaining was more intense than that of ERR $\alpha$ . When expression of ERR $\beta$  was compared with that of ER $\alpha$  using double fluorescent immunohistochemistry cell-specific patterns of expression were revealed. For example, endothelial cells were ERR $\beta$  + / ER $\alpha$ - and whilst most stromal cells were ERR $\beta$  + / ER $\alpha$  + there was also a population that were ERR $\beta$  + / ER $\alpha$ - that may represent one (or more) of the immune cell types that are prominent during the secretory phase.

In conclusion, we have demonstrated for the first time, that ERRs are expressed in the human uterus during the normal menstrual cycle. Future studies will use endometrial cells (primary and immortalised) to determine the impact of ERRs on oestrogen-regulated gene expression in the endometrium.

#### P761

##### Rapid glucocorticoid effects on insulin sensitivity

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##### Background

Although it is well known that glucocorticosteroids induce insulin resistance, probably due to postreceptor defects, the exact time scale is still a matter of debate.

##### Objective

The aim of the present study was to determine the time scale of effects of therapeutic doses of glucocorticoids on insulin sensitivity.

##### Methods

Study group consisted of 20 patients with Graves ophthalmopathy who were treated with corticosteroids for the first time. Infusion consisted of 500 mg of methylprednisolone suspended in normal saline and infused at a constant rate for four hours. In group I (10 patients; 8 female, 2 male, 50  $\pm$  11 years, BMI 24.1  $\pm$  4.4 kg/m<sup>2</sup>) insulin sensitivity was determined using euglycemic-hyperinsulinemic clamp, before, during the first methylprednisolone infusion and after two months of glucocorticoid treatment. Insulin clamp started two hours after the beginning of methylprednisolone infusion, and lasted for two hours (until the end of corticosteroid infusion). In II group II (10 patients; 7 female, 3 male, 45  $\pm$  11 years, BMI 22.3  $\pm$  2.2 kg/m<sup>2</sup>) insulin sensitivity was determined by insulin tolerance test (ITT). ITT started 15 minutes after beginning of methylprednisolone. We calculated insulin sensitivity as a slope of glucose disappearance from 3 to 15 minutes. Results

There were no significant differences in regard to age, gender and BMI between the two groups. Group I: Steady state insulin concentration was not significantly different between the observed period (161  $\pm$  56 vs. 141  $\pm$  49 vs. 156  $\pm$  33 mU/l,  $P$  = 0.589). However, there was a significant reduction in the whole body glucose disposal rate four hours after the infusion beginning ( $P$  = 0.001). Two months after the whole body glucose disposal rate was still significantly reduced compared to the period before

glucocorticoids ( $P$  = 0.012). Group II: Insulin sensitivity measured using ITT was not different between the first 30 min of the glucocorticoid infusion ( $P$  = 0.622).

##### Conclusion

It is suggestive that glucocorticoids exert full effects on the insulin sensitivity during the first few hours of the treatment, but with no change in the first 30 minutes.

#### P762

##### Differential regulation of Cyp4A isoforms in mouse kidney during the development of mineralocorticoid, salt-sensitive induced hypertension

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Arachidonic acid (AA) metabolites derived from cytochrome P450 enzymes regulate vascular tone and renal tubular function. The cytochrome P450 4A (Cyp4A) enzymes are responsible for the synthesis of 20-hydroxyeicosate-traenoic (20-HETE), the most abundantly produced AA Cyp P450 metabolite in the kidney. Cyp4a expression and the production of 20-HETE, have been implicated in the development of hypertension. Here we have investigated the expression of various renal isoforms of Cyp4A in a mouse model of mineralocorticoid induced hypertension. This model develops a 30 mmHg rise over 15 days compared to controls there was also a progressive rise in renal weight, (significant by day 13) but not heart weight. We have also tested whether bezafibrate, an inducer of Cyp4a metabolism and PPAR $\alpha$  agonist, affected the development of hypertension in this model. Male C57BL6 mice were uninephrectomised, infused with aldosterone (750  $\mu$ g aldosterone/kg/day by mini pump for up to 21 days) and were fed a diet containing 1% Na. At days 6, 13 and 21, kidneys and blood samples of mice ( $n$  = 6) were collected for analysis, including in situ hybridisation. In the kidney, by real-time PCR we found a 3-fold increase in Cyp4a14 ( $P$  < 0.01) and a significant early decrease in Cyp4a12 ( $P$  < 0.001) as HT develops. Here also, we describe for the first time, a fourth isoform which showed significant down-regulation compared with controls ( $P$  < 0.001). To induce 20 HETE production, a treatment group were injected with bezafibrate (50 mg/kg/day i.p.), controls were injected with vehicle over the hypertensive time course. Strikingly the bezafibrate treatment prevented the expected rise in BP ( $n$  = 6, 113  $\pm$  3 mmHg) present in mDOCA mice ( $n$  = 6, 134  $\pm$  2 mmHg)

These studies make the novel observation that Cyp4a metabolism and PPAR pathways in the development and maintenance of hypertension especially when there is salt sensitivity as in mDOCA mice.

#### P763

##### Glucocorticoid receptors exert a profound anti-proliferative and anti-survival effect on human small cell lung cancer cells

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Glucocorticoids (Gcs) act via the glucocorticoid receptor (GR) to inhibit proliferation of many epithelial cell types, and induce apoptosis in others, notably lymphoblastic leukemia. Resistance to Gcs occurs in human small cell lung cancer (SCLC), manifest clinically as dysregulated secretion of ACTH-related peptides. Currently nothing is known of the biological consequences such resistance causes. We have previously shown that a panel of human SCLC cell lines secrete ACTH related peptides, and are globally resistant to glucocorticoid action. These cell lines are a tractable model for a major, rapidly lethal human disease.

Overexpression of wild-type GR restores Gc sensitivity to transfected reporter genes in the SCLC cells. Therefore we analysed the effects of such restoration of Gc action on cell phenotype. Initial studies used classical stable transfection, and revealed three striking findings. Transfected cells proliferated more slowly, ACTH peptide secretion was inhibited by Gcs, and Gc sensitivity was rapidly lost. To generate sufficient cell numbers for comprehensive analysis, the SCLC cells were subject to retroviral infection with GR-EYFP expression constructs. The retroviruses conferred glucocorticoid sensitivity to another Gc resistant human cell line, HEK293, with appropriate nuclear translocation, GR phosphorylation, and regulation of both endogenous and transfected reporter genes. The retroviruses similarly conferred Gc sensitivity to two different Gc resistant SCLC cell lines. However, in these cells, expression of GR caused massive cell loss with 80% of GR expressing cells appearing apoptotic, compared to cells infected with a control virus.



We show, for the first time, a profound anti-survival effect of GR expression on human small cell lung cancer. Therefore evasion of Gc signalling, confers a survival advantage to the cells. Understanding how glucocorticoids work in SCLC may lead to novel therapeutic approaches.

## P764

### Quantitative analysis of mifepristone (RU38486) in plasma by HPLC triple quadrupole mass spectrometry

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Mifepristone (RU38486) is a progesterone and glucocorticoid receptor antagonist which is used clinically to induce medical abortion, and as a research tool, e.g., in metabolic syndrome and depression. We aimed to develop a sensitive method to detect and quantify mifepristone in human plasma.

Analyses were developed using a Surveyor HPLC system, with a mobile phase of methanol:ammonium acetate (5 mM) (70:30; 0.4 mL/min) and a C18 column (5 µm; 2.1 × 50 mm). The eluate was delivered into a TSQ Quantum Discovery triple quadrupole mass spectrometer in positive electrospray ionisation mode. Mass spectra were gathered by selective reaction monitoring using argon (collision) and nitrogen (sheath, auxiliary) gases.

The major ion formed from mifepristone was the protonated molecular ion, with *m/z* 430. Under collisional activation (30V), the major fragment ion was *m/z* 372, corresponding to loss of the hydroxyl and propionyl group from C17. Alfaxalone was used as an internal standard and yielded a protonated molecular ion at *m/z* 333 and a major breakdown ion, *m/z* 297. Mifepristone was extracted in diethyl ether from plasma (containing 100 ng alfaxalone) of healthy male subjects (18–31y; *n* = 5, BMI 23.1 (sd 2.4) kg/m<sup>2</sup>, WHR 0.88 (sd 0.08)) 12 h post-dose (200–800 mg po). Recovery of mifepristone and alfaxalone were 30 ± 1% and 70 ± 2%, respectively. The standard curve was linear over the range 0.5–500 ng (*r* > 0.98). Inter-injection reproducibility was 7.1%CV (*n* = 3) and intra-assay reproducibility was 8.3%CV (*n* = 3). The limit of quantitation was 1 ng injected in 10 µL, allowing mifepristone to be detected in plasma (100 µL) at concentrations ranging between 2–20 µM.

LC/MS/MS with electrospray ionisation is a robust method for quantitative analysis of mifepristone in small volumes of plasma.

## P765

### NaB affect the local regulation of vitamin D in colorectal cancer

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#### Introduction

The development of colorectal cancer is influenced by dietary as well as growth factors. Butyrate, (NaB) one of the metabolic by-products of bacterial fermentation of dietary fibre is a primary source of energy for colonic mucosa. It has growth-promoting effects on normal colonic epithelial cells, induces cell cycle arrest, differentiation and apoptosis in colorectal cancer cells. Since it has been suggested that the apoptotic effects of NaB may be due to its synergistic action with vitamin D (VitD), we hypothesized that NaB may be involve in the regulation of vitamin D axis locally.

#### Methods

Biopsies from patients undergoing colonoscopy for irregular bowel habit without colonic pathology were incubated with 5 mM NaB for up to 48 hours. HT29 cells were cultured for up to 72 hrs in serum free media with 5 mM NaB. Assessment was made of proliferation by the colorimetric MTS assay and direct cell counting using trypan blue; apoptosis by FACS analysis using propidium iodide and annexin staining. mRNA expression levels to the Vitamin D axis genes (1αOHase, 24OHase and VDR) and c-Myc were quantified by real-time RT-PCR.

#### Results

In HT29 cell line, NaB induced apoptosis in HT29 CRC cells line. Colonic explants gene expression varies between patients in response to NaB. NaB up-regulates both 1αOHase and 24OHase mRNA expression and down-regulate c-Myc and VDR mRNA levels. Similarly, in HT29 cells exposed to NaB, 1αOHase mRNA expression levels increased steadily to more than 700% of control levels after 72 hours. 24OHase mRNA expression was rapidly induced in NaB treated cells, rising from 400% by 24 hr to more than 1000% of control by 72 hours. Exposure of cells to NaB progressively inhibited VDR mRNA levels (by 50%) and c-Myc mRNA copy number by more than 70%.

#### Conclusions

Our findings suggest that there is a local regulatory pathway for both activation and further metabolism of VitD by the colon.

## P766

### Bone mineral density in patients with cushing's disease, the impact of impaired glucose homeostasis

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Decreased bone mineral density (BMD) and disturbances in glucose metabolism are common complications of Cushing's disease. Many recent studies have shown that hyperinsulinemia in type 2 diabetes mellitus (DM) may lead to higher BMD as compared to healthy individuals. The aim of the present study was to investigate the possible relationships between BMD, serum markers of bone metabolism and plasma cortisol concentrations. The second objective was to determine whether the presence of impaired glucose metabolism could have an impact on BMD in Cushing's disease.

The study included 66 patients with Cushing's disease (14 males, 52 females; mean age 33.4 ± 12.5 years). BMD measurements were performed by DEXA at the lumbar spine (LS), left femoral neck (FN) as well as at the total proximal femur (TF). Of the 32 patients without unequivocal DM, oral glucose tolerance test (oGTT) showed normal oGTT in 18 patients (32%) (subgroup 1, SG1), impaired glucose tolerance in 14 patients (24%) and DM in 25 patients (44%). The latter two subgroups were considered as subgroup 2 (SG2).

In the whole group of patients, mean BMD z-scores (± SD) were decreased at all regions (LS, -1.60 ± 1.04; FN, -1.12 ± 1.06; TF, -0.99 ± 1.01). There were no correlations between BMD z-scores at any site and plasma cortisol concentrations at 08 h, 24 h, or after a low-dose dexamethasone test (LDDT). In contrast, significant negative correlations were found between osteocalcin levels and cortisol concentrations at 08 h (*P* < 0.001), at 24 h (*P* < 0.05) and after LDDT (*P* < 0.001). Patients in SG2 had significantly higher BMD z-scores at TF (*P* < 0.01) and lower osteocalcin concentrations (*P* < 0.001) compared with patients in SG1.

Our results suggest that the severity of bone disease in patients with Cushing's disease fails to correlate directly with plasma cortisol concentrations. It is possible that the presence of insulin resistance often present in patients with Cushing's disease may have an anabolic effect on bones.

## P767

### Bone mineral density and its correlation with plasma cortisol concentrations in patients with inactive adrenal adenomas and in patients with subclinical and overt Cushing's syndrome

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Many previous studies have shown decreased bone mineral density (BMD) in patients with endogenous hypercortisolism, although data on the severity of bone disease are contradictory. There are very sparse data about the correlations between plasma cortisol concentrations and BMD.

The study included 188 patients with clinically inactive adrenal adenomas (IAA) and 30 patients with ACTH-independent Cushing's syndrome (CS) due to adrenocortical adenomas. All patients underwent a detailed hormonal evaluation including measurements of plasma cortisol at 08 and 24 h, as well as low-dose dexamethasone testing (LDDT). BMD was measured by DEXA at the lumbar spine (LS), left femoral neck (FN), as well as at the total proximal femur (TF).

ROC analysis performed for plasma cortisol at 24h and after LDDT in patients with IAA and CS indicated that the optimal cut-off value which discriminated between IAA and CS was 6.0 µg/dl for midnight plasma cortisol and 3.6 µg/dl for LDDT. Among patients with IAA, a subgroup of patients without overt CS had plasma cortisol levels >6.0 µg/dl at 24h and >3.6 µg/dl after LDDT, and these patients were considered as having subclinical hypercortisolism (SH, n = 9, 4.8% of total).

Patients with IAA had normal BMD at LS, FN and TF. Patients with SH had significantly lower ( $P < 0.01$ ) z-scores at FN ( $-0.57 \pm 0.81$  vs.  $+0.27 \pm 1.00$ ) and at TF ( $-0.48 \pm 1.43$  vs.  $0.29 \pm 1.03$ ) but not at LS compared to IAA patients without SH. Patients with CS had decreased BMD ( $P < 0.01$ ) at all region ( $-0.90 \pm 1.07$  at LS;  $-0.43 \pm 1.07$  at FN and  $-0.38 \pm 1.01$  at TF) compared to those with IAA. Significant negative correlations were found between plasma cortisol concentrations at 08h, 24h as well as after LDDT and BMD z-scores at FN and at TF but not at the LS in the combined group of patients with IAA and CS.

These results indicate that even mild forms of endogenous hypercortisolism lead to decreased BMD at TF. Plasma cortisol concentrations are not directly correlated with BMD at LS.

## P768

### Phenotypic variability in P450 oxidoreductase deficiency may be caused by differential effects of P450 oxidoreductase mutations on steroidogenesis

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Without adequate treatment patients with acromegaly die prematurely from cardiovascular disease (CVD); however the contribution of atherosclerosis in this process is controversial. Increased carotid IMT is an early morphological marker of atherosclerosis and predictor of subsequent cardiovascular events. Contradictory data exist regarding IMT in patients with acromegaly.

We measured carotid IMT in 79 patients with acromegaly (47 male, mean age  $55 \pm 14$  years) and 22 age-matched healthy controls (12 males,  $57 \pm 11$ ) [ $P = 0.5$ ]. 32 patients had HT, 16 DM, 8 IHD, 2 PVD, 19 hyperlipidaemia (on treatment) and 16 smoked; 8 controls smoked. Three measurements were taken bilaterally: at the carotid bifurcation and 1 cm above and below, the mean was calculated for each side.

Median IGF-I was 255 ng/ml (62–1155) and SDS 2.0 ( $-2.85$ – $+5.76$ ) in patients and 148 ng/ml (67–201) and 0.54 ( $-1.68$ – $+1.70$ ) respectively in controls [ $P < 0.0001$ ]. Median BP was similar in patients (130/78) and controls (139/69). Total cholesterol and LDL were 5 mmol/L (3–8) and 3 mmol/L (1–6) in patients and 6 mmol/L (4–7) and 4 mmol/L (2–4) in controls respectively [ $P = 0.07$ ,  $P = 0.06$ ].

Median IMT did not differ in patients: 0.75 mm (0.43–1.17) compared to controls: 0.71 mm (0.45–1.03) [ $P = 0.3$ ]. When comparing IMT in patients with high IGF-I levels and patients with normal IGF-I levels no difference was found [ $P = 0.6$ ]. No correlation was found with IGF-I or BP and IMT. 17 (21.5%) patients had 1/more plaques present and 4 (18.2%) controls [ $P = 1$ ].

In summary, carotid IMT does not differ in patients with acromegaly when compared to controls. The lack of increase in IMT is against the development of premature atherosclerosis in this patient group.

## P769

### Insulin-Like Factor 3: a new circulating marker for the polycystic ovary syndrome-type of ovarian dysfunction

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Insulin-like factor 3 (INSL3), a member of the relaxin-insulin family, is produced in the Leydig cells and at reduced levels in ovarian thecal cells. As ovaries from most of PCOS are characterized by an hyperplasia of the theca interna, we hypothesized that INSL3 could be overproduced in PCOS women and therefore that circulating levels of this hormone could become a new marker for the syndrome. Forty lean and obese women with PCOS and 20 controls comparable for age and weight were enrolled. Basal blood samples for androgens, SHBG, estradiol, LH, FSH, glucose and insulin

determinations were collected. All participants underwent also an oral glucose tolerance test, whereas PCOS women only performed also an ACTH<sub>1-24</sub> test (250 µg ACTH<sub>1-24</sub> i.v. with blood taken at 0 and 60 min) and a GnRH agonist test (100 µg GnRH agonist s.c. with blood taken at 0 and 24h) after 4 days of treatment with dexamethasone (2 mg orally daily) to suppress adrenal androgen production. INSL3 serum concentrations were measured by a radioimmunoassay method. We found that INSL3 concentration was: i) significantly higher in PCOS patients respect to controls ( $226.0 \pm 89.2$  vs.  $164.5 \pm 43.6$  pg/mL,  $P = 0.005$ ); ii) positively correlated with total ( $r = 0.431$ ,  $P < 0.001$ ) and free testosterone ( $r = 0.251$ ,  $P = 0.043$ ), androstenedione ( $r = 0.260$ ,  $P = 0.035$ ) and 17OH-progesterone ( $r = 0.246$ ,  $P = 0.049$ ) levels in the entire population; and iii) positively correlated with suppression of free testosterone by dexamethasone ( $r = 0.459$ ,  $P = 0.005$ ) and with the responsiveness of androstenedione ( $r = 0.453$ ,  $P = 0.003$ ) and 17OH-progesterone ( $r = 0.490$ ,  $P = 0.001$ ) to GnRH agonist (markers of ovarian androgen function) in PCOS. These data strongly suggest that INSL3 could be used as a new circulating marker for the PCOS-type of ovarian dysfunction.

## P770

### Transcriptional control of differential glucocorticoid receptor expression: determining tissue specific expression and protein isoforms

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The 5' UTR of the glucocorticoid receptor plays a key role in determining tissue specific expression, and protein isoforms. Analysis of the 5' UTR of the hGR has revealed 11 splice variants of the hGR exon 1, based on 7 exon 1 s, 4 of which (1-D to 1-F and 1-H) were previously unknown. All of the exon 1 variants have unique splice donor sites and share a common exon 2 splice acceptor site. Due to an upstream in-frame TGA stop codon the predicted translation from all splice variants is identical. The four new exon 1 s show remarkable similarity with their rat homologues. Exon 1-D starts and finishes 17- and 36bp upstream of the corresponding ends of the rat exon 1<sub>4</sub>. Exon 1-E is only 6bp longer than its homologue 1<sub>5</sub>. Exon 1-F contains two short inserts of 11- and 6bp when compared to the rat 1<sub>7</sub>. 1-H is 18bp longer than the corresponding rat 1<sub>11</sub>. In addition to these new exons, we found that the human exon 1-C occurs as 3 distinct splice variants, covering the region homologous to the rat exons 1<sub>9</sub> and 1<sub>10</sub>. All of the alternative hGR exons 1 s presented here were found to be transcribed in human tissue. The human hippocampus expresses mRNA of all the exon 1 variants, whilst the expression of the other exon 1 s seems to be tissue-specific. While exon 1-D is only in the hippocampus, exons 1-E and 1-F are also detected in the immune system, and exon 1-H additionally in the liver, lung and smooth muscle. The 5' region of the hGR is more complex than previously thought, and we suggest that each of these untranslated first exons have a distinct proximal promoter region, providing additional depth to the mechanisms available for tissue specific expression of the hGR isoforms.

## Thyroid

### P771

#### In healthy women increasing tsh concentrations within the normal range are associated with increasing cardiovascular risk

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#### Objective

There is currently extensive debate regarding elevated TSH (Thyroid Stimulating Hormone) concentrations and associated cardiovascular risk. This study investigated whether correlations exists between TSH concentrations within the accepted range of normality and increasing cardiovascular risk.

#### Design

We performed a cross-sectional study of 728 asymptomatic women aged 45–60 years attending for thyroid screening over a twelve month period. Patients found to be biochemically hyper (TSH < 0.5 ( $n = 14$ )) or hypothyroid (TSH > 4.5 ( $n = 53$ )) were excluded. Only those patients whose TSH concentrations were found to lie within the reference range (0.5–4.5 mU/L) were included in the final analysis ( $n = 635$ ). Subsequently, correlations between TSH levels and risk parameters for cardiovascular

disease were examined. Additionally, ten-year risk of both ischemic heart disease (IHD) and fatal cardiovascular event was calculated using Framingham risk-stratification and SCORE index calculation respectively. Results

This study demonstrates that within the reference range, increasing TSH concentrations correlate significantly with risk parameters for the development of cardiovascular disease. Results show statistically significant correlations of increasing TSH concentrations and waist-circumference (Pearson  $R = 0.096$ ,  $P = 0.017$ ), BMI, (Pearson  $R = 0.083$ ,  $P = 0.03$ ), fasting glucose (Pearson  $R = 0.103$ ,  $P = 0.01$ ), triglycerides (Pearson  $R = 0.136$ ,  $P = 0.001$ ) and systolic blood pressure (Spearman's  $R = 0.081$ ,  $P = 0.041$ ). However, no significant correlation with either risk of developing coronary heart disease or risk of a fatal cardiovascular event with in the next ten years was demonstrated.

#### Conclusion

This study demonstrates that increasing TSH concentrations within the normal reference range are associated with increased risk of cardiovascular morbidity. These findings add weight to the call for tightening of the reference range for TSH and may have clinical implications for the management of hypothyroid patients.

### P772

#### A case of delayed diagnosis of pituitary resistance to thyroid hormone (PRTH)

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Resistance to thyroid hormone is a dominantly inherited condition of impaired tissue responsiveness to the action of thyroid hormone. Thyroid hormone levels are elevated with failure to suppress TSH. The presentation is highly variable. In individuals with generalized resistance to thyroid hormone (GRTH) appear to have eumetabolic state with compensatory high thyroid hormone levels. In contrast, those who appear to have hyper metabolic features but may have similar thyroid hormone and TSH levels are classified as selective pituitary resistance to thyroid hormone (PRTH). The case presented with symptoms of Attention Deficit Hyperactivity Disorder since birth, childhood asthma and recurrent sinusitis. He was diagnosed hyperthyroidism at age 29 when thyroid function tests showed total T4 201 nmol/L (60–140), total T3 3.6 nmol/L (1.3–3), thyroid hormone distribution index 2.2 (2) and free thyroxine index 4.4 (1.3–3.2). Clinically he was thyrotoxic with a small goitre. Initially he was treated with carbimazole followed by sub total thyroidectomy after 18 months for uncontrolled thyrotoxic symptoms. Histology showed thyroid hyperplasia. He was started on thyroxine for post op hypothyroidism 10 years later. At age 54, he was referred to the endocrine clinic with thyrotoxic symptoms for several years with TSH 12.1 mIU/L, Free T4 28.9 pmol/L and total T3 2.7 nmol/L. He was tachycardic and had a small diffuse goitre. Thyroid antibodies were negative. TSH-oma was excluded with normal MRI pituitary and alpha subunit. TSH concentration increased after TRH administration. Sequencing of thyroid hormone receptor  $\beta$  gene showed heterozygous mutation for P453T. One of his children (29 female), who has the same phenotype is awaiting genetic evaluation.

#### Conclusion

Although this man has had features of PRTH through out his life, a diagnosis was delayed for many years, partly due to a lack of sensitive TSH assay, but also having high index of suspicion for this rare disorder.

### P773

#### Relationship between sodium iodine symporter and thyroid peroxidase expression and cytokines production by thyrocytes from patients with immune and non-immune thyroid disorders

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The course of Graves' disease is associated with the inflow of lymphocytes to the thyroid gland and dysregulation of the immune system characterized by reaction to thyroid antigens (peroxidase, thyroglobulin, TSH receptors and  $\text{Na}^+/\text{I}^-$  symporter).

The aim of this study was to estimate sodium iodine symporter (NIS) and thyroid peroxidase (TPO) expression in thyrocytes which release cytokines INF- $\gamma$  and IL-4 from young patients with Graves' disease (GD) and no-toxic multinodular goitre (NTMG). The investigation was performed on thyroid cells isolate from postoperation thyroid tissues from 12 patients aged 11–18 years old with GD and 12 cases aged 13–18 years old with NTMG. Detection of NIS and TPO in thyrocytes performed by immunohistochemistry using mAb-47 and anti-NIS antibodies in DAB chromogene visuality and marked by Mayer's haematoxylin. Additionally, TPO identified by Western Blot method with mAb-47. Analysis of cytokines from isolated and cultured thyrocytes was performed using antibodies to IL-4 -PE and IFN- $\gamma$ -PE by flow cytometry.

The analysis of expression of NIS and TPO in thyrocytes was higher in patients with GD in compared to their detection in patients with NTMG. In addition, degree of thyroid antigen expression positive correlated with amount of cytokines production (INF- $\gamma$ ,  $P < 0.05$ ; IL-4,  $P < 0.02$ ). Furthermore, detection of IL-4 and INF- $\gamma$  was significantly higher in thyrocytes from Graves' patients ( $P < 0.01$ ;  $P < 0.05$ , respectively) in comparison to the NTMG patients.

We conclude that elevated expression of NIS and TPO in Graves' disease is associated with higher stimulation and activation of thyroid follicular cells in inflammatory process within thyroid gland.

Approved by the Local Ethical Committee.

### P774

#### The reference ranges of maternal thyroid hormon (thyrotropin and free thyroxin) at the second and third trimesters of pregnancy: impact of FT4 assays

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The aim of our study was to evaluate the reference ranges of thyrotropin (TSH) and free thyroxin (FT4) assayed with 5 methods at the second and the third trimesters of pregnancy for women with iodine intake at the lower limit of the normal range. This study used 297 sera (42 controls without pregnancy with age <40 years, 139 women at the second trimester and 116 at the third with mean age to 27 years in both groups). The assays were for TSH (Elecsys Roche), for thyroperoxidase antibodies (BRAHMS), for FT4 (Dia-Sorin (DS), Elecsys (E), DPC (I), Bayer (ACS), Tosoh biomedical (AIA)). Fourteen women (5.5%) have detectable levels of TPOAbs and these sera were eliminated of the study. After to verify the normality or the Log-normality of the distributions, the reference ranges were calculated. By comparison with the controls (1.64 mIU/l), the TSH concentrations rise at the second trimester (1.68 mIU/l) and at the third trimester (1.99 mIU/l,  $P = 0.021$  versus C,  $P = 0.004$  versus 2 T). The FT4 concentrations decreased during the pregnancy but in variables proportions depending to the assays. We noted a significant mean decrease to 24.8% at the second trimester (extreme 15–33) and to 28.7% at the third (extreme 18–37). For 3 FT4 methods (DS, E, ACS) the use of appropriated ranges at the third trimester decrease the percentage of values under the normal range in mean from 32% to 2%. For one method all levels were in the normal range however the term and the norms (I). With the last method we noted 52% of low FT4 levels at the third trimester what decreased to 0% with appropriated normal range (AIA). In conclusion, at the second and the third trimester of the pregnancy, the TSH concentrations rise when the FT4 decrease was variable with the assays. Like this, all thyroid results during pregnancy must be interpreted in accordance with reference ranges established for each trimester and each FT4 assays.

### P775

#### The effect of high calcium on activity of thyroid gland in rat

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The effects of goitrogens on the thyroid gland have been much studied and calcium is considered as a goitrogen in some cases. Since the level of calcium is high in drinking water of some cities of Khuzestan province, the effects of high calcium intake on the function of thyroid gland is investigated in this study. For this purpose the concentration of thyroid hormones is measured in rats.

The study was carried out on 73 male and female rats. The animals were divided into 3 groups, control group with normal diet ( $n = 25$ ), group2 ( $n = 24$ ) consumed diet with high calcium (1 g/kg diet) and group3 ( $n = 24$ ) had diet with high calcium and iodine (50  $\mu$ g/kg diet). Animals had free access to diet and water. The rats were decapitated after 1.5 and 3 months, blood samples were collected, centrifuged and the sera stored at  $-20^{\circ}\text{C}$ . Hormones (T3, T4 and TSH) and T3 uptake were measured by radioimmunoassay method, using commercially available kits (Kodac, England). The results indicated that the amount of T3 in the test group was not significantly different from that of control group. The results showed that in the groups which consumed diet containing high calcium and iodide, mean total T4 concentration was significantly higher than control ( $P < 0.05$ ). Measurement of T3 uptake showed that in the test groups, T3 uptake was significantly lower than control. This may be due to increased synthesis of proteins, less hormone synthesis, or abnormality of the protein binding to the hormones. In general the results are predictable with the known concepts.

#### P776

##### Safety and efficacy of administering 0.2 mg of recombinant human TSH for two consecutive days as an adjuvant to low-dose radioiodine therapy in out-patients with large non-toxic multinodular goitre

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Recombinant human TSH (rhTSH) has recently been used as an adjuvant to the treatment of non-toxic multinodular goitre (MNG) by means of <sup>131</sup>I. The aim of our study was to evaluate the efficacy of 0.2 mg rhTSH or placebo administered im on 2 consecutive days before a fixed therapeutic dose of 14 mCi of <sup>131</sup>I. Thirteen elderly patients ( $71 \pm 7$  years) with large non-toxic MNG (group 1) were treated with rhTSH plus <sup>131</sup>I. While a control group of 8 patients, matched for age and non-toxic MNG volume, was treated with placebo plus <sup>131</sup>I (group 2). In all patients, surgery was either contraindicated or refused. Examinations were performed before and 3–180 days after the first rhTSH or placebo administration and then 6–24 months after therapy. Before and after <sup>131</sup>I therapy thyroid volume (TV) was evaluated by ultrasound. The number and severity of side-effects was similar in both groups of subjects. On final examination, the number of patients symptomatic for goitre was significantly lower in group 1 than in group 2 ( $P = 0.03$ ). In group 1, TSH levels peaked to  $40.3 \pm 9.5$  mU/L on day 3 from the baseline value of  $0.5 \pm 0.1$  mU/L ( $P < 0.001$ ). While a marked increase in f-T3, f-T4 and thyroglobulin ( $P < 0.001$ ) was noted in both groups during the first 2 weeks of treatment, peak values were much higher in group 1 than in group 2. At the end of the study the percentage of patients who did not need therapy to control TSH secretion was higher ( $P = 0.06$ ) in group 1 (83%) than in group 2 (38%). In group 1, TV was reduced from  $78 \pm 11$  ml to  $49 \pm 13$  ml ( $P = 0.004$ ) and from  $90 \pm 25$  ml to  $67 \pm 20$  ml in group 2. Median TV reduction from baseline values was 48% and 28% in groups 1 and 2, respectively. In conclusion, this long-term controlled study demonstrates that 0.2 mg administered on 2 consecutive days increases the efficacy of low <sup>131</sup>I (14mCi) doses in the treatment of non-toxic MNG in elderly subjects.  $\beta$ -blockers generally prevent side-effects due to short-term but marked thyrotoxicosis, which is enhanced by rhTSH administration. The most important effect of rhTSH administration before <sup>131</sup>I is to reduce TV.

#### P777

##### Clinical aspects and diagnostics of follicular thyroid tumours

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The aim of the study

To compare FNA cytological examination and histological examination results of follicular thyroid tumors (FTT) and to define clinical features of benign and malignant FTT.

##### Materials

516 cases of patients with FTT, who underwent thyroid surgery in the Head Research Center for Endocrinology during the period 1998–2003, were studied retrospectively. The results of FNA and histological examination were evaluated particularly.

##### Results

The frequency of follicular adenomas (FA) was 91.1%, 8.9% cases of all FTT were follicular cancer (FC). 85% patients with FA and FC were females. At histological examination in 86% cases FTT were associated with nodular/multinodular goiter, in 5.1% cases with lymphoid thyroiditis and in 8.9% cases with Graves disease.

In 34.8% cases FC were a solitary thyroid nodules and in 65.2% cases were associated with multinodular goiter (MNG). FA were solitary nodules in half cases. Preoperative FNA results were evaluated retrospectively. In 30% cases cytological diagnosis was colloid nodular proliferating goiter. Practical difficulties in preoperative cytological evaluation were related to prevailing of MNG cases among operated patients.

##### Conclusion

The frequency of FC among patients with FTT was 8.9%. If FTT are associated with MNG the sensitivity of FNA for cytological verification of follicular neoplasia is about 40%.

#### P778

##### Prevalence of thyroid dysfunction in old residents in the province of Pavia, northern Italy

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Thyroid dysfunction affects a considerable number of elderly subjects. However the role of screening and treatment of thyroid disorders in geriatric population is still debated. The aim of this study was the evaluation of the prevalence of thyroid dysfunction and its impact on comorbidity and comedication.

Two independent populations were evaluated: 2001 residents in 21 federated nursing homes in the province of Pavia (Northern Italy), age  $>60$  y, and 1275 age-matched hospitalized subjects. In the former population functional status, drugs currently administered on a scheduled basis and clinical diagnosis from patients' chart were available, while in the latter also thyroid hormones serum levels were evaluated.

Age and sex distribution were similar in the two groups with a remarkable predominance of the female gender.

In elderly subjects residents in nursing homes the overall prevalence of diagnosed thyroid dysfunction was 2.6% for hypothyroidism and 0.7% for hyperthyroidism. In hypothyroid patients the prevalence of implanted pacemakers, hypostenic-hypocinetic syndromes and autoimmune arthritis was higher than in euthyroid subjects. In hyperthyroid patients a clear increase of atrial fibrillation, heart failure, ischemic heart disease and cognitive impairment was found. In both cases the number of comedications was significantly higher than euthyroid controls.

In hospitalized patients, in whom hormonal evaluation was available, the prevalence of hypothyroidism, new or previously diagnosed, was 8.15%, while the prevalence of hyperthyroidism (TSH suppressed or suggestive for non-thyroidal illness or current antithyroid therapy) was 6.9%. In hospitalized patients the number was similar in euthyroid and hypo- or hyperthyroid patients.

Our findings suggest that in elderly subjects many cases of hyper- and hypothyroidism are undiagnosed probably because symptoms are subtle and attributed to normal aging. The lack of an appropriate diagnosis seems to be associated to a more severe comorbidity and comedication.

#### P779

##### Thyroid function in patients with atrial fibrillation

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##### Background

Thyrotoxicosis is a common disorder with a prevalence of 3% in females and 0.3% in males in iodine replete areas. The prevalence may be higher in areas of iodine deficiency. It is known to induce many cardiovascular



effects. The most important complication of thyrotoxicosis is atrial fibrillation. We aimed to assess thyroid function in patients with atrial fibrillation and determine the frequency of thyrotoxicosis in this group.

#### Methods

We studied medical history of 107 patients with atrial fibrillation (34 males and 73 females, mean age  $61.7 \pm 9.7$  yr). The time interval was January 2004 to August 2005. Thyroid function was assessed by serum TSH concentration (normal range 0.4 – 4.0) and data of ultrasonographic examination.

#### Results

11.2% of patients had thyrotoxicosis, TSH  $0.1 \pm 0.12$  mU/L. 13 patients (12.1%) had hypothyroidism, TSH  $9.1 \pm 6.4$  mU/L. 82 patients (76.7%) had euthyroidism (normal serum TSH values). Among 12 subjects of the first group were 8 (66.6%) with a nodular goiter.

#### Conclusion

The frequency of thyrotoxicosis in patients with atrial fibrillation was 11.2%. A nodular goiter was found in 66.6% subjects of this group. Serum level TSH level should be measured in all patients with atrial fibrillation.

### P780

#### Hyperthyreosis treated with thyroid artery embolization

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#### Aim

Evaluation of efficacy and safety of thyroid artery embolization procedure as a method of treatment of hyperthyreosis.

#### Patient and methods

Since May 2004 to July 2005 the thyroid artery embolization procedures have been performed in 9 pts (7 women and 2 men) in mean age 62.3 (44 – 77). The reason for such a treatment were: hyperthyroid goiter with the other organs compression in 5 cases, Graves disease in 2 and thyrotoxicosis type I after amiodarone medication in 2 cases. The local Ethical Committee approval has been obtained before.

#### Results

There weren't observed any serious adverse events after the thyroid artery embolization procedures. The most frequent adverse symptom was transient neck pain (in 8 pts – 89%). In one case (11%) transitory fever, and in two pts (22%) passing asymptomatic hypocalcemia (min. Ca conc. 8.1 mg/dl) occurred. During the first week after the embolization maximum increase of thyroid hormones concentration was observed in every case (FT<sub>4</sub> mean max. 3 – fold and FT<sub>3</sub> mean max. 2.5 – fold). There weren't observed any significant clinical symptoms of hyperthyreosis except one case – 54 y.o. man with unstable angina in a course of type I amiodarone – induced thyrotoxicosis. He was treated with two plasmapheresis procedures and with thiamazole, propranolol, methylprednisolon i.v. Eight patients (89%) was euthyroid 3 months after the thyroid arteries embolization. One patient with Graves disease needed L-thyroxin supplementation due to hypothyreosis and one patient with hyperthyroid autonomic nodular goiter needed thiamazole medication. The thyroid volume decreased by mean 37% (evaluated in CT scans). In every patient with a huge hypothyroid goiter the withdrawal of the other organs compression by goiter were achieved.

#### Conclusion

Thyroid artery embolization is a promising method in treatment of hyperthyreosis, especially in patients with a huge goiter.

### P781

#### Psychological wellbeing correlates with free T4 but not free T3 levels in patients on "adequate" thyroid hormone replacement

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Both thyroid dysfunction and psychological morbidity are common in the general population. Association between mood disorders and overt thyroid dysfunction are well established. Hypothyroid patients are more prone to depression and anxiety is a frequently reported symptom in thyrotoxicosis. However, the relationship between thyroid hormones in the normal range and psychological well being has not been established.

In this study we analysed the relationship between psychological wellbeing and thyroid hormones (thyroxine – T<sub>4</sub>, triiodothyronine – T<sub>3</sub>, thyrotropin – TSH, reverse triiodothyronine – rT<sub>3</sub>) in 697 patients who were on a stable

dose of thyroxine replacement as part of the baseline assessment of a randomized controlled trial of T<sub>3</sub>/T<sub>4</sub> therapy. Psychological well being was assessed by the General Health Questionnaire – 12 (GHQ-12). A Higher GHQ-12 score indicated increased psychological morbidity. Local ethical committee approval was obtained.

All the patients were euthyroid on thyroxine (median TSH – 0.9mU/L, mean free T<sub>4</sub> – 20.98 pmol/L and mean free T<sub>3</sub> – 3.85 pmol/L). While fT<sub>4</sub> showed a strong correlation to the GHQ-12 scores (b: -0.16, P = 0.005), the correlation with TSH was weak (b: 0.663, p = 0.04) and no correlation was seen with fT<sub>3</sub> and rT<sub>3</sub> (fT<sub>3</sub> – b:0.32, P = 0.28; rT<sub>3</sub> – b:0.09, P = 0.95). In addition, there was no correlation seen between the GHQ scores and rT<sub>3</sub>/T<sub>3</sub> & T<sub>4</sub>/rT<sub>3</sub> ratios.

We conclude that free T<sub>4</sub> concentrations may predispose to psychological morbidity even within the reference range in treated hypothyroid patients. However, the relationship of thyroid hormones and the mood in patients without thyroid dysfunction remains to be established.

### P782

#### Correlation between endogenous thyroxin and A, G, and M immunoglobulins in dysthyreosis

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#### Introduction

The thyroid is a multifunctional endocrine gland which, by its hormones, affects all the organs, metabolisms and nonspecific defence of the body. The aim of this study is to establish if endogenous thyroxin (FT<sub>4</sub>) is involved in the nonspecific defence of the organism.

#### Material and methods

94 patients were included in the study, 47 with hyperthyroidism and 47 with hypothyroidism, 81 women, 13 men aged 7–78. FT<sub>4</sub> was determined by ECLIA and IgM, IgG, IgA by nephelometry.

#### Results

We noticed a reversed relation between FT<sub>4</sub> and IgM in patients with hyperthyroidism and hypothyroidism irrespective of age and sex. We found IgG results almost identical in both forms of dysthyreosis. IgA oscillates within normal limits irrespective of sex, age, kind of dysthyroidism.

#### Conclusions

IgM concentration is significantly higher in hypothyroid patients than in the hyperthyroid ones. This reversed relation is more evident in hypothyroid women. In our research the anabolic effect of FT<sub>4</sub> is evident. The decrease of FT<sub>4</sub> determines the increase of IgM level. From this point of view hypothyroidism can be regarded of beneficial state for the body. Within the research groups the IgG normal values appear higher or lower in almost identical percentage. The conclusion is that this proteic fraction with in important part in the nonspecific defence is not sensitive to the thyroxin action in any concentration and it is not modified in dysthyreosis. IgA isn't sensitive either to the action of endogenous thyroxin.

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### P783

#### Long-term results of decompression surgery in thyroid-associated ophthalmopathy

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Graves'ophthalmopathy is an autoimmune disorder with orbital tissue inflammation. Decompression surgery is considered when conservative treatment fails. Indications for this operation are optic neuropathy, severe inflammation with pain and proptosis.

The aim of the present study is to describe the long-term results of decompression surgery in patients with thyroid-associated ophthalmopathy (TAO). This study includes retrospective analysis and clinical re-examination of TAO patients with comparison to healthy controls. The study protocol was approved by the Ethics Committee of University Central Hospital.

Seventy-eight patients who had undergone orbital decompression by transantral or endonasal technique between the years 1985 and 2000 were invited for re-examination. For comparison, 79 randomly selected healthy age- and sex-matched controls underwent a similar examination. Patients' median age was 54 years (range 22–77) and median follow-up time was 5.2 years (IQR 3.0–8.0). In comparison to the patients' preoperative state, proptosis was reduced 4.7 (2.6) mm (mean (SD)) in the right and 4.4 (2.9) mm in the left globe ( $P < 0.0001$ ) but did not reach the level of controls' globe ( $P < 0.0001$ ). In the right and left eye of patients, visual acuity improved in 44 to 55%, remained stable in 27 to 36%, and worsened in 18 to 20%, but remained significantly worse than in controls ( $P = 0.02–0.001$ ). Diplopia was the most frequent complaint ( $N = 39$ , 50%) in patients. Sensory disturbances were significantly more frequent in patients ( $P < 0.0001$ ). Among patients and controls, maxillary sinusitis and facial neuralgias were equally common. Although overall satisfaction with present eye status measured by VAS was lower (median 7, IQR 5 to 8) for the patients than the controls (median 8, IQR 7 to 9) ( $P < 0.0001$ ), the majority of the patients (91%) considered the operation helpful.

