Endocrine Abstracts

April 2007 Volume 14
ISSN 1470-3947 (print) ISSN 1479-68-48 (online)

9th European Congress of Endocrinology

28th April – 2 May 2007 Budapest, Hungary

9th European Congress of Endocrinology ece 2007 Budapest - Hungary - 28 April - 2 May

European Society of Endocrinology

Online version available at
www.endocrine-abstracts.org

Published by BioScientifica

Endocrine Abstracts (www.endocrine-abstracts.org)

Endocrine Abstracts (ISSN 1470-3947) is published by BioScientifica, Euro House, 22 Apex Court, Woodlands, Bradley Stoke, Bristol BS32 4JT, UK.
Tel: +44 (0)1454-642240; Fax: +44 (0)1454-642222;
E-mail: editorial@endocrinology.org;
Web: www.bioscientifica.com.

Subscriptions and requests for back issues should be addressed to *Endocrine Abstracts*, Portland Press, PO Box 32, Commerce Way, Whitehall Industrial Estate, Colchester CO2 8HP, UK. Tel: +44 (0)1206-796351; Fax: +44 (0)1206-799331.

Subscription rates 2007

	Annual	*Single part*
North & South America	$145	$110
Rest of the World	£80/€120	£60/€90

There are two regular issues per year plus occasional additional issues. Each issue is a separate volume.

Claims and communications
All claims or communications regarding issues lost or damaged in transit should be addressed to Portland Press in Colchester (see above for address). This applies to both institutional and personal subscribers. No claims can be entertained if they are later than 3 months after the date of despatch.

Disclaimer
The material contained in each issue of the journal has been prepared and written by named authors. Accordingly, neither the conference, BioScientifica Ltd nor their officers, employees or agents are responsible for the accuracy or otherwise of any abstracts or other articles and shall have no liability for any claims, damages or losses howsoever arising from the contents or any use to which they may be put by any person. It is not possible to guarantee that the abstracts printed in this issue will be presented at the conference.

Cover design by Rumba Graphic Design Ltd, Bristol, UK.

Typeset and printed in Europe by the Alden Group, Oxfordshire, UK.

Printed on acid-free paper.

Advertisements
Applications for advertisement space should be sent to Advertisement Department, BioScientifica Ltd, Euro House, 22 Apex Court, Woodlands, Bradley Stoke, Bristol-BS32 4JT, UK. Tel: +44 (0) 1454-642240; Fax: +44 (0)1454-642222; E-mail: sales@endocrinology.org. Copy is required 6 weeks before publication date. Rates are available on request.

USA Postmaster: send address corrections to *Endocrine Abstracts*, c/o Mercury International, 365 Blair Road, Avenel, New Jersey 07001. Periodicals postage is paid at Rahway New Jersey and at additional mailing offices.

All despatches outside the UK are sent by air-speeded service.

Citing *Endocrine Abstracts*
When citing abstracts from this publication please include the first author, year of publication, abstract title, name of this publication i.e. *Endocrine Abstracts*, volume and abstract number: e.g. Stewart P 2001 A tale of two enzymes. *Endocrine Abstracts* **2** SP2.

Endocrine Abstracts

Volume 14

April 2007

9th European Congress of Endocrinology

28 April–2 May 2007, Budapest, Hungary

Abstract Book

The ESE would like to thank the kind generosity of their corporate members:

ESE Corporate Members:
CMM Global
Eli Lilly
Ferring
Ipsen
Novartis
Novo Nordisk
Pfizer
Roche
Sanofi-aventis
Serono

ESE Secretariat
Euro House
22 Apex Court
Woodlands
Bradley Stoke
Bristol BS32 4JT, UK

Contact: Andrea Davis
Tel: +44 (0)1454 642247
Fax: +44 (0)1454 642222
E-mail: info@euro-endo.org
Web site: www.euro-endo.org

ESE 2007 Secretariat
Blaguss Ltd. Congress Bureau
PO Box 42, 1364 Budapest 4
Hungary

Tel: +36 1 371 7030
Fax: +36 1 312 1582
E-mail: ece2007@blauguss-congress.hu
Web site: www.ece2007.com

CONTENTS

9th European Congress of Endocrinology 2007

PRIZE LECTURES AND BIOGRAPHICAL NOTES

PLENARY LECTURES

SYMPOSIA

ORAL COMMUNICATIONS

Prize Lectures and Biographical Notes

European Journal of Endocrinology Prize Lecture

Brian Walker, UK

Brian Walker is Professor of Endocrinology at the University of Edinburgh in Scotland where he is administrative head of a 60-strong multidisciplinary research group in the Centre for Cardiovascular Science.