Orbital decompression seems an effective and safe treatment. Regardless of technique used, patient satisfaction with decompression surgery was high.

#### P784

##### Fixed dose radioactive iodine therapy for hyperthyroidism: the west of Ireland experience

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Radioactive iodine (RAI) is a common treatment for hyperthyroidism, but the dosage and cure rates vary between centres. We administer 370 MBq  $^{131}\text{I}$  in all cases unless a large goitre is present, and repeat the dose until hyperthyroidism is cured. Patients are considered cured if euthyroid or hypothyroid one year after the last dose of RAI.

We audited the outcome of RAI treatment in our institution between January 2000 and March 2002 to 112 patients (13 male) whose records were available. We excluded 12 patients who received larger doses of RAI for large goitres. Average patient age was 51. The cause of hyperthyroidism was toxic nodular goitre (TNG) in 35%, Graves' disease in 23%, and undetermined in 41%. Average time from diagnosis to RAI was 19 months. Of 87 patients followed until they were cured, 15 (17%) required more than one dose, 9 patients needed 2, 3 needed 3, 2 needed 4, and 1 needed 6 doses. There was no association between aetiology of hyperthyroidism, time from diagnosis to treatment or previous medication and the need for more than one dose of RAI. One year after successful treatment 40 patients (46%) required thyroxine replacement (66.7% of those with Graves' disease and 17% of those with TNG).

Of 17 cases of thyroid eye disease 4 deteriorated after RAI. One further patient developed ophthalmopathy, and one developed atrial fibrillation. There were no other complications.

RAI was well-tolerated, but 17% of patients required more than one dose. 46% of patients required thyroxine replacement after one year, 66.7% of those with Graves' disease versus 17% with Toxic Nodular Goitre.

#### P785

##### Pathogenic mutations in gene RET in patients with medullary thyroid carcinoma found in Polish population

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##### Aim

The aim of this paper is to summarise the seven's years of investigations on the frequency and kind of mutations which occurred in gene *RET* in patients with medullary thyroid cancer.

##### Methods

Within 330 subjects studied, there were 235 patients with RRT and 95 their healthy relatives. DNA was isolated from peripheral blood lymphocytes.

The following exons of the gene *RET* were amplified by the PCR method: 10, 11, 13, 14, 15 and 16 and then they were subjected to direct sequencing. Results

Pathogenic mutations found in gene *RET*.

No.	Diagnosis	Exon	Codon	Number of subjects
1	FMTC	10	609,618,620	26 (38.8%)
2	FMTC/MEN2A	11	634,649	19 (28.3%)
3	FMTC	13	791	14(20.9%)
4	FMTC	14	804,819,844	3 (4.5%)
5	FMTC	15	891	2 (3.0%)
6	FMTC/MEN2B	16	912,918	3 (4.5%)

##### Conclusions

There were 46 patients with RRT, 1 with Adenocarcinoma Renis, 1 with PTC and 19 healthy relatives within 67 patients with mutation in gene *RET*. The most frequent mutations were found in codons 620 (23.9%), 634 (26.8%) and 791(20.9%). Additionally a few new mutations were found: TGC 609 TTC (Cys/Phe), GAG 819 AAG (Glu/Lys), CGG 844 CAG (Arg/Gln), CGG 912 CCG (Arg/Pro) in gene *RET*.

#### P786

##### Epidemiology of endemic goiter in mountain region of Adjara Autonomy Republic (Georgia)

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2.788 inhabitant (726 men 1192 women and 870 children) were examined in mountain region of Adjara on thyroid gland pathology at the age from 1 to 75 (2003–2005 years). The investigated population was selected by "randomized method". The data of ultrasonography, clinical and laboratory tests (defining T3, T4, TSH hormones, antibodies and iodine excretion in urine) were diagnostic criteria's of thyroid gland pathology. To study risk factors of thyroid gland pathology development the examined population was asked by special Ask-tests. Obtained data were treated by means of computer program Epi info. Spread of thyroid gland pathology in examined population is 53.6% (41.6% men, 50.8% women and 67.4% children). The main form of endemic goiter is euthyroid diffuse goiter (specific share is 59.5%). The highest spreading of goiter in women of the age 21–30 was 58.3%. Index of Lenz-Bauer was 0.82. Spreading of nodular goiter in examined adult population is 6.8% (8.2% men and 8.2% women). Nodular goiter starts in women 3–4 years earlier than in men. Together with age increases the frequency of the nodular goiter. Date of iodurie median among children corresponds to middleweight of iodine deficiency disorders (4.3 mkg%). Concentration of iodine in water was 2.0 mg/l and soil was 0.3 mg/kg its evidence to iodine deficiency in environment. High index of endemic goiter spreading in mountain region of Adjara is caused by iodine deficiency.

#### P787

##### Epidemiologic analysis of thyroid fine needle aspiration biopsies over a period of 18 years (1987–2004)

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##### Introduction

Fine Needle Aspiration Biopsy (FNA) is a method widely used in the assessment of thyroid nodules. The main aim of this 18-year retrospective study was the investigation of the diagnostic value of FNA in the approach of thyroid malignancy.

##### Subjects and methods

1376 patients underwent 1938 FNAs from 1987 to 2004 in the Department of Endocrinology, "Hippocraton" General Hospital, Thessaloniki, Greece.

178 of these patients subsequently underwent total or subtotal thyroid resection and a pathology report was available.

#### Results

Pathology reports are the "gold standard" for the diagnosis of thyroid malignancy. FNA shows a sensitivity of 76.2% and a specificity of 90.5%, with a significant degree of agreement between the two methods (Cohen's method,  $P < 0.05$ ). There was a considerable improvement in the diagnostic value of FNA during the sub-period 1996–2004 as compared to the sub-period 1987–1995, probably due to increased experience of the persons performing and interpreting the FNA.

#### Conclusions

1) FNA is a reliable diagnostic method in the initial assessment of thyroid malignancy, 2) a non-diagnostic FNA should always be repeated, 3) even after a cytological diagnosis of benign hyperplasia, meticulous follow-up is necessary and 4) increased experience of the persons performing or interpreting the FNA leads to improvement in the diagnostic value of FNA in the approach of thyroid malignancy.

### P788

#### Differentiated thyroid carcinoma: experiences from a centre in south India

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#### Aim

To analyse the clinical profile in patients with differentiated thyroid carcinoma.

#### Materials & methods

A retrospective analysis of case records of 232 patients who had undergone treatment for differentiated thyroid carcinoma from year 2000–2004 in Christian Medical College, Vellore was done and the intended data obtained using spss data.

#### Results

A preoperative FNAC suggested thyroid neoplasm in 60%. Total thyroidectomy was done in 97% with 82% of the subjects being diagnosed to have papillary carcinoma. About 54% had unilateral involvement. In those aged less than 45 years, Papillary carcinoma was more common (61.3%). In biopsy proven Papillary carcinoma, 9%(20) had a follicular variant and one patient had a diffuse sclerosing variant. Lymphocytic thyroiditis was reported in 9%. The extent of the malignancy did not have significant relation with the presence of thyroiditis or variants. The sites of metastasis were lymph nodes (42%), followed by lung (9.0%), bone (8%) and multiple sites were involved in 9%. Lymph node metastasis was predominant in Papillary carcinoma (93%;  $P = 0.000$ ). Distant metastasis was seen more in follicular carcinoma (36.6%,  $P = 0.000$ ). Local infiltration was seen in 33% and found to be high in papillary carcinoma. The highest recorded Thyroglobulin value of any given patient had a significant association with metastasis ( $P = 0.000$ ) and with the number of ablations given ( $P = 0.039$ ).

#### Conclusion

The presence of variants or lymphocytic thyroiditis histologically did not influence the extent of the disease. Peak thyroglobulin value was a predictor of disease extent and number of ablations.

### P789

#### Ultrasound thyroid changes: Moscow population screening results

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#### Objective of the study

To determine the prevalence of thyroid tissue changes in mild iodine deficiency region.

#### Materials and methods

The 17th month-lasting study was carried out in Moscow colleges, factories and offices. 1520 people (1403 female, 117 male) median age – 34 years were screened by thyroid ultrasonography with liner probe 7.5 MHz.

#### Results

Thyroid ultrasound disorders have been demonstrated to be high prevalent in women – 37.6% (527/1403) vs men – 13.7% (16/117). As a male cohort was not large enough, only female screening results were evaluated. Nonpalpable foci of thyroid tissue under 1 cm in diameter were found in 12.5% (176 patients). Thyroid nodules larger than 1 cm were observed in 10.3% (144/1403) patients, 88 (6.3%) – had solitary thyroid nodules,

56 (4.0%) – had multinodular goiters. Diffuse hypoechogenic thyroid tissue was found in 8.7% (122) patients. Diffuse goiter (with normal echogenic tissue) was revealed in 6.1% (85) patients.

#### Conclusion

In Moscow (mild iodine deficiency region) the overall prevalence of ultrasound thyroid abnormalities were found 37.6%. We revealed that nonpalpable foci of thyroid gland under 1 cm are the most prevalent pattern (12.5%) in all age groups. Our study confirms the common established trend: the relative decrease of diffuse goiter prevalence in comparison with nodular goiter (with multinodular goiter predominance) in elderly people (>45 years old).

### P790

#### Quality of life, health status, symptoms and treatment satisfaction in subclinical hypothyroidism: a double-blind 12-week cross-over study of L-thyroxine versus placebo

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#### Background

It is controversial whether symptoms of hypothyroidism and quality of life (QoL) in people with subclinical hypothyroidism (SCH) are impaired and whether treatment with L-thyroxine alleviates these. Previous studies of L-Thyroxine therapy have shown conflicting results. No previous study has simultaneously investigated effects of treatment on quality of life (QoL), health status, symptoms and treatment satisfaction.

#### Methods

One hundred patients, aged between 18 to 80 years with SCH were identified from the laboratory database and from primary care practices. Exclusion criteria were previous thyroid disease, pregnancy, psychiatric disorders, chronic diseases, and medications affecting thyroid hormone levels. Ethical committee approval was obtained. All the patients were randomised in a double-blind cross-over manner to either L-Thyroxine (100mcg) or matching placebo for a period of 12-weeks each. Assessments were made by validated patient-completed questionnaires: disease-specific instruments for QoL (ThyDQoL), symptoms (ThySC), and treatment satisfaction (ThyTSQ), and generic health status (SF-36 v2).

#### Results

Ninety-nine patients completed the study. Compared to placebo, L-thyroxine reduced TSH from 6.1 to 1.5 mIU/L ( $P < 0.001$ ), free T4 and free T3 levels increased significantly ( $P < 0.01$ ), and both sex-life (ThyDQoL) and overall QoL (ThyDQoL) were less negatively impacted by hypothyroidism [difference in means (95%CI)] 0.41 (0.04 to 0.78,  $P < 0.04$ ) and 0.18 (0.01 to 0.35,  $P < 0.05$ ), respectively. ThySC symptom bother scores did not show any benefit of L-Thyroxine but there was a significant improvement in the frequency of tiredness (ThySC) - from 89% to 78%,  $P < 0.04$ , NNT = 5. Health status and treatment satisfaction did not show any significant change.

#### Conclusions

Treatment of individuals with SCH with 100 mcg of L-thyroxine improves overall QoL and sex-life. The number of patients feeling tired reduces significantly but there is no improvement in any respondent ratings for symptom bother, perceived health status or satisfaction with treatment with L-thyroxine over and above that of placebo.

### P791

#### Loss of heterozygosity and genetic association analyses within 11q13.5-q14 chromosomal region in papillary thyroid carcinoma

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#### Introduction

Papillary Thyroid cancer (PTC) accounts for 80% or more of all thyroid malignancies and genetic alterations associated with the development and progression of this type of carcinoma remains largely unknown. Chromosomal band 11q13 seems to be one of the most frequently amplified regions in human cancer. Allelic imbalance involving EMSY, CAPN5 and PAK1 loci in thyroid cancer, have never been investigated. The aim of our study was to investigate the existence of allelic imbalance at EMSY, CAPN5 and PAK1, as candidate genes within 11q13.5-q14 region, by SNP-based LOH analysis.



#### Patients and methods

To investigate LOH for this region, we selected a total of 9 SNPs, that were analysed by Pyrosequencing technology among 41 samples of PTC tissue and 41 corresponding normal thyroid tissue. In addition, 178 unselected controls were genotyped to estimate population frequencies of SNPs, genotypes and haplotypes in our population. The study protocol was approved by the Ethics Committee of the reference hospital.

#### Results

We found a complete absence of LOH at the EMSY, CAPN5, and PAK1 loci in this series of PTC patients. In addition, we used a genetic association study to determine if variation in EMSY, CAPN5 and PAK1 were associated with thyroid cancer risk. We observed differences in EMSY haplotype distribution of patients with PTC compared to general population ( $P = 0.02$ ,  $\chi^2 = 5.40$ ; OR = 2.00 [1.06-3.74]). When CAPN5 and PAK1 haplotype frequencies were compared between cases and controls, no statistical differences were observed.

#### Conclusions

We conclude that our studies do not support findings of LOH at EMSY, CAPN5 and PAK1 at least in thyroid cancer. We found evidence of association between EMSY haplotype and papillary thyroid cancer, suggesting that EMSY gene could be of relevance in the pathogenesis of papillary thyroid carcinoma.

### P792

#### Environmental and socio-economic risk factors of thyroid cancer in Olsztyn and the Warmia and Mazury region, Poland

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#### Aim

The aim of the study was to assess the effect of environmental and socio-economical factors on the increase in the incidence rate of thyroid cancer observed in Olsztyn and the Warmia and Mazury Region in the years 1994–2003.

#### Material and methods

In the group of patients registered in the standardized thyroid cancer register their place of residence, education level, place of parents descent, exposure to ionizing irradiation during the Chernobyl accident, iodine prophylaxis during irradiation, family history of thyroid diseases, thyroid diseases in childhood, lifestyle (smoking, drinking), nutritional habits, number of pregnancies and also contraception (used by women) were assessed based on specially designed questionnaire. Control group consist of healthy volunteers. They answered the same questionnaire.

#### Results

Among the patients who answered the questionnaire there were 261 women, average age 49.7 and 33 men, average age 51.4; 149 persons had elementary education, 220 patients were city inhabitants. 100% patients were exposed to ionizing irradiation during the Chernobyl accident, 31% received iodine prophylaxis during irradiation. Twenty seven patients (9%) had thyroid diseases in childhood and 96 patients (33%) had family history of thyroid diseases. 46% patients were on diet rich in milk and average in salt. The study population reported average cabbage and very low fish consumption.

#### Conclusions

1. Exposure to ionizing irradiation during the Chernobyl accident was the main risk factor in the increase in the incidence rate of thyroid cancer in study region. 2. The possible environmental factors were: the iodine deficiency caused by lack of iodine prophylaxis in the eighties and low fish consumption, diet rich in cruciferous vegetables. 3. Mothers of Bielarusia descent were the probably genetic risk factor.

### P793

#### Reverse Transcriptase (RT) inhibitors down regulate tumor growth, induce cell differentiation and re-establish iodine uptake in human thyroid anaplastic carcinoma *in vitro* and *in vivo*

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RT-coding genes are expressed at very low levels in differentiated, non pathological tissues, while are significantly up-regulated in undifferentiated and transformed cells, suggesting a direct correlation between the level of expression of RT and the proliferative state of the cell. Either nevirapine and efavirenz, two RT inhibitors, used in the therapy of AIDS, reversibly down-

regulate cell proliferation and induce differentiation in several human tumor cell lines.

We tested the pharmacological modulation of the endogenous RT activity as anticancer treatment in human thyroid anaplastic tumors. Efavirenz and nevirapine reversibly inhibited cell proliferation with no induction of apoptosis or necrosis in undifferentiated thyroid carcinoma ARO and FRO cells, two cell lines characterized by high levels of endogenous RT. This effect correlated with the accumulation of cells in G0/G1 phase of cell cycle, the appearance of morphological differentiation and a significant reprogramming of gene expression. Indeed, pharmacological inhibition of RT restored TSH signaling since induced the up-regulation of TSH receptor, thyroglobulin, TPO and pendrin genes, the TSH-dependent activation of NIS gene and re-established iodine uptake in response to TSH. Efavirenz reversibly down-regulated tumor growth in mice xenografts of ARO cells and restored iodine uptake *in vivo*, as well. Finally, nevirapine treatment up-regulated iodine uptake in a case of advanced undifferentiated thyroid tumor, insensitive to radiometabolic therapy. These findings suggest that endogenous RT inhibitors may represent a novel treatment in human undifferentiated thyroid tumors able to re-establish or improve the sensitivity to conventional radiometabolic therapy.

### P794

#### The effect of block & replacement regime following radioiodine therapy for thyrotoxicosis on thyroid function in the post-radioiodine period

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#### Introduction

Radioiodine (Iodine-131) is a widely used treatment for thyrotoxicosis. Fluctuation in thyroid hormone levels following radioiodine treatment is common. Hypothyroidism occurs in approximately 5.5% of patients per month in the first 6 months, with onset time hard to predict. In contrast, some patients remain hyperthyroid either due to persistent pre-radioiodine thyrotoxicosis or transient exacerbation following radioiodine. We examined whether the block & replacement treatment (B&R; thionomides plus thyroxine) following radioiodine helped prevent fluctuation of thyroid hormone levels immediately post-radioiodine.

#### Methods

A retrospective case-note audit of 71 patients, undergoing radioiodine treatment for thyrotoxicosis in a 2-year period (2002–3). 25(35%) patients started B&R (carbimazole 40 mg and thyroxine 100 mg daily) 7 days after radioiodine for 6 months (B&R group). 46 (65%) patients were treated with thyroxine or thionomide, depending on thyroid function levels (non-B&R group). Thyroid function tests at 6 weeks post-radioiodine were compared in these groups.

#### Results

Of 71 patients, 61(86%) were women. 24 (34%) had a diagnosis of Graves' disease (positive thyroid antibodies, diffuse thyroid uptake or the presence of ophthalmopathy), 11 (15%) had toxic multinodular goitre (patchy thyroid uptake), and in 36 (51%) the diagnosis of thyrotoxicosis was undetermined. 5 (7%) previously had thyroid surgery. 6(8%) previously had radioiodine. Before radioiodine, 45 (63%) were on thionomides; 17 (24%) on B&R; 28 (40%) on thionomide alone. Medication stopped 7 days prior to treatment for both groups. Post radioiodine, 25 patients were treated with B&R and of the 46 patients in the non-B&R group, 12 were subsequently treated with thionomides. At 6 weeks, thyroid function tests in the B&R group showed that 12(48%) had biochemical euthyroidism, 10 (40%) hyperthyroidism and 3(12%) hypothyroidism, as compared to 31 (67%) euthyroidism, 10 (21%) hyperthyroidism and 5 (11%) hypothyroidism in the non-B&R group ( $P = ns$ ).

#### Conclusions

Block & replacement treatment following radioiodine does not help to prevent fluctuation of thyroid hormone levels immediately post-radioiodine.

### P795

#### Prevalence of amiodarone- induce thyroid disorders in iodine deficiency region

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Uzbekistan is area of low dietary iodide intake (45.5% of population with iodide deficiency) and high goiter prevalence (more than 40%)

#### Objective

The aim of this study was to investigate the prevalence of amiodarone- induce thyroid disorders among patients residence in iodine deficiency region.



**Patients and methods**

We observed 46 patients with supraventricular or ventricular arrhythmias, aged  $53 \pm 12$  years. The mean duration of treatment with amiodarone was 20 (3–36) months. Control group consists 20 patients without thyroid diseases. The research program consisted physical examination, thyroid palpation, ultrasound, reflexometry, FNAB. TSH, fT3, fT4 was estimated by RIA method.

**Results**

Normal TSH and thyroid hormones were found in 33 (48.1%) of examined patients. Abnormal TSH, fT3, fT4 hormones values were seen in 13 patients. Hypothyroid patients had previously goitre. Mean age of this group was 44.6 years. Patients with Hyperthyroidism were consist 10 (21.7%) aged 38.0 years.

Group	TSH (mM/l)	fT3 (nM/ml)	fT4 (nM/ml)	Mean age
Euthyroid (n = 33)	1.99 ± 0.19	1.99 ± 0.05	123.6 ± 3.54	44.2
Hypothyroid (n = 3)	5.2 ± 0.61	0.7 ± 0.1	50 ± 1.73	54.6
Hyperthyroid (n = 10)	0.17 ± 0.03	3.13 ± 0.07	199.2 ± 9.91	38.4

**Conclusion**

In iodine deficiency region more than 1 year amiodarone intake lead to development of amiodarone-associated thyroid dysfunction in 28.2% of patients (hypothyroidism and thyrotoxicosis in 6.5% and 21.7% respectively). Previously thyroid diseases predispose to amiodarone-induced hypothyroidism. Amiodarone-associated hypothyroidism increased in elderly.

**P796****Correction of oxidative metabolism in myocardium by oligocrine during experimental thyrotoxicosis**

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Numerous number of studies indicate significant role of oxidative stress in the pathogenesis of thyrotoxicosis. The aim of this work was to estimate oxidative metabolism changes in myocardium and effectiveness of oligocrine in the experimental model of thyrotoxicosis.

**Methods**

Thyrotoxicosis was induced in mature white wistar rats by daily administration of L-thyroxine (100 µg/100 g). We have conducted 3 series of experiments studying: (1) effects of L-thyroxine administered for 10, 15 and 20 days; (2) therapeutic effect of oligocrine on 16th and 21 days of administration against a background of L-thyroxine injections; (3) therapeutic effect of oligocrine on 16th and 21 days of administration (L-thyroxine injections terminated on 10th day). We have studied thyroid status (TSH, FT3, FT4) of animals, oxidative metabolism and NO production in myocardium (by Electron Paramagnetic Resonance and spin-trap methods with X-band radio spectrometer ESR-231). Animals were anaesthetised by sodium ethamonal.

**Results**

Animals in 1 series present significantly elevated levels of free NO and disturbance in mitochondrial electron-transport chain on NAD.H ubiquinon-oxidoreductase site revealed by enhanced production of semiquinones and nitrosylation of NAD.H dehydrogenase Fe-S centers and leading to increased production of superoxide radicals and lipid peroxides. Oxidative metabolism disturbance results in lowered energogenesis of myocardiocytes and affects heart muscle function. In rats treated with oligocrin mitochondrial electron transport in myocardiocytes is recuperated, though remains moderately intensified, correlating with FT3 and FT4 levels. Free NO and lipid peroxide levels are moderately elevated.

**Conclusions**

Increased free radical production during thyrotoxicosis occurs due to thyroid status disturbance resulted in hypercatabolism and intensification of mitochondrial electron transport. Protective effect of oligocrine consists in facilitating both elimination of hypermetabolism and stabilization of thyroid status.

**P797****The metabolic syndrome and the thyroid in euthyroidism**

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**Introduction**

Thyroid disease is associated with cardiovascular disease and many cardiovascular risk factors cluster within the metabolic syndrome. It could therefore be hypothesised that thyroid function is associated with the metabolic syndrome.

**Aim of the study**

To investigate the relationship of thyroid function -in the euthyroid range- with serum lipid concentrations and with insulin resistance.

**Methods**

2703 adult inhabitants of a middle-sized city in the Netherlands, participated in this cross-sectional study. Subjects on thyroid hormone replacement therapy or thyroid blocking agents, and subjects taking medication for dyslipidemia and/or diabetes were excluded. Since most patients with the metabolic syndrome will have normal thyroid function, we also excluded aneuthyroid subjects. The HOMA index for insulin resistance was calculated on the basis of fasting glucose and insulin levels.

**Results**

Significant, but weak positive correlations (all  $P < 0.05$ ) were found between TSH and HDL-C ( $r = 0.06$ ) and triglycerides ( $r = 0.06$ ). Significant negative correlations were found between: FT4 and total cholesterol ( $r = -0.06$ ), LDL-C ( $r = -0.05$ ), TG ( $r = -0.08$ ) and Apo B ( $r = -0.05$ ); FT3 and total cholesterol ( $r = -0.08$ ), HDL-C ( $r = -0.05$ ), LDL-C ( $r = -0.06$ ) and Apo B ( $r = -0.06$ ). FT4 (but not FT3) was negatively correlated with HOMA index ( $r = -0.14$ ). The metabolic syndrome (according to NCEP ATP III criteria) was present in 21.0% of women and 16.6% of men and increased with age. FT4 in subjects with a metabolic syndrome was significantly lower than in subjects without a metabolic syndrome ( $12.6 \pm 1.7$  vs.  $12.9 \pm 1.8$  pmol/L;  $P = 0.04$ )

**Conclusion**

We demonstrated an association between thyroid function and lipid levels in subjects classified as being euthyroid, in accordance with the earlier observed association between (sub)clinical hypothyroidism and hypercholesterolemia. Moreover, low normal FT4 levels were significantly associated with increased insulin resistance. This implicates that subjects with low-normal thyroid function already have increased cardiovascular risk.

**P798****Thyroid disease prevalence and incidence in a Dutch population survey**

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**Introduction**

Thyroid disease, both clinical and subclinical, is a common disorder. Various studies, however, differ in reported prevalences of abnormal thyroid function.

**Aim of the study**

To investigate 1. the prevalence of abnormal thyroid function and anti-thyroidperoxidase antibodies (TPOab) in a random sample of the inhabitants of a middle-sized city in the Netherlands; 2. the relationship of the presence of TPOab with TSH concentration in the euthyroid range; and 3. the relationship of positive TPOab and incidence of thyroid dysfunction.

**Methods**

2703 adult inhabitants of the city of Groningen, the Netherlands, participated in this prospective population survey. Details about the use of thyroid hormone replacement therapy (THRT) and thyroid blocking agents were present in 2611 subjects. Incidence of thyroid disease was determined from a questionnaire taken 5 years after the baseline visit. TSH (reference range 0.35–4.94 mIU/L) and FT4 (reference range 9.14–23.81 pmol/L) were determined using a microparticle enzyme immunoassay (Architect and AxSYM respectively) at baseline.

**Results**

1.4% of 2611 subjects used thyroid medication (age  $58 \pm 12$  yrs; 70% female), mostly THRT. Of all the subjects not taking thyroid medication 2.0% had increased TSH and 2.4% decreased TSH. TPOab were present in 10.5% of 2703 subjects, with highest prevalence in older women (23% in women aged 56–65 yrs). Prevalence of TPOab increased with higher TSH, up to 50% in subjects with the highest TSH levels in the euthyroid range.

Incidence of thyroid disease after 5 years of follow-up ranged from 1% in TPOab negative subjects with low normal TSH concentration to 25% in TPOab positive subjects with the highest TSH levels in the euthyroid range.

**Conclusion**

The prevalence of abnormal thyroid function in the general population is substantial. Even in the euthyroid range, presence of TPOab is positively correlated with TSH concentration, and associated with future thyroid disease.

**P799**

**Increased lipid peroxidation in critically ill patients with low-T<sub>3</sub> syndrome**

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Low-T<sub>3</sub> syndrome (euthyroid sick syndrome) develops in critically ill patients. Due to the catabolic state, accompanying critical illnesses, enhanced oxidative damage to macromolecules is expected to occur in subjects suffering from the low-T<sub>3</sub> syndrome.

The study aimed at evaluating the level of lipid peroxidation products in blood serum, collected from critically ill patients with the low-T<sub>3</sub> syndrome and at estimating the relationships between lipid peroxidation level and biochemical parameters and the survival rate.

The procedures, used in the study, were approved by the local Ethical Committee. Sixty (60) critically ill patients (among whom 20 patients met the criteria for the low-T<sub>3</sub> syndrome, and in 40 – thyroid hormones remained in normal ranges), as well as 20 healthy subjects (controls) were enrolled in the study. Peripheral blood was collected at different time points, depending on hospitalization periods in intensive care unit and the survival time. The concentration of malondialdehyde +4-hydroxyalkenals (MDA + 4-HDA), as the index of lipid peroxidation, was evaluated in blood serum. The results were analyzed with respect to all the measured biochemical parameters, as well as to the further course of disease.

The level of lipid peroxidation was approximately twice as high in blood serum, collected from critically ill patients with the low-T<sub>3</sub> syndrome as in healthy subjects. Lipid peroxidation was also increased in the other group of critically ill patients, without low-T<sub>3</sub> syndrome, however, to a weaker extent than in the case of euthyroid sick syndrome. The level of lipid peroxidation corresponded with the mortality rate, which was – expectedly – higher in patients with the low-T<sub>3</sub> syndrome than in other critically ill patients.

**Conclusions**

Increased lipid peroxidation in critically ill patients, especially in those with the low-T<sub>3</sub> syndrome, may indicate enhanced oxidative damage to macromolecules, which may further contribute to organ disturbances.

**P800**

**Asymmetric dimethyl arginine levels in thyroid diseases**

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In terms of mechanism by which thyroid diseases lead the endothelial dysfunction, several aspects have been taking into consideration. One potential factor may be variation in plasma asymmetric dimethylarginine (ADMA) levels. ADMA, which is derived from the catabolism of proteins containing methylated arginine residues, competitively inhibits NOS. The relationship between thyroid disease and variation in plasma ADMA levels is still a potential research area. The aim of this study was to investigate the plasma ADMA levels in patients with thyroid dysfunctions. Three groups (25 healthy subjects, 25 hyperthyroid and 23 hypothyroid subjects) were studied. Concentrations of plasma ADMA were measured by high-performance liquid chromatography (HPLC).

Comparisons of multiple means were made by ANOVA followed by a Fisher's protected least significant difference test. Probability values of  $P < 0.05$  denote statistical significance.

Plasma ADMA levels were significantly higher in both patients with hyperthyroidism ( $0.890 \pm 0.284 \mu\text{mol/L}$ ) and hypothyroidism ( $0.665 \pm 0.364 \mu\text{mol/L}$ ) than control subjects ( $0.403 \pm 0.077 \mu\text{mol/L}$ ).

In conclusion, thyroid dysfunctions increased the ADMA concentration and increased ADMA levels may be one of the potential factor for developing of endothelial dysfunction in both hyper and hypothyroidism.

**P801**

**Results of fine-needle aspiration biopsy of thyroid nodules in region of mild iodine deficiency**

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The occurrence of thyroid diseases is determined by interplay between genetic and environmental factors. The major environmental factor that determines goiter prevalence is iodine status.

Thyroid nodules are present in up to 30% of the Georgian population. The causative role of iodine deficiency which is still endemic in this country has long been established.

Fine-needle aspiration biopsy (FNAB) of the thyroid gland is the cost-effective examination in the evaluation of thyroid nodules.

The aim of this study was to present results of FNA in out-patients of endocrinology department in the period of 2003-2005. Ultrasound-guided FNA was performed in 231 patients of whom 94.5% were females.

The cases were classified according to diagnosis into four groups: benign/negative 145 (62.8%), primary carcinoma 19 (8.2%), suspicious/in-determinate 61 (26.4%) and non-diagnostic 6 (2.6%). These results are in accordance with the already published data in the international literature.

In conclusion, we suggest that FNAB is an effective screening test in the evaluation of the necessity for surgical treatment in patients with thyroid nodules.

**P802**

**Thyroid cancer: diagnostic role of ultrasound and ultrasound-guided fine needle aspiration biopsy (US-FNAB) evaluation**

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Aim of this study was the evaluation of the accuracy of ultrasound and US-FNAB in the diagnosis of thyroid cancer in a population of subjects submitted to US-FNAB of all thyroid nodules, with no prior selection. 1803 patients (1447 females & 356 males, age range 11–87 years, mean  $49.6 \pm 13.7$ , with a total of 3378 nodules) were submitted by the same physician (first author) to ultrasound evaluation and US-FNAB of all thyroid nodules. Results. The nodules were 3 to 90 mm in diameter ( $17.9 \pm 10.9$ ); solitary in 708 cases (39.3%), 2 or more nodules in 1095 cases (60.7%); echographic pattern: hypoechoic in 51%, isoechoic in 29%, hyperechoic in 7%, anechoic in 5%, mixed in 8%; alo sign was present in 39.3%; microcalcification in 10.8% of cases. Cytology was negative in 3024 nodules (89.5%), suspicious or indeterminate in 97 (2.9%), positive in 111 (3.3%), non-diagnostic in 146 (4.3%). 170 patients underwent to surgery (9.4%): 79 carcinomas (71 papillary, 2 follicular, 3 anaplastic, 2 medullary, 1 Hurthle cells), often plurifocal, and 91 struma/adenomas. A total of 345 nodules were finally examined: 188 hyperplastic (54.4%), 33 adenomas (9.6%), 124 carcinomas (36%); 113 papillary, 2 follicular, 5 anaplastic, 2 medullary, 2 Hurthle-cells). US-FNAB had 87% sensitivity, 91% specificity and 90% accuracy. Malignant nodules were solitary nodules in 26.6%; more than one nodule in 73.4%; benign nodules were solitary in 17.6%, 2 or more in 82.4% ( $P < 0.05$ ). Malignant nodules were hypoechoic in 79%, isoechoic in 10.6%, hyperechoic in 3.2%, anechoic in 4%, mixed in 3.2%. The percentages for benign nodules were respectively: 44.7%, 34%, 8.6%, 4.1%, 8.6% ( $P < 0.0005$ ). Hypoechoic pattern had 79% sensitivity, 56% specificity and 64% accuracy in the diagnosis of cancer. Alo sign was present only in 13.7% of malignant nodules vs 46.2% of benign nodules ( $P < 0.0005$ ). The absence of alo sign had 86% sensitivity, 46% specificity and 61% accuracy in the diagnosis of cancer. Microcalcifications were present in 25% of malignant and 11.3% of benign nodules ( $P < 0.001$ ) (sensitivity 14%, specificity 89%, accuracy 66%). Diameters were not statistically different ( $19.6 \pm 13.3$  vs  $20.7 \pm 11.4$  mm). Our data confirm that there isn't any echographic sign that has sufficiently high sensitivity and specificity to substitute US-FNAB in the diagnosis of thyroid cancer.

**P803****Parathyroid hormone determination in fine needle aspirates (PTH-FNAB) allows the differentiation between thyroid and parathyroid lesions**V Triggiani, E Guastamacchia, F Resta, C Sabba & E Tafaro  
University of Bari, Bari, Italy.

Parathyroid lesions are often occasionally discovered during thyroid ultrasound evaluation (incidentalomas); in other cases there is a previous diagnosis of hyperparathyroidism. In many cases it is difficult or even impossible to distinguish these lesions from those of thyroid origin, because they often share common ultrasound features. Scintiscan and cytology are also frequently inadequate. The aim of this study is to evaluate the contribute of PTH determination in the aspirates. 46 patients (37 female and 9 male, age  $54.1 \pm 12.6$ , range 24–83 yrs) out of 1,870 consecutive patients with neck nodular lesions submitted to FNAB in three years by the same physician (first author) were suspected to have one or more nodule(s) of parathyroid origin. The ultrasound examination of the neck of these patients, in fact, showed one or more nodules placed in the posterior aspect of thyroid lobes. Furthermore, 13 of these patients showed a laboratory findings suggestive for primary hyperparathyroidism, 6 of which with clinical evidence. The nodules were submitted to ultrasound-guided-FNAB. For 55 lesions suspected to be of parathyroid pertinence, the needle used to perform the aspirate was then washed using 1 ml of normal saline and PTH determination (immunoradiometric assay) on the fluid obtained was done. In case of cystic lesions, PTH determination was performed directly in the liquid aspirated.

**Results**

The shape of the nodules suspected to be of parathyroid pertinence was round in 24 cases and oval in 31 cases. The echo-pattern was hypoechoic in 43 (78%) cases, isoechoic in 3 cases, cystic in 8 cases and mixed in 1 case. The range of volume was 0.7–33.4 ml. In 6 cases a water-clear liquid was obtained from the aspiration of cystic nodules, suggesting a parathyroid origin. In these cases the cytological examination lead to a non-diagnostic result due to the scant number of cells. The aspiration of solid lesions gave sufficient material but cytology did not lead to a clear distinction. The results of PTH determination in the needle washings ranged from 6.7 to 16640 pg/ml. 16 patients underwent surgical intervention: histological examination of the 23 lesions submitted to PTH-FNAB showed 7 thyroid nodules, 11 parathyroid adenomas and 5 hyperplastic parathyroid lesions. In one case the lesions aspirated (low PTH in the aspirates) resulted thyroid nodules at histology, but a parathyroid adenoma in ectopic location was found. There was a strong positive correlation ( $P < 0.0005$ ) between high levels of PTH-FNAB (more than 190 pg/ml) and the histological finding of parathyroid lesions. PTH-FNAB was also high in 4 out of 6 water-clear liquid containing cysts.

**Conclusions**

PTH-FNAB can be considered the gold standard for verification of parathyroid tissue.

high doses of thyroxine (as adaptation period) take place significant activation of sympathetic and parasympathetic parts of autonomic nervous system and neurohumoral system. Afterwards, a period of dysadaptation begins with considerable lowering of heart rate variability. Also we found significant prolongation of corrected Q-T interval (QTc) in patients with long time suppression with thyroxine. QTc, together with low values of SDNN and HRV-triangular index determine increased risk of severe complications and arrhythmic death. Follow-up of this cohort this all allow us to identify high-risk groups who should be targeted for therapeutic intervention.

**P805****Clinical value of the assessment of neopterin in Graves' ophthalmopathy**A Baldys-Waligorska<sup>1</sup>, B Kusnierz-Cabala<sup>2</sup>, E Plackiewicz-Jankowska<sup>1</sup> & B Huszno<sup>1</sup><sup>1</sup>Collegium Medicum, Jagiellonian University, Dept. of Endocrinology, Krakow, Poland; <sup>2</sup>Collegium Medicum, Jagiellonian University, Dept. of Clinical Biochemistry, Krakow, Poland.**Background**

In Graves' disease, immune cellular mechanisms and cytokine release, as well as auto-antibody production found in the serum, most likely contribute to the development of thyroid eye disease (TED). Biosynthesis of neopterin is closely associated with activation of the cellular immune system and could be a valuable marker of the severity of the disease.

**Objectives**

To assess whether serum neopterin concentrations in patients with TED are a reliable measure of the severity of the disease, to be used to monitor the effectiveness of treatment.

**Methods**

In a prospective study of 30 patients with TED (ophthalmopathy index, IO > 3) of mean age  $57 \pm 12$  years, levels of hTRAb, neopterin, CRP, sTNF $\alpha$ RII and IO were studied prior to and after anti-inflammatory glucocorticoid treatment and consecutive radiotherapy.

**Results**

The median values before and after treatment were: hTRAb 12.2 vs. 1.7 IU/L; neopterin 8.6 vs. 7.3 nmol/L; CRP 1.6 vs. 0.7 mg/L; sTNF $\alpha$ RII 2228.3 vs. 2109.4 ng/L and IO 7 vs. 4 respectively. The differences between these levels (Wilcoxon's test) were statistically significant for IO, CRP and hTRAb, but not for neopterin. Statistically significant correlations ( $r$  Spearman's factor) were found between the levels of CRP and sTNF $\alpha$ RII ( $r = 0.85$ ;  $P < 0.05$ ) prior to treatment, and IO and hTRAb levels ( $r = 0.55$ ;  $P < 0.01$ ) after treatment.

**Conclusions**

Serum neopterin concentrations were not markedly elevated initially and did not decrease significantly after the treatment. The clinical relevance of neopterin for evaluating and monitoring TED severity is therefore questionable. Other markers studied appear to be more useful in evaluating TED treatment: CRP – prior to treatment, and hTRAb post-treatment, as they correlate with sTNF $\alpha$ RII and IO, respectively.

**P804****Heart rate variability in thyroidectomized patients using suppressive therapy with thyroxine**AV Kovalchuk<sup>1</sup>, ST Zubkova<sup>1</sup> & VN Kovalchuk<sup>2</sup><sup>1</sup>Institute of endocrinology and metabolism, Kyiv, Ukraine; <sup>2</sup>National Medical University, Kyiv, Ukraine.

The suppression of serum TSH is a marker for increased risk of vascular mortality or death due to other causes are unknown. Nevertheless, patients who was operated on account of differentiated carcinoma of thyroid, had to take high doses of thyroxine for a long time to achieve suppression of TSH < 0.1 mU/L. Heart rate variability (HRV), a method of mathematical analysis of heart rhythm (computer analysis of 24 h Holter monitoring), was used to assess function of the autonomic nervous system and its central control. We recruited 18 patients (5 men, 13 women, age  $48.22 \pm 2.02$  years), who received suppressive dose of thyroxine and had a level TSH  $\leq 0.01$  mU/L. We found a considerable difference of heart rate variability which turned us to divide patients into two groups. 1-group ( $n = 14$ ) with significant lowering of time domain measurements and frequency domain measurements comparing to health control. 2-group ( $n = 4$ ) characterized with time domain measurement close to health control but significantly ( $P < 0.01$ ) increased frequency domain measurements comparing both to 1-group and health control. Also longevity of thyroxine suppressive therapy intake varied – 1 group – 3–7 years, 2-group – 0.5–1 year. We consider that obtained results are stages of the same process. During short-term use of

**P806****Maternal iodine deficiency alters learning ability and emotional state of the progeny**A Sarishvili<sup>1</sup>, N Lazrshvili<sup>2</sup> & D Metreveli<sup>1</sup><sup>1</sup>I. Beritashvili Institute of Physiology, Department of Functional Neuroanatomy, Tbilisi, Georgia; <sup>2</sup>Georgian State Medical Academy, Department of Endocrinology, Tbilisi, Georgia.

Deficient production of thyroid hormones in maternal organism may have a drastic impact on brain development of the offsprings. Among the people originated from the regions with essential deficiency of iodine in foodstuff there is a high chance for prevalence of mental retardation and neurological cretinism with all possible complications.

The present study analyses changes in emotional state (open field testing), learning ability and memory processes (maze testing) of the progeny of dams suffered from iodine deficiency of different severity during 3 months before mating. Control group of rats received standard diet.

The diet with very low iodine content resulted in a low level of thyroxin in maternal serum and development of neurological deficiency in progeny. The offsprings manifested decreased motor activity and that was clearly demonstrated in open field testing as well as during maze testing. Besides,



decreased learning ability was revealed in experiments with maze. More pronounced changes in both mentioned experimental conditions were observed in the progeny of dams with more restricted iodine diet (addition of KClO<sub>4</sub> to the basic diet) – they show significant decrease in the number of crossed squares, in the number of entering into the central squares and vertical standings as well as increased frequency of grooming. In this group of animal's learning disability during maze testing was revealed clearly. Addition of the iodine to the diet prevents development of all mentioned changes in animal's behavior in open field and the maze. The data obtained lead to the conclusion that offsprings of the dams suffered from iodine deficiency of different severity produced decrease motor activity and learning disability. The intensity of these abnormalities depends on the extent of iodine deficiency.

## P807

### Quantitative and qualitative evaluation of the impact of a ten-year iodine intervention programme in rural areas of Tehran

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#### Introduction

The integrated quantitative and qualitative method was used to evaluate the impact of a ten-year iodine intervention programme on school children's health status in rural areas of Tehran.

#### Methods

In survey, 188 students aged 6–15 years of two villages, Kiga and Ahar, were studied. These villages that are situated near the Tehran, were known for severe endemic goitre in previous studies. The clinical and biochemical measurement carried out and results were compared with data from our previous study in 1989. The focus group discussion (FGD) was used to complement the clinical and biomedical evaluation. The subjective views and experiences of parents ( $n = 51$ ) were explored in six FGDs; four groups of women ( $n = 36$ ) and two groups of men ( $n = 15$ ). These parents were asked whether changes in children's health and any changes in goitre rate were due to iodine supplementation or other socio-economic factors. Data were tape-recorded, transcribed and analysed using content analysis.

#### Results

The survey results showed a significant decrease in goitre grades ( $P < 0.001$ ). The studied variables such as urinary iodine excretion and thyroid hormones concentrations were within the normal range in all school children. Based on FGDs findings, parents believed the administration of iodised oil and the subsequent use of iodine salt was mainly responsible for reduction in goitre and prevention of iodine deficiency. They reported that increased knowledge of the causes of goitre, and changes in the pattern of food consumption; were also contributory factors in improving children's health status.

#### Conclusion

This study showed the importance of collecting subjective as well as objective data for the evaluation of any long-term nutrition intervention programme.

## P808

### Effect of oral administration of propylthiouracil during pregnancy and lactation period on isolated aorta response of their adult male off springs

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Thyroid Hormones have extensive effects on differentiation, development, and growth of different organs. In this study the effect of the administration of PTU on pregnant and lactating rats on isolated aorta response of their adult offsprings has been investigated. Three groups of female rats were selected. In the first group (fetal group) observation of vaginal plug after mating was considered as the first day of pregnancy, then PTU was added to their drinking water until the end of the gestation period. In the second group PTU was added to the drinking water of their mothers from laboring time for 25 days (neonatal group). The third group was the control group which consumed only drinking water. In all three groups Total Thyroxine (tT4), Free Thyroxine (fT4), Total triiodothyronine (tT3), Free Triiodothyronine

(fT3) and TSH immediately after discontinuing of the drug were measured in the sera obtained from the mothers. The results indicate that the levels of the above mentioned hormones (except TSH) in fetal and neonatal groups were significantly lower than control group ( $P < 0.05$ ) and TSH on fetal and neonatal group were significantly higher than control ( $P < 0.001$ ). After two months the adult of off springs were anesthetized, dissected and isolated aorta response was examined against KCl and phenylephrine. Results of this study indicated that responsiveness of aorta in fetal group was significantly decreased compared to the control group ( $P < 0.05$ ), but neonatal group had no significant difference with the control group. It can be concluded that hypothyroidism in fetal period has significant effects on differentiation and development of vascular bed (aorta) in a way that can be still observed during adulthood.

## P809

### Study of the prevalence and mechanisms of action of TSH receptor and Gs protein alpha-subunit mutations, in toxic multinodular goiter and toxic adenoma from Galicia (Spain)

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#### Aims

To study (1) the prevalence of TSHr and Gsa mutations in TMNG and TA from Galicia, (2) the clonality of sequenced samples, (3) the constitutive activity of the identified mutations.

#### Material and methods

(1) Mutations search. 62 thyroid samples were obtained at surgery: TA (31), TA within MNG (20) and TMNG (11). TSHr exons 10 to 1 and Gsa exons 8 and 9 were PCR amplified followed by sequencing using dRhodamine. Identified mutations were confirmed by enzymatic restriction analysis or TA-cloning plus sequencing. (2) Clonality was performed by HUMARA assay, performing fragment analysis with 6FAM-labeled primer. (3) Constitutive activity. Mutants D633Y, T632I, F631L, L629F, Δ619, D619G, I568T were cloned in pSVL-TSHr; COS-7 cells were transfected with mutants plus a CRE-LUC reporter and incubated with and without hrTSH.

#### Results

(1) Mutations were found in 32 samples: 19 TA (65.5%), 13 TA within MNG (56.5%), and 0 TMNG; 28 mutations (87.5%) were found in TSHr exon 10, one in exon 9 and none in exons 1 to 8; only 3 mutations were in Gsa. TSHr D727E was found in 9%, P52T in 7.5% and 3 silent mutations were at aa 459. (2) 68% of samples were polyclonal, and 3) mutants had 2.5–6 times higher CRE-Luc activity than TSHr WT.

#### Conclusions

(1) In Galicia, 53% of toxic goiters have TSHr and Gsa mutations, (2) 68% of our samples were polyclonal, probably due to contamination by non-tumoral tissue, (3) tested mutants shown higher constitutive activity than TSHr-WT, suggesting that they are the cause of the hyperthyroidism.

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## P810

### The relationship between echocardiographic left atrial size and atrial fibrillation in patients with Graves' disease

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#### Background and aim

Atrial fibrillation (AF) is a relatively common complication of thyrotoxicosis. The aim of this study was to estimate the association between echocardiographic variables and AF in patients with Graves' disease.

#### Patients and methods

Standard echocardiographic examination was performed in 166 patients with Graves' disease (40 men and 126 women, mean age  $\pm$  SD  $43.9 \pm 12.8$  years) referred to the endocrinological department. 81 of them had paroxysmal (35) or chronic (46) AF due to thyrotoxicosis. Patients with concomitant heart diseases were not included in the study. 18 patients with chronic AF spontaneously reverted to sinus rhythm after they became euthyroid and remained in sinus rhythm during the follow-up (not less than 12 months).



**Results**

Mean maximal left atrial diameter (LAD) was significantly higher in patients with chronic AF than in patients with sinus rhythm ( $4.40 \pm 0.63$  cm vs  $3.37 \pm 0.53$  cm correspondingly, Mann-Whitney  $U = 615.5$ ;  $P < 0.001$ ). The presence of chronic AF significantly correlated with LAD after controlling for age and sex (partial correlation coefficient 0.539;  $P < 0.001$ ). No significant correlation was found between the echocardiographic variables and the presence of paroxysmal AF ( $P > 0.05$ ).

In multiple regression analysis LAD was the best echocardiographic predictor of the restoration of sinus rhythm in patients with chronic AF after they became euthyroid ( $R^2 = 0.460$ ;  $P = 0.002$ ). Discriminant analysis allowed to classify patients with chronic AF into 2 groups – those who reverted to sinus rhythm after achieving euthyroidism (LAD  $< 4.31$  cm) and those who did not (LAD  $> 4.31$  cm) with the probability of correct classification 82.6%.

**Conclusion**

Echocardiographic LAD can be used for the prognosis of the spontaneous reversion to sinus rhythm in patients with chronic AF related to thyrotoxicosis after achieving euthyroid state.

**P811****CIC-5 does not affect megalin expression and function in the thyroid gland**

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Megalín is a member of the LDL receptor family expressed by several epithelial cells where it mediates endocytosis of ligands. In the thyroid megalín is responsible for transcytosis of hormone-poor thyroglobulin (Tg) molecules. This process favors hormone release by preventing hormone-poor Tg to enter the lysosomal pathway, where hormones are cleaved from hormone-rich Tg molecules. Accordingly, megalín KO mice have a distinct thyroid phenotype with primary hypothyroidism. In renal proximal tubule cells megalín expression and function are reduced when the gene encoding CIC-5 is deleted or mutated. Here we investigated whether disruption of CIC-5 affects megalín expression and function also in the thyroid gland. For this purpose, we used the model of CIC-5 KO mice. By Western blotting of tissue extracts, CIC-5 was found to be expressed in the thyroid of WT mice, but not of CIC-5 KO mice. No differences were found in thyroid size, weight or histology between CIC-5 KO and WT mice. Expression of megalín, as detected by Western blotting in thyroid extracts, did not differ between CIC-5 KO and WT mice. In addition, serum levels of Tg, used as a measure of megalín-mediated transcytosis, were similar in the two groups of mice, suggesting that megalín function was unaffected by CIC-5 deficiency. Accordingly, serum levels of FT<sub>4</sub> and TSH, unlike in megalín KO mice, were similar in CIC-5 KO and WT mice. Therefore, we concluded that, unlike in the kidney, CIC-5 does not affect megalín expression and function in thyroid epithelial cells, suggesting that expression of megalín undergoes different pathways of regulation in the two organs.

**P812****Rap (LDL receptor-associated protein) expression in thyroid epithelial cells: evidence for TSH-dependence *in vivo* and *in vitro***

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RAP (LDL receptor associated protein) is a 44 kDa endoplasmic reticulum (ER) resident molecular chaperone. In the thyroid RAP is required for expression of megalín, an endocytic receptor responsible for transcytosis of thyroglobulin (Tg), but it also binds to Tg itself, suggesting it may affect thyroid function in various manners. Indeed, findings in RAP KO mice indicate that disruption of the RAP gene results in impaired Tg storage into the colloid, suggesting that RAP serves as a Tg chaperone. Because expression of proteins involved in thyroid homeostasis is TSH-dependent, here we investigated whether also RAP is regulated by TSH. By immunofluorescence RAP was found to be expressed intracellularly in

FRTL-5 cells cultured in the presence of TSH, and to co-localize with a known ER-resident protein, namely GRP78 (BiP), showing its correct ER location. RAP expression in FRTL-5 cells was then analyzed following TSH deprivation for 24–72 hours, or culture with various concentrations of TSH. At 24 hours, TSH did not affect RAP levels, as observed by immunofluorescence and Western blotting. However, at 48 and 72 hours TSH up-regulated RAP in a concentration-dependent manner. We then studied RAP expression *in vivo*, using the model of methimazole and perchlorate treated mice. As expected, treatment resulted in a reduction of serum FT<sub>4</sub> and an increase of serum TSH. This was associated with a remarkable increase of RAP expression in thyrocytes, as observed by immunohistochemistry and Western blotting. Therefore, based on findings in FRTL-5 cells and *in vivo*, we concluded that RAP is expressed by thyrocytes in a TSH-dependent manner, which supports a thyroid specific function of this molecular chaperone.

**P813****Liver enzymes alterations during high dose intravenous glucocorticoid pulse therapy for graves' ophthalmopathy: frequency and putative risk factors**

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Recently, we have reported a few cases of acute liver damage in patients with Graves' ophthalmopathy (GO), during or following high dose intravenous (iv) glucocorticoid (GC) pulse therapy. In the present study we analyzed retrospectively liver enzymes (LE) in 294 consecutive patients with GO who underwent ivGC. LE were measured before or during ivGC, and in 91 patients also within 6 months after ivGC. An asymptomatic increase in LE (AST peak: 67–88 U/L, ALT peak: 75–228 U/L) was observed in 5/294 patients (1.7%). In 2/294 patients (0.68%) this occurred during ivGC, whereas in 3/91 patients (3.3%) it occurred 1–4 months after ivGC. LE returned spontaneously within the normal range in 4/5 patients, whereas no follow-up data are available for one patient. LE alterations were significantly ( $P = 0.016$ ) more frequent in patients with pre-existing high serum cholesterol and/or triglyceride levels (4/75 = 5.33% vs 1/219 = 0.45%), and also, but not to a significant extent ( $P = NS$ ), in patients with pre-existing obesity (3/102 = 2.94% vs 2/192 = 1.04%), diabetes (1/42 = 2.38% vs 4/252 = 1.58%), high blood pressure (2/60 = 3.33% vs 3/234 = 1.28%), previous exposure to hepatitis B virus (2/38 = 5.26% vs 3/256 = 1.17%) or liver steatosis at ultrasound (2/15 = 13.3% vs 0/12 = 0.00%). We conclude that transient, asymptomatic increase in LE can occur in GO patients during ivGC with a relatively high frequency. Several pre-existing conditions are likely to enhance the risk of LE alterations. Our findings, together with the previous reported cases of severe liver damage, prompt a strict selection and a careful monitoring of patients to be subjected to ivGC.

**P814****High-intensity focused ultrasound (HIFU) treatment for thyroid nodules: first clinical study**

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The aim of the study was to evaluate the safety, feasibility and efficacy of HIFU (High Intensity Focused Ultrasound) for the destruction of thyroid nodules in patients who are indicated for thyroid surgery.

**Material and methods**

13 patients were treated by a HIFU device 2 weeks before thyroidectomy for euthyroid multinodular goiter. Ultrasound (US) examination was performed before and at 3, 8 and 15 days post treatment. Free T<sub>3</sub>, T<sub>4</sub>, TSH, thyroglobulin were measured before and after HIFU treatment. Only one thyroid nodule per patient was targeted. The included nodules were solid or

mixed, with mean diameter  $\geq 8$  mm, located at least at 3 mm apart from the trachea, the esophagus, the carotid artery and the skin as evidenced by ultrasonography. Thyroidectomy was performed at 2 weeks followed by histopathological examination.

**Results**

The treatment was well tolerated, but became uncomfortable as the energy was increased. Subsequently the last patients received a local anesthesia. A superficial and reversible skin blister on patient 8th was observed. Design of treatment head was subsequently modified to eliminate such risk. Post HIFU US examination showed changes in echogenicity, a decrease in volume and vascularization at power Doppler examination in 10, 1 and 3 cases respectively. Thyroglobulin level increased in 1 case. Macroscopic and histological lesions were observed, and were precisely located in the targeted nodule without affect to the neighboring structures. The type of lesions in the treated nodule were central thrombosis in 1 case, diffuse lesion with cavitation, coagulative necrosis, haemorrhage and disappearance of the nuclei.

**Conclusion**

This study confirmed the feasibility and safety of the HIFU procedure. No serious adverse event was observed. The histological lesions were clearly visible in 10 cases. To obtain the complete nodule destruction in the next patients, the pulse energy must still be increased.

**P815**

**Digital infra-red orbital thermography in the assessment of thyroid eye disease**

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Thyroid Eye Disease (TED) is an autoimmune inflammatory condition of the orbit occurring in the setting of thyroid disease. Prevention of the distressing physical and psychological sequelae of TED depends on accurate quantification of disease activity to allow selection of the most appropriate treatment modality. Current practice is based on the use of a Clinical Activity Score (CAS). As an alternative approach, and with the potential benefits of reproducibility and ease of use, we have examined the utility of orbital infra-red thermography in quantifying TED inflammatory activity. Patients were imaged with eyes open and closed. Open-eye images were analysed with a temperature profile along the corneal centres line of intersection. This profile showed, per eye, two main peaks (infra-red emission from the conjunctiva overlying the medial [MR] and lateral [LR] recti insertions) and one trough (infra-red emission from the cornea [CA]). Closed-eye images were described with the maximum temperature in the periorbital region (Tmax) and the rate of eyelid heating following localised cooling by fan ( $\Delta E$ ). In an ethically approved study of 49 age- and sex-matched subjects (active TED  $n = 15$ ; inactive TED  $n = 9$ ; normal control  $n = 25$ ) we found (1) lateral:medial rectus insertion temperature ratios (LR-CA)/(MR-CA) correlate significantly with TED patient status ( $P = 0.0002$ , 2-tailed Student's t-test); (2) there is a trend toward greater Tmax and  $\Delta E$  values in active versus inactive TED; (3) Tmax correlates significantly with the classical CAS in inactive/active TED patients (Pearson's  $R = 0.433$ ,  $P = 0.034$ ). In conclusion we describe the use of orbital thermography as an imaging modality in the clinical assessment of TED. Our experiments describe methods to obtain thermography-derived parameters useful in the investigation of ocular/orbital pathology and we show these to compare favourably with the current methods of TED disease assessment. Further experiments to ascertain the predictive value of thermography in TED are planned.

**P816**

**Prognostic factors for persistent or recurrent disease from a series of 81 patients with oncocytic thyroid carcinoma**

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Much controversy persists over aggressiveness of oncocytic thyroid carcinoma (OTC) and its optimal treatment. The study purpose was to determine prognostic factors for persistent or recurrent disease (PRD) and discuss therapeutic directions.

We reviewed the medical records of 81 consecutive patients with OTC among a total of 1729 (4.7%) epithelial thyroid cancers, operated from 1983 to 2003 in our center. Follow-up information was updated. Age ranged from 17 to 86 years (median, 52). Univariate and multivariate analysis were performed to determine prognostic factors for PRD and calculate disease-free survival (DFS).

With a median follow-up of 7.9 years, 62 (77%) patients never relapsed and had no residual tumor at last follow-up and 19 (23%) had persistent disease or experienced recurrence of their disease. With univariate analysis, ten variables were significantly associated with PRD: age  $\geq 45$ , malignant solitary or dominant nodule, compressive symptoms, tumor size  $> 40$  mm, malignancy diagnosed intraoperatively, radical modified neck dissection performed following frozen section findings, major vascular invasion, moderate or poor differentiation, pT4 and synchronous distant metastases. With multivariate analysis, only pathological parameters were independently associated with PRD: major vascular invasion (OR = 1.64), moderate or poor differentiation (OR = 6.69) and pT4 (OR = 2.66). Among the 19 patients with PRD, 5 patients died of thyroid cancer, 10 are alive with disease progression, 2 demonstrated decreasing thyroglobulin to iterative radioiodine treatment, and 2 were cured after reoperation.

Our findings suggest that pT4 tumor, moderate or poor differentiation and major vascular invasion independently predict higher risk of persistent or progressive disease and shorten DFS; their presence should prompt completion of thyroidectomy if needed and radioiodine adjuvant therapy to optimize follow-up. Residual disease should indicate further medical or surgical intervention in a curative effort, since selected patients may be cured or stabilized and long term survival can be expected. effort, since selected patients may be cured or stabilized and long term survival can be expected.

**P817**

**Association of CTLA-4 exon 1 polymorphism with Graves' disease in Spanish patients**

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**Introduction**

Graves disease (GD) is inherited as a complex multigenic disorder. One of the most promising genes for susceptibility to GD is cytotoxic T lymphocyte antigen-4 (CTLA4), a negative regulator of T-cell activation.

**Objective**

The aim of this study was to determine whether A/G polymorphism in exon 1 of the CTLA-4 gen was associated with GD in Spanish patients.

**Patients and methods**

Fifty one adult GD patients and 25 unrelated controls were analyzed. GD was defined as hyperthyroidism together with following criteria: diffuse goiter, thyroglobulin and/or thyroid peroxidase abs and/or ophthalmopathy. Polymorphism were analyzed using a restriction enzyme digestion with *BbvI* of polymerasa chain reaction (PCR) amplified genomic.

**Results**

Forty three women (84%) in the total of 51 patients studied. The medium age was 41.25 years (18–75). The distribution of genotype frequencies and the frequencies of the A and G alleles differed between GD subjects and controls (see Table). In the control group we have not detected homozygous for the G allele.

**Table**

A49G Mutation	Graves	Controls	P
Genotype distribution			
A/A	21(41)	24(58)	ns
A/G	18(35)	21(42)	ns
G/G	12(24)	0	0.005
Alleles frequency			
A	30(59)	41(82)	0.01
G	21(41)	11(22)	0.04

**Conclusions**

Our data show that G-carrying genotypes of polymorphism CTLA4 A/G are associated with increase in risk of GD in our region. The G allele and the GG genotype were increased among Spanish patients. We not found association between AG genotype and GD like other previous studies in Caucasian patients. CTLA-4 polymorphism varies according to ethnic group and environmental variables and it is important its identification than in future can influence the selection of treatment of GD.

**P818****Reference intervals of maternal free thyroxine (FT4) at the second and third trimesters of pregnancy using the Beckman Coulter's Access<sup>®</sup> free T4 assay**

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Pregnancy causes significant physiological changes that can alter the results of thyroid function testing. The changes in circulating plasma-binding protein (TBG) concentrations interfere with FT4 estimate methods, and the hCG thyrotropic effect impacts TSH activity estimation. A TSH assay is the most sensitive test to assess thyroid status. T4 levels have been implicated as associated with fetal neurodevelopment. During pregnancy, differences in FT4 concentrations have been described depending on the method used.

The objective of this study was to establish pregnancy-specific reference intervals for the Access<sup>®</sup> Free T4 assay. Reference intervals for the Access<sup>®</sup> HYPERSensitive hTSH assay and for the Access<sup>®</sup> Free T4 assay were defined in a previous evaluation (Clin. Chem. Lab. Med. 2005; 43(1): 102–105).

Concentrations of FT4 were measured in sera from 364 women with no goiter, no history of thyroid dysfunction and on no medication that could interfere with thyroid function: 109 nonpregnant (<40 years), 139 during the 2nd trimester and 116 during the 3rd trimester of pregnancy (mean age of 27 years). Assessment of TPO-antibodies was systematically done and the positive samples were excluded (6%). The Access<sup>®</sup> Free T4 assay is a two-step competitive assay. FT4 distribution was calculated using the Sharipo-Wilkes test.

In the control group, the central 95% range between the 2.5th and the 97.5th percentiles was 0.4 to 4.2 mIU/L for TSH, and 7.85 to 13.2 pmol/L for FT4 (mean = 10.53, SD = 1.34). A decrease of 24% and 28.3% in FT4 concentrations from nonpregnant levels was observed in the second and in the third trimester, respectively. FT4 trimester-specific reference intervals defined as central 95% range, were 5.84 to 10.2 pmol/L (mean = 8.02, SD = 1.09) and 5.51 to 9.60 pmol/L (mean = 7.55, SD = 1.02) for the 2nd and the 3rd trimester, respectively.

During pregnancy, thyroid function tests should be interpreted according to trimester-specific reference intervals defined for the method used.

**P819****Bioavailability of iodine bound in humic substances in drinking water and the impact on thyroid function**

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Both humic substances (HS) and iodine may influence the thyroid gland. Tap water is a major source of iodine intake in some areas, where iodine has been shown to be bound in HS.

We investigated tap water and old subjects from two areas with and without iodine bound in HS in tap water, to assess their importance for urinary iodine excretion and for the thyroid. Analysis of tap water, measurement of iodine in urine and thyrotropin (TSH), thyroid peroxidase antibody (TPO-Ab) and thyroglobulin (TG) in serum from old people living in Skagen and Randers, Denmark were done. Thyroid disease, medication, smoking, and alcohol and vitamin use were evaluated with questionnaires. Average tap water iodine content in Randers/Skagen was 2.0/140 µg/L. The 430 participants were 130/82 women/men aged 78 years in Randers, and 134/84 aged 75-80 years in Skagen.

Median (25; 75 percentiles) urinary iodine content of spot urine samples from participants not taking vitamins was 42 (28; 58) µg/L in Randers and 146 (116; 206) µg/L in Skagen ( $P < 0.001$ ). TSH < 0.4 mIU/L was more frequent (17.1% vs. 3.9%,  $P < 0.001$ ), and TSH > 3.6 mIU/L slightly less frequent (7.0% vs. 12.3%,  $P = 0.074$ ) in Randers than in Skagen, without gender differences ( $P = 0.18$ ). TPO-Ab were present in serum in 27.9% women and 8.7% men ( $P < 0.001$ ) with no difference with areas ( $P = 0.87$ ), smoking habits ( $P = 0.11$ ), alcohol use ( $P = 0.40$ ) or use of iodine in supplements ( $P = 0.77$ ). Among TPO-Ab positive subject, the level tended to be higher among women than among men (495 vs 201,  $P = 0.09$ ). TG-Ab were present in serum in 37.1% (88) of women and 22.9% (36) of men ( $P = 0.003$ ). Serum TG was markedly higher in Randers compared to Skagen dwellers (14.8 vs. 7.7,  $P < 0.001$ ).

In conclusion, iodine in drinking water is bioavailable despite binding to HS, and HS do not corrupt iodines influence on the thyroid gland.

**P820****Application of positron emission tomography (PET) in the study of cerebral glucose uptake in hypothyroid rats: effect of T3 administration**

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Thyroid hormone (TH) plays a major role in nervous system development. Its deficiency causes severe brain malfunction, which in the human results in cretinism. With the recent availability of new imaging techniques, as small animal PET, it is now possible to study cerebral metabolism *in vivo*. The aim of this work was to study the *in vivo* effect of TH on the brain of hypothyroid rats in terms of metabolic function, using PET scanning. For the experiment we used the YAP-(S)PET small animal scanner. As radiotracer we used 2-deoxy-2-(<sup>18</sup>F)fluoro-D-glucose (FDG). 19 Wistar rats (10 F, 9 M) were maintained in a climate-controlled room on a normal 12h light/dark cycle with food and water available ad libitum. Thyroid hormone deficiency was induced in 14/19 by drinking water with antithyroid drugs (MMI 0.02% and KClO<sub>4</sub> 1%). To study the effects of TH on the brain, 7/14 rats were treated with 50 µg of T3 injected i.p. for 3 consecutive days in 5 rats and for 6 consecutive days in 2 rats. The PET scanning was done the 4th and 7th day. The day before the experiment the rats were fasted but allowed free access to water. The rats were anaesthetized with a mixture of ketamine and xylazine. In each rat we injected i.v. 1.4 mCi of [<sup>18</sup>F]FDG. We started PET scanning 30 minutes after [<sup>18</sup>F]FDG injection. The images showed a clear resolution of several brain structures: we distinguished the cerebral cortex, the neostriatum, the thalamus, the cerebellum and the olfactory bulbs. The [<sup>18</sup>F]FDG uptake value obtained in different brain areas were normalized to body surface and brain weight of the animals. The TSH levels were high in hypothyroid rats and not detectable in T3-treated rats ( $P = 0.002$ ). There was a statistically significant difference between cerebral glucose uptake in hypothyroid rats with respect T3-treated rats but, contrary to what expected, the glucose uptake was higher in hypothyroid rats. No differences were found between male and female and the duration of treatment with T3. In conclusion, a) we developed a new methodology to study the effects of TH on the brain *in vivo*; b) contrary to what expected, the glucose uptake was higher in hypothyroid rats than in T3-treated rats.

**P821****The development of a symptom-based clinical activity score for thyroid eye disease**

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**Background**

Mourits' clinical activity score (CAS) is commonly used in assessing disease activity in patients with thyroid eye disease (TED). However measurements must be taken on more than one occasion, and specialised ophthalmic equipment is needed.

**Aim**

To develop a symptom-based TED clinical activity score.

**Methods**

The Mourits Clinical Activity Score (CAS) was modified to produce a CAS based only on symptoms. 15 patients were recruited from a TED clinic at an Eye Hospital. Patients were asked categorical questions to produce a symptom based CAS. Clinical activity was then assessed by a second, blind, observer using Mourits' CAS. The conventional and modified clinical activity scores were compared. Local Ethical Committee approval was obtained before commencing the study.

**Results**

The mean conventional CAS was 1.2 (range 0.0–6.0) and the mean modified CAS was 3.87 (range 0.0–10.0). There was no significant correlation between the 2 scores,  $P = 0.07$ .

**Conclusion**

The modified CAS did not correlate with the conventional CAS and cannot therefore be recommended for clinical use.



## P822

### A qualitative study of patients' experience of thyroid eye disease

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#### Background

Thyroid Eye Disease (TED) is known to affect vision and appearance. Although the signs and symptoms are well documented, little research has been done to establish patients' experience of their symptoms.

#### Aim

To document patients' descriptions of TED symptoms using qualitative research methods.

#### Methods

Fifteen patients were recruited from a hospital TED clinic. Patients with a range of disease severity were sampled. Interviews were conducted using open and topic-lead questioning methods. The same researcher conducted all interviews. Interviews were recorded on audio-tape and transcribed. Emerging themes were provisionally coded, and then refined using an iterative process. Counting of descriptive categories was then performed. Local Ethical Committee approval was obtained before commencing the study.

#### Results

Patients' descriptions of disease symptoms were broader than those traditionally recognised by clinicians. Symptoms relating to sensitivity to light, psychological effects of changed facial appearance and limitation of activities were particularly prominent.

#### Conclusions

Patients with TED experience a wide range of symptoms, many of which have not previously been well documented. Symptoms that patients are less likely to volunteer should be specifically enquired about. It should be appreciated that TED has a major impact on patients' lives.

## P823

### Etiology of congenital hypothyroidism in children with a normal located thyroid gland

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Congenital hypothyroidism (CH) with a normal located thyroid gland may be transient or permanent and environmental, iatrogenic, immunologic or genetic factors can be involved. PAX-8, TSH receptor and THOX2 gene mutations have been identified in cases of CH with a gland of normal size.

In this study we performed genetic analysis of PAX8, TSH receptor and THOX2 genes in 14 children with CH and a normal sized eutopic gland. Genomic DNA was extracted from lymphocytes and the coding-region of the genes was analyzed by direct sequencing. No mutations were identified in PAX8 and TSHr genes, but only polymorphisms of the TSHr gene. With respect to the THOX2 gene, in 3 children a monoallelic deletion of a GTTC at position 2895–2898 in exon 21 was identified. This deletion introduces a frame shift generating a termination signal in exon 22. The same deletion was found in all 3 fathers who were euthyroid. 1 of the fathers showed a mild organification defect at the perchlorate discharge test (KClO4). This deletion was also present in 1/60 euthyroid adult subjects who was affected by nodular goiter. When the 3 children were 3 yr old, the L-T4 was stopped to perform the KClO4. 1 child was euthyroid with a borderline test, 2 were hypothyroid with no organification defect. 3 other children had 2 heterozygous point mutations at exon 16 of THOX2 gene, causing a H678R substitution and a R701Q substitution. The same mutations were found in 8/60 controls. In another child we found a C1052Y substitution at exon 23, together with a S911L substitution at exon 20. These mutations were not present in any of the 60 controls.

In conclusion, in 14 children with CH and normal eutopic thyroid gland, THOX2 gene modifications were the most frequent alterations identified. The phenotypic variability of THOX2 gene alterations may be explained by the presence of other genes involved in the iodine organification process.

## P824

### Clinical characterisation and genetic analysis of a large euthyroid family with TSH-receptor germline mutation (N372T) and a hyperthyroid index patient with an additional somatic tsh-receptor mutation (S281N) on the second TSH-receptor allele

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#### Objective

Twenty-one different activating germline mutations have been identified up to date. We describe a large euthyroid family with a new TSH-receptor(TSHR) germline mutation where the hyperthyroid index patient also carries a somatic TSHR mutation.

#### Method

Mutation analysis of the exon 9 and 10 of the TSHR gene from the peripheral blood samples of the members of this family and toxic thyroid nodules of the index patient were performed by denaturing gradient gel electrophoresis in addition to direct sequencing. Local Ethical Committee approval has been obtained.

#### Results

We report a novel germline TSHR (N372T) mutation in blood sample of a 53 year-old man who presented with thyrotoxic storm. This mutation was also detected in six siblings and seven children of this patient. This mutation causes different phenotypic expression in this family ranging from normal thyroid to diffuse or nodular goiter with euthyroidism. Interestingly an additional somatic mutation (S281N) was also identified in the dominant toxic thyroid nodules of the index patient in the allele of the TSHR not affected by the germline mutation.

For both the single mutants N372T and S281N and the mixture of the two mutants N372T/S281N, basal cAMP level was higher in cells expressing the mutated receptors than in cells transfected with wild-type construct. Specific constitutive activity of the somatic mutation (S281N) was higher than the double mutant (N372T/S281N) and it was lowest in the germline mutant TSHR (N372T).

#### Conclusion

This family presents a new TSHR germline mutation located in the extracellular domain. The most likely explanations for euthyroidism are either the iodine deficiency or an unknown functional specificity of this mutation, since previously reported TSHR germline mutations with a similar low constitutive activity did cause hyperthyroidism. The only patient with hyperthyroidism in this family presented the first hot nodule with compound heterozygosity for two constitutively activating TSHR mutations

## P825

### Estimation of the optimal diagnostic complex of the treatment of the nodular euthyroid formations of thyroid

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#### Background

The ultrasound investigation in combination with fine-needle aspiration biopsy is one of the precise methods of the diagnostic of the thyroid nodular formations.

#### Aim

To assess the possibilities of the diagnostic methods in case of the euthyroid nodular formations of the thyroid.

#### Materials and methods

Two hundred twenty patients with nodular euthyroid formations of the thyroid were investigated, from which: 189 were women and 31 were men. Their age varied between 18 and 65 years. In all of the patients the ultrasound of thyroid was done.



## Results

After the ultrasound investigation of 220 patients in 207 (94.09%) subjects the nodule was determined, the false positive answer in 11 cases, false negative answer in 2 cases. The sensitivity of this method according to our data was 99%, specificity – 54%. In 220 patients fine-needle aspiration biopsy was done in 91 patients. The biopsy was done under the ultrasound control. In 12 cases (13%) we could not receive enough material for the verification of the diagnosis, in 5 (6.9%) patients the malignant tumor was diagnosed, in 3 patients (3.7%) follicular cancer, in 2 patients (2.5%) papillary cancer. The benign tumor of the thyroid was detected in 32 subjects (40%). After the histological examination of the operated material the following divergences were detected: papillary cancer in 1 case was follicular adenoma, also in 1 case cystic goiter was – cystadenomatous goiter. In rest of the cases the diagnosis were confirmed.

## Conclusions

In case of the nodular formations of the thyroid the revealed nodules should be assessed with circumspection. If the suspect of tumor is, the ultrasound and fine-needle aspiration biopsy should be implemented.

## P826

### The diagnostic value of Doppler ultrasonography in determining the malignancy potential of thyroid nodules

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#### Objective

It is very important to determine the malignancy risk in thyroid nodules for to improve therapeutic strategies. In this study we attempted to evaluate the relationship of doppler (DUS) and B- mode ultrasonographic (BUS) features of thyroid nodules with fine needle aspiration (FNA) findings.

#### Method

One hundred and eleven patients which have been followed between March and September 2004 were evaluated with ultrasonography and ultrasound guided FNA was performed in 124 nodules. The size, number and echogenicity of the thyroid nodules were studied with BUS. The vascularity of the thyroid nodules was assessed by (DUS). Flow characteristics was classified between Type 1 and 4, peak systolic velocity, resistive index values were calculated and were correlated with cytopathological findings.

#### Results

Eighty-eight of the patients were female (80%), 23 of the patients were male (20%). In evaluation of 124 nodules with FNA, 110 nodules were diagnosed as benign (90.7%), 10 nodules as malignant (8.5%), 4 nodules (0.35%) as indeterminate. The results of the BUS yielded: In 68% of the patients with benign nodules and 100% of the patients with malignant nodules had more than one nodule. 64% of benign nodules and 20% of malignant nodules were less than 2 cm. When these nodules were evaluated with DUS type-4 blood flow (80%) in malignant nodules and type-2 blood flow (52.7%) in benign nodules were observed. Peak systolic velocity values under 50 cm/sec were diagnostic for benign nodules, whereas values over 50 cm/sec were diagnostic for malignant nodules. Resistive index (RI) values were <0.75 in 93.5% of benign nodules and >0.75 in 60% of malignant nodules.

#### Conclusion

The nodules which are over 2 cm in size have more malignant potential. Also nodules which have increased intranodular blood flow, peak systolic value over 50 cm/sec, RI values over 0.75 carries high risk for malignancy.

## P827

### Favourable outcome of Graves' ophthalmopathy (GO) after glucocorticoids (GC) in patients treated with total thyroid ablation (TTA): results of a randomized clinical trial

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Whether TTA affects GO remains to be established. Here we studied the effects of TTA in 90 patients with mild-moderate GO randomized into 3 groups of thyroid treatment: MMI (methimazole), TX [near-total thyroidectomy (NTTX)], TTA (NTTX plus 131-I). Patients were treated with ivGC and evaluated at 3 and 9 mo. GO was considered improved or worsened when at least 2 of the following criteria changed, either positively or negatively: i) proptosis and/or eyelid width by at least 2 mm; ii) clinical

activity score by at least 2/7 points; iii) diplopia: progressed, reduced, appeared or disappeared. GO was otherwise considered stable. At baseline, TX and TTA groups were homogeneous for thyroid volume, serum Tg, TgAb, TPOAb, TBII, degree and activity of GO. However, for unknown reasons, MMI patients had significantly lower thyroid volumes and proptosis. Because these parameters affected GO independently, this group was excluded. At the end of the study a random sample of TX and TTA patients withdraw LT4 and underwent RAI uptake and Tg assay. Complete ablation in the TTA group was confirmed by the lower mean RAI uptake (3 h: 0.7 vs 1.5%,  $P = 0.018$ ; 24 h: 0.28 vs 2.9%,  $P = 0.0006$ ) and Tg levels (0.3 vs 4.0 ng/ml,  $P = 0.008$ ). 3 mo. after GC, GO improved in 33.3% of TTA and 22% of TX patients, but the difference was not significant. At 9 mo., GO improved in a significantly ( $P = 0.0189$ ) greater proportion of TTA (55.5%) than TX patients (20.0%). GO worsened in a lower proportion of TTA patients both at 3 (7.4% vs 14.8%) and 6 mo. (7.4 vs 16%), but the difference was not significant. In conclusion, TTA has a beneficial effect on the short term outcome of GO following GC compared with TX. Thus, TTA should be a first choice thyroid treatment in GO patients undergoing thyroid surgery.

## P828

### Lipids profile and carotid scan atherosclerotic parameters in newly diagnosed hypothyroid females

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Dyslipidemia is frequently registered in the patients with hypothyroidism (HLP type 2a, 2b, 4). Its atherosclerosis acceleration role is well known.

#### Objective

To evaluate lipids profile in newly diagnosed hypothyroidism and to assess atherosclerosis features by carotid scan morphological parameters.

#### Material and methods

Study involved 30 newly diagnosed hypothyroid females ( $54 \pm 10$  years). After the completion of lab (including lipids profile) and ultrasound thyroid evaluation, carotid Doppler scans were performed.

#### Results

Mean TSH level was  $44.9 \pm 45.6$  mIU/L. Positive thyroid antibody screening was observed in 16 patients, but 97% patients had typical ultrasound presentation of Hashimoto as well the same percentage of some HLP (18 patients HLP 2a). Clinically significant increase in RI (>75%) and intima-media thickness ( $\geq 1.1$  mm) was registered in 3 and 15 patients respectively. We didn't find significant influence of hypothyroidism severity (TSH >30 mIU/L) and intima-media thickness on RI. Carotid branch stenosis (mean  $38 \pm 5\%$ ) was confirmed in 6 patients. 83% patients with stenosis had HLP2a and all of them had significant intima-media thickness. No one of the patients had cerebrovascular or cardiovascular accident.

#### Conclusion

Nearly all evaluated patients had dyslipidemia. Incipient atherosclerotic carotid scan changes were registered in more than a half of patients. Despite high cardiovascular and cerebrovascular mortality rates in hypothyroid population, could decreased body metabolism, beside many factors, be protective in this way at the onset of disease?

## P829

### Safety of pharmacological treatment of thyroid diseases during pregnancy

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Pregnant women may require treatment of hypo- or hyperthyroidism, L-thyroxine (LT4), propylthiouracil (PTU) and methimazole (MMI) being the most frequently used drugs. Aim of this study was to verify the consequences on both the mother and the fetus of pharmacological treatment during pregnancy. We retrospectively evaluated 292 pregnancies: 63 patients under MMI treatment, 8 of whom (7 Graves' (GD) and 1 toxic nodular goiter) still hyperthyroid in spite of treatment (H-MMI) and 55 euthyroid (E-MMI); 26 GD patients under PTU, 6 of whom still hyperthyroid (H-PTU) and 20 euthyroid (E-PTU); 139 women under LT4 therapy, suppressive (SUP) for nodular goiter or replacement (REP) for hypothyroidism. These two last groups were further subdivided in adequate REP or SUP or non-adequate REP or SUP on the basis of TSH serum levels.

We also included 64 untreated (EU) patients with thyroid disease: 41 Graves' euthyroid after MMI, PTU or  $^{131}\text{I}$  treatment, 16 multinodular goiter and 7 chronic autoimmune thyroiditis. The prevalence of miscarriages and fetal abnormalities, newborns' weight and length and neonatal TSH values were evaluated. Results were analyzed by Student t-test. Miscarriage occurred in: 7/55 (12.7%) E-MMI, 2/8 (25%) H-MMI, 3/20 (15%) E-PTU, 3/74 (4.1%) adequate REP, 1/17 (5.9%) non-adequate REP, 1/21 (4.8%) adequate SUP and 6/64 (9.4%) EU. 1 E-PTU and 2 EU underwent voluntary miscarriage for a prenatal diagnosis of Down (2) or Klinefelter (1). Neonatal TSH values, weight and length at time of birth did not present significant differences between all the groups and normal pregnancies. All except 9 newborns (4 from adequate REP, 2 from EU, 2 from E-MMI mothers and 1 from E-PTU) were born at term. In 1 H-PTU a fetal goiter occurred, in 1 adequate-SUP a genital malformation and in 1 EU a renal malformation occurred. In summary, neonatal TSH values, weight and length were not different between groups and the prevalence of miscarriages and fetal malformations was not higher than that reported in the literature. These results indicate the safety of LT4, MMI and PTU treatment during pregnancy.

### P830

#### Increase of L-thyroxine requirement during pregnancy

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In pregnant women with thyroid diseases an increased amount of LT4 may be required for the correction of hypothyroidism or treatment of nodular goiter. Aim of this study was to assess the amount of the variations of LT4 requirement in pregnant women with thyroid diseases. To address this issue, we retrospectively evaluated a cohort of 107 women treated with LT4 divided in two groups: 42 euthyroid (E) (affected by nodular goiter (NG) treated with LT4 suppressive therapy) and 65 hypothyroid (H). This last group was divided in two subgroups: women with a residual functioning thyroid tissue (R-H) (31 with chronic autoimmune thyroiditis, 14 with post- $^{131}\text{I}$  for Graves' disease, 1 post-methimazole treatment) and women without residual thyroid tissue (NR-H) (2 thyroid agenesis, 12 post-total thyroidectomy). In E pregnant women the goal was to maintain TSH serum level between 0.1 and 0.4 mU/L, while in H pregnant women the goal was to maintain the TSH serum level between 0.4 and 4.0 mU/L. 16 E and 46 R-H and 14 NR-H pregnant women respected these criteria during the entire pregnancy. Only 3 out of 16 (23%) E had to increase LT4 in order to maintain serum TSH in the appropriate range. The mean increase was 112% at 3rd trimester with respect to pre-gravidic dose. In 34 out of 46 (74%) R-H an increase of L-T4 was necessary to maintain serum TSH in the appropriate range. The mean increase was  $142 \pm 25\%$  at 3rd trimester with respect to pre-gravidic dose. In 12/14 (86%) NR-H an increase in LT4 dose was required to maintain serum TSH in the appropriate range. The mean increase was  $136 \pm 42\%$  at 3rd trimester with respect to pre-gravidic dose. In conclusion, a rise in LT4 dose is required in the minority of pregnant women with NG under suppressive therapy. We observed that a rise in LT4 dose is required in the majority of hypothyroid women, especially in those without a residual tissue, in order to maintain TSH serum level in the appropriate range. The increase of LT4 requirement to obtain the appropriate serum TSH levels is higher in hypothyroid with respect to NG pregnant women.