He graduated in medicine in Edinburgh in 1986 and completed his clinical training in Glasgow and Edinburgh. Since 1996 he has practised as an honorary consultant in Diabetes & Endocrinology at the Western General Hospital in Edinburgh, recently transferring his activities to the Royal Infirmary following the opening of the Queen's Medical Research Institute at the new Little France campus.

Brian's research interest in cortisol and cardiovascular disease began in 1989 as an MRC Training Fellow, and developed as a Lecturer and British Heart Foundation Senior Research Fellow. The focus of his group has been on translating findings in animal models into detailed mechanistic experiments in humans. He published the original studies elucidating the role of 11β-HSD type 1 as a therapeutic target in obesity and diabetes, and was influential in the studies linking activation of the HPA axis with low birthweight and adult cardiovascular risk factors.

Previous awards include the Dorothy Hodgkin Lecture from Diabetes UK, the 'Hot Topic' Plenary Lecture at the Nutrition Society, and the Society for Endocrinology Medal.

Cortisol and cardiovascular disease

Brian R Walker
Endocrinology Unit, Centre for Cardiovascular Science,
University of Edinburgh, UK

Similarities between the metabolic syndrome and Cushing's syndrome, and reversibility of the features of Cushing's syndrome, suggest that cortisol may contribute to pathophysiology in both conditions and that reducing cortisol action may provide a novel therapeutic approach in metabolic syndrome.

There is substantial evidence that circulating cortisol concentrations are higher in people with hypertension and glucose intolerance. The basis for this activation of the hypothalamic-pituitary-adrenal (HPA) axis remains uncertain, but it may be attributable to 'programming' effects of events in early life since it is associated with low birth weight.

In people who become obese, intracellular cortisol levels within adipose tissue are further amplified by increased local re-generation of cortisol by the enzyme 11β-HSD type 1. Recent evidence highlights the role of nutrition and inflammation in regulating 11β-HSD1 in rodents and in humans. In mice, transgenic manipulations of 11β-HSD1 have potent effects on obesity and associated features of the metabolic syndrome. Promising pre-clinical data suggest that novel 11β-HSD1 inhibitors will have a role in lowering intra-cellular cortisol levels as a treatment for metabolic syndrome.

In addition to their metabolic effects, glucocorticoids act in the blood vessel wall. Pharmacoepidemiological studies suggest that glucocorticoid excess is an independent risk factor for cardiovascular disease. Recent data in rodents suggest that 11β-HSD1 within the blood vessel wall influences vascular remodelling and angiogenesis, for example in the myocardium following coronary artery occlusion.

Thus, HPA axis hyperactivity may provide a lifelong susceptibility to metabolic syndrome which is amplified by altered cortisol metabolism in obesity. Glucocorticoid signalling provides a potentially tractable system to influence both risk factors for, and the outcome of, type 2 diabetes and cardiovascular disease.

Geoffrey Harris Prize Lecture

Hubert Vaudry, France

Dr Hubert Vaudry is Director of Research at the Institut National de la Santé et de la Recherche Médicale (INSERM), the French National Institute for Health, and Director of the Laboratory of Cellular and Molecular Neuroendocrinology at the University of Rouen. He was born in February 1946 in Le Havre, Normandy, and obtained his PhD at the University of Rouen in 1974. He then worked in Canada for two years as a post-doctoral fellow, at Queen's University (Kingston, Ontario) and Laval University (Quebec). He obtained a Doctor of Science degree in 1979 at the University of Rouen and has developed one of the most productive groups in the field of neuroendocrinology.

Dr Vaudry is involved in a number of International Committees and Advisory Boards. He is the author of 800 publications in first rank scientific journals and has presented over 1450 communications or lectures in international congresses. Previous awards include the Descartes-Huygens Prize for scientific cooperation between France and the Netherlands, and the Prize of the Académie Nationale de Médecine. He has been appointed as Invited Professor in several Universities including the Catholic University of Nijmegen, Netherlands (1982–1983), Waseda University in Tokyo, Japan (1986), and the University of Turin, Italy (1989).

Dr Vaudry is the Chairman of the European Institute for Peptide Research, a major multidisciplinary institute working in the field of biologically active peptides. He is also the Chairman of the Research and Education Network for Neuroscience (LARC-Neuroscience network). He is a former President of the International Federation of Comparative Endocrinology Societies (1997–2001), the European Society for Comparative Endocrinology (1998–2002) and the Société de Neuroendocrinologie (2001–2004).