### P831

#### Congenital thyroid hemiagenesis in association with multinodular goitre: a case report

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Congenital morphological thyroid abnormalities can be classified as hemiagenesis or total agenesis, lingual thyroid, substernal thyroid, thyroglossal duct remnant. Hemiagenesis with absence of one lobe or lobe and isthmus is very rare, it was found in 4 of 7000 scans in one study. The left lobe is absent four times as often as the right lobe, and women are about three times more likely than men to have this developmental defect. Usually diagnosing this condition is not very easy, because it can be confused with a hyperfunctioning thyroid nodule with a marked suppression of all other thyroid tissue. Generally it presents with symptoms of hyperthyroidism, but hypothyroidism or euthyroidism can also be observed.

Our 18 year old female patient presented with symptoms of palpitation, dysphagia, insomnia. On physical examination her thyroid was palpable. She had no neck or thyroid operation in the history. Laboratory data revealed euthyroidism, thyroglobuline antibody and thyroid peroxidase antibody testing were all negative. Ultrasonography revealed left lobe and isthmus agenesis, enlargement of the right lobe with multiple cystic nodules in different sizes. Scintiscan findings with technetium Tc 99 m pertechnetate also showed left lobe and isthmus agenesis. We wanted to report this condition because of its rare occurrence and diagnostic problems. Generally it is diagnosed when there are other thyroid abnormalities. In the patients who have unilateral radionuclide uptake on the scintiscan, a doppler ultrasonography of all the thyroid tissue must be performed for to prevent the futile therapeutic interventions.

### P832

#### Insulin-like growth factor-1 counteracts $^{131}\text{I}$ -induced thyroid stunning by stimulating the transcription of the sodium/iodide symporter (NIS)

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Thyroid stunning means reduced radioiodine uptake in thyroid tissue or tumour following a diagnostic  $^{131}\text{I}$  test dose. This may compromise subsequent  $^{131}\text{I}$  radiotherapy. Recently, we found that  $^{131}\text{I}$  inhibits iodide uptake in cultured thyroid cells at non-lethal absorbed doses. Here, we have investigated whether  $^{131}\text{I}$  irradiation affects the TSH-stimulated transcription of the sodium/iodide symporter (NIS) gene, and whether insulin-like growth factor-1 (IGF-1) previously known to stimulate iodide transport is able to prevent  $^{131}\text{I}$ -induced thyroid stunning.

#### Methods

Confluent pig thyrocytes grown in bicameral culture systems were single or co-stimulated with TSH (1 mU/ml) and IGF-1 (10 ng/ml) and simultaneously irradiated with 7.5-Gy  $^{131}\text{I}$  for 48 hours. pNIS mRNA was quantified by real-time RT-PCR using 18S as reference. Transepithelial (basal-to-apical) iodide transport was monitored using  $^{125}\text{I}$  as tracer. [ $^3\text{H}$ ]Thymidine incorporation and total DNA content were also measured.

#### Results

TSH increased the NIS mRNA expression 35–50-fold after 2 days and onwards. In irradiated cells the NIS transcript level was reduced by 45% and 75% after 2 and 5 days, correlating with inhibited iodide transport. IGF-1 *per se* had a small and delayed stimulatory effect, but further enhanced the TSH-stimulated NIS expression and iodide transport 4–6-fold. Co-stimulation with IGF-1 fully prevented the inhibitory effect of  $^{131}\text{I}$  on both parameters.  $^{131}\text{I}$  blocked the IGF-1 stimulated DNA synthesis, ruling out that the protective effect of IGF-1 was caused by increased cell number.

#### Conclusions

$^{131}\text{I}$  inhibits thyroidal iodide transport by blocking NIS mRNA expression. IGF-1 exerts a radioprotective effect by rescuing both NIS transcription and iodide transport in  $^{131}\text{I}$ -irradiated thyroid cells.

### P833

#### A case of Riedel's thyroiditis successfully treated with glucocorticoids

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A 38-year-old woman with chronic autoimmune thyroiditis and hypothyroidism treated with levothyroxine (125 µg/day) was referred to our outpatient clinic with a history of sudden increase of goiter, weakness, dysphagia, dyspnea and suffocating sensation. Physical examination showed an enlarged, stony thyroid gland (three times of normal), without palpable cervical lymph nodes. Biochemical evaluation showed the following abnormalities: TSH 6.0 µU/ml (0.4–4.3), free-T4 11 pg/ml (7–17), free-T3 2.9 pg/ml (2.7–5.7), TgAb >2000 U/ml (<1.0–30), TPOAb >1000 U/ml (<1.0–10) and TSH receptor antibodies <1 UI/l (<2). Thyroid ultrasound showed an enlarged diffusely (VT = 109 ml) and hypoechoic gland, with absent vascularization at color-power-Doppler, without cervical lymph nodes. At Computed tomography (CT) was found a hypodense, enlarged thyroid gland (right lobe: 50 × 30 × 65 mm, left lobe: 52 × 27 × 65 mm, and isthmus 20 mm) with tracheal compression and esophageal dislocation. Fine needle aspiration biopsy was not diagnostic. The patient underwent thyroid biopsy, which showed a T and C lymphocyte infiltration with

extensive fibrosis, suggesting the Riedel's thyroiditis diagnosis. With the aim to support this diagnosis, further, imaging studies were made. Magnetic resonance imaging showed thyroid hypointensity in all pulse sequences with homogeneous enhancement after gadolinium administration; fludeoxyglucose F-18 positron emission tomography (FDG-PET) revealed an abnormal thyroid uptake, indicating an increased glucose metabolism. The dose levothyroxine was increased at 137.5 µg/day to correct hypothyroidism and prednisone (75 mg/day) was started. The prednisone dose was tapered off gradually and withdrawn after seven months. The clinical conditions improved dramatically in few weeks of treatment. At the end of therapy, ultrasound and CT showed a normal thyroid volume (right lobe 20 × 12 mm and left lobe 21 × 13 mm), while no uptake of FDG-PET was found. Six-months after withdrawal prednisone treatment, the patient is in good health.

#### P834

##### An audit of radioiodine treatment for thyrotoxicosis in Cambridge

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Radioiodine (RAI) is widely used for the treatment of thyrotoxicosis. The efficacy and hypothyroidism rate following a single dose of RAI is variable and the optimum administered dose – sufficient to achieve remission but with an acceptable low hypothyroidism rate, is still debated.

Our audit sought to compare the results of local practice with published rates of success and hypothyroidism following RAI. We also examined the relationship between treatment success and hypothyroidism rate at one year, with parameters such as age, gender, fT<sub>4</sub> at diagnosis, pre-treatment with antithyroid drugs (ATD) and dose of RAI.

We randomly selected 105 patients over a two year period, undergoing RAI treatment for either Graves Disease (GD) or toxic nodular disease (TND) (Toxic Adenoma, and Toxic Multinodular Goitre). Patients with GD were younger than those with TND (GD 47.8 ± 1.67 yrs vs TND 70.7 ± 1.76 yrs;  $P < 0.001$ ). The median dose of RAI was 400 MBq (range: 180–800) for GD, and 600 MBq (range: 400–800) for TND. The success rate for GD was 73.8%, and for TND 77.5% – comparable to published success rates of 66–84%. The early hypothyroidism rate was 60% in patients with GD (20–76% in the published literature) and 22.5% in patients with TND (11.4–34% in the published literature).

Using multivariate regression analysis, fT<sub>4</sub> at diagnosis was the only factor related to a higher rate of treatment failure ( $P < 0.001$  for GD,  $P < 0.014$  for TND). Pre-treatment with ATD ( $P < 0.001$ ), dose of RAI ( $P = 0.013$ ), and gender ( $P = 0.026$ ), predicted a higher rate of hypothyroidism.

Our audit of RAI therapy showed similar success, but also high hypothyroidism rates at one year, compared to the published literature and confirms the efficacy of this treatment modality for both GD and TND. Our local experience suggests that patients with GD in particular need to be counselled about a risk of early hypothyroidism requiring lifelong thyroxine replacement.

#### P835

##### On-levothyroxine measurement of thyroglobulin is not a reliable screening test for patients at high risk of remnant/recurrent differentiated thyroid carcinoma

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At the present time, the most widely accepted tool for follow-up management of differentiated thyroid cancer (DTC) patients consists of serum thyroglobulin (Tg) measurement. It is not uncommon to measure the serum Tg level while the patient taking thyroid hormones (on-treatment Tg measurement). The purpose of the study was to evaluate the accuracy of on-treatment measurement of serum Tg in detecting remnant/recurrent or metastatic disease in high risk DTC patients.

Patients and methods

We retrospectively analyzed the medical records of 26 high risk DTC patients and compare the on-treatment and off-treatment Tg levels of these patients. All patients were anti-Tg negative. Using off-treatment measurement of Tg as the gold standard, the results of on-treatment measurement of Tg in diagnosis of remnant/recurrent disease were analyzed for sensitivity, specificity, negative predictive value (NPV) and positive predictive value (PPV).

#### Results

The median serum Tg level under thyroid hormone suppressive therapy (on-treatment Tg) was 16.5 ng/ml and after withdrawal of thyroid hormone suppressive therapy (off-treatment Tg) was 95.0 ng/ml ( $P$  value = 0.001). In 6 patients (23%) the on-treatment Tg level missed the recurrence of the disease. Regarding the off-treatment Tg as the gold standard, sensitivity, specificity, PPV and NPV of the on treatment Tg measurement was 72.7%, 100%, 100%, and 40%, respectively.

#### Conclusion

Diagnostic Tg measurement without TSH-stimulation (on-treatment) is useless in the follow-up of DTC patients with high probability of residual/recurrent or metastatic disease.

#### P836

##### Prevalence of somatic TSHR and GS alpha mutations in toxic thyroid nodules (TTNs) in endemic and nonendemic goiter areas of turkey

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#### Objective

Differences in iodine intake could account for the variable prevalences reported for somatic TSHR and Gs alpha mutations in TTNs. However, this question has never been settled, since no study has yet determined the TSHR mutation prevalence in TTNs in regions with and without iodine deficiency in the same population. Therefore we studied the prevalence of somatic TSHR mutations in TTNs by investigating TTNs from patients who lived in endemic and nonendemic goiter regions in Turkey.

#### Methods

We screened 78 TTNs from 59 patients for somatic TSHR mutations. Exon 9–10 of the TSHR and 7-8 of the Gs alpha were screened by denaturing gradient gel electrophoresis (DGGE). A PCR-based clonality assay was used for the clonality analysis of the mutation positive and negative nodules. Local Ethical Committee approval has been obtained.

#### Results

TSHR mutations were identified in 56 (71.8%) of 78 TTNs. Gs alpha mutation was identified in only one case. Four new TSHR mutations were detected (A428V, A627V, I640K and I486N). The D727 polymorphism was identified in 11 (18.6%) patients. No significant differences for the frequency of the TSHR mutation in endemic (70%) and nonendemic (74%) goiter regions of Turkey were found ( $\chi^2$ : 0.751  $P$ : 0.57). The frequency of non-random-X- chromosome inactivation was similar in endemic (85.7%) and non endemic goiter (75%) regions and in TSH-receptor mutation positive (69.5%) or negative (69.3%) hot nodules. Conclusion: These findings suggest that TTNs in both areas predominantly arise from aberrant growth of a single cell. Moreover, our results suggest that neither the proportion of TSHR mutations nor the proportion of the monoclonal origin of TTNs is related to iodine supply in the diet. This would imply that iodine more likely plays a role in determining the number of events that lead to thyroid autonomy rather than deciding the molecular signature of TTNs.

#### P837

##### The role of autoimmunity and thyroid function on pregnancy outcomes in euthyroid women with autoimmune disease undergoing assisted reproduction

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#### Context

Euthyroid women positive for thyroid antibodies are prone to develop subclinical/overt hypothyroidism during gestation and seem to suffer from a poor outcome.

#### Objective

Assessing if patients with autoimmune thyroid disease undergoing Assisted Reproduction Technologies (ART) are afflicted by poor pregnancy and/or delivery rate and if the outcome is conditioned by pre-ART thyroid status.

#### Design

Prospective study since January 1999 until June 2006. Women undergoing ART were screened for TSH, FT4, TPOAb.

#### Setting

Division of Physiopathology of Human Reproduction.

#### Patients

A total of 464 euthyroid women were selected; 52 (11.2%) were TPOAb (+).

#### Main outcomes

The endpoints were pregnancy rate, miscarriage rate and obstetrical complications.

#### Results

No difference in pregnancy rate was observed between women with and without antibodies. The rates of miscarriage and pre-term delivery were higher in TPOAb (+) than TPOAb (-) [ $P = 0.049$ ;  $RR = 1.1$  (95%  $CI = 0.98-1.25$ );  $P = 0.023$ ;  $RR = 1.2$  (95%  $CI = 0.98-1.4$ ), respectively]. In TPOAb (+), women who miscarried displayed higher TSH values before ART (2.8 mIU/L) compared to the ones who delivered or failed to become pregnant (1.6 and 1.7 mIU/L;  $P = 0.032$  and  $P = 0.018$  respectively).

#### Conclusions

In euthyroid women undergoing ART the pregnancy rate is not affected either by the presence of TPOAb or thyroid function. TPOAb (+) showed significantly increased number of miscarriages and pre-term deliveries; thyroid function before ART appears to play a pivotal role in determining the gestation outcome. These results show that thyroid autoimmunity and even a mild thyroid impairment represent risk factors for obstetrical complications (miscarriage and pre-term delivery). Further studies are required to ascertain possible benefits of levothyroxine in such patients.

### P838

#### Value of routine measurement of serum calcitonin concentrations in patients with nodular goiter: a multicenter study

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#### Context

The routine measurement of serum calcitonin (CT) has been proposed for patients with nodular goiter (NG), to detect unsuspected medullary thyroid carcinoma (MTC) before surgery.

#### Objective

To assess the prevalence of hypercalcitoninemia and MTC in NG patients; to compare the ability of CT measurement and fine needle aspiration cytology (FNAC) to predict MTC; to identify age groups of NG patients who should be better candidates than others to undergo routine measurement of CT.

#### Design, setting and patients

1425 consecutive patients with NG, referred to 4 Italian endocrine centers due to NG, were grouped depending on age, and underwent basal and, in some cases, Pentagastrin (Pg)-stimulated CT measurement, FNAC and, when indicated, surgery. Serum CT concentrations were measured by an immunoluminometric assay (ILMA). Results Hypercalcitoninemia was found in 23 of 1425 patients. MTC was discovered in 9 patients, all belonging to age groups 2-4 and showing high CT levels. Sensitivity of basal and Pg-stimulated CT to predict MTC before surgery was 100% for both tests, whereas specificity was 95% and 93%, respectively. FNAC showed an overall 86% sensitivity. When >10 mm MTC nodules were considered, FNAC sensitivity approached 100%. On the contrary, a correct cytological diagnosis was obtained in only 1/5 patients with <10 mm MTC nodules (microMTC); in 1 patient with histologically proved microMTC, FNAC demonstrated a benign lesion. Hypercalcitoninemia or MTC were associated with chronic thyroiditis in 30% or 33% of cases, respectively. C-cell hyperplasia was found in 57% of hypercalcitoninemic patients without MTC.

#### Conclusions

Basal CT measurement detects elevated CT values in 1.6% of NG patients. Although CT is not a specific marker of MTC, its routine measurement represents a useful tool in the pre-operative evaluation of NG patients, particularly those older than 40 years presenting with nodules smaller than 10 mm, even when FNAC does not show malignant features. To our knowledge, this is the first trial using ILMA to assess the ability of pre-operative CT measurement to predict MTC in a large series of NG patients.

### P839

#### Association between patient- and tumor-related factors and the gene expression profile of papillary thyroid cancer

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We performed the analysis of correlation between gene expression profile of the papillary thyroid cancer PTC tumors and known clinical factors influencing the outcome of the disease. Gene expression profile was assessed on Human Genome U133A array (Affymetrix). We examined tumors samples obtained from 49 patients diagnosed with PTC. For further evaluation we included following factors: sex, age, tumor size, capsule invasion, multifocality, vascular invasion, lymph node and distant metastases. We used corrected Welch t-test estimation to analyze how many genes are associated with each factor. We found out that poor differentiation/early recurrence (2 tumors) is connected with a prominent difference in gene expression profile (812 genes changed). For the further analysis we excluded these 2 samples (because of the large scale of difference between two poorly differentiated tumors and the rest of PTCs) and performed the analysis on the remaining 47 well-differentiated PTCs. The strongest factor in this group was sex with 10 sex-related genes. Large difference appeared for distant metastases: 1486 transcripts showing moderate level of statistical significance. To rank the remaining clinical factors, with low significance, we performed the analysis of uncorrected Welch t-test p-values. In such classification distant metastases were the strongest factor. We compared gene expression profile of 8 patients with metastases to 39 samples who did not present metastases within the short follow-up time. We selected 150 genes differentiating both groups of patients. By Support Vector Machine approach this dataset correctly predicted the status (metastases present or absent) in 45/49 patients. Our conclusions: 1) Some clinically relevant features in PTC (poor differentiation, sex) are strongly and significantly associated with gene expression profile. 2) We revealed that presence of distant metastases is related to gene expression and that it is possible to specify the metastatic signature in PTC.

### P840

#### Levothyroxine suppressive therapy in thyroid nodules

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The efficacy of Levothyroxine suppressive therapy in thyroid nodules is still controversial; the aim of this study is to evaluate the effect of levothyroxine on nodule volume.

#### Subjects and methods

Ninety three euthyroid patients (92 females, mean age = 43.7 years) harboring multinodular ( $n = 53$ ) or solitary ( $n = 40$ ) nodules have been treated for at least 6 months with thyroid hormones (Levothyroxine), the nodule size is assessed by its maximal diameter on sonography, a decrease of 20% or more is considered as significant.

#### Results

After a mean period of treatment of 18 months, 32% of the patients showed a significant decrease in nodule volume with a complete disappearance of the nodule in 11% despite an insufficient dosage as evidenced by thyrotropin which wasn't well suppressed, the response rate is better in solitary nodules than in multinodular goiter.

#### Conclusion

We conclude from this study that hormonal suppressive therapy is an effective treatment at least in some patients in reducing nodule volume.



**P841****Levothyroxine suppressive therapy and body composition**

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Reputedly, thyroid hormones make thinner and change the body composition. The aim of this randomized and prospective study was the assessment of the effect of levothyroxine suppressive therapy on body composition in women with benign thyroid nodule.

**Method**

Thirty seven euthyroid and non obese women (18–60 years old) with benign thyroid nodule (fine needle cytology and calcitonin measurement) were randomized into 2 equal groups. The first group received gradually 1.7 µg/kg of levothyroxine during one year and the second (control group) was not treated. The body composition was evaluated by bioelectric impedance and biphotonic absorptiometry, body density by plethysmography, energy expenditure by indirect calorimetry and muscular function was evaluated by effort test.

**Results**

At the end of the study, TSH was lower in the treated group than in the no treated group ( $0.58 \pm 0.30$  mU/l and  $1.21 \pm 0.50$  N:  $0.35-3.5$ ). The body composition didn't change. There was no difference in weight ( $62 + /9.51$  vs  $61.74 \pm 6.98$  kg,  $P > 0.5$ ), nor body density ( $1.04 \pm 0.019$  vs  $1.04 \pm 0.02$  kg/l,  $P > 0.5$ ). The energy expenditure wasn't different between the 2 groups during the study ( $1376$  kcal/j in the treated group vs  $1374$  kcal/j in the no treated group,  $P > 0.5$ ), circulating leptine level was the same in the 2 groups too ( $12.7$  vs  $12.4$  ng/ml) and the muscular function was not altered by levothyroxine therapy (equal decrease oxygen consumption in the 2 groups).

**Conclusion**

There was no modification of body composition, muscular function and energy expenditure in women received levothyroxine suppressive therapy for thyroid benign nodule, during one year. The treatment doesn't appear deleterious.

**P842****Differential regulation of soluble Fas in patients with autoimmune thyroid disease**

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**Introduction/Objective**

Fas-mediated thyroid cell apoptosis is differentially regulated in autoimmune thyroid disease (AITD) being up-regulated in Hashimoto's thyroiditis (HT) and down-regulated in Graves' disease (GD). The soluble form of Fas (sFas), lacking the transmembrane domain due to alternative splicing may inhibit apoptosis by binding to Fas ligand. In this preliminary study we examined whether sFas is also differentially expressed in patients with AITD.

**Materials and methods**

We studied 32 newly diagnosed patients, 17 with HT in subclinical hypothyroidism ( $35.47 \pm 13.74$  years), 6 with hypothyroid HT ( $35.67 \pm 14.88$  years) and 9 with GD ( $32.5 \pm 10.67$  years). We also examined 9 age-matched healthy controls ( $30.22 \pm 11.18$  years). In all subjects we measured serum sFas by ELISA together with thyroid function indices and thyroid antibodies.

**Results**

There was a non significant decrease in sFas levels in patients with subclinical hypothyroidism ( $2.37 \pm 0.66$  ng/ml) compared to controls ( $2.8 \pm 0.75$  ng/ml) but not in patients with clinical hypothyroidism ( $3.25 \pm 1.15$  ng/ml). However, in patients with GD, mean sFas levels ( $4.47 \pm 1.0$  ng/ml) were significantly higher compared with controls ( $P = 0.001$ ) and patients with HT ( $P = 0.005$ ).

**Conclusion**

sFas is differentially regulated in patients with AITD. The elevated sFas in GD may reflect reduced full length membrane Fas and may have a role in the inhibition of Fas-mediated apoptosis of thyrocytes in this condition.

**P843****Application of new tecnology of anti-DNA antibodies detection in diagnostics of autoimmune thyroiditis**

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The mechanism of autoimmune destruction of thyrocytes in pathogenesis of Hashimoto thyroiditis remain unclear. It has been showed that the HT patients produce antibodies to DNA, which are directed against various structures of the nucleus and probably may be involved in process of apoptosis.

In order to show that the increased production of anti-DNA antibodies is intrinsic property of HT, but not of another non-autoimmune thyroid diseases, we have measured the levels of antibodies to single-stranded (ssDNA) and double-stranded DNA (dsDNA) in the sera from eu-, hypo- and hyperthyroid patients with HT (30 subjects in each group), 30 patients with euthyroid endemic goiter (EEG) and 30 healthy donors. The diagnosis was confirmed according to the standard criteria. The concentrations of free thyroid hormones, TSH, antibodies to thyroglobulin (TG), thyroid peroxidase (TPO) and levels of anti-ssDNA and anti-dsDNA antibodies were measured by immunometric enzyme immunoassay (ELISA).

All patients with HT are characterized by reliable ( $P < 0.01$ ) elevation of all types of anti-DNA antibodies in comparison to donors and patients with EEG. It was strong positive correlation between concentrations of anti-DNA antibodies and levels of anti-TG and anti-TPO antibodies in HT, especially in patients with thyroid dysfunction. The elevation of antibodies to dsDNA ( $17.9 \pm 0.35$  IU/ml) in HT patients was reliably ( $P < 0.01$ ) more significant than antibodies to ssDNA ( $10.6 \pm 0.23$  IU/ml).

Additionally anti-dsDNA antibodies were tested by piezoelectric quartz immunosensor (PQIS) in the same groups and in 10 patients with systemic lupus erythematoses (SLE). PQIS demonstrated reliable ( $P < 0.01$ ) increase of anti-dsDNA antibodies in HT ( $124.5 \pm 3.1$  Hz) and SLE ( $144 \pm 2$  Hz) patients in comparison to donors ( $7.4 \pm 2.5$  Hz). Since the PQIS proved to be more sensitive in detection of anti-DNA antibodies in HT patients than ELISA, it can be proposed as additive diagnostic method.

**P844****Thyroxine replacement dose in patients with hashimoto disease: a potential role for interleukin-6**

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**Objective**

To investigate the potential association between serum inflammatory cytokine levels and thyroxine replacement dose in patients with Hashimoto disease.

**Patients and methods**

The study included 40 patients (12 men) with a mean age of  $56.52 \pm 6.12$  years who had hypothyroidism due to Hashimoto disease. Serum Interleukin-1b (IL-1b), Tumour Necrosis Factor-alpha (TNF-alpha) and Interleukin-6 (IL-6) levels, as well as TSH,  $fT_3$  and  $fT_4$  were measured (ELISA).

**Results**

Serum IL-6 showed a significant positive correlation both with total thyroxine replacement dose ( $r = 0.551$ ,  $P = 0.001$ ) and with dose per kilogram of body weight ( $r = 0.482$ ,  $P = 0.002$ ). There was also a significant negative linear correlation between serum IL-6 and  $T_3$  ( $r = -0.322$ ,  $P = 0.043$ ), as well as between serum IL-6 and  $T_3/T_4$  ratio ( $r = -0.332$ ,  $P = 0.036$ ). A further significant ( $r = 0.419$ ,  $P = 0.007$ ) positive association was demonstrated between IL-6 and TNF-alpha. However, no association was found between  $T_3$  or  $T_3/T_4$  ratio and TNF-alpha or IL-1b.

**Conclusions**

In patients with Hashimoto disease serum IL-6 levels are positively associated with thyroxine replacement dose and negatively associated with  $fT_3$  and  $fT_4$ . These results are possibly attributable to the inhibitory effect of IL-6 on deiodination of  $T_3$  and imply a role for IL-6 in determining thyroxine replacement dose among these patients.

**P845****The glutathione peroxidase 3 promoter is activated by Pax8**

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The selenoenzyme plasma glutathione peroxidase (GPx3, pGPx) is highly expressed in thyroid, kidney, placenta and bronchio-epithelial tissue. In the thyroid gland, the enzyme is thought to regulate thyroid hormone production by controlling the availability of H<sub>2</sub>O<sub>2</sub>, the cosubstrate for thyroperoxidase (TPO) and to protect the thyrocyte against H<sub>2</sub>O<sub>2</sub>-induced oxidative damage. To explain the high thyroidal expression levels, we studied GPx3 promoter regulation. Sequence analysis of the GPx3 5'URS revealed putative binding sites for the thyroid-specific transcription factors Pax8 and Nkx2.1 (TTF-1). In electrophoretic mobility shift assays using nuclear extracts from the rat thyroid cell line FRTL5, the putative Pax8 binding site displayed the same binding pattern as the Pax8 site of the well characterized TPO promoter. 2530, 1824, 1271 and 578 bp fragments of the human GPx3 5'URS were inserted into the luciferase reporter vector pGL4 and analyzed by transient transfection of the thyroid carcinoma cell lines FTC-133, FTC-238 and ML1, the kidney cell line HKC-8 and the hepatocarcinoma cell line HepG2, all of human origin. Depending on the cell line, induction of luciferase activity by the constructs was 20 to 30 fold vs. the empty vector, but this basal activity decreased with increasing 5'URS fragment length in all cell lines. Cotransfection of an expression plasmid coding for wildtype Pax8 further increased GPx3 promoter activity significantly, which, in contrast, was not observed after cotransfection of two expression plasmids coding for Pax8 loss of function mutants. Our results provide a molecular basis for the high GPx3 expression levels in the thyroid gland, however, they are also relevant for regulated GPx3 expression in the kidney, since Pax8 is highly expressed in renal cells as well. Further experiments will demonstrate the effect of Nkx2.1 on GPx3 promoter activity, possibly even in concert with Pax8 as it is described for other thyroid-specific genes like TPO and Tg. Supported by DFG Priority Programme.

#### P846

##### Similarities in the role of FoxE1 in thyrocytes and keratinocytes?

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Patients with non-functional FOXE1 (forkhead transcription factor) display congenital hypothyroidism, 'spiky' hair and other abnormalities. In the thyroid, FoxE1 controls migration of the developing gland and adult expression of thyroid specific genes. Our earlier studies demonstrated FOXE1 protein expression in keratinocytes of the epidermis and hair-follicle outer root sheath.

We aimed to characterise the expression and functional activity of FOXE1 during proliferation and differentiation of HaCaT, a spontaneously immortalised human epidermal keratinocyte cell-line.

Keratinocytes cultured in low Ca<sup>2+</sup> proliferate, elevating the Ca<sup>2+</sup> concentration above 1.0 mM induces differentiation. HaCaT cells were plated in 0.06 or 0.15 mM Ca<sup>2+</sup> and increased to 1.8 mM when they reached 80% confluence. Cultures were maintained for a further 6 days. mRNA was extracted at various time points, reverse transcribed and transcripts for FOXE1 and the APRT housekeeper gene measured using SYBR green and a Stratagene MX3000. Variations in FOXE1 functional activity were measured in a subclone of HaCaT cells that expresses a FOXE1 responsive luciferase reporter.

Even in low Ca<sup>2+</sup> medium, cell contact with increasing confluence induced differentiation and a 2-fold increase in FOXE1 transcripts. The calcium-shift produced a transient dip in FOXE1 transcripts which then continued to rise achieving levels 3 to 5 fold higher than at the outset, before finally declining. This was accompanied by temporal expression of differentiation markers, keratin 10 and filaggrin, evaluated by IMF. Similar results were obtained at both plating Ca<sup>2+</sup> concentrations and in the HaCaT subclone, which also displayed a 3-fold increase in luciferase. Subsequent studies revealed that TIMP-3 (promoter contains FoxE1 consensus) transcripts were upregulated 4 to 5 fold overall and also displayed a temporal reduction after adding Ca<sup>2+</sup>. FoxE1 transcripts and activity increase with keratinocyte differentiation and target at least one gene implicated in modification of the extra-cellular matrix, a situation of potential relevance to hair-follicle migration.

#### P847

##### Inhibin B/inhibin binding protein complex is differentially expressed in normal and nodular human thyroid tissue

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Inhibins A and B, peptides belonging to TGF- $\beta$  superfamily, have an ubiquitous antagonist action on activin signalling. In thyroid gland, TGF- $\beta$ 1 and activin mainly exert an inhibitory action on growth and function. In human thyroid, beside the presence of activin  $\beta$  subunits and its transductional elements (ActRI/II, SMAD), there is no evidence for  $\alpha$  subunit expression, an essential component for inhibin A/B gathering ( $\alpha$ - $\beta$ A/ $\alpha$ -BB), nor for its transductional cascade. The identification of mRNA expression for inhibin  $\alpha$  subunit and inhibins transductional elements in normal and nodular human thyroid tissue was the aim of this study. We therefore analyzed by competitive RT-PCR 10 samples from goitrous thyroid, 3 of whom from hyperfunctioning nodules, and 3 normal samples as control. Beside the expected mRNA expression for  $\beta$  subunits we detected for the first time thyroidal expression of  $\alpha$  subunit mRNA both in normal and nodular samples, although with a differential pattern. Interestingly,  $\beta$ B subunit was constantly overexpressed in goitrous tissue, while  $\alpha$  subunit levels were increased in hyperfunctioning nodules. We next evaluated the presence of mRNA for ancillary proteins (coreceptor  $\beta$ -glycan [ $\beta$ gly], Inhibin Binding Protein [IBP] and Follistatin [FS]) that agonize inhibin binding to ActRII, interfering with activin signalling.  $\beta$ gly and FS were regularly expressed in all samples, suggesting that human thyroid could be a high affinity target for inhibin. On the contrary, IBP was only expressed in nodular tissue. Since activin signal may be overridden by a specific interaction between inhibin B and IBP, their simultaneous presence suggest a role for this complex in goitrogenesis. These preliminary results show that human thyroid may be a source and a target for inhibin. Also, we suggest that inhibin B/IBP complex may play an autocrine role in the control of thyroid growth.

#### P848

##### Towards the development of a valid quality of life questionnaire for patients with thyroid diseases: Which questions shall we ask?

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##### Background and aim

In clinical populations, generic quality of life (QoL) questionnaires should be supplemented with disease-specific questionnaires, which may have higher sensitivity and clinical relevance. For the purpose of developing a comprehensive thyroid QoL questionnaire, we have evaluated the relative importance of various QoL aspects to thyroid patients.

##### Methods

A list of problems (issues) related to thyroid diseases, previously identified through literature review and expert-interviews, was presented to 80 outpatients with treated and untreated thyroid disease (n: non-toxic goiter 15, nodular toxic goiter 12, Graves' 21, TAO 17, autoimmune hypothyroidism 15). For each issue, the patients indicated whether they had experienced it at any time during their disease and rated the importance. An importance score was derived by multiplying prevalence and importance. For each patient category, the 15 most important issues were included.

##### Results

Forty-three issues were included, covering aspects of fatigue, anxiety, emotional susceptibility, cognitive complaints, cosmetic concerns and symptoms related to hyper- or hypothyroidism, eyes or goiter. Thus, there was an extensive overlap across diagnoses: General fatigue was among the 15 most important in all patient groups and 42% of the 43 issues were selected by more than one patient group. The five most important issues overall were general fatigue (mean rank 7.7), dyspnea (8.0), feeling of unrest (11.0), reduced coping (11.3) and physical fatigue (11.9).

##### Conclusion

Fatigue was very important and various aspects of mental well-being were as important as symptoms of thyroid disease. The level of agreement across patient groups was high. Therefore, developing a comprehensive thyroid QoL questionnaire encompassing all thyroid diseases seems preferable. This would also be advantageous in longitudinal studies, where thyroid state may shift. Based on these ratings, and similar ratings from endocrinologists, such a questionnaire is being developed and tested among 2000 Danish and American patients.

**P849****The efficacy of levothyroxine therapy in the nontoxic multinodular goiter**AM Mihai, AE Ranetti, C Spiroiu, A Mazilu & C Ranetti  
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There is no ideal treatment for the simple goiter. The treatment options in NMG is: surgery,  $I^{131}$ , L-thyroxine or continuous monitoring without therapy. From 2000–2003 we evaluated 150 patients (120 women and 30 men) having their agings between 20 and 80 years with L-T<sub>4</sub> treatment for nontoxic multinodular goiter. Duration of therapy was 3 years. The patients was evaluated by clinical examination, level of TSH, free-T<sub>4</sub>, anti-TPO, thyroid sonography, scintigraphy, FNAB (in the evaluation of solitary nodule or a dominant nodule), ECG, DEXA (in postmenopausal woman. 28% (32 patients) were responders (reduction of the total nodular volume with >50%); 22% (33 patients) were partial responders (reduction of the total nodular with 20–50%). ~1/3 developed symptoms of thyrotoxicosis (atrial fibrillation and bone mineral loss).

**Conclusion**

L-T<sub>4</sub> therapy have beneficial effect in patients with smaller nodules, but have side effects: subclinical hyperthyroidism with increased risk of atrial fibrillation and reduced bone density in elderly patients. L-T<sub>4</sub> therapy for NMG have a low efficacy and is a life long treatment.

**P850****Association of the FCRL3 gene with Graves' disease in the UK Caucasian population**MJ Simmonds, JM Heward, J Carr-Smith, JA Franklyn & SCL Gough  
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Recently, linkage between chromosome 1q23 and rheumatoid arthritis within the Japanese population has been narrowed down to association of four single nucleotide polymorphisms (SNPs), *fcr13\_3*, *fcr13\_4*, *fcr13\_5* and *fcr13\_6*, within *FCRL3*, a known regulator of B cell signalling. Of these four SNPs, *fcr13\_3* was shown to disrupt *FCRL3* expression on B cells, suggesting a potential etiological role. Association of *fcr13\_3* was also replicated in a Japanese Graves' disease (GD) cohort. The aim of this study was to investigate these four *FCRL3* SNPs within a large UK Caucasian GD dataset. In total 1056 patients with GD and 864 control subjects were genotyped for each SNP. All subjects gave informed written consent, and the project was approved by the local ethics committee. Association was found between GD and *fcr13\_3* (OR = 1.19, 95% CI = 1.03–1.37), *fcr13\_5* (OR = 1.18, 95% CI = 1.04–1.35) & *fcr13\_6* (OR = 1.20, 95% CI = 1.05–1.36), although at a significantly lower level than previously reported. Only a trend towards significance was detected for *fcr13\_4* ( $P = 0.059$ ). No evidence for an interaction with the previously established HLA class II, *CTLA-4* and *PTPN22* GD-associated alleles was detected for any of these SNPs. Similarly there was no evidence for association with any specific GD sub-phenotype. Linkage disequilibrium (LD) analysis revealed LD between *fcr13\_4*, *fcr13\_5* and *fcr13\_6* but not *fcr13\_3*. Further replication in independent datasets and fine mapping of the surrounding gene regions in UK Caucasians is now needed to confirm the magnitude of the effect and location of the etiological variant(s) present within this gene region.

**P851****Treatment options in progressive medullary, follicular, and papillary thyroid carcinomas: Evaluation of chemotherapy with doxorubicin**A Matuszczyk<sup>1</sup>, A Bockisch<sup>2</sup>, P Veit<sup>3</sup>, K Mann<sup>1</sup> & S Petersenn<sup>1</sup>  
<sup>1</sup> University Duisburg-Essen, Division of Endocrinology, Essen, Germany;  
<sup>2</sup> University Duisburg-Essen, Department of Nuclear Medicine, Essen, Germany;  
<sup>3</sup> University Duisburg-Essen, Department of Radiology, Essen, Germany.**Aim**

Progressive medullary (MTC) or iodine-negative papillary (PTC) or follicular (FTC) thyroid carcinomas present a challenge due to limited treatment options. The aim of this study was to evaluate the response to chemotherapy with doxorubicin.

**Methods**

22 patients (12 female, 10 male, mean 61 years) with PTC or FTC received chemotherapy with doxorubicin. Tumors were histologically classified as follicular in 14 (64%) patients, including 6 (27%) oncocytary tumors, and

papillary in 8 (36%) patients, including 1 (5%) papillary-oncocytary and 1 (5%) poorly differentiated tumor. In addition, 9 patients (5 female, 4 male, mean 51 years) with MTC received doxorubicin. Treatment consisted of either 8 cycles 15 mg/m<sup>2</sup> weekly or 3 cycles 60 mg/m<sup>2</sup> every 3 weeks, repeated once depending on response and side effects. Prior to chemotherapy, progressive disease was established during follow-up over 7.0 ± 5.6 (X ± SD, range 1–23) months. Effect of therapy was evaluated by radiographic imaging, <sup>18</sup>F-DG-PET, and bone scans. Treatment response was defined according to WHO criteria.

**Results**

In patients with PTC or FTC, 5.3% had a partial regress over 6 months, followed by stable disease >16 months (until today), 42.1% had stable disease for 8.8 ± 5.9 (3–22) months, and 52.5% had continuous progression established over 4.3 ± 2.8 (1–8) months. Three patients died before completing chemotherapy. In patients with MTC, 11.1% had a partial regress for 6 months, followed by stable disease for 2 months, 11.1% had stable disease over 7 months, and 77.8% demonstrated progressive disease, established over 6 ± 3.3 (2–12) months.

**Conclusions**

Whereas nearly half of the patients with PTC or FTC and established progressive disease had some benefit of chemotherapy with doxorubicin, most patients with MTC did not respond to such treatment. Due to the lack of alternatives, doxorubicin may be used as a palliative option in the treatment of patients with progressive PTC or FTC.

**P852****Staging of progressive papillary, follicular, or medullary thyroid carcinomas: Comparison of various staging procedures to define the extent and progress of disease**A Matuszczyk<sup>1</sup>, S Petersenn<sup>1</sup>, A Bockisch<sup>2</sup>, S Sheu<sup>3</sup>, P Veit<sup>4</sup> & K Mann<sup>1</sup>  
<sup>1</sup> University Duisburg-Essen, Division of Endocrinology, Medical Center, Essen, Germany; <sup>2</sup> University Duisburg-Essen, Department of Nuclear Medicine, Essen, Germany; <sup>3</sup> University Duisburg-Essen, Department of Pathology, Essen, Germany; <sup>4</sup> University Duisburg-Essen, Department of Radiology, Essen, Germany.**Aim**

Metastatic medullary (MTC) or iodine-negative papillary (PTC) or follicular (FTC) thyroid carcinomas present a challenge due to limited treatment options. We prospectively compared various staging procedures that may be necessary to define extent and progress of the disease.

**Methods**

31 patients were included (9xMTC, 8xPTC, 14xFTC). Staging procedures included CTs of chest, abdomen, and CNS, <sup>18</sup>F-DG-PET, and bone scan. Tumor spread was defined by evidence of tumor detected in any of the methods. In MTC, tumor distribution included neck (56%), lung (100%), liver (44%), other abdominal organs (22%), CNS (22%), and bone (78%). In PTC and FTC, tumor was detected in neck (50%, 43%), lung (100%, 100%), liver (25%, 0%), other abdominal organs (25%, 7%), CNS (13%, 0%), and bone (50%, 36%), respectively. For comparative evaluation, procedures had to be performed within 2 weeks.

**Results**

In MTC, CTs localized all tumor masses except bone metastases (71%). PET identified 89%, 60%, 75%, and 43% of the thorax, neck, liver, and bone metastases. The bone scan detected all bone metastases. Disease progress became evident by CTs, PET, and bone scan in 86%, 86%, and 71% of patients, respectively. In PTC and FTC, both CTs and PET identified all metastases except for bone. Bone metastases were detected by CTs, PET, and bone scan in 75%, 75%, and 88% of patients, respectively. Disease progress was seen in PET (100%) and CT (90%). CNS metastases was identified by CCT only.

**Conclusion**

In MTC, CTs of the chest, abdomen, and CNS, combined with a bone scan are required to determine the full extent of the disease, and may therefore also be used for follow-up. In PTC and FTC, <sup>18</sup>F-DG-PET correctly identified tumor spread and progress in most patients, with the addition of CT for the CNS. A bone scan provided some additional information.

**P853****The effects of orlistat and sibutramine on serum thyroid hormone levels in obese subjects**L Keskin<sup>1</sup>, I Sahin<sup>1</sup>, R Bentli<sup>1</sup>, S Senel<sup>1</sup> & C Taskapan<sup>2</sup>  
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#### Background

Studies on thyroid hormones in obese adult patients are contradictory. Moreover, little is known about the effect of weight loss on thyroid functions. Thus, we aimed to investigate thyroid hormone status, and possible effect of weight loss on thyroid hormones in obese patients.

#### Methods

We study population consisted of 39 (35 female, 4 male) euthyroid obese patients with mean age  $42.76 \pm 10.52$  years. Of these 19 patients were morbid obese. Weight, BMI, waist circumference, thyroid functions were evaluated initially and after 3 month of orlistat (120 mgr, three times a day) and sibutramine (15 mgr, once a day) treatment period.

#### Results

Morbid obese patients had higher total T4 ( $9.40 \pm 1.7$  vs.  $8.7 \pm 1.9$  ug/dl, respectively,  $P = 0.048$ ) and total T3 ( $162.06 \pm 36.9$  vs.  $151.49 \pm 36.4$  ng/dl, respectively  $P = 0.039$ ) levels compared to those of obese; however TSH, free T3 and free T4 levels were similar in obese and morbid obese patients. There were no statistically significant difference between the orlistat and sibutramine groups with regard to age, sex and BMI. Weight loss with orlistat therapy (mean  $6.59 \pm 4.62$  kg/3 month) decreased free T3 levels ( $4.17 \pm 2.13$  vs.  $3.08 \pm 0.63$  pg/dl, respectively,  $P = 0.042$ ). However, TSH, total T4, total T3 and free t4 were not significantly changed. Weight loss with sibutramine therapy (mean  $8.59 \pm 4.92$  kg/3 month) were not significantly changed TSH and any other thyroid hormone levels. In correlation analysis, weight was positively correlated with total T3 ( $r = 0.280$ ;  $P = 0.043$ ) levels. BMI was positively correlated with total T3 ( $r = 0.318$ ;  $P = 0.022$ ) and total T4 ( $r = 0.281$ ;  $P = 0.044$ ). In addition, waist circumference was positively correlated with total T3 ( $r = 0.434$ ;  $P = 0.001$ ) levels.

#### Conclusion

Degree of obesity may effect thyroid hormone status. The effects of sibutramine and orlistat on thyroid hormone status may be different beyond lowering body weight.

### P854

#### The research of IQ level in school children who live in the regions with iodine deficiency

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SI Endocrinology Research Centre RAMS, Medical Academy named by I.M. Sechenov, Moscow, Russia.

#### Purpose

To evaluate the level of intellect according to tests data in the regions with iodine deficiency.

#### Methods

In the frame the "Thyromobil" project that was held during 2003–2005 y in 18 regions of Russian Federation were done standartized epidemiologic research. The research included clinical examination, palpation thyroid, ultrasonic examination of thyroid, definitions of ioduria. We used Cattell test, free of cultural influence (Culture Fair Intelligence Test, CFIT), modification CF 2A (for children from 8 to 12 years) to detect the IQ level (the index of intellectual development). We examined 3902 school children, 2078 school children were tested by Cattell test.

#### Results

In the majority of regions the iodine supply was't lower than the normal level.

The mediane of iodine concentration in urina was within variabilities from 23.2 to 88.9 mkg/l. This indicated the light or medium degree of iodine deficiency. In some regions was found the destrics with hard iodine deficiency. The frequency of goiter according to ultrasonic data was from 3.9 to 42.6%. The average IQ index according to the results of test were from 77 to 93.2 (and only in some districts was higer than 95).

#### Conclusions

The iodine deficiency was found in the most regions, at least in light degree. Besides, the IQ level in most of this regions was lower than optimal level, but didn't go out the low bound of IQ diapason, which appropriated the light parabound degree of intellectual insufficiency

### P855

#### Unmasking of central hypothyroidism following growth hormone replacement in adult hypopituitary patients

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The effect of growth hormone (GH) replacement on thyroid function in hypopituitary patients has only been studied in small groups of children and adults with conflicting results.

We aimed to define the effect and clinical significance of adult GH replacement on thyroid status in a large cohort of 243 patients with hypopituitarism due to various causes. 84 patients were considered euthyroid before GH treatment and 159 patients had central hypothyroidism and were receiving thyroxine replacement. All patients had severe GH deficiency and the GH dose was titrated over 3 months to achieve serum IGF-1 concentration in the upper half of the age-adjusted normal range.

In the euthyroid group, we observed a mean reduction of serum free thyroxine (FT4) concentration of  $2.6$  pmol/l ( $P < 0.001$ ) and mean increase of total triiodothyronine (TT3) of  $0.09$  nmol/l ( $P = 0.06$ ) but a non-significant change in serum TSH. 30/84 patients (36%) became hypothyroid and needed initiation of thyroxine therapy. Patients who became hypothyroid had lower baseline serum FT4 concentration ( $13.9 \pm 3.3$  pmol/l) compared with patients who remained euthyroid ( $16.3 \pm 3.3$  pmol/l),  $P = 0.02$  and were more likely to have multiple pituitary hormone deficiencies (44% vs 16% respectively,  $P = 0.01$ ). In patients receiving thyroxine at baseline, we observed a mean reduction in FT4 of  $1.2$  pmol/l ( $P = 0.11$ ) and mean increase in TT3 of  $0.1$  nmol/l ( $P = 0.017$ ). 25/159 patients (16%) needed an increase in thyroxine dose.

GH deficiency masks central hypothyroidism in a significant proportion of hypopituitary patients and this is exposed after GH replacement. We recommend that hypopituitary patients with GH deficiency and serum free thyroxine concentration below  $14$  pmol/l should be considered for thyroxine replacement

### P856

#### Somatostatin receptor scintigraphy with $^{99m}\text{Tc}$ -HYNIC-TATE in visualization of non-radioiodine-avid differentiated thyroid carcinoma

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#### Introduce

Lack of radioiodine uptake in Differentiated Thyroid Carcinoma (DTC) is a huge diagnostic and therapeutic problem. This sign is associated with worse prognosis. In patients with elevated thyroglobulin levels and with no evidence of disease on radioiodine scintigraphy, scintigraphy with somatostatin analogs can be an alternative imaging modality.

#### The aim

Assessment of scintigraphy with the somatostatin analog  $^{99m}\text{Tc}$ -HYNIC-TATE to visualization of non-radioiodine-avid DTC. All patients gave written informed consent to participate in the study. The local Ethical Committee approval has been obtained before.

#### Material and method

Four patients with metastatic non-radioiodine-avid DTC (3 with papillary thyroid carcinoma (PTC) and 1 with follicular thyroid carcinoma (FTC) underwent neck, chest and upper abdomen scintigraphy with the somatostatin analog  $^{99m}\text{Tc}$ -HYNIC-TATE produced by OBRI POLATOM Świerk/Poland. The scintigraphy image acquisition was performed with a double head gamma camera Varicam Elscint in 10 min, 2–3 h and 24 h after i.v. injection of  $^{99m}\text{Tc}$ -HYNIC-TATE preparation in dose 15–20mCi. The SPECT images were reconstructed with use of HERMES (Nuclear Diagnostic, Sweden) processing workstation.

#### Results

A pathological uptake of  $^{99m}\text{Tc}$ -HYNIC-TATE was found in every patient with PTC.

In patient with FTC there wasn't observed any accumulation of this radiopreparation.

#### Conclusion

Non-radioiodine-avid Differentiated Thyroid Carcinoma can be visualized with  $^{99m}\text{Tc}$ -HYNIC-TATE preparation.

### P857

#### A novel test with recombinant human TSH for the differential diagnosis of congenital hypothyroidism in pediatric age

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Congenital hypothyroidism (CH) affects 1:2,000–3,000 newborns. In most cases, the cause is a developmental defect (dysgenesis) or an arrested migration (ectopia) of the thyroid gland. In the remaining cases TSH resistance or defects in iodide transport or thyroid hormonogenesis account for CH. The differential diagnosis is aimed to recognize permanent CH forms and to achieve an etiologic diagnosis for accurate management and genetic counselling. Appropriate investigations are in most cases performed at 3–4 years of age, after 1 month L-thyroxine withdrawal. In this study, a novel rhTSH (Thyrogen<sup>®</sup>) protocol has been tested after approval by Institutional Ethical Committee and informed consent in 8 patients, aged 17 months–12 years, during L-T4 treatment. In the presence of a normally positioned thyroid gland at ultrasound, 2 i.m. injections (0.004 mg/kg) were administered at days 1 and 2, and thyroid uptake with <sup>125</sup>I + perchlorate test (200–500 mg of KClO<sub>4</sub>) were performed at day 3. In the case of apparent agenesis, 3 rhTSH injections were administered at days 1–3, with neck scintigraphy uptake at days 3 and 4. In all cases, blood was sampled at baseline and at day 3 for TSH and thyroglobulin (Tg) levels. Side effects were not observed in any patients. Basal TSH levels were normal at baseline and peaked at day 3 (range: 15–47 mIU/l). Tg levels increased after stimulation in 7/8 patients. At scintigraphy, in a patient previously classified as affected with agenesis, a sublingual thyroid (ectopia) was revealed. Two patients had a large thyroid with a positive perchlorate test (discharge = 100%, indicating a total organification defect), while 5 patients had a normally positioned gland with normal uptake and negative perchlorate test (indicating transient CH or partial TSH resistance). In conclusion, we report a novel rhTSH protocol for the differential diagnosis of CH performed during L-T4 treatment in childhood. This test not only led to the correct diagnosis in all cases but also allowed the specific patient management and the targeted genetic analyses. rhTSH test represents a valid alternative to L-T4 withdrawal, avoiding untoward effects of transient hypothyroidism in CH infants.

#### P858

##### The use of recombinant human thyrotropin (rhTSH) in amiodarone-induced hyperthyroidism

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##### Introduction

An increase of thyroid iodine uptake (Tup) is one of the symptoms of thyrocyte stimulation by means of recombinant human thyrotropin (rhTSH). Low Tup, characteristic of iodine induced hyperthyroidism, creates serious problems, when <sup>131</sup>I is considered, making this kind of treatment difficult, if not impossible.

##### Material and methods

We report a case of a 74 years old patient, with hyperthyroidism, induced by amiodarone administration, applied because of paroxysmal atrial fibrillation (AF). The failure of antiarrhythmic therapy (after amiodarone withdrawal) in preventing AF, was the reason to attempt <sup>131</sup>I treatment. Initial Tup of 4% after 24 hours rose to 36% following stimulation with rhTSH. Thyrogen (Genzyme) administered i.m. in a single dose of 80 µg resulted also in an increase of thyroid hormones concentration (see Table 1).

**Table 1** Concentrations of thyroid hormones and Tup values before and after rhTSH administration.

The time point of study	FT <sub>3</sub> (pg/mL)	FT <sub>4</sub> (pmol/L)	TSH (µIU/mL)	Tup (%)
Initial values	4,9	24,6	0,18	4,4
12 h after rhTSH administration	5,2	32,2	24,8	
24 h – <sup>131</sup> I diagnostics	3,7	16,8	14,8	
48 h – <sup>131</sup> I therapy	6,1	38,3	4,05	36,0
96 h	6,6	41,6	0,62	
192 h, AF, thyrostatics	5,3	35,1	0,02	

Following the administration of <sup>131</sup>I in therapeutic activity (888 MBq), despite increased thyroid hormone concentrations, no clinical symptoms of

thyrotoxicosis were observed, except AF, which normalised within 12 h after increased propranolol dose.

##### Conclusion

Recombinant human TSH may enable <sup>131</sup>I therapy, especially in cases of iodine induced hyperthyroidism.

#### P859

##### Thyrotropin receptor (TSHR) activation has variable effects on the TNFα axis in preadipocyte and osteoblast-like cell lines

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In addition to its central role in the control of thyrocyte growth and function, the TSHR is expressed during the differentiation of mesenchymal precursors. To explore further, we have developed an *in vitro* model in which TSHR activation is achieved by introducing constitutively active (M453T, L629F) TSHR, using retroviral vectors.

We aimed to investigate the effect of TSHR activation on body composition, via the production of TNFα and expression of its receptor, TNFαR1, in preadipocyte and osteoblast-like cell lines.

Preadipocyte (NIH3T3LI,WAT; PAZ6, BAT) and osteoblast-like (MG63) cell lines, non-modified or stably expressing the WT, L629F or M453T TSHR were cultured in DMEM supplemented with FCS. NIH3T3LI were also cultured in medium to induce differentiation. TNFα released into the medium was measured by ELISA. RNA was extracted; reverse transcribed and transcripts for TNFαR1 and a housekeeping gene measured with Sybr green and a Stratagene MX3000.

##### Results

Number of cells required to produce 1 pg/ml TNFα in 18 hours.

**Table 1**

	Non/WT	L629F/M453T
NIH3T3LI	1.3 × 10 <sup>5</sup>	5 × 10 <sup>5</sup> (P < 0.01)
NIH3T3LI diff.	3.3 × 10 <sup>4</sup>	1.1 × 10 <sup>4</sup> (P < 0.01)
PAZ6	1 × 10 <sup>5</sup>	2.5 × 10 <sup>5</sup> (P < 0.05)
MG63	8 × 10 <sup>5</sup>	4.4 × 10 <sup>5</sup> (P < 0.05)

In NIH3T3LI, TSHR activation induces a 4–5 fold decrease in TNFα and a 10 fold increase in TNFαR1 expression. Differentiation induces a 5 fold increase in TNFα which is increased 3 fold further by TSHR activation. In PAZ6, TSHR activation induces 2–3 and 10 fold decreases in TNFα and TNFαR1 respectively. In MG63, TSHR activation induces 2–3 and 2–8 fold increases in TNFα and TNFαR1 respectively. TSHR activation has opposing effects on the production of TNFα and on the potential to respond to this ligand in pre-adipocytes and osteoblast-like cells. Since TNFα inhibits adipogenesis but promotes osteogenesis, TSHR activation may impact body composition. It may also provide a mechanism for the increased inflammation of Graves' disease.

#### P860

##### Clinical parameters and echographic patterns are poorly predictive of malignancy in thyroid nodules with a cytological diagnosis of follicular and Hürthle cell neoplasia

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Aim of the study was to evaluate whether clinical parameters and echographic patterns may be predictive of malignancy in thyroid nodules with a cytological diagnosis of follicular (FOL) and Hürthle cell (HC) neoplasia. The study included 495 patients (388 F: mean age 45 ± 14 yr; 107 M: mean age 46 ± 14 yr) with FOL (n = 418) and CH (n = 77) nodules, "cold" at scintiscan and undergone to thyroidectomy. Clinical parameters considered were: sex, age, goiter association and nodule size. Echographic patterns previously described as suggestive of malignancy were evaluated with real time ultrasound apparatus (Technos, Esaote, SPA) using a 7.5 MHz linear probe. Histological diagnosis of malignancy (CA) was performed in 124 cases and benign nodule (BN) in 371 cases.

	Benign (n)	N = 371 (%)	Carcinoma (n)	N = 124 (%)	P
Clinical parameters					
Sex (m/f)	76/295	21	31/93	25	0.29
Age > 40/<40 yr	240/131	65	85/39	68	0.4
Single/multiple nodule	230/141	62	71/33	57	0.3
Echographic patterns					
Goiter (VT > 21/ <20 ml)	184/187	50	52/72	42	0.1
Size > 1.6/<1.5 cm	311/60	84	105/19	85	0.3
Solid/cystic aspect	356/15	96	117/7	94	0.45
Dis/homogeneous	228/143	61	78/46	63	0.7
Hypo/isoechoogenicity	223/148	60	80/44	66	0.38
Absent/present halo	125/246	34	52/72	42	0.09
Absent/present	103/268	28	50/74	40	0.008
Microcal					

In conclusion, sex, age, goiter association and single nodularity are not predictive of malignancy. Among the echographic patterns, only the presence of microcalcifications is significantly associated with malignancy. Hypoechoogenicity, at difference of what previously observed in the whole group of nodules, has no predictive value in thyroid nodules with a cytological diagnosis of follicular and H<sup>2</sup>rtthle cell neoplasia.

### P861

#### The use of konjac glucomannan to lower serum thyroid hormones in hyperthyroidism

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#### Objective

Patients with hyperthyroidism occasionally need rapid restoration to the euthyroid state. In view of the increased enterohepatic circulation of thyroxine (T<sub>4</sub>) and triiodothyronine (T<sub>3</sub>) in thyrotoxicosis, and metabolic effects of konjac glucomannan in gastrointestinal system, we aimed to determine the activity of glucomannan in treatment of hyperthyroidism.

#### Methods

A prospective, randomized, placebo-controlled, one-blind study design was used with newly diagnosed 48 hyperthyroid patients (30 patients with Graves' disease and 12 with multinodular goitre). They were assigned to one of the following treatment groups: I) methimazole 2 × 10 mg, propranolol 2 × 20 mg, and glucomannan (Propol) 2 × 1.3 gr daily for two months; II) methimazole 2 × 10 mg, propranolol 2 × 20 mg, and placebo powder daily for two months.

#### Results

No differences were detected from the point of view of the baseline thyroid hormone levels between groups ( $P > 0.05$ ). Further analyses revealed that the patients receiving glucomannan at the end of the second, fourth and sixth weeks of the study had significantly lower serum T<sub>3</sub>, T<sub>4</sub>, FT<sub>3</sub> and FT<sub>4</sub> levels than the patients who received placebo ( $P < 0.05$ ). TSH was not different between the two groups at any specific time ( $P > 0.05$ ). At week 8, thyroid hormone levels were not shown any differences. The glucomannan-treated group had a more rapid decline in all four serum thyroid hormone levels than the placebo-treated group.

#### Conclusions

We believe our preliminary results indicate that glucomannan may be a safe and easily tolerated adjunctive therapeutic agent in the treatment of thyrotoxicosis. This combination therapy seems most effect during first weeks of treatment of a hyperthyroid patient.

### P862

#### Comparison of the effects of thyrotropin receptor (TSHR) activation on preadipocyte cell lines from white (WAT) and brown (BAT) adipose tissue

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Adipogenesis is induced when intracellular cAMP increases and follows cell cycle arrest. During preadipocyte differentiation expression of the TSHR, which signals via cAMP and the inositol phosphate cascade, is upregulated.

To investigate the role of TSHR activation in adipogenesis, we have developed an *in vitro* model in which constitutively active TSHR mutants (L629F, M453T) are introduced by retroviral vectors (RV).

We aimed to compare the effects of TSHR activation on preadipocyte cell lines derived from brown (BAT) and white (WAT) adipose tissue.

NIH3T3L1 (murine WAT) and PAZ6 (human BAT) cell lines, non-modified or stably expressing the WT, L629F or M453T human TSHR were studied. Proliferation was assessed by direct counting (Coulter). Basal cAMP was measured by RIA. Spontaneous lipid accumulation was determined as the absorbance (490 nm) following oil red O stain.

Expression of activating TSHR mutants induced morphological changes. 3T3-L1 had a more rounded appearance, accompanied by significant increase in population doubling time (PDT). Proliferation of PAZ6 cells was severely inhibited, with WT and L629F expressing cells ceasing to grow as monolayers and forming aggregates, M453T barely proliferated.

	PDT (hours)		Lipid(% nonmod)	
	3T3-L1	PAZ6	3T3-L1	PAZ6
Non-mod	21	26	100	100
Wild type	21	72**	100	140
L629F	23*	118**	240**	170*
M453T	25*	ND	170*	280**

\* $P < 0.05$ ; \*\* $P < 0.001$

In both cell types, the mutant TSHR expressing cells demonstrated increased spontaneous lipid accumulation. cAMP increased 127–220% in activating mutant expressing 3T3-L1 compared with non-mod & WT.

The results indicate that TSHR activation stimulates WAT and BAT adipogenesis – possibly via cell cycle arrest consequent to elevated cAMP. The model could be improved by using inducible RV, to vary TSHR expression, and resemble more closely the *in vivo* situation. Our preliminary results with such a system are promising.

### P863

#### Effects of increment of endogenous TSH levels on the effective half-life time of <sup>131</sup>I and iodine uptake by thyroid remnants in patients after thyroidectomy

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The effectiveness of thyroid remnants ablation with <sup>131</sup>I after thyroidectomy for differentiated thyroid carcinoma (DTC) depends mainly on absorbed radiation dose, which is proportional to <sup>131</sup>I uptake (Tup) and its effective half-life time (EHL) in the remnants.

#### Aim

Assessment of endogenous TSH level increment effects on EHL of <sup>131</sup>I and Tup in thyroid remnants after surgery for DTC.

#### Material

Twenty-one patients were studied (20 women, 1 man), the age: 30–79 years (mean: 48 y.); 10 patients with tumour - pT1a, 1 patient - pT1b, 6 - pT2, 4 with pT4 (UICC 1997).

#### Methods

Diagnostic doses of 4MBq <sup>131</sup>I were orally administered to each patient 10, 20 and 30 days after thyroidectomy and Tup was measured 2 h, 4 h, 24 h, 48 h and 240 h after the administration. TSH level was determined before each <sup>131</sup>I administration. EHL and the products of Tup and EHL were calculated. The differences between TSH, Tup, EHL and Tup\*EHL were assessed at each time-point (paired Wilcoxon test).

#### Results

	Median at day			Difference between days (P)		
	10	20	30	10 vs 20	10 vs 30	20 vs 30
TSH (μIU/ml)	6.7	29.0	53.2	<0.0001	0.00004	0.00004
Tup (%)	3.2	3.4	4.8	0.033	0.063	0.420
EHL (days)	4.0	4.6	3.6	0.030	0.120	0.013
Tup*EHL	12.9	15.6	9.9	0.006	0.92	0.17

## Conclusions

1. Between the 10th and the 20th day after surgery the rise of TSH was accompanied by a rise of both Tup and Tup\*EHL while no further increment of Tup or Tup\*EHL was observed between the 20th and the 30th day. EHL did not change significantly during the study period. 2. No improvement of dosimetric parameters (EHL, Tup) was noted during the long (30 days) vs the short (20 days) TSH stimulation.

## P864

Surgery should be considered equally with I<sup>131</sup> and thionamide treatment as first line therapy for thyrotoxicosisT Sathyapalan<sup>1</sup>, SL Atkin<sup>2</sup> & RJA England<sup>1</sup><sup>1</sup>Hull Royal Infirmary, Hull, East Yorkshire, United Kingdom; <sup>2</sup>Hull York Medical School, Hull, East Yorkshire, United Kingdom.

## Objectives

To analyse the outcome of 100 consecutive patients treated for thyrotoxicosis by total thyroidectomy, and compare the efficacy of this treatment modality to I<sup>131</sup> therapy and thionamide therapy.

## Design

Data collected prospectively from a tertiary referral multidisciplinary thyroid clinic on 100 consecutive patients operated on for thyrotoxicosis by one thyroid surgeon. Patient demographics, reasons for selection of surgical treatment, preoperative treatment and postoperative complications were recorded. Histological diagnoses were also recorded. All patient data was anonymised.

## Participants

All patients who underwent surgery for thyrotoxicosis through the thyroid clinic between November 2000 and June 2004. Data collection was suspended once 100 sequential patients were included.

## Results

71% of patients underwent surgery out of preference. The haematoma rate was 4%. There were no permanent recurrent laryngeal nerve palsies. The temporary hypocalcaemia rate was 12%, the permanent hypocalcaemia rate was 2%. These complication rates with the 100% cure rate compare very favourably with the other two treatment modalities. In addition four malignant histologies were discovered (three T1 papillary carcinomas and one T4 multicentric papillary carcinoma).

## Conclusions

The risks from surgery for thyrotoxicosis are lower than appreciated. A surprising number of patients opt for surgery for definitive treatment of the condition when given the choice suggesting that the choice is not offered sufficiently frequently. A 4% malignancy rate was evident in the series and these cancers would have presented later if the patients had not opted for surgery. Surgery should be considered equally with thionamide therapy and I131 therapy as first line management of thyrotoxicosis.

## P865

## Thyrotoxicosis – a risk factor for subsequent development of osteoporosis?

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## Purpose

Thyrotoxic patients have an increased bone turnover and decreased Bone Mineral Density (BMD) at diagnosis. During antithyroid drug treatment (ATD) the patients improved their BMD but earlier studies have not been able to determine if BMD normalize or how long it will take.

## Methods

We have prospectively examined 32 females (approved by Danish Ethical Committee) with newly diagnosed Graves' disease (antilog10, mean age (CI 95%): 39 years (34–45) and as controls 20 females with Hashimoto's thyroiditis (42 years (35–49)) and 20 healthy females without thyroid disease (43 years (38–50)). There were no significant differences between age, weight or height between the 3 groups. We measured T-scores of BMD and BMC by DEXA scans (Norland, XR-26 MarkII/HS) and PTH, Ca<sup>2+</sup>, total and free T3 and T4, TSH, TSH-receptor autoantibodies (TSAb), anti-TPO and lipid profile at baseline and after 3 and 18 months of treatment with thiamazole (Thycapzol<sup>®</sup>) and after additionally 21 months follow-up without ATD.

## Results

Graves' patients had a significantly lower BMD (0.928 g/cm<sup>2</sup> (0.880–0.970)) compared to the Hashimoto patients (1.016 (0.975–1.059)) and the

healthy controls (1.017 (0.976–1.060)) ( $P < 0.01$ ). After thirty-nine months follow-up there were no significant differences between the 3 groups for BMD of the spine, hip or total body. In addition, T-scores (columna) were for Graves' patients, Hashimoto patients and normal controls:  $-0.2$  ( $-1.2$ – $0.8$ ),  $-0.4$  ( $-0.9$ – $0.1$ ) and  $+0.1$  ( $-0.5$ – $0.9$ ), respectively. Compared to baseline, BMD (total body) increased significantly from 0.928 g/cm<sup>2</sup> (0.880–0.970) to 0.998 g/cm<sup>2</sup> (0.964–1.034) ( $P < 0.02$ ) in the Graves' patient. A multiple regression analysis was performed and for the Graves' patients the strongest predictors for BMD were T3 ( $B = 0.29$ ,  $P < 0.01$ ) and anti-TPO ( $B = -0.21$ ,  $P < 0.03$ ) and for BMC T3 ( $B = 1.18$ ,  $P < 0.03$ ). The strongest predictor for BMD and BMC for the control group was TSH ( $B = -0.61$ ,  $P < 0.01$  and  $B = -0.45$ ,  $P < 0.05$ ).

## Conclusion

This study showed that thyroid patients are still osteopenic 39 months after initiation of ATD, and we therefore suggest regularly DEXA scans to detect development of osteoporosis after thyrotoxicosis.

## P866

## Relationship between cardiac arrhythmias and subclinical hyperthyroidism

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Atrial fibrillation (AF) and, in general, cardiac arrhythmias are among the most frequent complications during clinical thyrotoxicosis. However to-day the most frequent conditions of hyperthyroidism are subclinical and more subtle is the relationship between this last condition and cardiac arrhythmias.

In order to evaluate this topic, we considered two groups: A) patients admitted into the hospital for AF; B) patients affected by subclinical hyperthyroidism (SH) (low TSH, less than 0.1 mIU/ml, normal FT4 and FT3). Patients of group A were evaluated for thyroid dysfunction and those of group B for cardiac rhythm changes by ECG-Holter.

A condition of SH, unknown before AF episode, that brought to the hospital admission, was diagnosed in 10% patients of group A, while a 4% showed a clinical hyperthyroidism. In the follow-up the treatment of subclinical hyperthyroidism has determined a prophylaxis of FA. In the group B we did not observed any case of FA, but only an increased mean Cardiac Frequency (FCm) (Table):

Patients	n.	mean age	Subclin. Iper.	Clin. Iper.	FA	FCm
Group A	149	65 yrs	10%	4%	100%	87b/m
Group B	85	62 yrs	100%	0%	0%	99b/m

FA is complex phenomenon from pathogenetic point of view, not well explained. It is the concurrence of many factors, grouped in three classes:

Trigger, cardiac and extracardiac factors, unbalance of vagal and sympathetic tone. These observations suggest on one hand subclinical hyperthyroidism is per se a determinant cause of FA, as the result in the first group could demonstrate; in the other hand it is a factor that necessitate of others to precipitate cardiac arrhythmias, as the result of second group could suggest.

Another explanation is in peripheral modulation of thyroid hormones (TH) at nuclear receptors of miocytes, that can respond in different way to acute or chronic exposition of TH.

## P867

## Cardiovascular disease in patients with and without hypothyroidism

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## Aims

To evaluate the cardiovascular morbidity in patients with and without hypothyroidism admitted to a central hospital, between 1989 and 2004.

## Materials and methods

Data from the Hospital Coding Centre were retrospectively analysed according to International Classification of Diseases (9th version) criteria. We have studied the distribution by age, sex, causes and duration of admissions. Statistical analysis was performed with Student's t-test,  $\chi^2$  test

or Fisher exact test. Results are expressed as means  $\pm$  SD or percentages. A two-tailed *P* value  $<0.05$  was considered significant.

**Results**

A total of 730487 admissions were registered during the studied period. There were 211 admissions with a diagnosis of cardiovascular disease and hypothyroidism and 46042 admissions with a diagnosis of cardiovascular disease without hypothyroidism. There were no significant differences in the age of the patients with and without hypothyroidism ( $61.1 \pm 12.5$  vs  $66.7 \pm 10.5$ , *P* = NS). The duration of hospitalisation was significantly higher in hypothyroid patients ( $21.4 \pm 5.3$  vs  $14.0 \pm 2.4$  days, *P* = 0.04). Patients with hypothyroidism had an incidence of cerebrovascular disease (12.8%) that was not significantly different from that in patients without hypothyroidism (12.7%). The incidence of coronary artery disease was significantly higher in patients with hypothyroidism than in patients without hypothyroidism (23.3% vs 17.5%, *P* = 0.02).

**Conclusions**

Patients with hypothyroidism appear to have a higher risk of coronary artery disease. Concerning the impact of hypothyroidism in cardiovascular risk factors, the study of the interrelations of thyroid's function with lipidic profile and with the immune and inflammatory tracers could contribute to the enlightenment of etiopathogenic mechanisms involved in the endothelial dysfunction, enabling the definition of new therapeutic or preventive strategies.

**P868**

**Clinical parameters and echographic patterns are poor predictive of malignancy in follicular and Hürthle cell nodules at cytology**

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Aim of the study was to establish the diagnostic value of nuclear atypia thyroid nodules with cytological diagnosis of follicular (FOL) and Hürthle cell (HC) neoplasia. 1039/23,313 fine needle aspiration (FNA) performed in January 2002 June 2005 in the Department of Endocrinology, University of Pisa had cytological diagnosis of FOL e CH. FNA was performed under echo guidance, using 23 gauge needle attached to 10 mL syringe. The material was air-dried and stained with Papanicolaou e Giemsa. Cytological criteria used for diagnosis of follicular nodule included hypercellularity with small, uniform cells and poor and watery colloid content. The presence of nuclear enlargement and hyperchromic nuclei with irregular / coarse chromatin was defined as atypia. 495 patients (388 F: mean age  $45 \pm 14$  yr; 107 M: mean age  $46 \pm 14$  yr) with "cold" nodule at scintiscan underwent thyroidectomy at the Department of Surgery, University of Pisa. Histology was made according to the World Health Organization guide lines.

**Results**

418/495 patients had a cytological diagnosis of FOL: 82 with nuclear atypia and 336 without nuclear atypia; 77/495 had HC diagnosis: 18 with nuclear atypia and 59 without nuclear atypia. CA histological diagnosis was made in 45/100 (45%) nodules with nuclear atypia and in 79/395 (20%) nodules without nuclear atypia ( $X^2 = 26.5$ ; *P*  $<0.0001$ ).

**Table 1**

	Atipia + (n = 100)	Atipia (n = 395)	P
Follicular			
Benign	43	260	
CA	39	76	$<0.0001$
Hürthle			
Benign	12	56	
CA	6	3	$<0.0001$
Total		316	
Benign	55	79	
CA	45		$<0.0001$

In conclusion, nuclear atypia characterized by nuclear enlargement and hyperchromic nuclei with irregular/coarse chromatin is a pattern predictive of malignancy in follicular/ Hürthle neoplasia at cytology.

**P869**

**Identification of housekeeping genes useful for the normalization of rna in studies of gene expression in thyroid carcinomas**

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Housekeeping genes (HK) are commonly used as controls for the normalization of the RNA amount in quantitative RT-PCR experiments. The question whether their expression is influenced by the metabolic state of the patient and/or the tumour transformation is still unresolved and until now, HK genes have been used in quantitative studies without considering that their expression could be different in tumor with respect to normal tissue. Aim of this study was to identify HK genes equally expressed in the tumor and in the normal tissue of the same patient.

We evaluated the mRNA expression of 5 HK genes (GAPDH, HPRT,  $\beta$ -2-microglobulin,  $\beta$ -actin and cyclophilin A) in 30 papillary thyroid carcinoma (PTC) tissues and in the relative normal thyroid, by Real-time RT-PCR.

RNA was extracted from the tissues and reverse transcribed into cDNA. After validating the good quality of the cDNA using primers for a specific thyroid gene (PAX-8), samples were used for Real-time RT-PCR using specific primers. The statistical analysis was done by coupling the value of the threshold cycle (Ct) relative to the expression of any single gene in the tumor and in the normal controlateral tissue (t-test paired).

Our results showed that GAPDH and HPRT are equally expressed in the tumor and in the controlateral normal tissue. In particular the Ct values were similar in tumor and benign tissues of 26/30 cases (*P* = 0.25 and *P* = 0.16 respectively). On the contrary,  $\beta$ -2-microglobulin,  $\beta$ -actin and cyclophilin A were significantly more expressed in the normal tissue than in the tumor (*P* = 0.045, *P* = 0.0008 and *P* = 0.033 respectively).

In conclusion, our data suggest that GAPDH and HPRT can both be used for RNA normalization of quantitative studies of gene expression in PTC. At variance,  $\beta$ -2-microglobulin,  $\beta$ -actin and cyclophilin A can not be used since their expression seems to be modulated by tumoral transformation.

**P870**

**Identification of a novel germ-line point mutation of the ret gene (Met848Thr) in a patient affected by medullary thyroid carcinoma and castleman's syndrome**

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Mutations in the RET proto-oncogene are responsible for multiple endocrine neoplasia type II. Somatic RET mutations were described in 50% of medullary thyroid carcinoma (MTC). We describe here a novel germline mutation of the RET gene detected in an apparently sporadic MTC.

The index case was a 67 years old patient who arrived at our observation for a bilateral laterocervical linfadenopathy. The patient was clinically evaluated for thyroid function and morphology. The thyroid ultrasound showed the presence of a 10 mm anechoic nodule and a solid nodule with 6 mm iperchoic spots, both in the left lobe. The measurement of thyroid hormones revealed a normal function of the gland, while the basal calcitonin (CT), was elevated (29 pg/ml). CT after pentagastrin (PG) stimulation test shows a peak of 240 pg/ml. We suspected the presence of an MTC, that was confirmed by cytological analysis of FNAB of the 6 mm nodule. In addition a cytological evaluation of FNAB from suspicious lymphnodes showed several degrees of atypia and an histological examination was suggested. The patient underwent total thyroidectomy with central and bilateral lymphadenectomy. The histological evaluation confirmed the diagnosis of MTC in the 6 mm nodule while the laterocervical limphonodes presented the typical features of the Castleman's-syndrome. We performed genetic analysis for RET mutations by sequencing exons 10, 11, 13, 14, 15, 16. The analysis revealed the presence of a novel germline mutation ATG- > ACG at the codon 848 of the exon 14.(Met848- > Thr). The 28 years old daughter was also found to be positive for the same mutation, but negative for clinical/biochemical examination. In conclusion, although the lymphnode disease was unrelated to thyroid, it allowed to identify a case of familial MTC. Since the association between the Castleman's-syndrome and the MTC has been never described, it is likely that they were fortuitously associated.

**P871**

**Usefulness of molecular analysis of BRAF mutation and RET/PTC rearrangements in fine needle aspiration (FNA) of thyroid nodules with nondiagnostic cytology**

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About 10–15% of FNA cytologies are nondiagnostic, because of inadequate material or indeterminate (i.e. follicular neoplasms). Aim of this study was to analyse the practical usefulness of the analysis of BRAF mutations and RET/PTC rearrangements, known to be associated to papillary thyroid carcinoma (PTC), in FNA-material.

We analysed 100 thyroid nodules with a nondiagnostic cytology: 75 follicular nodules and 25 cases with inadequate material. Fifteen cases with a cytological diagnosis of PTC were used as controls. Thyroid aspirates were used for cytology and the needle was then washed in the solution for the extraction of RNA. A housekeeping gene (GAPDH) and a thyroid specific gene (PAX-8) were amplified by RT-PCR to assess the follicular origin of the RNA. BRAF V600E mutation was analysed by direct sequence analysis of PCR products. RET/PTC rearrangements were analysed by RT-PCR using specific primers and southern blot analysis with specific oligoprobes. In 17/100 (17%) nodules with nondiagnostic cytology, we found BRAF mutation, TRK and/or RET/PTC rearrangements. In particular, 7 BRAF mutations, 6 RET/PTC3 and 3 RET/PTC1 rearrangements and 1 TRK rearrangement were revealed. In 1 of these cases both BRAF mutation and RET/PTC3 rearrangement were present. The histology revealed 33/100 (33%) PTC, 11 of whom were positive for BRAF ( $n=6$ ) mutation, RET/PTC rearrangements ( $n=4$ ), both BRAF mutation and RET/PTC rearrangement ( $n=1$ ) and TRK rearrangement ( $n=1$ ). Four benign thyroid nodules (4/67, 5.9%) were positive for RET/PTC rearrangements (3 RET/PTC3, 1 RET/PTC1). No BRAF mutations were found in benign nodules. In agreement with the literature, we also found 8/15 (53.3%) PTC harboured a BRAF mutation [6/15 (40%)] or RET/PTC1 [3/15 (20%)] rearrangement.

Our data confirm that the molecular analysis of BRAF mutation and RET/PTC rearrangements in fine needle aspirates is feasible and may be useful in improving the presurgical diagnosis in those cases with a nondiagnostic cytology.

#### P872

##### Does Selenium deficit trigger post partum thyroiditis

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Selenoproteins play a major role in the redox-systems of the body. Glutathionperoxidase is important for the enzymatic reduction of hydroperoxidases in the thyroid gland. Selenium (Se) is able to capture radicals, one of which is oxygen originating from thyroxin-peroxidase (TPO) activity. It has been shown that Se supplementation is associated with a decrease of anti-TPO-titers. Women with Hashimoto's were shown to have lowered serum Se concentrations.

We estimated Se concentrations in pregnant women upon detection of the pregnancy. If Se was decreased ( $<1 \mu\text{mol/l}$ ), a daily dose of 100  $\mu\text{g}$  sodium selenite was substituted. Serum Se was re-evaluated between weeks 32 and 40 of pregnancy and it was also measured in the cord blood of the new-born.

In the first trimester of pregnancy serum Se levels were significantly ( $P < 0.05$ ) decreased in Hashimoto's ( $0.65 \pm 0.08$ ,  $n=7$  vs. controls  $0.97 \pm 0.11$ ,  $n=13$ ). At term serum Se concentrations were  $0.72 \pm 0.1$  ( $n=15$ ) without and  $1.13 \pm 0.2$  following Se Supplementation ( $P < 0.05$ ,  $n=20$ ). Serum cord levels were  $0.55 \pm 0.07$  ( $n=15$ ) and  $0.78 \pm 0.09$  ( $P < 0.05$ ,  $n=20$ ) respectively.

The results indicate that serum Se decreases during pregnancy for about 25%, most probably due to the requirement of the fetus. To prevent a lack of this trace element, a daily supplementation dose of 100  $\mu\text{g}$  appears adequate. It is well established that a deterioration of Hashimoto's occurs in women post partum with known immunthyroiditis in up to 60% (Lazarus 2000). There is also a fair number of women where manifestation of Hashimoto's is occurring for the first time during the post partum period. Whether a deficiency of Se is responsible for this disease, cannot profoundly be proven, but it seems to be a logical hypothesis.

#### P873

##### 17-AAG and DIDS increase the iodide retention time in thyroid cancer cells transfected with NIS

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#### Background

One of the major limits of gene therapy with sodium iodide symporter (NIS), which enables cells to be subjected to radioiodine therapy, is that NIS transfected cells rapidly release the intracellular iodine.

#### Materials and Methods

We transfected two human anaplastic (FRO) and medullary (TT) thyroid cancer derived cell lines, unable to take up iodine, with human NIS cDNA. The possibility of increasing the iodine retention time by treating cells with myricetin, lithium, 17-AAG and DIDS was explored.

#### Results

We obtained 19 FRO and 16 TT clones stably transfected with NIS; 12/19 FRO and 9/16 TT clones expressed the full length NIS mRNA; 11/12 FRO and 2/9 TT clones were able to specifically take up radioiodine and correctly expressed NIS protein on the plasma membrane. Kinetic analysis of iodide uptake in the two clones with the highest uptaking (FRO-19 and TT-2) activity revealed that the plateau was reached after 30 minutes by FRO-19 and 60 minutes by TT-2. The  $t_{1/2}$  of the iodide efflux was 9 minutes in FRO-19 and 20 minutes in TT-2. The treatment of the two cell lines with four different drugs revealed that DIDS and 17-AAG significantly increased the intracellular iodide retention time in FRO-19, but not in TT-2.

#### Conclusions

We showed that 17-AAG and DIDS prolong the retention time of  $^{131}\text{I}$  in thyroid tumoral cells transfected with NIS, thus increasing the possibility of using efficiently this approach for future clinical application, especially in those patients with dedifferentiated thyroid carcinoma no longer responsive to conventional therapy.

#### P874

##### Demethylating treatment with azacytidine induces retinoic acid receptor RAR beta expression in human thyroid cancer cells

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We previously found that two cell lines (ARO and FRO) derived from anaplastic thyroid cancer and one (TT) deriving from medullary thyroid cancer did not respond to the retinoic acid (RA) treatment. We supposed that it was related to the lack of the RA receptor RAR beta mRNA expression. It has been suggested that the promoter methylation was one possible cause of loss of RAR beta expression.

We analyzed the methylation status of RAR beta promoter in ARO, FRO, TT, NPA and WRO (deriving from poor differentiated papillary carcinoma and follicular carcinoma) cell lines. We also analyzed the induction of thyroid specific genes expression (NIS, Tg, TSH-R, TPO, Pax-8, TTF-1) and of RAR alpha, beta and gamma genes expression, after treatment with the demethylating drug azacytidine.

The methylation of RAR beta promoter was analyzed by methylation specific PCR (MSP). Thyroid specific genes and RAR alpha, beta and gamma expression was studied by quantitative real time RT-PCR.

We observed that RAR beta promoter was methylated in ARO, but not in FRO and TT cells, which did not express RAR beta, nor in NPA and WRO, which expressed RAR beta mRNA. However, azacytidine treatment induced RAR beta mRNA expression in ARO, FRO and TT. The analysis of thyroid specific gene expression after azacytidine treatment showed the induction of TTF-1 in FRO and TT, TPO and NIS in TT cells.

In conclusion, in this study we showed that although methylation could explain the lack of RAR beta mRNA expression only in ARO, the azacytidine treatment induced RAR beta expression also in FRO and TT cell lines. These results open the possibility that a treatment with both azacytidine and RA may induce the re-expression of thyroid specific genes, not allowed by the treatment of either drug alone.

#### P875

##### Free thyroxine (FT4) is the analyte of choice in early gestation

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Thyroid physiology in early pregnancy is characterised by an increase of thyroxine binding globulin. This has been taken into account when using total T4 (TT4) if a 'T3 uptake test (TU<sub>p</sub>)' is used to derive the FTI. The

American Thyroid Association Guidelines (1990) recommend ceasing the use of uptake tests, although in the USA they are still widely used. The aim of this study is to compare the TT4 and TUp to the FT4 in early gestation. TT4, TUp, FT4 and TSH were measured by ADVIA Centaur (Bayer) in 980 samples from 11–16 weeks gestation.

Results indicated a curvilinear relationship between TT4 and TUp for TT4 concentrations between 0.1 and 15.0 µg/l. A plot of FTI vs FT4 showed 24 samples with low FTI but normal FT4 (up to 18 pmol/l). TSH plotted against FT4 showed a closer relationship than TSH vs FTI.

It is concluded that thyroid binding protein increase in gestation is not a single linear function (especially at extremes of thyroid function). Therefore, the FTI is not always compensated for by the TUp test. FT4 is therefore the analyte of choice for determining thyroid function in early pregnancy.

## P876

### Comparison of TPOAb assays in early pregnancy

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The presence of thyroid peroxidase antibodies (TPOAb) is a marker of autoimmune thyroid disease and, when present in early pregnancy, is predictive of postpartum thyroiditis. In the course of undertaking the Controlled Antenatal Thyroid Screening Study we have taken the opportunity to measure TPOAb in a large number of samples from 11–16 weeks gestation by 3 different assays.

The assays were (1) an in-house ELISA; (2) Bayer ADVIA Centaur anti-TPO assay, and (3) Abbott AXSYM anti-TPO assay.

Of 923 samples measured by in-house and Bayer 50 were abnormal by both assays, 14 abnormal by in-house and 11 by Bayer. The correlation between the two assays was 0.711. In 836 samples (a different group) assayed by in-house and Abbott, 90 were positive to both assays, 32 by in-house alone and 23 by Abbott alone. The correlation was 0.727. The correlation between Bayer and Abbott assays in 688 samples was only 0.2 possibly because there were very few positives in this group by either technique.

It is concluded that, while the performance of each method was satisfactory up to 5% of samples may give different results depending on the assay. Further exploration of the antigen antibody interaction in this regard is warranted.

## P877

### Age and gender differences and phenotypes of patients with autoimmune thyroid disease – The UK AITD consortium

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The cohort comprised 2296 patients with Graves' disease (GD) (1920 females, 376 males) and 361 with Hashimoto's thyroiditis (HT) (313 females, 48 males) recruited using standard diagnostic criteria for investigation of genetic susceptibility to AITD. We investigated variation in disease phenotype with age and gender, examining factors including biochemical severity, presence of goitre and presence of thyroid eye disease (TED) classified by NOSPECS score.

The median age at diagnosis was lower in GD females (40y [IQR 31-51]) than males (44.5[33-56],  $P < 0.001$ ), with a similar age difference for HT (females 41.0 [31-52], males 49.0[37.5-58],  $P = 0.01$ ). Biochemical severity (presentation serum  $fT_4$ ) was higher in both male and female subjects with GD and goitre compared with those without thyroid enlargement (females with goitre: median  $fT_4$  45.0 pmol/L[30.4-66.6] vs. 36.0[25-52],  $P < 0.001$ ; males with goitre:  $fT_4$  44.9[33-68.1] vs. 36.0[26-55],  $P = 0.009$ ), but there was no difference in severity of hypothyroidism (presentation serum TSH) in those with HT divided according to presence or absence of goitre (females with goitre: TSH 12.3 mIU/L[7.3-50] vs. 11.5[7.2-25.3],  $P = 0.178$ ; males with goitre: 20.5[17.0-38.0] vs. 19.4[9.6-100],  $P = 0.99$ ). Amongst those with GD, males were over-represented in

those with severe TED (NOSPECS score 4 or more) (males 116 of 365, 31.8% vs. females 410 of 1861, 22.0%,  $P < 0.001$ ) and the median age of males with severe TED was higher than females with severe TED (52.0[40-59], vs. 43.0[32-54],  $P < 0.001$ ). There was also a higher prevalence of severe TED in those presenting at older age with GD (males <40y 19.8% vs. males >40y 38.8%; females <40y 18.2% vs. females >40y 24.7%,  $P = 0.003$ ). AITDs are diagnosed older in males than females. Males with GD have biochemically more severe disease, and more severe TED. Gender thus has a major impact on disease phenotype in AITD with potential consequences for therapeutic management.

## P878

### Influence of family history on the age of presentation of AITD – the UK AITD consortium

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Autoimmune thyroid diseases (AITD), Graves' disease (GD) and Hashimoto's thyroiditis (HT) cluster in families. We investigated whether having a family history (FH) of AITD altered age of diagnosis. The cohort comprised 2296 GD (1920F,376M) and 361 HT (313F,48M) recruited according to standard diagnostic criteria. We compared age at diagnosis of GD or HT in those with or without a FH of overt hyper- or hypothyroidism (ascertained using a standardised questionnaire).

In males and females with GD, any FH of thyroid dysfunction reduced median age of diagnosis compared with no FH (females: 38.0y [IQR 30–48.25],  $n = 900$  vs. 43[32–53],  $n = 1022$ ,  $P < 0.001$ ; males: 41.0[31.25–53],  $n = 148$ , vs. 47.0[34.25–58],  $n = 228$ ,  $P = 0.008$ ). For HT, difference in age of diagnosis according to FH was females 39.0[29–50.8] vs. 45.0[34–56] ( $P = 0.002$ ), males 40.0[34.5–50] vs. 54.0[46.8–66], ( $P < 0.001$ ). For females with GD, a parental FH reduced age at diagnosis (parent with hyperthyroidism: 37.5 [31–46], parent with hypothyroidism: 35.0 [28–45], no FH 43.0[32–53],  $P < 0.001$ ). For GD males and HT probands, only parental hypothyroidism (not hyperthyroidism) reduced age at diagnosis (male GD parent with hyperthyroidism: 44.0[36.5–53.5], parent with hypothyroidism: 36.5[28–47], no FH: 47.0[34.5–58],  $P = 0.002$ ; female HT parent with hyperthyroidism: 42.0[32.5–53.5], parent with hypothyroidism: 35.0[26–46.5], no FH: 45.0[34–56],  $P < 0.001$ ; male HT parent with hyperthyroidism: 40.0[40–40], parent with hypothyroidism: 42.0[29.5–50], no FH: 54.0[46.8–66]). For GD and HT probands, FH in mother or father exerted a similar influence. Diagnosis of thyroid dysfunction in siblings did not affect age at diagnosis of AITD in probands. The greater the number of relatives with thyroid dysfunction, the lower the age of diagnosis of AITD (GD females, 0 relatives: 43.0[32–53], 1 relative: 39.0[29–49], 2 relatives: 38.0[30.5–49], 3 or more relatives: 35.0[27.26–45],  $P < 0.001$ ). FH of thyroid dysfunction (especially in parents) exerted a marked influence on age at diagnosis of both GD and HT, which could reflect ascertainment bias or genetic anticipation.

## P879

### Prevalence thyroid dysfunction and other autoimmune disorders within the families of subjects with autoimmune thyroid disease – the UK AITD consortium

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The association of autoimmune thyroid diseases (AITD) with other autoimmune disorders is well documented. Familial prevalence of thyroid dysfunction and other autoimmune diseases was examined in our cohort of 2296 with Graves' disease (GD) (1920 females, 376 males) and 361 with Hashimoto's thyroiditis (HT) (313 females, 48 males), probands being

recruited using standard diagnostic criteria. FH was ascertained using a standardised structured questionnaire.

A high proportion of GD and HT probands had parents with thyroid dysfunction (GD females,  $n = 363$ , 18.9%, GD males,  $n = 71$ , 18.9%; HT females,  $n = 95$ , 30.4%; HT males,  $n = 13$ , 27.1). The prevalence of siblings with thyroid dysfunction was similar in GD and HT (GD females,  $n = 241$ , 12.5%; GD males,  $n = 42$ , 11.2%; HT females,  $n = 41$ , 13.1%; HT males,  $n = 6$ , 12.5%). Risk to the siblings ( $\lambda$ (s)) of probands with AITD was derived using data from the Whickham survey (GD  $\lambda$ (s) = 8.50; HT  $\lambda$ (s) = 8.98).

Autoimmune diseases were more prevalent in AITD index cases than reported general population prevalence including type 1 diabetes (GD:  $n = 25$  cases, 3.2 fold higher prevalence; HT:  $n = 3$ , 2.4 fold higher), pernicious anaemia (GD:  $n = 31$ , 10.4 fold higher; HT:  $n = 12$ , 25.6 fold higher) and rheumatoid arthritis (GD:  $n = 67$ , 5.3 fold higher; HT:  $n = 12$ , 6.0 fold higher). Increased risk of Addison's disease was observed, especially in HT probands (HT:  $n = 10$ ; 308 fold higher; GD:  $n = 6$ , 29.0 fold higher). Age of diagnosis of AITD was decreased if family members had type 1 diabetes (GD females: 40.0y [30–49],  $n = 252$  vs. 43.0 [32–54],  $n = 716$ ;  $P = 0.009$ ), type 2 diabetes (GD females: 39.0 [30–49],  $n = 569$  vs. 43.0 [32–54],  $n = 716$  or rheumatoid arthritis (GD females: 39.0 [30–50],  $n = 507$  vs. 43.0 [32–54],  $n = 716$ ;  $P < 0.001$ ) when compared to probands without FH of autoimmune diseases. Striking family associations are consistent with a common genetic aetiology, across a wide range of autoimmune disorders.

## P880

### Do SNPs within the PTPN22 gene contribute to autoimmune disease via different mechanisms?

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Graves' disease (GD) is an autoimmune disorder of the thyroid gland. Autoimmune diseases cluster within families and individuals, leading to the hypothesis of common autoimmunity genes being shared between diseases. This has been confirmed through studies demonstrating association of the HLA region, the CTLA-4 gene and the PTPN22 gene with many disorders including GD and rheumatoid arthritis (RA). We and others have confirmed highly significant association of the R620W SNP of the PTPN22 gene with GD. Recent analysis of 37 SNPs in or near the PTPN22 gene in a cohort of patients with RA, revealed strong associations of further SNPs, some of which seem to be independent of the association seen with the R620W SNP. The aim of this study was to assess these novel RA-associated SNPs with GD in a case control cohort consisting of 768 GD patients and 768 controls. All subjects were UK white Caucasians and all gave informed written consent. The study was approved by the local ethics committee. Five haplotype tagged SNPs were selected for this study and were genotyped using Taqman<sup>®</sup> technology on an ABI 7900HT. No association of any of these SNPs was seen with GD at either the allelic ( $P = 0.292-0.815$ ) or genotypic ( $P = 0.458-0.7$ ) levels despite having greater than 99% power to detect an effect if present. Lack of replication may be attributed to the different geographical origins of the datasets used. Whilst both were white Caucasian, those in the RA study were from North America, whereas our dataset consisted of patients from the UK. However, it seems more likely that, although the PTPN22 gene appears to be acting as a general autoimmunity locus, the association of different SNPs with RA and GD suggests the mechanisms by which PTPN22 confers susceptibility may in part be disease specific.

## P881

### Screening of autoimmune thyroid disorders in pregnancy in a highland district

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On their first antenatal visit (median 10th gestational week), 977 pregnant women in one district were assayed for TSH (IRMA, non-pregnant normal range 0.15-5 mIU/l) and TPO-Ab (RIA). Abnormal values were found in

154 women (15.8%): positive TPO-Ab in 92 (9.4%), TSH  $< 0.15$  in 35 (3.6%), TSH  $> 5$  in 49 (5.0%). Of them, 128 women were examined in our endocrine clinic (median 15th week), incl. thyroid ultrasonography (7.5 MHz, Doppler), and assayed for TSH, free T4, free T3, and antibodies against TPO, TG and TSH-receptor. Diagnoses of Graves' disease (GD) and Hashimoto's thyroiditis (HT) were evaluated, and treatment was started in those with confirmed abnormal thyroid function. Positive predictive values (PPV) of the screening abnormalities for these diagnoses and for treatment initiation were calculated.

No active GD was found. Suppressed TSH could always be explained by HCG surge in early pregnancy. In contrast, HT was found in 99/128 (69.5%) positively screened, suggesting ca. 11% prevalence in our pregnant population. L-thyroxine treatment was indicated in 46/128 (35.9%) positively screened, corresponding to ca. 5.7% prevalence. Of the screening parameters, TSH  $> 5$  was a good predictor both of HT (PPV 33/40 = 82.5%) and treatment indication (PPV 28/40 = 70%), while positive TPO-Ab were better predictor of HT (PPV 71/81 = 87.7%) but less perfect for treatment initiation (PPV 32/81 = 39.5%).

TSH (non-pregnant range) and TPO-Ab combined made a cheap and useful screening tool for HT and hypothyroidism detection, bringing 16% pregnant women for further evaluation, with a high yield of HT and hypothyroidism. As in pregnancy TSH suppression is rarely caused by GD hyperthyroidism, and upper normal limit may be close to 2.5 mIU/l, our screening strategy may be modified – labelling as “positive” those with TSH  $> 2.5$  and omitting those with TSH  $< 0.15$ . Together with TPO-Ab, this would label “positive” 320 of our 977 pregnant women (32.8%).

The study has been approved by local Ethical Committee and supported by research project MZO 00179906.

## P882

### Gene expression profile in functioning and non-functioning nodules of autonomous multinodular goiters from an area of iodine deficiency

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Toxic multinodular goiter is a heterogeneous disease producing hyperthyroidism frequently found in iodine-deficient areas. The aim of this study was to evaluate the gene expression profile of functioning and non-functioning thyroid nodules present in the same thyroid gland from two patients affected by autonomous multinodular goiter by using the Affymetrix technology. Total RNA was extracted from nodular and non nodular tissues, retrotranscribed, and the double strand cDNA was synthesized. Biotinylated cRNA was hybridized on U133 set arrays. The acquired images were analyzed to obtain the expression levels of transcripts. The expression levels of a subset of genes were also evaluated by real time PCR.

In functioning nodules about 16% of genes were modulated while in non-functioning nodules only 9% of genes were modulated. In functioning nodules of both patients an up-regulation of cyclin D1 was observed. Interestingly the cyclin-dependent kinase inhibitor 1 gene was also up-regulated, suggesting a feedback from increased proliferation. In functioning nodules a marked decrease in blood cells (suggested from a decrease in blood cell antigens like FY and CXCR4) and a decrease of complement components (suggesting a decrease of macrophages) were observed. An increase of cellular fibronectin precursor gene was also found in functioning nodules suggesting an increase of endothelial cells. In both functioning nodules a reduced expression of Frizzled 1 gene of the Wnt signaling was observed.

In conclusion, the gene expression profile of functioning and non-functioning thyroid nodules co-existing inside the same gland demonstrated that these profiles are very similar, with an increase of genes implicated in cell proliferation, a decrease of blood cells and an increase of endothelial cells in functioning nodules.

## P883

### Comparison of efficiency of iodine-prophylaxis in healthy pregnant women and pregnant women with non toxic goiter, living in region with mild iodine-deficiency

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**Aim**

To estimate efficiency of 200 mkg/day iodine for prevention of development of hypothyroxinemia in pregnant women with non toxic goiter and without it, who were living in region with mild iodine - deficiency.

**Subjects and methods**

study group consists of 32 pregnant women: 16 - with non toxic goiter (8 from them took 200 mkg of iodine), 16 - without thyroid disease (8 from them took 200 mkg of iodine).

**Results**

pregnant women with goiter, who took iodine, had lower TSH level and smaller frequency of hypothyroxinemia in 3rd trimester, than pregnant women, who didn't take iodine (TSH – 0.8 mU/ml vs 1.8 mU/ml,  $P < 0.05$ ; fT<sub>4</sub> less than 10 percentiles 25% vs 87.5%,  $P < 0.05$ ). There wasn't difference between TSH and frequency of hypothyroxinemia in 3rd trimester in healthy pregnant women, who took iodine or didn't take it. But in 2nd trimester fT<sub>4</sub> less, than 10 percentiles, was determined in 25% of women without goiter and without iodine and in women with goiter. Nobody of pregnant women without goiter, taking iodine, had hypothyroxinemia in 2nd trimester. The frequency of threat of abortion also was dependent on iodine. It was determined in 50% of pregnant women with goiter taking iodine and 75% without iodine. In pregnant women without goiter the threat of abortion was 28.6% with iodine and 60% without iodine. The weights and highs of offsprings from women taking iodine was some greater, than without iodine.

**Conclusions**

addition of 200 mkg of iodine effectively prevent development of hypothyroxinemia in the 2nd trimester in pregnant women without goiter. But pregnant women with non toxic goiter need to higher doses of iodine for notice of hypothyroxinemia in region with mild iodine - deficiency. The iodine had favourable effect on pregnancy and weight and high of offspring.

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**P884**

**Gene expression profile in orbital fibroblasts from a tao patient before and after adipocytic differentiation**

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Thyroid associated ophthalmopathy (TAO) is a chronic autoimmune disorder characterized by an increased volume of the adipose/connective tissue in the human orbit. Fibroblasts from the connective orbital tissue are able to differentiate into mature adipocytes if grown in particular culture conditions. Aim of the present study was to determine the gene expression profile of orbital fibroblasts after adipocytic differentiation. Fibroblasts in primary culture were obtained from orbital connective tissue from a TAO patient. For adipocytic differentiation, fibroblasts were grown in the presence of biotin, pantothenic acid, transferrin, T3, insulin, cPGI<sub>2</sub>, dexamethasone and IBMX. Total RNA was extracted from orbital fibroblasts and double strand cDNA and biotinylated cRNA were synthesized. cRNA was hybridized on U133 set arrays following the Affymetrix protocol. The acquired images were analyzed to obtain the expression levels of different transcripts. The GeneMAPP software was used to insert each gene in the context of metabolic pathways. In differentiated fibroblasts 409 modulated genes were identified, the majority being down-regulated (97%) with respect to control fibroblasts. Among the down-regulated genes numerous factors involved in the inflammatory process and many chemotactic cytokines like macrophage inflammatory protein 2-alpha, interleukin-8, small inducible cytokine B6, macrophage inflammatory protein 2-beta were identified. Among the genes which were up-regulated, genes coding for nuclear protein involved in the control of DNA replication (DNA replication licensing factor MCM4) and in RNA synthesis (RNA polymerase II elongation factor ELL3) were identified. The gene that mainly increased its expression in differentiated fibroblasts was 60S acidic ribosomal protein P2 coding for a protein involved in protein synthesis. In conclusion, 97% of genes from fibroblasts subjected to adipocytic differentiation were down-regulated and they were represented mainly by genes involved in the inflammatory process. An increased expression of genes implicated in DNA and RNA synthesis was observed.

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**P885**

**Is subclinical hyperthyroidism associated with blood pressure and hypertension?**

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**Objectives**

While evidence for an increased risk of hypertension in both overt hyperthyroidism and overt hypothyroidism is consistent, the relation between subclinical hyperthyroidism and blood pressure has not yet received sufficient attention. We aimed to investigate possible associations of decreased serum thyrotropin levels and subclinical hyperthyroidism with blood pressure, pulse pressure, and the risk of hypertension.

**Design**

The population-based cross-sectional Study of Health in Pomerania.

**Setting**

We recruited a sample of general adult population of West Pomerania, a previously iodine-deficient region in Northeast Germany.

**Participants**

A study population of 4087 subjects (2050 females) without overt hyperthyroidism or increased serum thyrotropin levels was available for the present study.

**Main outcome measures**

Systolic and diastolic blood pressures were measured three times at the right arm of seated subjects. Systolic and diastolic blood pressures of  $\geq 140$  mmHg and  $\geq 90$  mmHg, respectively, were considered increased. Serum thyrotropin levels  $< 0.25$  mIU/l and  $< 0.1$  mIU/l were considered decreased and suppressed, respectively.

**Results**

Multivariable analyses revealed lower adjusted mean values for systolic blood pressure in subjects with decreased (132.9 mmHg, 95%-confidence interval 131.1 to 134.8 mmHg) versus normal serum thyrotropin levels (135.0 mmHg, 95%-confidence interval 134.4 to 135.6 mmHg,  $P = 0.04$ ). The adjusted mean values for diastolic blood pressure and pulse pressure did not differ significantly between both groups. There was also no association of suppressed serum thyrotropin levels with any of the endpoints investigated.

**Conclusions**

We conclude that subclinical hyperthyroidism as evidenced by decreased as well as suppressed serum thyrotropin levels and serum free thyroid hormone levels within the reference range is not associated with hypertension.

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**P886**

**Thyrotoxicosis in Childhood: UK and Ireland Surveillance Study 2004–2005**

SM Williamson & SA Greene

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Abstract withdrawn.

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**P887**

**PKA II mediates cAMP-dependent phosphorylation of CREB and activation of gene transcription in differentiated rat thyroid cells**

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Thyroid stimulating hormone (TSH) is the key modulator of thyroid cell function. Binding of TSH to its receptor (TSHR) results in an increase of cAMP intracellular concentration, which activates protein kinase A (PKA) to phosphorylate CREB and other substrates. Eventually, this leads to gene transcription modification associated with both proliferation and differentiation. Two major types of PKA (PKA I and II) exist in mammal cells, but their specific role in TSH signaling is poorly understood so far. Therefore, the aim of this study was to dissect PKA I/II effects on gene transcription in thyroid cells. In order to selectively activate PKA I or II, pairs of cAMP analogs with different affinities for sites A and B of PKA regulatory subunits were used. The effect of PKA I or II activation on the transcription of early genes, genes involved in cell replication and genes associated with thyroid



differentiated function was evaluated in FRTL5 cells by quantitative real-time PCR. The results indicate that PKA II, but not PKA I, stimulation can mimic the effect of TSH treatment on these genes. In addition, pre-treatment with a PKA II inhibitor was able to abolish the effect of TSH. Since CREB is the major transcriptional mediator of cAMP signal, we also evaluated the effect of PKA I or II activation on CREB phosphorylation. Also in this case, only PKA II activation lead to efficient CREB phosphorylation, and TSH-dependent activation was abolished by pre-incubation with the PKA II inhibitor. Finally, silencing of PKA regulatory subunit 2B (Prkar2b) by RNA interference resulted in down-regulation of proliferative genes. These results indicate that PKA II is the principal mediator of cAMP transcriptional effects in thyroid cells. Further studies are needed to evaluate the role of PKA I, which could play a role in post-transcriptional modifications rather than directly affecting gene transcription.

## P888

### Transcriptomic analysis of the oncocyctic signal in vesicular thyroid tumours

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Vesicular thyroid tumours evolve from benign to malignant states, respectively called adenomas and carcinomas by respect to the World Health Organisation classification. Borderline cases, atypical adenomas, cannot be clearly classified. When an abnormal cytoplasmic mitochondrial proliferation occurs into the tumour cells, these tumours called oncocytomas, are more aggressive. Oncogenic mechanisms and signal pathways of these tumours are not well known and the origin of the oncocyctic signal is uncertain. Early detection of this feature is needed to choose appropriate treatments for patients.

We studied the oncocyctic signal of 166 thyroid tissues by statistically analysing data from microarrays containing more than 9000 genes. We analysed normal thyroid tissues, micro and macrovesicular adenomas, oncocytic adenomas, atypical adenomas, as well as vesicular and oncocytic variant of vesicular carcinomas.

We propose a new classification of vesicular thyroid tumours using the false discovery rate method that increases the statistical power of multiple comparisons from microarrays data. The 368 genes that lead to distinguish the vesicular classes show different metabolic profiles on gene ontology analysis. We show that oncocyctic feature occurs before the oncogenetic signal by contrast to the current generalized hypothesis. Interestingly, the oncocyctic signal can be detected by the gene-expression levels of only few genes. This allows us to propose a gene-expression-based diagnostic predictor that may be useful for routine screening of oncocyctic tumours.

## P889

### Application of MRI in the differential diagnosis of different forms of Hashimoto's thyroiditis in patients from the iodine deficient region

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The purpose of this study was to describe MRI signs of Hashimoto's thyroiditis (HT) in patients from the iodine deficient region.

30 euthyroid patients with HT from the iodine deficient region and 5 healthy individuals were examined by MRI in FSE T1 and T2 pulse sequences. The diagnosis was confirmed according to the standard criteria.

The atrophic form of HT (2 cases) showed the reduction of thyroid volume with denominated lobularity of its structure and prevalence of low signal intensity on T1- and T2-weighted images. The focal form of HT (2 cases) demonstrated blurring sidebars and structure of smitten lobe with slightly hyperintensity on T2- and insignificant hypointensity on T1-mode. The hypertrophic form of HT (26 cases), as well as diffuse (20 cases) and diffuse-nodular (6 cases) variants noted 2 types of changes. The first type showed the increased thyroid volume, blurring and inhomogeneity of its structure with hyperintensity on T2 mode (5 cases). The second type was distinguished by presence of multiple hyperintensive spots in T1 and T2 pulse sequences with signs of the first type (11 cases). Obtained diversity of MRI appearances may be explained by existence of several histological forms of HT. Presence of hyperintensive spots on T1- and T2- weighted images can be explained by colloid accumulation in patients with HT, developed on the background of

endemic goiter (morphologically it was defined as chronic lymphocytic strumitis). All patients (11 persons) with the second type of changes were treated with physiological doses of iodine. Iodine assumption resulted in reliable reduction of thyroid volume and disappears of multiple hyperintensive spots in T1 and T2 pulse sequences.

MRI-tomography of thyroid may be useful in differential diagnosis of various forms of HT. Patients with HT defined as chronic lymphocytic strumitis should be treated with physiological doses of iodine.

## P890

### Immunohistochemical staining for thyroid peroxidase (TPO) of tru-cut biopsies from scintigraphically cold thyroid nodules

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#### Background

Scintigraphically cold nodules are hypofunctioning areas in the thyroid gland. These nodules are common and as the risk of malignancy is low, surgery is only necessary in very few cases. Fine Needle Aspiration Cytology (FNAC) is the standard method for detecting malignant tumours. However, FNAC is a difficult diagnostic procedure which often gives insufficient working material.

#### Materials and methods

A prospective study was designed to calculate the diagnostic sensitivity and specificity of tru-cut based diagnosis and TPO immunostaining for patients with a post-operative diagnosis. All patients with a scintigraphically, solitary cold thyroid nodule or a dominant cold nodule in a multinodular thyroid gland where included. The study was conducted during a 5-year period. All patients underwent a tru-cut biopsy. The patients were subsequently allocated to either thyroidectomy or follow-up. The tru-cut based diagnosis was evaluated by comparison with the post-operative diagnosis. The diagnostic sensitivity and the diagnostic specificity were calculated.

#### Results

A total of 427 patients with a cold thyroid nodule where included. The TPO staining of tru-cut biopsy was compared with tru-cut based diagnosis for all patients. 141 patients underwent thyroidectomy and 286 patients were followed-up during the 5-year study period. For the 141 operated patients the diagnostic concordance between tru-cut based diagnosis and post-operative diagnosis was 100% for Papillary- (17), Follicular- (1), Medullary- (1) and Undifferentiated- (3) Carcinomas. Only Adenomas and Minimally Invasive Follicular Carcinomas were not reliably diagnosed as either benign or malignant by tru-cut biopsy. The diagnostic sensitivity and specificity were 89% and 99%, respectively.

#### Conclusions

Histology of the nodule from tru-cut biopsy was easily determined. TPO-staining of tru-cut biopsic material had a diagnostic sensitivity of 89%, what made the difference was the Minimal Invasive Follicular Carcinomas (3). The diagnostic specificity was 99%, the only inconsistency was for Adenoma (1).

## P891

### Regulation of fibroblast growth factor receptor-1 (FGFR1) by thyroid hormone (T3): identification of a thyroid hormone response element (TRE) in the murine *Fgfr1* promoter

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T3 is essential for skeletal development and T3-actions in bone are mediated mainly by the nuclear receptor TR $\alpha$ 1. We previously identified that FGFR1 is a T3-responsive gene in osteoblasts by subtraction hybridization. In mice that harbor a dominant negative mutation PV, FGFR1 mRNA expression is reduced in TR $\alpha$ 1<sup>PV</sup> mutants that exhibit skeletal hypothyroidism and increased in TR $\beta$ <sup>PV/PV</sup> mice with skeletal thyrotoxicosis. In this study, primary calvarial osteoblasts from wild-type and TR $\beta$ <sup>PV/PV</sup> littermates were cultured in the absence and presence of T3. Quantitative RT-PCR revealed FGFR1 mRNA expression was increased 25-fold in TR $\beta$ <sup>PV/PV</sup> osteoblasts relative to wild-type cells maintained in T3, but elevated only 2.3-fold in the absence of T3. These data indicate that, although FGFR1 mRNA expression is increased in TR $\beta$ <sup>PV/PV</sup> osteoblasts in the absence and presence of hormone, the mutant cells remain exquisitely sensitive to T3, demonstrating

that T3-action in TR $\beta$ <sup>PV/PV</sup> osteoblasts is mediated via the unaffected TR $\alpha$ 1 protein. We next performed electrophoretic mobility shift assays to identify and characterize a TRE between positions -279/-264 of the *Fgfr1* promoter that showed strong and specific binding with TR/RXR heterodimers but weaker specific binding with TR/TR homodimers. Mutational analysis of the putative binding site attenuated TR/RXR and TR/TR specific binding and confirmed the identity of the TRE, which consisted of a classical direct repeat-4 (DR4) motif. We further performed transient transfection studies using T3-treated CV-1 cells co-transfected with TR $\alpha$ 1 or TR $\beta$ 1 and a range of CAT-reporter gene constructs linked to 5'-deletion mutants of the *Fgfr1* gene 5'-flanking region. Initial results indicate that truncation of the *Fgfr1* 5'-flanking region containing the putative TRE leads to a loss of T3-responsiveness without affecting basal promoter activity. These data indicate that the effects of T3 in the skeleton are mediated, at least in part, via TR $\alpha$ 1-dependent transcriptional regulation of FGFR1 expression.

## P892

### Subclinical hypothyroidism – how soon to treat?

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A lot of debate has been going on over when to treat subclinical hypothyroidism. Some studies plead to lower the consensus TSH cutoff value of 10mUI/L in order to improve general well-being and cardiovascular risk factors with early LT4 treatment. Our study aim was to assess progression of subclinical to overt hypothyroidism and to identify factors that would benefit from early treatment. A cohort of 76 patients with subclinical hypothyroidism was studied retrospectively, over a period of 36 months. We assessed general well being, features of metabolic syndrome, thyroid function tests, ATPO, cardiac function before and after treatment with levothyroxine. Subclinical hypothyroidism was classified as grade I (TSH 4.5-9.9 mUI/L), grade II (TSH 10–14.9 mUI/L), grade III (TSH 15–20 mUI/L), and sub-subclinical hypothyroidism (TSH 2.5-4.4 mUI/L associated with an high titres of ATPO). Substitutive treatment was commenced at a value of TSH above 6mUI/L. Results: Initial TSH value was a strong predictor for disease progression seen in 25% of patients with sub-subclinical hypothyroidism, 35% with grade I subclinical hypothyroidism, 43% with grade II and 69% with grade III. Patients with negative ATPO titres had a significant lower incidence of developing overt hypothyroidism than patients with positive titres. General well being improvement was reported in 52%, 16% felt worse and 32% reported no change in symptoms. There was no significant improvement in BMI and slight improvement in serum lipids (-3.8% for total cholesterol and -7.9% for LDL). The lipid lowering effect of LT4 was greater in the subset of patients with pre-treatment hypercholesterolemia. Myocardial contractility and diastolic function were subtly impaired in 15 patients with grade II and III subclinical hypothyroidism and improved with LT4 treatment. Substitutive treatment in subclinical hypothyroidism grade II and III has benefits on symptoms, lipid profiles and cardiac function. No evidence was found that grade I and sub-subclinical hypothyroidism would benefit from early treatment.

## P893

### Cytostatic effect of plitidepsin (Aplidin<sup>TM</sup>) in human undifferentiated (anaplastic) thyroid carcinoma

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Undifferentiated thyroid carcinoma is a highly aggressive human cancer with poor prognosis. A strong association has been observed between undifferentiated thyroid carcinoma and *TP53* mutations. Plitidepsin (Aplidin<sup>TM</sup>) a novel anticancer compound from a sea tunicate, has been reported to induce apoptosis independently of *TP53* status. We investigated the actions of plitidepsin in human thyroid cancer cells. Initially experiments using primary cultured cells from a differentiated (papillary) carcinoma, we found that 100 nM plitidepsin induced apoptosis, while lower doses were cytostatic. Since our aim was to study the effects of plitidepsin at clinically relevant concentrations, subsequent experiments were performed with a dosage regime reflecting plasma concentrations observed in previously reported clinical trials: 100 nM for 4h, followed by 10 nM for 20h (4100/2010 Plitidepsin). This plitidepsin dosage regime blocked the proliferation of a primary undifferentiated/anaplastic thyroid carcinoma culture obtained in our laboratory and of a commercial cell line (8305C) from an undifferentiated thyroid carcinoma; however, it did not induce apoptosis. The proportion of cells in the G1 phase was greatly increased, and the proportion in the S/G2-M phases greatly reduced, suggesting that plitidepsin blocks G1-to-S transition. Levels of the cyclinD1/cdk4/p21 complex proteins were decreased and levels of unphosphorylated Rb1 increased. Finally, we performed experiments to assess how rapidly tumor cells return to normal behavior after 4100/2010 plitidepsin treatment. Cells from undifferentiated tumors needed more than 3 days to recover logarithmic growth, and after 7 days cell number was still significantly lower than in control cultures. Together, our data show that plitidepsin is able to block *in vitro* cell-cycle progression at concentrations similar to serum concentrations observed *in vivo*, and that this effect is persistent for several days after plitidepsin removal. Whether plitidepsin will prove to be clinically useful in the treatment of undifferentiated thyroid cancers remains to be established.

## P894

### A case of severe thyrotoxic hypercalcaemia

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A 25-year-old teacher presented acutely unwell with palpitations, breathlessness, nausea and vomiting. 4 years previously, she was treated for Graves' disease with carbimazole for 15 months, and had since remained euthyroid. On examination, she was tachycardic but normotensive, tachypnoeic, had tremors of out-stretched hands, retraction of eyelids, and moderate sized smooth diffuse goitre with bruit. Initial blood tests revealed hyperthyroidism together with hypercalcaemia - the maximum measured corrected calcium level being 3.36 mmol/L (normal range: 2.08–2.49 mmol/L), raised phosphate 1.87 mmol/L (range: 0.75–1.55 mmol/L) and alkaline phosphatase 135 U/L (range: 35–125 U/L). Renal function was normal. She was treated with intravenous fluids, intravenous anti-emetics, carbimazole 60 mg/day and propranolol 160 mg/day. Further investigations included positive TSH – receptor antibodies at 17.8 u/L (range: 0–1 u/L), suppressed parathyroid hormone 3.0 pg/mL (range: 11–65 pg/mL), hypercalciuria 13.83-mmol/24 hr (range: < 7.5 mmol/24 hr), normal chest x-ray and negative myeloma screen. The metabolic abnormalities resolved after being rendered euthyroid.

Significant hypercalcaemia related to thyrotoxicosis alone is rare while milder elevations of serum calcium are well documented. However, the pathophysiology is not fully understood. Reduced intestinal calcium absorption, enhanced urinary and faecal calcium excretion, accelerated bone turnover and negative calcium balance seen in hyperthyroidism, all point towards bone as being the source of hypercalcaemia. A direct stimulatory effect of increased thyroid hormones on bone cells is the commonly proposed causative mechanism for hypercalcaemia; catecholamines'-induced bone resorption may also play a role. A combination of anti-thyroid medication and beta-adrenergic blockade was effective in our patient. The degree of thyrotoxic hypercalcaemia observed is possibly the highest identified in the contemporary literature.

## P895

### Efficacy of high dose radioiodine in the treatment of elevated serum thyroglobulin in patients with differentiated thyroid carcinoma and negative whole body iodine scan

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**Introduction**

In the management of patients with differentiated thyroid carcinoma (DTC), serum thyroglobulin (Tg) levels are often well correlated with whole-body radio-iodine scanning (WBS) results. However, occasionally a mismatched result, increased Tg with negative WBS, is encountered. High doses of radio-iodine (RAI) have been suggested as a therapeutic choice with controversial results. Our aim was to evaluate the therapeutic benefit of this approach.

**Method**

We studied 32 DTC patients (12M and 20F; mean age: 42.3 yr) with elevated Tg level and negative WBS who had been treated with high-dose RAI. With a mean follow-up of 25.6 months (all follow-ups > 11months), serum Tg, anti-Tg and TSH levels, WBS results, clinical, radiographical and pathological findings following treatment were recorded.

**Results**

The mean pre-therapy serum Tg with the patient off-treatment was  $152 \pm 119.0$  ng/ml. Although there was a mild trend toward the increase in serum Tg level in the first post-treatment year, the difference did not reach statistical significance. 53.8% of patients had foci of radiotracer uptake in the post-treatment scans. At the end of the follow-ups, 22 patients (68.7%) categorized as non-respondent to RAI therapy (any change or elevation in Tg level or radiological and pathological evidences of progression), four patients (12.5%) as partial-respondent (transient reduction but not normalization of serum Tg) and six patients (18.7%) as respondents to RAI therapy (normalization of serum Tg with no other evidences of remnant disease). In nine of these ten partial- and complete-respondents, the reduction or normalization of serum Tg had occurred in the first post-treatment year.

**Conclusion**

We recommend that in DTC patients with elevated Tg and negative WBS, only one course of RAI therapy should be examined and if satisfactory response (reduction or normalization of serum Tg) was not achieved, repeated RAI therapies are not logical and other therapeutic methods should be applied.

**P896****Isometric tension studies in a transgenic mouse model of thyroid hormone resistance**

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Thyroid hormone resistance (THR) is associated with elevated FT4 and FT3 with inappropriately high TSH levels. T3 action is mediated by two types of thyroid hormone receptor, encoded by alpha and beta genes. We evaluated the endothelial function of a THR transgenic mouse model with a targeted mutation in the thyroid hormone beta receptor gene *ex vivo*.

Isometric tension of aortic rings of 10 mice of each genotype (TRb<sup>PV/PV</sup>, TRb<sup>PV/+</sup>) were compared with controls (TRb<sup>+/+</sup>) after being mounted in Krebs' buffer in a myograph. After resting a dose response to contraction with phenylephrine [PE] (half log increments from  $10^{-9}$  to  $10^{-6}$  M) was measured. After maximal contraction, relaxation to acetylcholine [ACH] ( $10^{-9}$  to  $10^{-5}$  M) was measured and a further dose relaxation response to sodium nitroprusside [SNP] ( $10^{-9}$  to  $10^{-6}$ ) was performed.

A difference in the peak contraction obtained with PE ( $P = ns$ ) was found between genotypes. The average relaxation to ACH (an endothelium dependent relaxation) was lower in TRb<sup>PV/PV</sup> (62.1%) compared to both TRb<sup>PV/+</sup> (70.6%;  $P < 0.01$ ) and TRb<sup>+/+</sup> (74.9%;  $P < 0.001$ ). Relaxation to SNP, (endothelium independent smooth muscle relaxation) differed between TRb<sup>PV/PV</sup> and TRb<sup>PV/+</sup> (105%, 130%  $P < 0.01$ ).

The EC<sub>50</sub> (concentration required to achieve 50% relaxation) to ACH was higher ( $1.1 \times 10^{-6}$   $P < 0.05$ ) in TRb<sup>PV/PV</sup> compared to TRb<sup>PV/+</sup> or TRb<sup>+/+</sup> ( $1.2 \times 10^{-7}$ ,  $1.1 \times 10^{-7}$   $P = ns$ ). In contrast in TRb<sup>PV/PV</sup> mice in response to SNP  $10^{-9}$  to  $10^{-5}$  EC<sub>50</sub> was not different between genotypes. T4 levels were 10 fold higher in TRb<sup>PV/PV</sup> c.f. TRb<sup>+/+</sup> and TSH levels 110 fold higher in the same genotypes.

The impaired vascular response of THR may be partly due to the beta mutation though the role of T4 and TSH levels needs further exploration.

**P897****The GH response to ghrelin in humans is reduced in conditions of hyper- and hypothyroidism**

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Both hyper- and hypothyroidism show reduced spontaneous and GHRH-stimulated GH secretion. Although impaired GHRH secretion and activity as well as reduced GH pituitary synthesis have been reported in these pathological conditions, a definitive description of the underlying pathophysiological mechanisms have not been provided yet. Ghrelin elicits a potent GH-releasing effect partially mediated by a functional somatostatin antagonism and a synergic interaction with GHRH. Moreover, the functional integrity of GHRH neurons is essential for ghrelin's GH releasing effect. We aimed to investigate the GH releasing effect of ghrelin in conditions of naïve overt altered thyroid function in humans. To this aim, in 8 patients with primary autoimmune hypothyroidism (HYPO; age [mean  $\pm$  SEM]:  $41.8 \pm 6.9$  yr.; BMI:  $27.1 \pm 2.2$  kg/m<sup>2</sup>; TSH:  $16.5 \pm 5.8$  mU/ml; fT3:  $3.1 \pm 1.2$  ng/l; fT4:  $8.7 \pm 1.6$  ng/l), 8 patients with thyrotoxicosis due to Basedow Disease (HYPER; age:  $45.3 \pm 7.6$  yr.; BMI:  $23.5 \pm 3.0$  kg/m<sup>2</sup>; TSH:  $0.0 \pm 0.0$  mU/l; fT3:  $6.5 \pm 3.0$  ng/l; fT4:  $25.0 \pm 6.4$  ng/l; TRAb:  $47.5 \pm 7.6$  U/l) and 8 control subjects (NS; age:  $35.1 \pm 5.9$  yr.; BMI:  $22.1 \pm 1.9$  kg/m<sup>2</sup>) we studied the effects of the acute iv as a bolus administration at 0' of acylated ghrelin (AG;  $1.0$   $\mu$ g/kg) and placebo (saline; 3 ml) on circulating GH levels assayed every 15' up to 90'. The study had been approved by local Ethical Committee. In all the subjects, AG increased GH levels ( $P < 0.01$ ). Notably, both in HYPO ( $\Delta$ peak:  $30.4 \pm 6.2$   $\mu$ g/l;  $\Delta$ AUC:  $1458.7 \pm 364.0$   $\mu$ g/l/h) and in HYPER ( $\Delta$ peak:  $39.6 \pm 4.1$   $\mu$ g/l;  $\Delta$ AUC:  $1924.9 \pm 252.7$   $\mu$ g/l/h) the GH response to AG was lower ( $P < 0.01$ ) than in NS ( $\Delta$ peak:  $79.8 \pm 8.2$   $\mu$ g/l;  $\Delta$ AUC:  $4360.3 \pm 552.5$   $\mu$ g/l/h). This study shows that in presence of altered thyroid function, the GH response to ghrelin is reduced. Such an impairment could result from the above mentioned functional alterations of the GHRH/GH axis, nevertheless the existence of a specific reduced sensitivity to the GH releasing effect of ghrelin as an adjunctive mechanism of hyposomatotropism in thyroid disease is worthy being further investigated.

**P898****Multicenter evaluation of the Abbott ARCHITECT® anti-Tg and anti-TPO assays**

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The purpose of these studies was to conduct a multi-center evaluation of the performance of the new Abbott ARCHITECT Anti-Tg and Anti-TPO assays. Both assays are two-step, chemiluminescent, microparticle-based immunoassay for the quantitative determination of either the IgG class of thyroglobulin autoantibodies (anti-Tg) or the IgG class of thyroid peroxidase autoantibodies (anti-TPO) in human serum and plasma. The ARCHITECT Anti-Tg and Anti-TPO assays are designed to have a dynamic range of 0–1000 IU/mL and are standardized against WHO MRC 65/93 (Anti-Tg) and MRC 66/387 (Anti-TPO). Evaluation studies for these assays were conducted at four European sites. Analytical sensitivity, at 95% confidence, was determined to be 0.018 IU/mL (Anti-Tg) and 0.08 IU/mL (Anti-TPO) for 4 runs each. Total variability, determined using the 5-day NCCLS protocol EP10-A2 for low and high controls, ranged from 2.52–8.51% CV for Anti-Tg and 1.31–10.00% CV for Anti-TPO. For method comparison, at minimum 200 patient samples were tested on both ARCHITECT Anti-Tg or Anti-TPO assays and several competitor assays; Concordance was determined as follows: to Centaur® 92.8% for Anti-Tg and 96.6% for Anti TPO; to Autodelphia® 91.5% for Anti-Tg and 98.5% for Anti-TPO, DPC Immulite® xx% for Anti-Tg and xx% for Anti-TPO, Liason® xx% for Anti-Tg and xx% for Anti-TPO, AxSYM® xx% for Anti-Tg and xx% for Anti-TPO and Brahms xx% for Anti-Tg and xx% for Anti-TPO. Based on our evaluation we conclude that the ARCHITECT Anti-Tg and Anti-TPO assays are sensitive, precise and accurate assays, that show good concordance to other test methods.



**P899**

**Autoantibody profile in patients with Graves' disease and thyroid-associated ophthalmopathy**

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Graves' disease (GD) and thyroid-associated ophthalmopathy (TAO) are often observed together. In purpose to elucidate the pathogenetic relationships between these two autoimmune disorders we investigated profile of different autoantibodies in patients with GD without TAO ( $n = 38$ , group 1), GD with TAO ( $n = 40$ , group 2), and in healthy people ( $n = 124$ , control group). Autoantibodies to thyroid gland, eye muscle cell membranes (Ab-EMCM) and skin fibroblasts were detected in sera. The frequency of detected antibodies to TSH-receptor was: group 1–89.1%, group 2–94.4%, and were not found in control group. The presence of antibodies to thyroidperoxidase was: group 1–77%, group 2–52.5%, control group 4.2%. The frequencies of antibodies to thyroglobulin were 48.2%, 35.7% and 6.4% respectively. AB-EMCM were found in 18% of patients from group 1, 81.1% – from group 2 and 8.5% – from control group. The frequencies of antibodies to fibroblasts were 45.7%, 36.1% and 7.2% respectively. The frequency of detected antibodies to TSH receptor, thyroidperoxidase, thyroglobulin and fibroblasts was significantly higher in groups 1 and 2 compared to control group ( $P < 0.005$ ), and there was no difference between groups without or with TAO. However, concentration of the antibodies to TSH receptor was significantly higher in group 1 compared to group 2 ( $P < 0.01$ ). In contrast, the frequency of AB-EMCM was significantly higher in group 2 than in group 1 and controls ( $P < 0.005$ ). There was a direct correlation between concentration of AB-EMCM and the severity of TAO clinical symptoms ( $r = 0.8$ ,  $P < 0.01$ ). In conclusion, antibodies to fibroblasts are probably not specific for TAO and their appearance reflects polyclonal activation of antibodygenesis in patients with Graves' disease. According to our data, we suggest that antibodies to eye muscle cell membranes may play an important part in the pathogenesis of thyroid-associated ophthalmopathy.

**P900**

**Treatment of disseminated non-radioiodine - avid papillary thyroid cancer with <sup>90</sup>Y-DOTA-TATE preparation**

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**Introduction**

Lack of radioiodine uptake in Differentiated Thyroid Carcinoma is a huge diagnostic and therapeutic problem. This sign is associated with worse prognosis. DOTA-TATE preparation is a somatostatin analogue coupled with <sup>90</sup>Y which emits  $\beta^-$  radiation. Its use in a treatment depends on excessive expression of somatostatin receptors (SSTR) in malignant tumours.

**The aim of the study**

to present a possibility of treatment of non iodine – avid disseminated papillary thyroid cancer with <sup>90</sup>Y-DOTA-TATE preparation. The local Ethical Committee approval has been obtained before.

**Material and methods**

73 y. o. male with disseminated non-radioiodine - avid papillary thyroid cancer has been treated by <sup>90</sup>Y-DOTA-TATE. Presence of SSTR was confirmed with use of <sup>99m</sup>Tc-HYNIC-TATE (Tektrotyd) preparation earlier. Both of these radiopreparations are produced by POLATOM – Swierk/Poland. In March, April and July 2005 this patient was treated with the <sup>90</sup>Y-DOTA-TATE three times (3.7 GBq per dose). Nephroprotection was obtained by 10 hours infusion of 1000 ml 10% amino acids preparation with max. speed 120 ml/h.

**Results**

There weren't observed any serious adverse events after <sup>90</sup>Y-DOTA-TATE treatment. Non significant, transient decrease of thrombocytes and lymphocytes was observed. Thyreoglobuline serum concentration increase was considered as a sign of cancer destruction. The reduction of neoplasm infiltrations in site of thyroid removal and in mediastinum and lungs in CT scans was observed two months after the third <sup>90</sup>Y-DOTA-TATE injection. Histopathological estimation of excised the new superficial meta tumours of the neck region showed mainly necrosis and calcifications.

**Conclusions**

<sup>90</sup>Y-DOTA-TATE preparation seems to be useful and promising in treatment of non – radioiodine – avid papillary thyroid cancer.

**P901**

**Hashimoto's thyroiditis in the children: clinical features at presentation**

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Hashimoto's thyroiditis (HT) is the most frequent thyroid disease in children, in areas of the world with sufficient iodine intake.

**Aim**

To study the clinical features of HT in children at presentation.

**Material and method**

We studied 99 children with HT, 76 (76.8%) girls and 23 (23.2%) boys, with mean chronological age  $10.7 \pm 2.6$  ys (3.2–15.6 ys). The diagnosis of HT was established from the high titers of antithyroid antibodies (aTPO-Ab, aTg-Ab) and the absence of other thyroid disease associated with positive antibodies. Thyroid volume was estimated with ultrasound, and compared to WHO reference values. T4 and TSH were measured in all children.

**Results**

Thirty six children (36.4%) were prepubertal and 63 (63.6%) pubertal. Euthyroidism was present in 70 (70.7%) children, subclinical hypothyroidism in 20 (20.2%), hypothyroidism in 6 (6.1%) and hyperthyroidism in 3 (3%). aTPO-Abs were positive in 78 (78.8%) and aTg-Abs in 82 (82.8%) children. In 6 (6.2%) children the thyroid volume by ultrasound was less than the 50th centile for their age, in 46 (46.4%), between the 50th and 95th centile and in 47 (47.4%) greater than the 95th centile. Girls significantly outnumbered boys,  $\chi^2 = 28.3$ ,  $P < 0.001$ .

**Conclusion**

HT in children usually presents with euthyroidism. Subclinical hypothyroidism is present in 1/5 of the children. Hypothyroidism and hyperthyroidism are rare at presentation. About half of the children present with significant increase in thyroid gland volume, greater than the upper range for their age.

**P902**

**Corticotherapy alone or corticotherapy combined with orbital radiotherapy in severe active Graves' ophthalmopathy: therapeutic outcome in 905 patients**

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The aim of the study was to compare efficacy and tolerability of systemic corticotherapy alone to corticotherapy combined with orbital radiotherapy in severe active Graves' ophthalmopathy. We studied 905 patients, aged 35-73 years (mean  $58.2 \pm 12$ ), with clinical activity score above 4 and ATA class 3c to 6. Therapeutic outcome was assessed by change in ophthalmopathy index. For 4 weeks all subjects received corticotherapy: 869 - oral prednisone 60–100 mg daily and 36 patients with optic neuropathy -methylprednisolone iv 1000 mg for 7 days followed by oral glucocorticoids. Afterwards the subjects were randomized to group 1 (331 cases) – receiving gradually reduced corticoids for 20 weeks and group 2 (574 cases) - treated with glucocorticoids and retrobulbar megavoltage orbital radiotherapy of 20 Gy. Corticotherapy alone resulted in significant reduction of ophthalmopathy index (7.4 vs. 4.4;  $P < 0.001$ ) with special effectiveness on soft tissue and optic nerve (80%, 81% of patients respectively) and unimpressive proptosis diminution and ocular motility improvement (52%, 50% respectively). In group 2 ophthalmopathy index decreased similarly (8.0 vs 4.7;  $P < 0.001$ ). Recurrence rate of severe phase during the first year of follow-up was 54% in group 1 and 15% in group 2. In those cases courses of corticotherapy were repeated. Side effects occurred in 42% patients during the first course of corticotherapy and in 59% during repeated courses ( $P < 0.001$ ). In particular, diabetes occurred in 13% of cases. Early postradiation exacerbation of soft tissue inflammation was noticed in 15.5% of patients. The combined therapy enabled reduction of treatment time by 30% and glucocorticoid dosage by 50%. We conclude that, compared to glucocorticoids alone, corticotherapy combined with orbital radiotherapy results in a better long-term effectiveness and tolerability in treatment of severe active Graves' ophthalmopathy.



**P903****The effects of treatment with L-thyroxin on level of prolactin, CRP and insulin in premenopausal women with subclinical hypothyroidism**

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We examined the effects of L-thyroxin on level of prolactin (PRL), CRP (C-reactive protein) and insulin in premenopausal women with subclinical hypothyroidism (SH). The study included 21 women ages  $41.3 \pm 3.5$  years with SH (TSH > mIU/ml with normal level of T3 and T4).

Laboratory evaluation included basal hormone (serum free T3, free T4, TSH, PRL, insulin, cortisol, ACTH, GH, FSH, LH, E2, thyroid antibodies and TGL), level of CRP and level of lipids.

Radiological investigation included thyroid echosonography and scintigraphy. Percentile, average and correlation analysis have been utilized in statistical analysis. Eleven patients had hyperprolactinaemia, 14 patients had fasting hyperinsulinaemia and 8 patients had amenorrhoea. All patients were treated with low dose of L-thyroxin (25–50 ug). After the six months treatment, women had normal or limited TSH, level of PRL significantly decreased ( $839 \pm 145$  vs.  $421 \pm 89$  uIU/ml), level of CRP ( $5.9 \pm 1.4$  vs.  $2.7 \pm 0.9$  mg/l) and fasting insulin ( $231 \pm 85$  vs.  $153 \pm 42$  pmol/l) decreased as well. Six of eight women with amenorrhoea had regular menstrual cycles. The correlation between TSH and amenorrhoea was positive and significant ( $r = 0.43$ ). The correlation between TSH and PRL, TSH and CRP, TSH and insulin was positive, as well.

The normalization of TSH on one side resulted in decrease of level of PRL, FSH and LH and on the other side in increase of E2. Higher CRP associated with fasting hyperinsulinaemia before insulin resistance has been evidenced in most patients with SH. These data support an important role of treatment of SH in premenopausal women. In sum, the screening of TSH in all women older than 35 years is necessary.

**P904****Modifications in the frequency of presentation and the TNM stage at diagnosis of thyroid carcinoma in the last decade in Valencia (Spain)**

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**Aims**

The aim of the present study was to evaluate the relative frequency of histological categories of thyroid malignant neoplasms. Another aim was to evaluate the TNM stage differentiated thyroid carcinoma (DTC) at the time of initial presentation in Valencia Community (Spain).

**Methods**

Histopathology and TNM stage thyroid carcinomas diagnosed of thyroid carcinoma diagnosed between 1998 and 2005 in our endocrinology service, were recorded. The sample was divided in: 1978–1994 (107 cases) and 1994–2005 (216 cases).

**Results**

The frequency of histological subtypes during both periods is shown in the table below:

	1978-1994		1994-2005	
	Absolute frequency	Relative frequency	Absolute frequency	Relative frequency
Papillary*	80	74%	207	81%
Follicular	16	15%	15	6%
Hurtle	5	5%	10	4%
Medullary	3	3%	8	3%
Anaplastic	2	2%	3	1%
Lymphoma	1	1%	3	1%
Others	0	0%	10	4%
Total	107	100%	256	100%

Regarding: DTC, the stage at diagnosis during both period is shown in the table below:

	T1	T2	T3	T4
1978- 1994	77%	14%	6%	3%
1994-2005	90%	7%	2%	1%
	No	N1	Mo	M1
1978- 1994	81%	19%	96%	4%
1994-2005	91%	9%	99%	1%

Papillary-follicular carcinomas have been included in papillary carcinomas. Conclusion

We have found an increase in the relative frequency of papillar carcinoma over follicular carcinomas. The relative frequency of the other histological subtypes has not changed. DTC have been diagnosed earlier in the second period probably owing to an increase in cervical image testing performed for extrathyroid pathology. Our data support the growing utility of low-risk DTC protocols.

**P905****Pre-treatment with rhTSH allows effective <sup>131</sup>I therapy in patients with multinodular toxic goiter and low radioiodine uptake**

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**Aim**

Estimation of the effectiveness of a single low dose 0.05 mg rhTSH in increasing radioiodine uptake (RAIU) before <sup>131</sup>I therapy for multinodular toxic goiter with low initial RAIU.

**Patients**

24 patients (21 women, 3 men) with multinodular toxic goiter without prior antithyroid drug therapy. The initial 24 h RAIU was  $21.1 \pm 8.44\%$ ; range, 6–39% and thyroid volume  $64.14 \pm 22.49$  ml; range, 24.8–109 ml.

**Methods**

A single dose of 0.05 mg rhTSH (Thyrogen, Genzyme) was given im. 24 h later diagnostic activity of <sup>131</sup>I was administered and RAIU after 6, 24, 48 and 72 h was estimated. On the 4th day therapeutic dose of <sup>131</sup>I adjusted to the increased RAIU was given. Therapeutic dose was calculated to deliver 120–150  $\mu$ Ci <sup>131</sup>I/ml thyroid tissue retained at 24 h.

**Results**

The significant 3.2-fold increase in 24 h RAIU to  $66.9 \pm 13.05\%$  after 0.05 mg rhTSH was noted. It allowed to reduce <sup>131</sup>I therapeutic dose from the mean 46.6 mCi to 14.3 mCi. Shortly after rhTSH administration (24, 48 h) serum fT3 elevated from  $3.08 \pm 0.8$  pg/ml to peak value  $6.02 \pm 1.36$  pg/ml ( $P < 0.001$ ) and serum fT4 augmented from  $19.16 \pm 3.59$  pmol/l to peak value  $29.04 \pm 10.11$  pmol/l ( $P < 0.001$ ). Clinical signs of thyrotoxicosis exacerbation were mild to moderate. There was 1 case of painful thyroid enlargement without respiratory tract obstruction 24 h after rhTSH administration. Twelve months after <sup>131</sup>I therapy 75% of patients were euthyroid, 6.5% hypothyroid and 18.5% still thyrotoxic. There was 45% reduction of thyroid volume.

**Conclusions**

Administration of a single dose of 0.05 mg rhTSH enhances 3.2-fold RAIU in patients with multinodular toxic goiter. This method enables significant <sup>131</sup>I dose reduction but should be carefully undertaken because of the risk of thyrotoxicosis exacerbation and thyroid volume enlargement.

**P906****Repression of the human NIS upstream enhancer (hNUE) by PTTG**VE Smith, K Boelaert<sup>1</sup>, AL Stratford<sup>1</sup>, SR James<sup>1</sup>, R Susarla<sup>1</sup>, T Kogai<sup>2</sup>, MC Eggo<sup>1</sup>, JA Franklyn<sup>1</sup> & CJ McCabe<sup>1</sup><sup>1</sup>Department of Medicine, University of Birmingham, Birmingham, United Kingdom; <sup>2</sup>Department of Medicine, UCLA School of Medicine, Los Angeles, United States.

The sodium iodide symporter (NIS) mediates the uptake of iodide into thyroid follicular cells. The pituitary tumor transforming gene (PTTG) is a multifunctional oncogene which stimulates expression of fibroblast growth factor-2 (FGF-2) via the PTTG binding factor (PBF). PTTG, FGF-2 and PBF are all up-regulated in thyroid cancer. PTTG and FGF-2 inhibit NIS mRNA expression and iodide uptake in rat thyroid FRTL5 cells. We have

shown previously that both PTTG and PBF repress NIS mRNA expression and iodide uptake in primary human thyroid cultures. To determine whether the regulation of NIS by PTTG and PBF is a direct transcriptional event, we co-transfected primary human thyroid cultures and FRTL5 cells with PTTG and PBF plasmids along with luciferase reporter constructs containing (1) the human NIS proximal promoter, (2) an 879 bp fragment including the human NIS upstream enhancer (hNUE) coupled to the human NIS proximal promoter and (3) the hNUE fragment coupled to the SV40 promoter. In FRTL5 cells, PTTG repressed promoter activity via the hNUE element, whether driven by the SV40 (30% repression;  $P < 0.001$ ;  $N = 9$ ) or the NIS proximal promoter (19% repression;  $P < 0.001$ ;  $N = 9$ ). In primary thyroid cells, PTTG similarly repressed promoter activity via the hNUE element when coupled to the SV40 promoter (45% repression;  $P = 0.003$ ;  $N = 6$ ). PBF also repressed hNUE activity when coupled to either the SV40 (29% repression;  $P = 0.04$ ;  $N = 6$ ) or the NIS proximal promoter (30% repression;  $P = 0.016$ ;  $N = 6$ ). In conclusion, the repression of NIS by PTTG and PBF in human thyroid cells occurs via its upstream enhancer region, hNUE. In thyroid cancer, where PTTG and PBF are up-regulated, NIS activity, and hence radioiodide uptake, are likely to be reduced via a promoter-specific mechanism.

### P907

#### Comparison of cytogenetic damage in patients with differentiated thyroid cancer receiving ablative $^{131}\text{I}$ therapy in the hypothyroid status versus the euthyroid status (rhTSH assisted treatment)

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The long-term risk of carcinogenic and genetic damage following the treatment with  $^{131}\text{I}$  for differentiated thyroid cancer (DTC) is quite low. The hypothyroid status (HS), at the time of  $^{131}\text{I}$  administration, decreases the renal clearance of  $^{131}\text{I}$  and increases the whole-body, blood and bone marrow irradiation, unlike the euthyroid status (ES). Chromosome aberrations (CA) in peripheral lymphocytes reflect blood cells exposure to irradiation. This study aimed at comparing CA following ablative  $^{131}\text{I}$  therapy in 20 patients with DTC:

#### Group A

9 patients (8 f, 1 m), aged 28–67 yr (median 48) treated after L-T4 withdrawal;  $^{131}\text{I}$  activity: 3.7 GBq  $\times$  6 pt, 4.8 GBq  $\times$  2, 5.5 GBq  $\times$  1.

#### Group B

Patients (6 f, 5 m), aged 19–75 yr (median 52) treated after rhTSH standard protocol on L-T4 (3.7 GBq  $\times$  11 pt).

CA were scored in peripheral lymphocytes obtained before and on the 15th and 45th day after the treatment according to conventional cytogenetic. Lymphocytes were stained routinely (100 cells each time).

We observed unstable anomalies (dicentric, chromosome breaks, chromatid breaks, premature centromeric divisions) and numeric anomalies (polyploidy, aneuploidy). No correlation was found between the observed frequency of CA and the dose to the bone marrow per unit of administered radioactivity. The percentage of total CA increased significantly after the treatment in both groups, but the net increase of CA and unstable anomalies on the 15th and 45th day after the treatment were not statistically significant between the two groups. The percentage of numeric anomalies, not statistically different before the treatment, became significantly lower on the 15th ( $P < 0.005$ ) and on the 45th ( $P < 0.005$ ) day after the treatment in group B.

#### Conclusions

This study shows that the dynamics of radiation-induced CA *in vivo* is influenced by the thyroid hormone status and the kinetic of  $^{131}\text{I}$  parameters.

### P908

#### Correction of hypomagnesaemia improves the symptoms of hypocalcaemia after thyroidectomy

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IGF-I is an important measure of disease activity in states of GH deficiency and excess and therefore it is interesting that a significant proportion of AsGHD have normal IGF-I levels. In an attempt to understand the factors

determining IGF-I values in patients with severe AGHD we have compared age-, gender- and BMI-matched GHD patients (defined by the international consensus criteria, peak GH  $< 3$  ng/mL) with low and IGF-I (Norms:  $n = 5$ , 3 males, age 42 (31–54), BMI = 32.8 (22.4–33.3) kg/m<sup>2</sup>; Los:  $n = 5$ , 2 males, age 43 (38–53), BMI 36.5 (30.9–39.9) kg/m<sup>2</sup>).

Mean GH was calculated from a 24 h profile (20 minute sampling). GH was assessed using a sandwich-type immunoassay (intra- and inter-assay CV  $< 7\%$ , limit of detection 0.1 ng/mL). IGF-I was measured by immunoradiometric assay (sensitivity 4.4–5.2%, specificity of 5.7–7.4%, lower limit of detection 6 mcg/l). Statistical analysis was performed using GraphPad Prism software.

There was no difference between the groups in peak GH during the diagnostic stimulation test (0.3 ( $< 0.3$ –1.2) ng/mL v 0.3 ( $< 0.3$ –0.8) ng/mL,  $P = 0.84$ ), age of onset (Norms and Los childhood/adult = 3/2), prior irradiation status (Norms = 3, Lows = 2) and current DEXA body composition (total fat 31106(20322–45725) g v 35548(29159–46072),  $P = 0.22$ ; %trunk fat 33.5(30.4–46.9)% v 39.2(28.9–42.2)%,  $P = 1$ ; lean mass 50183(46656–66119) g v 55299(45239–74339) g,  $P = 0.84$ ). However, Los had more other pituitary hormone deficiencies than Norms (3 (1–3) vs 1 (0–1),  $P = 0.03$ ).

Mean 24 h GH tended to be lower in patients with low IGF-I levels (Norms: median 0.1, range  $< 0.1$ –0.13 ng/mL v Los: all  $< 0.1$  ng/mL,  $P > 0.05$ ).

In conclusion, other than IGF-I levels the only differences between the cohorts were a tendency for more pituitary hormone deficiencies and lower 24 hr GH secretion in those with a low IGF-I. The significance of these observations requires further investigation.

### P909

#### The thyroid gland and its selenoenzymes are preferentially supplied in selenium-deficient transgenic mice

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Selenium (Se) is indispensable for enzymatic activities of glutathione peroxidases (GPx) involved in antioxidative defense and deiodinase isozymes (Dio) that control thyroid hormone (TH) action. Se deficiency can impact negatively on thyroid gland functioning and TH metabolism and thereby perturb development and metabolism. We have generated Se-deficient mice by genetic inactivation of Selenoprotein P (SePP), the main plasma Se carrier. SePP-KO mice display decreased serum and tissue Se levels and manifest growth defects and neurological abnormalities. Therefore we speculated that the HPT axis and TH metabolism are impaired causing hypothyroidism-like symptoms. Surprisingly, Dio1 and Dio2 were regularly expressed in liver, kidney, thyroid gland and brain, respectively. Thyroid Se levels were also comparable despite strongly reduced Se concentrations in plasma, kidney or even brain of SePP-KO mice. Thyroid glands appeared morphologically normal, thyroid GPx activity was at wild-type levels and serum levels of TSH, or T4 and T3 were at wild-type levels. Pituitary TSH $\beta$  transcripts and hepatic Dio1 mRNA levels as markers of intracellular T3 activity were unchanged. During development, cerebellar granule cell migration as a sensitive indicator of local T3 action in brain was undisturbed. Collectively, these findings demonstrate that low levels of serum Se or SePP in the absence of other challenges do not necessarily interfere with the HPT axis. This is surprising with respect to the phenotype of patients with inherited selenoprotein synthesis defects, and indicates a top priority of Se supply to the HTA axis in an otherwise healthy organism.

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### P910

#### Changes in thyroxine requirement in the long-term follow-up of hypothyroid patients

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**Background and methodology**

There are few data on changes in thyroxine requirement in the long-term follow-up of hypothyroid patients. We carried out a retrospective analysis to evaluate the long-term changes in thyroxine dose and its relationship with age, gender, thyroxine dose at registration and aetiology of hypothyroidism.

**Results**

We identified 5,029 patients from our hospital based thyroid register with a minimum of 4 years follow-up (88% female, 74% autoimmune hypothyroidism, and mean age at registration was 53 SD15 years). The mean thyroxine dose at registration was 115.5 SD 38 mcg/day (median 100). Patients with autoimmune hypothyroidism were on smaller doses (111 mcg versus 127 mcg;  $P < 0.01$ ). After a median follow-up of 8.7 years there was an overall increment in thyroxine requirement (to a mean of 122 SD 44 mcg; median 125). This increment was seen predominantly in patients with autoimmune diseases who were perhaps on smaller thyroxine dose at registration or had progressive thyroid failure.

During follow-up thyroxine dose remained unchanged in 42% and a further 29% required only a minor adjustment in dose (25 mcg). Patients on thyroxine 100–150 mcg at registration were least likely to require a dose change. On regression analysis the final thyroxine dose was related to age, gender and thyroxine dose at registration. Women on an average required 12.8 mcg less thyroxine than men (95% CI 10.2 to 15.5;  $P < 0.001$ ). As patients got older, thyroxine requirement fell by 0.73 mcg/year (95% CI 0.67 to 0.79;  $P < 0.001$ ). Thyroxine dose was unrelated to the aetiology of hypothyroidism

**Conclusions**

During long-term follow-up, thyroxine dose remained unchanged in most patients who were registered on 100–150 mcg. Factors such as age, gender and thyroxine dose may be useful in identifying patients who can be monitored by longer intervals between thyroid function tests during routine surveillance.

**P911****Problems of identifying independent non-class II susceptibility loci within the HLA region for Graves' disease**

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The HLA class II region, in particular *DRB1/DQA1/DQB1*, has been consistently associated with Graves' disease (GD) for over thirty years. Only recently has work within our own group made progress in narrowing down the etiological variant(s) present within *DRB1/DQA1/DQB1*, by excluding *DQB1*, and by mapping association at *DRB1* to nine amino acid positions present within the peptide binding domain, with position  $\beta 74$  being the most associated. Independent associations outside of the HLA class II region have been proposed although only recently have data been reported to support these associations. *BTNL2*, a co-stimulatory surface molecule involved in T cell activation, has been proposed as one such factor. Studies in patients with sarcoidosis have shown association of the A allele of the *BTNL2* rs2076530 G-A single nucleotide polymorphism (SNP), with the A allele further associated with disruption of *BTNL2* membrane localization. The aim of this study was to investigate rs2076530 within a large UK Caucasian GD dataset. In total 864 patients with GD and 864 control subjects were genotyped. All subjects gave informed written consent, and the project was approved by the local ethics committee. Association was found between GD and the A allele of rs2076530 (OR = 1.32, 95% CI = 1.14–1.52). When linkage disequilibrium (LD) was assessed between rs2076530 and *DRB1* position  $\beta 74$ , a  $D' = 0.81$  was generated, suggesting strong LD between these loci and lack of an independent effect. To be able to completely rule out an independent contribution to GD, mapping of all Tag SNPs within this region is needed, combined with logistic regression analysis. These data once again highlight the difficulties in detecting class II independent effects at HLA because of strong LD within this gene region.

**P912****Is  $^{131}\text{I}$  therapy for hyperthyroidism harmful for testis function?**

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Radioiodine-131 is commonly used for treatment of hyperthyroidism, however the effects on testis function are not known. This prompted us to study testis function in hyperthyroid men after  $^{131}\text{I}$  treatment. 15

hyperthyroid patients scheduled for  $^{131}\text{I}$  treatment underwent: testis US, hormone assessment and sperm analysis before and 45 days, 3, 6 and 10–15 months after  $^{131}\text{I}$ . The control group (for testis volume and semen analysis) was constituted by 19 normal subjects. Testis volume was similar in patients and in controls (31 and 32 ml, respectively). Basal hormone levels in hyperthyroid males were within the normal limit.

After  $^{131}\text{I}$  treatment, FSH did not change significantly. Only in 2 patients FSH increased; one of them was severely hyperthyroid and underwent a second course of  $^{131}\text{I}$ ; the other had small testes and basal FSH in the normal upper limit. Serum testosterone and testosterone/LH (T/LH) ratio were significantly reduced as compared to basal values from 45 days to 6 months after treatment and reverted to basal levels after one year. Semen analysis showed sperm concentration and percentage of normal form not different between patients and controls. Motility was significantly reduced ( $P = 0.008$ ). 10 out of 15 hyperthyroid patients (67%) were asthenospermic before treatment. No significant variation of sperm concentration and percentage of normal forms was observed after  $^{131}\text{I}$ . On the contrary, a significant increase of progressive motility was observed after  $^{131}\text{I}$  therapy ( $P = 0.01$ ).

**Conclusions**

Our results show that in males treated for hyperthyroidism with  $^{131}\text{I}$ , germinal epithelium and Leydig cell function undergo only marginal changes that might have some relevance in subjects with a pre-existing fertility impairment.

**P913****What is the most effective screening interval in the long-term follow-up of stable hypothyroid patients on thyroxine?**

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**Background and methodology**

In the long-term surveillance of treated hypothyroid patients, annual surveillance (with thyroid function testing) is widely recommended. This is largely based on consensus, as there is limited evidence to support this practice. Currently around 20,000 patients are registered in our hospital based thyroid register and the majority are on 18 monthly follow-up. We carried out a retrospective analysis to see if there is evidence to support increased frequency of testing.

**Results**

We identified 2,125 patients with a minimum of 10 years follow-up (89% female, 65% autoimmune hypothyroidism, and mean age at registration was 51 years). There were 2 groups: 1182 (56%) had been allocated to 18 monthly follow-up and the rest had annual surveillance. There was no significant difference between the groups in relation to baseline characteristics: age, sex and thyroxine dose. A slightly larger proportion of patients on 18 monthly follow-up (68% versus 62%;  $P < 0.05$ ) had autoimmune hypothyroidism.

The median duration of follow-up was 15.5 years (range 10–30). On statistical analysis we found no significant difference in the following outcomes between the 2 groups: initial and final thyroxine dose, number of screening interval changes, dose changes; death or the number of patients with hyperthyroid tests (FT4 > 25 pmol/L; normal range: 10–25 pmol/L or TSH < 0.01 mU/L). The number of patients with one or more hypothyroid tests (TSH > 4 mU/l or FT4 < 10 pmol/L) during follow-up was less in the 18-month group (52.5% versus 60%;  $P < 0.01$ ). As we carried out multiple hypothesis testing, a  $P$  value of <0.01 was considered significant.

**Conclusions**

Our results do not support a change to more frequent testing, which has cost implications. 18 monthly testing (surveillance) should be an option for stable hypothyroid patients when there is an adequate register or recall system for the follow-up of these patients.

**P914****Expression and signal transduction of the cAMP-binding protein Epac in thyroid cells and thyroid tumors**

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cAMP signaling systems play a critical role in regulation of thyroid cell proliferation and function. Besides the well known protein kinase A (PKA) cascade, Epac (exchange protein directly activated by cAMP) depicts a novel effector of cAMP. Epac is directly activated by cAMP and in turn stimulates kinase cascades like Akt kinase, phospholipase C $\epsilon$  and MAP kinases as well as the Ras-like GTPases Rap1 and Rap2. As data about the impact of Epac on cAMP signaling of thyroid cells are limited, we studied whether Epac may be involved in the signaling network of thyroid cells and thyroid tumors.

In thyroid carcinoma tissues, we showed a strong expression of Epac1 in aggressive subtypes of thyroid carcinomas: a more intense immunohistochemical staining was determined in tall cell papillary thyroid carcinomas (PTC) compared to follicular and classical PTC subtypes. Expression in follicular thyroid carcinomas (FTC) was more intense in oxyphilic types compared to non-oxyphilic FTCs. The role of Epac activation in thyroid cell models was studied using the Epac-specific cAMP analog 8-pCPT-2'-O-Me-cAMP. During stimulation with this cAMP analog, proliferation of the normal thyroid cell line FRTL5 as well as of the thyroid carcinoma cell lines FTC133 and SW1736 was enhanced, while proliferation of HTh74 thyroid carcinoma cells was not significantly affected. In FRTL5 cells, the proliferation-promoting effect was enhanced by the presence of calf serum or H5-hormone mix implicating a network of distinct signaling pathways. Using Rap-pull down assays with immobilized GST-Ras/Rap binding domain of RalGDS we additionally found that Epac activates the Ras-like GTPases Rap1 and Rap2 in FRTL5, FTC133, SW1736 and HTh74 cells.

In conclusion, Epac-dependent pathways are functionally active in thyroid cells and thyroid carcinoma cells and thus may contribute to regulation of proliferation and function of these cells by cAMP.

#### P915

##### **Orbital tissue expansion expressed as a number: usefulness in grading the severity of thyroid-associated ophthalmopathy (TAO)**

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In assessing TAO, orbital CT scan allows measurement of extraocular muscle and optic nerve dimensions but it is useful mainly for a qualitative analysis and cannot be used in the clinical score of the disease. Aim of the present study was to seek a number which could express the expansion of the orbital tissue and be used in grading the severity of TAO. 39 TAO patients and 24 control subjects were studied. We have measured the orbital area (OA) on the central axial section of the CT scan image, approximated to a triangle, and the area of the portion of the ocular globe within the orbit (ocular globe chord area), defined as ocular cord (OC). The areas of ROIs (in mm<sup>2</sup>) were obtained by manually contouring the image on the computer console. We have then calculated the OC/OA ratio as an additional parameter, in order to reduce the variability of the obtained measurements. The areas and the OC/OA ratio of patients and controls were compared. In addition, the correlation between the orbital parameters and ocular proptosis (mm), palpebral fissure (mm) and intraocular pressure (IOP, mmHg) were studied. We did not observe significant differences of the mean orbital area of TAO patients (783.6  $\pm$  12.1 mm<sup>2</sup>) and normals (758.5  $\pm$  20.4 mm<sup>2</sup>; ANOVA, *P* = NS); the value of OC in TAO patients was 130.2  $\pm$  11.5 mm<sup>2</sup>, significantly lower than that of controls (281.8  $\pm$  9.7 mm<sup>2</sup>; ANOVA, *P* < 0.0001). OC/OA ratio was also significantly lower in TAO patients compared to controls (0.16  $\pm$  0.01 vs 0.38  $\pm$  0.01; ANOVA, *P* < 0.0001). A significant correlation was found between OC/OA ratio and proptosis (*r* = 0.65, *P* < 0.0001), palpebral fissure (*r* = 0.35, *P* < 0.02) and IOP (*r* = 0.36, *P* < 0.03). Manual measurement on CT scan images yielded a very low error (<2%). In conclusion, by measuring OC/OA ratio, we have obtained a number which represents the degree of orbital involvement in TAO patients and can be used as a score of the severity of the disease. The significant correlation of OC/OA with proptosis confirms that this parameter is associated to TAO.

#### P916

##### **Radioiodine treatment for hyperthyroidism – do royal college guidelines suggest too high a radioiodine dose?**

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Radioiodine (RAI) has been used to treat hyperthyroidism for more than 60 years. However there is no absolute clarity in the appropriate dose or method of administering RAI. We evaluated the efficacy of RAI therapy and compared our RAI doses with the guide doses suggested by Royal College of Physicians (Graves' disease 400–550 MBq and Multinodular goitre (MNTG) > 550 MBq).

We audited 602 hyperthyroid patients treated with RAI between 1994–2004. They were etiologically classified into Graves' disease, MNTG and unspecified diagnosis. Although our patients received 120 Seiverts or 140 Seiverts of RAI using dosimetry, they received a wide range of absolute radiation doses. After RAI therapy patients were categorised as hypothyroid, euthyroid and relapsed hyperthyroidism.

41% of patients had Graves', 13.6% had MNTG and rest had unspecified diagnosis. Overall cure rate was 86% with a median RAI dose of 333 MBq (241–456). There was no significant difference in cure rates between Graves' and MNTG patients (90% Vs 82%; *p* not significant). The median dose of RAI for Graves' was lower than MNTG (332 vs 419 MBq, *P* < 0.0002).

Interestingly patients with Graves' disease had a higher cure rate with < 400 MBq RAI dose compared with > 550 MBq (93.1% vs 68%, *P* < 0.005) and higher cure with 400–550 MBq than > 550 MBq (90.5% Vs 68%, *P* < 0.05). Similarly patients with MNTG had a higher cure rate with < 400 MBq RAI dose compared with > 550 MBq (97% Vs 69.6%, *P* < 0.02) and higher cure with < 400 MBq than 400–550 MBq (97% Vs 66.7%, *P* < 0.02).

Using low dose of radioiodine (< 400 MBq) it is possible to achieve high cure rates for hyperthyroidism. There was no significant benefit in treating Graves' disease and MNTG with more than 400 MBq dose of radioiodine. Royal College of Physicians guidelines may suggest too high a dose of radioiodine.

#### P917

##### **Thyroid cancer management: are guidelines being met?**

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Thyroid cancer is the commonest malignancy seen in endocrine practice. Audits of clinical practice have revealed significant variations in management, and the British Thyroid Association have published guidelines (2002) to address this. The aim of our study was to assess the care-stage at which practice falls short of agreed standards. We performed retrospective case note review of 83 patients (72 female) followed in a specialist thyroid cancer clinic over 5 years. Histology distribution was as expected: 50 had papillary, 29 follicular, 2 anaplastic and 2 medullary carcinoma. Only 66 (82.5%) patients had cytology preoperatively. 80 patients had thyroid surgery. 54 had tumour size > 1 cm: 53 underwent total thyroidectomy and 1 lobectomy, 51 (94.4%) had radioiodine therapy, and all 54 received TSH-suppressive therapy. 26 patients with tumour size < 1 cm, eight (30.8%) had lobectomy alone (6 for micro-carcinoma), and 1 received TSH-suppressive therapy. 9 patients out of 80 had lymph node dissection recommended by the guidelines. After surgery 5 (6.3%) patients had impaired vocal cord function and 9 (11.3%) had hypocalcaemia (< 2.00 mmol/L), all remaining on calcium treatment 1 year later. At first post-surgery follow-up only 61 (76.3%) patients had thyroid function, 32 (40%) calcium and 29 (36.3%) thyroglobulin measured. For all patients subsequent management was undertaken in a specialist thyroid cancer clinic, where this monitoring is routine. At last review 75 patients were disease-free, 1 has recurrent disease, 5 were dead from thyroid disease and 2 from other causes.

This study shows that some guideline standards are not achieved; lymph-node sampling is not being performed as recommended, and pre- and post-operative laboratory assessment is incomplete. These observations indicate that closer adherence to guidelines is needed in the surgical stage of treatment, and that the provision of a specialist thyroid cancer clinic is not sufficient to ensure ideal care.



## P918

**Treatment of patients with Graves' disease and ophthalmopathy with the anti-CD20 monoclonal antibody rituximab**

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We are conducting an open study on the treatment of Graves' disease (GD) and thyroid-associated ophthalmopathy (TAO) with the monoclonal antibody to CD-20, rituximab (RTX) (MabThera, Hoffman-Roche, Basel). RTX induces depletion of B cells and GD is typically a B cell dependent autoimmune disease. We present preliminary results on 7 patients with GD and TAO at different clinical stages: 3 women with newly diagnosed GD and initial TAO; 1 woman with GD and moderate-severe TAO, not responsive to steroids; 1 man with severe TAO, which appeared 12 months after thyroidectomy; one man and one woman with GD and moderately severe, active TAO on antithyroid drugs withdrawn about a month before RTX. Patients were assessed by measuring thyroid function tests, serum autoantibodies and peripheral blood lymphocytes subpopulations, by orbital imaging and thyroid ultrasound, and by ophthalmologic evaluation with the attribution of the clinical activity score (CAS). We applied a standardized protocol of  $2 \times 1000$  mg RTX infusions at a 2-week interval. All but one patient attained total B cell depletion. In 6 patients with active TAO the CAS improved within 1–4 months from RTX infusion and remained low even after B cell return. In one patient submitted to thyroidectomy and orbital decompression after RTX we found B cells in the thyroid but no immune cells in the orbital tissue. She had severe TAO, high serum TRAb and a relapse of hyperthyroidism during B cell depletion. Remission of hyperthyroidism was observed in 3 patients, in one after adding methimazole (MMI), and only in 2 with normalization of serum TRAb. One patient at 3 months after RTX and no other therapy is still hyperthyroid with no change in serum TRAb. In conclusion, RTX therapy improves clinically active TAO and induces stabilization in 1–3 months. We have evidence that RTX may act by depleting the orbit of immune cells. RTX therapy does not affect hyperthyroidism in GD patients with elevated serum TRAb levels, unless MMI is added. It may induce progressive normalization of serum TRAb levels and remission of hyperthyroidism in GD patients with moderately elevated serum TRAb levels ( $< 10$  U/L).

## P919

**Withdrawal of alfacalcidol supplements following thyroidectomy: baseline plasma PTH predicts successful outcome**

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Permanent hypoparathyroidism is rare after total thyroidectomy. However, our experience is that often patients remain on alfacalcidol and calcium supplements long-term after thyroidectomy.

A study was initiated in our centre in 2004, whereby all patients on alfacalcidol or calcium supplements post thyroidectomy underwent a gradual alfacalcidol and calcium reduction programme. Of 57 patients thus enrolled, we report on 22 who were initially on alfacalcidol and had a minimum follow-up of 3 months (). There were 4 men and 18 women, 6 had benign and 16 malignant thyroid pathology. Median age was 45.5 years (range 19–90). Median interval since thyroidectomy was 76.4 months (range 19–552). Ten patients were able to come off alfacalcidol during a median follow-up of 13.5 months (range 3–21).

Age, sex, thyroid pathology, time since thyroidectomy, and corrected serum calcium concentration at entry did not correlate with ability to come off alfacalcidol supplements. A lower dose of alfacalcidol ( $0.67 \pm 0.27$  vs  $1.23 \pm 0.16$  mcg daily) and a higher plasma PTH ( $26.1 \pm 16.1$  vs  $8.9 \pm 4.7$  ng/l) at enrolment correlated with ability to withdraw alfacalcidol ( $P < 0.05$ ). A plasma PTH greater than 18 ng/l was associated with 66.7% sensitivity and 92.3% specificity and 100% positive predictive value.

Withdrawal of alfacalcidol should be attempted post thyroidectomy regardless of duration of treatment and is more likely to be successful in patients on small doses of alfacalcidol and plasma PTH above 18 ng/l.

## P920

**Treatment of pretibial myxedema with dexametazone injected subcutaneously by mesotherapy needles**

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Pretibial myxedema (PM) is a rare extrathyroidal manifestation of Graves' disease for which there is no specific treatment. When the clinical picture is markedly evident, topical treatment with steroid ointments is practised. There has been previous experience of subcutaneous injections with steroids by using standard needles, but this procedure caused nodular degeneration of the skin. Aim of the present study was to experiment a novel modality of treatment with the injection of a solution of dexametazone (DXM) in the subcutaneous area by needles used for mesotherapy. These needles are no more than 2mm long and allow injection of the drug within the dermis or the first layer of the subcutaneous fat. We have treated 5 patients, 4 with the diffuse and one with the elephantiasic form. We have carried out multiple injections of a solution of DXM, lidocaine and saline in the pretibial area either on the PM plaque or in the area surrounding the lesions, once a week for 3 consecutive weeks. Two patients, with the more severe forms, were submitted to another 2 cycles 4–6 weeks after the initial treatment. Patients were evaluated before and after treatment by clinical assessment and ultrasound scan of the pretibial skin, as previously reported. The treatment was well tolerated with only moderate pain upon injection of the solution. One month after treatment all patients showed improvement of PM at clinical assessment and reduction of the thickness of the pretibial lesions by ultrasound of about 15%, for both the dermis and the subcutaneous fat. Moreover, all patients reported amelioration of the leg appearance and were satisfied with the therapy. In one patient, subjected to 2 cycles of treatment, we could show a further reduction of the PM skin thickness. The present study, although preliminary, shows that intralesional steroid injection with mesotherapy needles in PM is effective and well tolerated, without producing undesired modification of the skin. Whether this treatment is able to modify the natural history of PM is still to be ascertained. Furthermore, more studies are warranted to determine the optimal dosage of the steroid to be administered and the number of therapy cycles.

## P921

**Radioiodine uptake test reduces duration of restrictions following radioiodine therapy for management of hyperthyroidism**

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Behavioural restrictions observed following Radioiodine therapy (RAI) administered for management of hyperthyroidism are becoming more stringent. Over the last two years we have administered a fixed 400 MBq RAI dose after performing a 24-hour RAI uptake test with a view to reducing post RAI restrictions e.g. for patients with a 50% uptake, restrictions applicable to 200 MBq were advised. The aim of our study was to compare the restrictions as advised by this method with what would have been advised if uptake test had not been performed. 53 patients were treated over the last 18 months with a fixed 400 MBq RAI dose. Mean age was 53 years, 43(81%) were females and mean uptake was 56%. Degree of reduction in restrictions is shown in the table. 15 randomly selected patients were visited at home, 24 hours after administration of the full RAI dose and uptake was calculated from the measured dose rate (mSv/hour) at one metre. In all cases the measured uptake of the test dose accurately predicted the calculated uptake of the total dose. Patient preference was sought between 2-day method incorporating the test dose with reduced restrictions with the 1-day method without test dose but with full restrictions. Only 3 patients (6%) preferred the 1-day method. In conclusion, we believe that RAI uptake prior to therapeutic RAI dose significantly and safely reduces the duration of post RAI restrictions and although it involves attending the hospital on two days, a majority of patients prefer this method.

## P922

### The distance between histotypes of differentiated thyroid cancer: gene expression profiling study

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We apply the oligonucleotide microarray technology to study the expression profile of differentiated thyroid cancer (DTC) and to select transcripts which differentiate between its subclasses. We use state-of-the-art bioinformatic techniques, based on Support Vector Machines algorithms, to select not only the single "solitary" markers but rather the sets of genes which are taken into account cooperatively to enhance the diagnosis accuracy. Simultaneously, we try to measure the molecular "distance" between various histotypes of DTC. We used high density oligonucleotide microarrays (HG-U133A, Affymetrix). We started our analysis from the simplest model, the comparison between papillary thyroid cancer and normal thyroid tissue. In total, 73 thyroid samples were investigated: 48 PTC samples and 25 samples of macroscopically unchanged thyroid tissue. We observed that the difference between PTC and benign thyroid was large, with thousands of genes differentiating these two classes. We further extended this analysis to the set of differentiated thyroid cancer samples (57 tumors: 11 FTC, 9 FA – follicular adenomas and 37 PTC, both classic and follicular variant). We used Singular Value Decomposition to select 579 major variability genes in this dataset. Based on these genes, we were able to classify 18 of 20 FTCs or FAs as "follicular" and 36 of 37 PTCs as papillary. Next, we included also datasets obtained by other groups and publicly available to evaluate the genomic distance between various histotypes of differentiated thyroid cancer.

#### Conclusion

The distance between molecular profiles of papillary and follicular thyroid cancer is large. This indicates that both histotypes differ not only by their initiating events but also by further steps of neoplastic transformation. Simultaneously, we show the heterogeneity of follicular variant of papillary thyroid ca.

## P923

### The risk of hypercholesterolemia in patients with subclinical hypothyroidism

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Subclinical hypothyroidism is defined as a biochemical abnormality characterized by an elevated serum level of sTSH with normal thyroid hormones (FT4) and no clinical symptoms. The objective of this study was to assess whether patients with subclinical hypothyroidism have a risk of hypercholesterolemia. We have evaluated 37 adults (34 female and 3 male), mean age  $49.20 \pm 9.65$  years, with laboratory confirmed subclinical hypothyroidism, sTSH value above of 4.5 mU/L and normal thyroxine. We divided them in two groups. 1st group: 22 persons (20 female and 2 male), mean age  $48.04 \pm 9.82$ , who did not previously have a diagnosis of hypothyroidism. 2nd group: 15 persons (14 female and 1 male), mean age  $51.00 \pm 9.49$  years, who were taking thyroid replacement medication. None of them had history of coronary heart disease, signs of liver and renal dysfunction or were previously treated with lipid lowering drugs. We determined serum cholesterol level in every person and a level above 5.12 mmol/L was considered elevated. Statistical analysis was done by Spearman correlation and Mann-Whitney U test. The results showed that 75.67% of examined persons with subclinical hypothyroidism had elevated serum cholesterol, mean  $6.75 \pm 1.11$  mmol/L. In the first group 76.20% had elevated cholesterol, mean  $6.66 \pm 1.13$  mmol/L, in the second 85.71%, mean  $6.87 \pm 1.13$  mmol/L. There was no significant difference ( $P > 0.05$ ) among the cholesterol levels between the groups and there was no significant correlation between the level of sTSH and cholesterol level in the whole group. The percentage of examined persons with subclinical hypothyroidism and hypercholesterolemia is significantly higher than the percentage of ones with normal cholesterol levels. Therefore, subclinical hypothyroidism seems to be associated with high cholesterol levels. We haven't found any correlation with sTSH level and a degree of cholesterol increase.

## P924

### Audit of management of hypothyroidism in a joint endocrine/antenatal clinic

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In euthyroid women normal pregnancy is associated with a rise in free thyroxine and fall in TSH levels attributable to high levels of hCG in the first trimester. In hypothyroid women free thyroxine tends to fall and TSH rises if the dose of thyroxine is not raised, due to further pregnancy - associated changes including a rapid rise in thyroxine-binding globulin and increased renal clearance and placental metabolism of thyroid hormone. Fetal thyroid development occurs by 12 weeks gestation and thyroid hormone secretion not till later. Organogenesis particularly of the nervous system is dependent on adequate thyroxine levels in the fetal circulation, implying transplacental transport of maternal thyroxine to the fetus. If this is inadequate potential impairment of various aspects of mental development ensues, best seen in areas of endemic iodine deficiency but also suggested in offspring of under or untreated hypothyroid women.

Since there is debate about how early appropriate changes in thyroxine dosage should be made we audited the practice in our recently established endocrine/antenatal clinic for the first 2½ years. 83 hypothyroid women were seen. The first visit to the joint clinic occurred after 12 weeks gestation in 50 women (60.2%), over the years of observation this appeared to increase (52.8%, 64% and 71.4%). Overall 9 women were seen at 12 weeks (10.8%) and 19 women (22.8%) were seen before 12 weeks gestation. At the first visit 56 women (67.4%) had their thyroxine dosage increased. These findings prompt concern that possibly important adjustments in thyroxine dosage were delayed beyond an optimal stage in pregnancy in many hypothyroid women. We plan to pilot preconception advice and early assessment in selected primary care centres and in the longterm to monitor what, if any, the timing of thyroxine dosage adjustment has on the outcome of children born to these women.

## P925

### Thyrotoxicosis is coupled with quantitative and qualitative sleep disruptions

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Despite the common clinical opinion that thyrotoxicosis is associated with quantitative and qualitative sleep alterations, at present, an objective evaluation of sleep quality and quantity in this clinical condition has never been performed so far. Based on this foregoing, in order to perform a qualitative and quantitative description of sleep in this clinical condition, we enrolled 6 normal-weighted patients suffering from naïve overt hyperthyroidism due to Basedow Disease (BD) and 11 healthy age-, sex- and BMI-matched control subjects (CS). In both groups, the presence of clinical conditions or drug therapies known to affect per se sleep architecture was considered as exclusion criteria. In all the subjects, sleep recording was performed with wrist actigraphy (Actiwatch, Mini Mitter Co., Inc.; Bend, OR, USA) on three consecutive days in free living conditions. In all BD patients TSH levels were lower ([mean  $\pm$  SD]  $0.0 \pm 0.0$  mU/l), while fT3 ( $8.7 \pm 2.1$  ng/l), fT4 ( $30.5 \pm 5.6$  ng/l) and TRAb ( $47.5 \pm 9.3$  U/l) were higher than the normal range. The study had been approved by local Ethical Committee. No significant differences between BD and CS were observed in terms of time in bed ( $7h44' \pm 0h47'$  vs  $7h54' \pm 1h03'$ ). In BD, however, actual sleep time ( $6h35' \pm 0h50'$ ) was significantly lower ( $P < 0.01$ ) than in CS ( $6h54' \pm 0h58'$ ), although sleep latency in the two groups were similar ( $9' \pm 5'$  vs  $12' \pm 8'$ ). Moreover, in BD, a significant impairment of sleep quality compared with CS was recorded, as indicated by an increase of fragmentation index ( $21.06 \pm 8.37$  vs.  $14.06 \pm 3.92$ ;  $P < 0.05$ ) and of moving time percentage ( $10.04 \pm 2.66\%$  vs  $7.20 \pm 1.26\%$ ;  $P < 0.05$ ). In conclusion, these data describe for the first time the presence of quantitative and qualitative sleep disruptions in patients with thyrotoxicosis. Taking into account the well-known modulatory effect of sleep on hormonal secretions and glucose metabolism, the present results deserve further investigations to better characterize the role of sleep alterations as potential adjunctive determinant of the metabolic and hormonal alterations in hyperthyroidism.

**P926****The Gods placed the diagnosis before the therapy: case reports of the Slovak families with resistance to thyroid hormones**P Vanuga<sup>1</sup>, E Mendelova<sup>1</sup>, G Fodor<sup>1</sup>, K Vitesnik<sup>1</sup>, F Savagner<sup>2</sup>, Y Malthiery<sup>2</sup> & M Pura<sup>1</sup><sup>1</sup>Institute of Endocrinology, Lubochna, Slovakia; <sup>2</sup>Inserm Laboratoire de Biochimie-Biologie Moléculaire, Angers, France.

Resistance to thyroid hormones (RTH), is with the exceptions caused by mutations in the gene coding  $\beta_1$ -subunit of the thyroid receptor (TR $\beta_1$ ) located on chromosome 3. More than 700 individuals with RTH belonging to about 250 families have been identified up to date. The presence of goiter, tachycardia and hyperactivity together with abnormal findings on routine thyroid testing usually lead to further investigation and, ultimately, the diagnosis of RTH. Characteristic thyroid function tests are elevated fT4 and fT3 concentrations with non-suppressed TSH levels. Approximately 1/3 of all reported patients with RTH had the data of some therapeutic approach with the aim of lowering the thyroid hormones levels (antithyroid drugs, radioiodine therapy, surgery) in consequence of the initial erroneous diagnosis of thyrotoxicosis.

Authors present four families with RTH. Two adult probands experienced iatrogenic therapeutic approach (repeated subtotal thyroidectomy in one patient, respectively antithyroid therapy with propylthiouracil in another patient), early accurate diagnosis in two children (also treated with antithyroid drugs) prevented potential irreversible outcomes of the surgery in future. Molecular analysis of TR $\beta_1$  demonstrated a novel heterozygous missense mutation F451L in exon 10 in 22-yr old male patient on long-term antithyroid therapy and his three clinically euthyroid siblings (two brothers and father). Already known mutation A317T was found in 62- old male patient after two operations on thyroid gland, any other siblings were available. Another two already known mutations V336M and R438C confirmed the diagnosis of RTH in 7-year old boy and his father, and in 5-year old boy and his mother, respectively.

Trends toward the radicality in indications to the surgery as well as in the operation radicality itself emphasize the importance of the watchfull differential diagnostic approach of the hyperthyroid states. Despite of relatively low incidence, in a case an elevated level of serum fT4 together with non-suppressed TSH levels it is of the importance to think of the diagnosis of RTH.

**P927****Epidemiology of thyroid cancer in Republic of Sakha during 1970–2001**

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Exposure to ionising radiation, changing levels of iodine nutrition, and increased pathologic diagnosis of clinically unimportant thyroid neoplasm have all been proposed as explanations for a world-wide rise in the incidence of thyroid carcinoma over the past 6 decades. Yakutia is geographically an area of endemic iodine deficiency (the population is about 1 million). According to previous research in the endemic areas there are more occasions of follicular thyroid cancer, whereas in the areas free from iodine deficiency - papillary cancer. Additionally, radioinduced thyroid cancers also belong to the same histological form - papillary cancer.

Aim of our study was to present evaluate the epidemiology of the new cases of thyroid in population of Republic of Sakha (Yakutia) during 30 years, stating from 1990 and up to 2000.

Materials and methods

We traced all new cases of thyroid cancer in the whole Republic of Sakha. Results

The statistics by each year of the study are shown in the:

Year	Total	Year	Total	Year	Total	Year	Total
1970	5	1978	4	1986	10	1994	32
1971	3	1979	5	1987	20	1995	20
1972	6	1980	10	1988	9	1996	33
1973	6	1981	18	1989	10	1997	40
1974	2	1982	11	1990	19	1998	39
1975	4	1983	6	1991	17	1999	41
1976	2	1984	6	1992	16	2000	42
1977	5	1985	9	1993	25	2001	44

During the period of study the number of new cases raise from 5 in 1970 to 44 in 2001.

Conclusion

Epidemiology of thyroid cancer in Republic of Sakha shows rapidly significantly increased incidence during last 32 years. Prevailing papillary cancer among the population of the endemic area (Republic of Sakha) may be connected with unfavorable ecological situations in this region.

**P928****Rate of decrease in antithyroid antibody levels after therapeutic intervention in patients with thyroid cancer**D Thomas<sup>1</sup>, I Kostoglou-Athanassiou<sup>1</sup>, E Vassiliou<sup>1</sup>, V Liakos<sup>1</sup>, N Mytakidis<sup>1</sup>, F Chatzimakou<sup>1</sup>, P Athanassiou<sup>2</sup> & Ph Kaldrymidis<sup>1</sup><sup>1</sup>Metaxa Hospital, Pireaus, Greece; <sup>2</sup>Asclepeion Hospital, Athens, Greece.

The detection of antithyroid antibodies, specifically of antithyroglobulin antibodies, in patients with differentiated thyroid carcinoma after near-total thyroidectomy, radioiodine therapy and thyroxine suppression presents difficulties in screening patients for residual disease or recurrence of the disease as it may interfere with thyroglobulin measurement. The aim of the study was to estimate the rate of decrease in antithyroid antibody levels in patients with differentiated thyroid carcinoma after near-total thyroidectomy, <sup>131</sup>I therapy and thyroxine suppression.

Patients with differentiated thyroid carcinoma,  $n = 75$ , age at diagnosis  $47.1 \pm 13.7$  years (mean  $\pm$  SD), median 48 years, were studied. The patients had differentiated thyroid carcinoma and they had been managed by near total thyroidectomy, <sup>131</sup>I therapy and thyroxine suppression. Before surgery they had abnormally elevated antithyroid [antimicrosomal (anti-TPO) and antithyroglobulin (anti-Tg)] antibody levels. Recording of antithyroid antibody levels was performed at least 9 months after <sup>131</sup>I therapy and until 192 months later.

Anti-TPO antibodies. In 50% of the patients with abnormally elevated levels of anti-TPO antibodies before surgery normal values of the respective antibodies were observed 75 months after surgery and <sup>131</sup>I administration. The decrease in anti-TPO levels was not correlated with sex, age at diagnosis, histology and size of the tumor. Anti-Tg antibodies. In 50% of the patients with abnormally elevated levels of anti-Tg antibodies before surgery normal levels of the respective antibodies were observed 41 months after surgery and <sup>131</sup>I administration. The decrease in anti-Tg levels was not correlated with sex, age at diagnosis, histology and size of the tumor. Anti-Tg antibody levels decreased to normal earlier than anti-TPO levels ( $P < 0.05$ ).

Anti-TPO and anti-Tg antibodies remain detectable a long time after complete destruction of thyroid tissue. Anti-Tg antibody levels seem to decrease earlier than anti-TPO levels.

**P929****Further results of treatment and outcomes in patients with subacute thyroiditis observed in the period from 1993 to 2005**

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Aim

To estimate further results of treatment and outcomes in patients with subacute thyroiditis observed in the period from 1993 to 2005 years.

Materials and methods

120 patients (84 women, 18 men) with confirmed De Quervain's subacute thyroiditis (SAT) were investigated and the findings were analysed retrospectively (clinical features, ultrasonography, laboratory tests). Ultrasound device (Hewlett Packard Image Point HX) with 7.5–10 MHz. Serum TSH, fT4, fT3, AbTPO were measured with an automated chemiluminescent immunoassay "Vitros".

Results

There were 102 patients in the cohort within the 13-yr period: the female-male ratio was 4.7; aged  $40 \pm 9.8$  yr. A history of upper respiratory tract infection was recorded in the majority of patients 98. Among 102 patients 76 received prednisone therapy. Therapy with nonsteroidal anti-inflammatory drugs (NSAIDs) was therapy for the rest of the patients. 16 patients developed the recurrence (16.3%). SAT recurred during the first year after the treatment in 14 patients. One patient had a clinical diagnosis of recurrence of SAT 4,5 yr after the initial treatment and the other in 10 yr after the initial treatment. Early transient hypothyroidism is common in

SAT: 18 patients (18.3%) of 102. Among them 15 received prednisone and 3 received NSAIDs. Permanent hypothyroidism is less common, and only 15 (15.3%) of the patients are receiving T4 therapy after 4 yr of follow-up. Among them 13 received prednisone and 2 received NSAIDs as initial treatment. Increased rate of AbTPO developed in all patients with hypothyroidism.

#### Conclusion

SAT is more frequently in women than in men. There is an evidence of relation of the disease with upper respiratory tract infection. Permanent hypothyroidism developed in 15 patients and required T4 therapy. The majority of these patients received prednisone as initial treatment. The recurrence of SAT is more likely in the first year after treatment.

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### P930

#### Cowden syndrome - a clinical entity to be aware of

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Cowden syndrome is an autosomal dominant disorder characterized by germline mutations in the PTEN tumour suppressor gene on 10q23.3 which mediates cell-cycle arrest and apoptosis. The diagnosis is primarily based on clinical findings including a combination of mucocutaneous lesions (trichilemmomas), macrocephaly, thyroid lesions (mainly follicular adenoma or carcinoma) and breast and endometrial cancer. The prevalence is estimated at 1/200 000. Lifetime risk for developing breast cancer is 50%; thyroid adenomas become malignant in 10%. Classical PTEN mutations can be identified in 80% of individuals who meet the diagnostic criteria.

We discuss two recently diagnosed non-related cases.

(1) 35-year-old woman who presented to the dermatologist with multiple papillomatous nodules and hyperkeratotic papules at forehead and cheeks. A skin biopsy confirmed trichilemmomas, a pathognomonic criterion for Cowden's disease. She was referred with a small nodular goitre. Clinical examination revealed macrocephaly and galactorrhea. Her breast examination and mammogram were negative. She was clinically and biochemically euthyroid. Her thyroid ultrasound showed multiple 11–16 mm nodules in both lobes.

(2) 56-year-old woman who attends our clinic with long standing hypothyroidism. She has a strong family history for both ovarian and breast carcinoma. Clinical examination showed multiple bilateral breast lumps, galactorrhea, macrocephaly, obesity. She previously underwent hysterectomy and bilateral oophorectomy which revealed ovarian cystadenoma and myometrial leiomyoma. Mammography showed bilateral fibro-adenomata. Both women are enrolled in regular breast and thyroid screening programs.

#### Conclusion

We currently investigate three further possible cases of Cowden's syndrome. This may suggest that the prevalence is underreported and patients with nodular thyroid disease should be assessed for a family history for breast, thyroid or endometrial cancer. There are no nationally agreed guidelines in the UK with regard to management and surveillance strategies for Cowden's syndrome. We will outline international recommendations.

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### P931

#### Fine-needle aspiration biopsy of thyroid nodules

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#### Background

The most common method for evaluation of a suspicious thyroid nodule is a fine needle aspiration biopsy (FNAB). Together with ultrasound examination, thyroid scanning using pertechnetate and functional thyroid testing it helps in distinguishing between benign and malignant disease.

#### Objective

Aim of this study is to assess accuracy of FNAB in the diagnostic evaluation of thyroid nodules.

#### Patients

We retrospectively analyzed the medical records of the 84 patients who underwent FNAB of thyroid nodules.

#### Results

Fifty five of 84 patients had solitary thyroid nodule and 29 had multinodular goiter. Size of nodules was from 12 mm to over 60 mm measured by

ultrasound. There were 27 cystic and 57 solid nodules. FNAB was performed on "cold" nodules (57 nodules were non-functioning; scanning was not performed on cystic nodules). FNAB was non-diagnostic in 11 patients (13.09%), 6 with cystic and 5 with solid nodules. Benign finding was in 56 (66.67%), 21 with cystic and 35 with solid nodules. Papillary carcinoma was found in 3 (3.57%) and follicular neoplasm in also 3 (3.57%) and they underwent surgery (diagnoses of carcinoma were confirmed on histological results). In 11 patients (13.09%) finding was atypical. Six of these patient had nodules bigger than 2 cm and underwent surgery, and for 5 with smaller nodules the decision was made to be followed up and to repeat FNAB eventually. Final histological diagnoses in 3 of these 6 patients were benign.

Echogenicity, echo structure and shape did not show any significant difference between benign and malignant nodules in this study. Thyroid scanning in all patients showed non-functioning nodules. All the patients were euthyroid (normal TSH and T4). There were no complications of FNAB.

#### Conclusions

When the literature is reviewed and compared with the results of this study, the use of FNAB as a decision tool to operate is valid.

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### P932

#### Palpation versus ultrasonography-guided fine-needle aspiration biopsy of thyroid nodules in endemic goiter area

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Fine-needle aspiration biopsy (FNAB) of the thyroid gland is the most effective examination in the evaluation of thyroid nodules. To describe our experience with FNAB of the thyroid and compare the results with palpation versus ultrasonography-guided (UG) methods in an endemic goiter area in Turkey.

#### Methods

We studied on 176 patients whom 140 were females and 36 males, submitted to FNAB of thyroid nodules at outpatient clinic of a university hospital in an endemic goiter area of southwest of Turkey. We performed FNAB to direct palpable thyroid nodules without ultrasonography guidance at outpatient clinic for one year. After this period we performed FNAB to all thyroid nodules (palpable and nonpalpable) with ultrasonography guidance for three months. We compared the results with palpation versus ultrasonography-guided FNAB of thyroid nodules.

#### Results

Of 176 individual 257 thyroid nodules FNAB were performed. The ratio of the overall multinodular goiter was 80%. Palpation-guided FNAB group was made up of 117 patients, aged 18–84 years. Ultrasonography-guided FNAB group was made up of 140 patients, aged 18–78 years. The mean nodule size was 20.6 mm in ultrasonography-guided FNAB group and 27.5 mm in palpation-guided FNAB group. Rates of nondiagnostic specimens after the single puncture were 32% in palpation-guided group and 29% in ultrasonography-guided group and the difference was not statistically significant. Rates of malignant and suspicious cytologic results were 2.5% in palpation-guided group and 4.3% in ultrasonography-guided group.

#### Conclusion

We recommended that FNAB of multinodular thyroid in endemic goiter areas should be done with ultrasonography guidance.

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### P933

#### Thyroid storm induced cardiomyopathy is reversible

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A 31-year-old Taiwanese lady presented with palpitations, anxiety, orthopnoea, peripheral oedema, diarrhoea and a swelling in her neck. She was taking no regular medication. Her paternal uncle had been treated for thyrotoxicosis. She smoked 20 cigarettes per day. Clinical examination revealed tachycardia (pulse rate between 115 and 150 beats/min), tachypnoea (respiratory rate 30 breaths/min), hypertension (BP 161/125 mmHg) and congestive cardiac failure. The thyroid gland was enlarged but examination of the eyes was normal. Investigations revealed



normal renal function, hypoalbuminaemia (albumin 27 g/l) but otherwise normal liver function tests, undetectable TSH and significantly elevated fT<sub>4</sub> 55.7 pmol/l (NR 9–26). Atrial flutter with variable block was confirmed on electrocardiogram. The chest radiograph was consistent with significant pulmonary oedema. An echocardiogram showed dilated right and left ventricles with a left ventricular (LV) ejection fraction of 52%, (55–75%), moderate to severe mitral regurgitation (MR) and moderate tricuspid regurgitation (TR). A diagnosis of thyroid storm with cardiomyopathy was made, and the patient was commenced on high dose propylthiouracil 250 mg every four hours, loop diuretic, fluid restriction, beta-blocker, ACE inhibitor and anti-coagulated with warfarin. Two weeks later she had reverted spontaneously to sinus rhythm and was no longer clinically in heart failure. She was discharged on carbimazole (initially 40 mg tds and titrated down to 5 mg od according to thyroid function tests) and ramipril 2.5 mg od. Two months later, she was not breathless on exertion and had the following results: TSH 0.06 mU/l, fT<sub>4</sub> 6.1 pmol/l, fT<sub>3</sub> 5.3 pmol/l. TSH receptor antibodies were positive, consistent with Graves' disease. There was a dramatic improvement in her echocardiogram which now showed good right and left ventricular function with a LV ejection fraction of 68%, only mild MR and no TR. This case illustrates that thyrotoxic cardiomyopathy and arrhythmias may resolve rapidly with anti-thyroid medication.

### P934

#### Medullary thyroid carcinoma – which test is the best?

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Medullary thyroid carcinoma (MTC) is a rare tumoral entity related to parafollicular C cells of the thyroid gland, characterized by peculiar morphological, functional and evolutionary features. The aim of this study was to characterize the cases of medullary carcinoma diagnosed in a university hospital in a 5 years interval (January 1999 – December 2003) and to evaluate the diagnostic methods. In this time interval, from a total of 171 thyroid malignancy, 12 (7%) were medullary carcinoma. Most of the MTC (66.6%) presented clinical expression and high levels of calcitonin. In 1/3 the diagnosis was made by the morphological examination, which made necessary the immuno-histochemical confirmation. The morphological study included cytology of the samples of fine needle aspiration (FNAB), performed in all cases, extemporaneous examination and paraffin sections. Although FNAB could not establish the precise diagnosis, 11 of the 12 biopsies showed a malignant aspect. The immuno-histochemical reactions confirmed the diagnostic (positive for calcitonin and chromogranin A, negative for thyroglobulin). The MTC surgical treatment is aggressive, consisting in neck dissection with lymph-node dissection. Total thyroidectomy was performed in all cases, in 11 cases by first intention and in one case as a secondary step. All cases were in IIIrd tumoral stage. Only 2 cases in other series (mother and daughter) presented MEN type II. Time monitoring of the patients demonstrated secondary lymph node in 5 patients in a 3–12 months interval. All but one patient with associated pathology are still in life and have a good quality of life. Conclusion: Clinical approach for medullary thyroid carcinoma, generally starting from a thyroid nodule, is based on pathological findings following fine needle aspiration biopsy. Calcitonin dosage and paraffin examination, completed with immuno-histochemistry are essential for the positive diagnostic. Early diagnosis and treatment are essential to improve life expectancy.

### P935

#### Glutamic acid decarboxylase (GAD) and anti-tyrosine phosphatase (IA2) antibody positivity in newly-diagnosed Graves' disease patients

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#### Background and aims

The type 1 DM patients who are GAD antibody positive have more risk of autoimmune thyroid disease than who are negative. In patients with autoimmune thyroid disease there is a significantly increased risk of anti-pancreatic autoimmune response as shown by increased GAD antibody

positivity compared to normal population. However, the linkage between IA2 and thyroid autoimmunity is evaluated in one studied. Our aim is to evaluate the frequency of presence of anti-GAD antibody and IA2 in a Graves' disease population.

#### Patients and methods

A total of 53 patients with newly-diagnosed Graves' disease were included in this study of whom % 71.7 (n = 38) were female (mean age = 45.3) and % 28.3 (n = 15) were male (mean age = 41.2). The fasting plasma glucose measurements of all of the patients were normal. The GAD antibody, IA2 antibody, TSH receptor antibody and anti-TPO levels were studied by ELISA technique.

#### Results

There were 3 patients (2 female and 1 male) positive for GAD antibody (% 5.7) while 5 patients (4 female and 1 male) were positive for IA2 antibody (% 9.4). All of the patients with IA2 antibody positivity were also positive for TSH receptor antibody while only 2 of the GAD antibody positive patients had TSH receptor positivity. None of the patients revealed co-positivity for GAD and IA2 antibodies.

#### Conclusion

Our findings suggest that there was a unique population in autoimmune thyroid patients who presented with only IA2 positivity and without GAD antibodies. We also found that Graves' disease patients have increased co-positivity of TSH receptor antibodies with IA2 compared with co-positivity with GAD. Further long term follow up are required to evaluate the clinical significance of IA2 positivity in this patient group.

### P936

#### Hyperthyroidism presenting as ventricular fibrillation

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A 42-year old lady, previously fit and well, presented to the Accident and Emergency department following a cardiac arrest. She had received four D.C. shocks by the ambulance crew prior to cardioversion to sinus rhythm from ventricular fibrillation. She was admitted to ITU and during this admission developed a grandmal seizure. Physical examination was unremarkable and CT scan of her head was normal. Blood tests including full blood count and biochemistry were all normal except for her thyroid function test, which revealed a TSH < 0.01 mU/l, fT<sub>4</sub> 32.3 pmol/l and fT<sub>3</sub> 12.2 pmol/l. ECG done at the time of admission was normal except for sinus tachycardia. She was commenced on Carbimazole 40 mg once a day.

The patient later underwent a left cardiac catheterisation and had an echocardiogram, both of which were unremarkable. She was discharged on 40 mg of Carbimazole and followed up at the Endocrine clinic. Repeat blood tests 4 weeks later showed a TSH < 0.01 mU/l, fT<sub>4</sub> 16.8 pmol/l and fT<sub>3</sub> 6.0 pmol/l. Dose of Carbimazole was increased to 50 mg and 8 weeks later her TSH came down to 3.18 mU/l and she was clinically euthyroid. In view of her normal blood results and cardiac investigations, it appears most likely that this patient's ventricular fibrillation was secondary to hyperthyroidism. This is a recognized but very rare complication and rarely reported in the literature. Hence hyperthyroidism should be excluded especially in a young patient presenting with ventricular fibrillation.

### P937

#### Heart rate dynamics in hyperthyroidism

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#### Background

Clinical manifestations of hyperthyroidism resemble those of hyperadrenergic state. Previous studies of linear analysis of heart rate variability (HRV) revealed cardiac autonomic dysfunctions in hyperthyroidism. In recent years, increasing attention is being directed to the nonlinear analysis of HRV to gain insight into neural modulation of heart rate dynamics. Based on the chaos and fractal theory, an attractor in phase space can characterize the dynamics of a chaotic system. Besides, the complexity of the system can be quantified by the properties of the attractor, one of which is the correlation dimension (CD).

#### Objective

We investigated whether it is possible to distinguish between hyperthyroid patients and normal control subjects based on CD analysis of HRV.

#### Subjects and methods

The study subjects comprised 15 newly diagnosed hyperthyroid Graves' disease patients and 15 sex-, age-, and body mass index- matched normal control subjects. All subjects received one-channel electrocardiogram recording for 30 minutes. We computed CD analysis of HRV from the sequence of normal R-R intervals by the Grassberger and Procaccia algorithm.

#### Results

The slope of the straight line of best fit in the linear scaling region of the plot of  $[\ln C(r) \text{ vs. } \ln r]$  is determined as the value of CD. We observed that the values of CD reached a constant value of saturation (which is the value of CD) with the increasing embedding dimension. The CD was significantly reduced in hyperthyroid patients compared with the normal control subjects ( $4.88 \pm 0.12$  vs.  $6.35 \pm 0.11$ , mean  $\pm$  SE,  $P < 0.001$ ).

#### Conclusions

These results suggest reduced complexity in hyperthyroidism. In analyzing the heart rate dynamics in hyperthyroid patients, the CD could be used to distinguish hyperthyroid patients from normal subjects. Thus, the CD analysis of HRV gives additional information about neural modulation of the cardiovascular system in hyperthyroidism.

### P938

#### Impact of TNM classification categories on thyroid gland carcinoma

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We have collected an extensive record of more than 8000 patients with a thyroid carcinoma over the years 1946 - 2005. In all cases, the diagnosis was done by the histology examination carried by a pathologist. Those records were classified by their dimension and the extent of the thyroid gland. This classification is used as a basis for the treatment decision. Tumors of the bigger size needs radiotherapy with I 131 whereas the smaller tumors are subject of observation and record only.

Inappropriate classification having too course classes can lead to a mistreatment. A critical region appears to be around the size of the carcinoma equal to 1 cm. This is supported by the experimental data get from our patients database. The results show that for the size of carcinoma bigger than 1 cm there is a substantial increase in the probability of the metastasis. The analysis reveals that close to 10% patients, in the group of carcinoma up to 1 cm, had metastasis developed on nodes, on bones and on lung. The number of patients with the diagnosis falling into this critical region grows steadily during recent years, it has exhibited 12% increase in last 20 years. Based on those results, we suggest to classify the carcinoma into finer classes. This would improve probability of correct treatment decision in comparison with the current classification which covers the whole region 0-2 cm by a single class.

### P939

#### Antiproliferative effects of SOM230 on orbital fibroblasts from active Graves' ophthalmopathy

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Recently it has been shown that somatostatin (SST) might be of therapeutic value in the treatment of active Graves' ophthalmopathy (GO). SST and somatostatin receptors (*sst*) transcripts are expressed and functional in cultured orbital fibroblasts deriving from GO patients (FGO). Octreotide, the SST analogue currently used is able to inhibit *in vitro* growth and activity of FGO. New analogues with wider *sst*-affinity are now available for *in vitro* experimental studies. In this study we investigated the effects of the new synthetic SST analogue SOM230 on the control of growth and apoptosis in FGO.

#### Methods

Cells, cultured in dMEM, were starved without FCS for 2 days, then treated. with 10 nM, 100 nM SOM230 for 48 h. For the analysis of proliferation and

apoptosis, cells were harvested after treatment, and analyzed by MTT and TUNEL techniques.

#### Results

FGO, after 48 h treatment with 100 nM SOM230, showed a significant cell growth reduction and induction of apoptosis in 15% of cells.

#### Conclusions

These data demonstrate that SOM230 is able to control cell growth and induce programmed cell death in FGO and might be another therapeutic tool available for active GO control.

### P940

#### Further results of treatment and outcomes in patients with subacute thyroiditis observed in the period from 1993 to 2005

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Russian Endocrinology Research Centre, Moscow, Russia.

#### Aim

To estimate further results of treatment and outcomes in patients with subacute thyroiditis observed in the period from 1993 to 2005 years.

#### Materials and methods

102 patients (84 women, 18 men) with confirmed De Quervain's subacute thyroiditis (SAT) were investigated and the findings were analyzed retrospectively (clinical features, ultrasonography, laboratory tests).

#### Results

There were 102 patients in the cohort within the 13-yr period: the female-male ratio was 4.7; aged  $40 \pm 9.8$  yr. A history of upper respiratory tract infection was recorded in the majority of patients 98. Among 102 patients 76 received prednisone therapy. Therapy with nonsteroidal anti-inflammatory drugs (NSAIDs) was therapy for the rest of the patients. 16 patients developed the recurrence (16.3%). SAT recurred during the first year after the treatment in 14 patients. One patient had a clinical diagnosis of recurrence of SAT 4.5 yr after the initial treatment and the other in 10 yr after the initial treatment. Early transient hypothyroidism is common in SAT: 18 patients (18.3%) of 102. Among them 15 received prednisone and 3 received NSAIDs. Permanent hypothyroidism is less common, and only 15 (15.3%) of the patients are receiving T4 therapy after 4 yr of follow-up. Among them 13 received prednisone and 2 received NSAIDs as initial treatment. Increased rate of AbTPO developed in all patients with hypothyroidism.

#### Conclusion

Permanent hypothyroidism developed in 15 patients and required T4 therapy. The majority of these patients received prednisone as initial treatment. The recurrence of SAT is more likely in the first year after treatment.

### P941

#### Age related changes of soluble Fas, Fas ligand and Bcl-2 in autoimmune thyroid diseases

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Apoptosis plays a pivotal role in the regulation of the immune mechanisms in the pathogenesis of autoimmune thyroid diseases. The aim of the study was to compare soluble Fas, FasL and Bcl-2 in Graves' disease (GD) and Hashimoto thyroiditis (HT) in relation to the age of the studied patients. The study was carried out in 5 groups of subjects: 1/14 patients with GD in euthyrosis on methimazol (euGD) 2/20 patients with hyperthyroid GD (hrGD) 3/15 patients with HT in euthyrosis on levothyroxine (euHT) 4/16 patients with hypothyroid Ht (hoHT) 5/12 healthy volunteers age and sex-matched to group 1-4. The serum levels of Fas, FasL, Bcl-2, aTPO and aTG were determined by the ELISA kit. aTSHR were measured by the RIA method. The statistical significance was estimated by the Mann-Whitney U-test. Spearman's test was performed to evaluate relationships between variables.

Level of sFas was the highest in hoHT individuals: 8.7 (7.2-9.8) ng/ml as compared to the controls: 6.6 (4.4-8.0) ( $P < 0.01$ ) and euHT patients: 7.7 (5.2-8.7) ( $P < 0.05$ ). We found positive correlations between sFas and age in GD patients ( $r = 0.35$ ,  $P < 0.05$ ). There were no significant differences in sFasL concentrations between studied groups of patients. However in GD patients we found a negative correlation between sFasL and age in all HT

and GD patients ( $r = -0.34, P < 0.01$ ). Levels of sBcl-2 were significantly increased in euHT: 31.0 (13.5–44.1) ng/ml as compared to the controls: 8.0 (5.0–18.9) ( $P < 0.05$ ) and euGD patients: 9.1 (6.6–19.0) ( $P < 0.05$ ). We found a negative correlation between sBcl-2 and age in HT patients ( $r = -0.42, P < 0.05$ ).

In summary our results suggest that mechanisms of apoptosis mediated by interaction of Fas and its ligand play an important role in the active stage of the development of autoimmune process both in pathogenesis of Hashimoto thyroiditis and Graves' disease. Fas/FasL and Bcl-2 signaling pathways seem to be age-related and may explain, at least in part, milder course of Graves' disease in elderly patients and increased prevalence of Hashimoto disease in this group of subjects.

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## P942

### Comparison of radioiodine with radioiodine plus lithium in the treatment of hyperthyroidism

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#### Introduction

Radioactive Iodine (RAI) is a well-established and effective treatment for hyperthyroidism. Lithium (Li) blocks the release of organic iodide and thyroid hormone from the thyroid gland without effecting thyroidal RAI uptake. Through decreasing the loss of RAI from the thyroid it has also been shown to reduce urinary RAI excretion. Its use as an adjunct to RAI in the therapy of hyperthyroidism has been postulated, but information on the subject is limited.

#### Objective

To evaluate the efficacy of RAI therapy alone and RAI combined with lithium for treatment of hyperthyroidism.

#### Methods

41 patients with hyperthyroidism were randomly assigned to treatment with RAI (controls) or RAI plus lithium and evaluated for changes in thyroid function on the day and weeks 1, 3, 9, 12 and at least 6 months post treatment. Urinary Iodine (UI) excretion measurements from 24-hour urine collections of 29 trial patients (14 Li vs. 15 Non-Li) were obtained at -7, 0 and +7 days from RAI therapy.

#### Results

Both groups were similar in age, sex and received equivalent mean doses of RAI. One of 22 patients treated with RAI plus lithium (4.5%) and 2 of 19 patients treated with RAI alone (10.5%) were not cured (euthyroid + hypothyroid) ( $P = ns$ ). UI excretion levels overall (Li + Non-Li) increased one-week post RAI (152 nmol/mmol) compared to levels measured immediately before RAI (52 nmol/mmol) ( $P = <0.001$ ). There was no significant difference in UI excretion in the Li vs. Non-Li group ( $P = ns$ ).

#### Conclusions

Lithium does not appear to improve the efficacy of RAI and hence is not translating into an improved outcome for patients. Lack of a significant difference might be due to the excellent cure rate achieved in both groups during this trial. A more definitive conclusion will be reached upon greater patient recruitment and completion of the continuing trial.

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## P943

### Monitoring of goiter and urinary iodine excretion in 6–10 year old school children in Ahvaz (2003–2004)

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Simple goiter is one of the most prevalent endocrine diseases all over the world. Iran and Khuzestan province were Endemic area for goiter before Iodine supplementation in 1990. This study was performed in 2002–2003, about 13 years after consumption of Iodized salt(40 ppm), to determine prevalence of goiter and urinary Iodine excretion in 1800 school children (6–10 years old) in Ahvaz, choosed by cluster sampling. Physical examination and goiter staging according to WHO criteria was done by a medical student trained in endocrine department. A questionnaire include: age, sex and Iodized salt consumption, was filled out for each person. Urinary Iodine excretion (by digestion method), was assessed in 75 person by random sampling. Thyroid function tests include: T3, T4, T3RU by RIA and TSH by IRMA method were assessed randomly in 75 person

52.5 percent of study group were male and 47.5 were female. 712 school children (39.6%) had goiter. (17.3% stage Ia, 18.4% Ib, 3.7% stage II). There were not any cases of stage III goiter. Prevalence was higher in girl, than boys (43.5% versus 35.9%) ( $P < 0.005$ ). 90.4 percent of study group consumed Iodized salts. Mean Urinary Iodine excretion was 31  $\mu$ g/dl in normal children, 26  $\mu$ g/dl in person with stage Ia. goiter, 21  $\mu$ g/dl in Ib goiter and 14  $\mu$ g/dl in stage II goiter. Urinary Iodine was significantly higher in normal children than children with goiters stage Ib or II. 9.3% of studied persons had urinary Iodine excretion lower than 10  $\mu$ g/dl, 18.7% between 10–20  $\mu$ g/dl and 72% had urinary iodine more than 20  $\mu$ g/dl. Mean T4 in normal and goiterous person was 7.7 versus 8.02  $\mu$ g/dl, mean T3 184 versus 186 ng/ml, mean TSH 32% versus 33% and mean TSH 2.5 versus 3.4 mIU/ml, of them only TSH had significant difference in normal and goiterous persons ( $P < 0.01$ ).

#### Conclusion

After 13 years consumption of iodized salt, although more than 90% of studied persons had urinary iodine excretion more than 10  $\mu$ g/dl, goiter has high prevalence (39.6%) in study group. There for other goitrogens and higher iodine supplementation dose should be studied in this area.

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## P944

### Prevalence of subclinical dysfunctions of thyroid gland in women older than 40 years

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Adjara is a region in Georgia with high level at iodine deficiency.

The aim of the present work was to study the subclinical dysfunctions of thyroid gland in women older than 40 years whose thyroid state hadn't been examined before.

#### Materials and methods

From 1998 to 2005 693 women aged 40–78 had been examined. Primary examination was done by different specialists.

All women were tested on thyreotropic hormone (TSH) by means of immunodiagnostic unit "Digiscan SD-1000" (AUSTRIA). Examination of thyroid gland on the unit Philips SD-800 was done to estimate the volume and echological structure.

The Patients with raised level of TSH (more then 4.1) and lowered of THS (less 0.3) had undergone additional testing on free T<sub>4</sub> and titers of antibodies to thyreoglobulin and thyreoperoxidase.

All patients were divided according to their age. The 1st group = n 372 from 10 to 55 (average age 44 + 1.3) and the 2nd group n = 321 from 56 to 78 (average age 61.5 + 2.3).

#### Results

High frequency of subclinical hypothyreosis has been found in women from the first group 7.9% as well as in the second group – 17.7%. Higher frequency of subclinical hypothyreosis was associated with the older age ( $P < 0.001$ ).

In the first group the level of subclinical thyreotoxicosis was lower – 2.2% and in the second group – 4.1%. Higher frequency of subclinical hypothyreosis was associated with the older age as well.

#### Conclusion

The results indicate that in older age group subclinical hypothyreosis is present more frequently than in younger population. It is reasonable to continue the study.

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