Neuroendocrine control of steroid biosynthesis within the hypothalamus

Hubert Vaudry[1], Jean-Luc Do Rego[1], Delphine Beaujean[1], Ludovic Galas[1], Dan Larhammar[2], Jae Young Seong[3], Van Luu-The[4], Georges Pelletier[4] & Marie-Christine Tonon[1], [1]INSERM U413, Univ. Rouen, France, [2]Dept Neuroscience, Univ. Uppsala, Sweden, [3]Lab. G Protein-Coupled Receptors, Korea Univ. College of Medicine, Seoul, Korea, [4]Lab. Molecular Endocrinology and Oncology, Laval Univ. Medical Center, Quebec, Canada

Neuroactive steroids synthesized in the brain, referred to as neurosteroids, have gained particular attention as they appear to be involved in the modulation of various neuroendocrine, behavioral and pathophysiological processes. Thus, the distribution of steroidogenic enzymes and the identification of the biochemical pathways leading to neurosteroid formation have now been almost completely elucidated in various groups of vertebrates. In contrast however, the neuronal mechanisms controlling the activity of neurosteroid-producing cells in the brain have received little attention. Therefore, we have investigated the effects of neurotransmitters and neuropeptides on the biosynthesis of neurosteroids, using the frog brain as an experimental model. We have first observed that steroid-synthesizing neurons express several subunits of the $GABA_A$/central-type benzodiazepine receptor (CBR) complex, and we have found that GABA, acting through $GABA_A$ receptors, inhibits the synthesis of neurosteroids. We have shown that glial cells containing the octadecaneuropeptide (ODN; endogenous ligand of CBR)-like immunoreactivity make contact with neurosteroid-producing neurons, and that ODN stimulates steroid biosynthesis in hypothalamic neurons in a dose-dependent manner through activation of CBR. Steroid-producing neurons are also innervated by vasotocin (VT)-containing fibers, and they are gathered in hypothalamic regions which actively express the V1a receptor subtype and mesotocin (MT) receptor (MTR). We have found that VT and MT, acting on V1a and MTR respectively, are potent stimulators of neurosteroidogenesis. Finally, we have shown that steroidogenic neurons are innervated by NPY and GnRH fibers, and that the nuclei where these neurons are located are enriched with NPY Y_1 and Y_5 receptors, and GnRHR1/3 receptors. We have observed that NPY, acting through Y_1 receptors, inhibits neurosteroid biosynthesis, while GnRH stimulates the production of neurosteroids probably via GnRHR1/3 receptors. Taken together, these data suggest that some of the activities exerted by neurotransmitters and neuropeptides in the brain may be mediated via the regulation of neurosteroid production.

Supported by INSERM (U413), the Regional Platform for Cell Imaging, a France-Québec exchange program (INSERM-FRSQ), a France-Korean exchange program (STAR) and the Conseil Régional de Haute-Normandie.

Symposia

Hypopituitarism–S1

S1.1

Traumatic brain injury-induced hypopituitarism: whom and when to test

Chris Thompson
Beaumont Hospital/RCSI Medical School, Dublin, Ireland.

A large body of evidence has accumulated to indicate that between 20–30% of survivors of acute traumatic brain injury (TBI) develop permanent pituitary dysfunction. Growth hormone (GH) deficiency is the commonest abnormality documented in most studies followed by ACTH and gonadotrophin deficiency and hyperprolactinaemia, with TSH deficiency least common. In contrast to other forms of pituitary disease, the classical hierarchy of pituitary hormone failure is not always seen and there is a higher proportion of single hormone defects. Many of the symptoms of chronic TBI are similar to those of untreated hypopituitarism, which suggests that identification and appropriate treatment of hypopituitarism offers a valuable service to survivors of TBI. Who should be tested? Most studies have been confined to survivors of moderate to severe TBI and the rationale for investigation is currently confined to this subgroup. There is little relationship between severity of TBI, neuro-imaging studies or operative intervention and the likelihood of hypopituitarism so until better guidance is available from prospective studies, all survivors should be tested. The choice of dynamic stimuli for ACTH and GH are centre-dependent. The timing of testing is important.
In the acute phase of TBI the key deficiency to identify is ACTH; patients who develop hypotension, hypoglycaemia or hyponatraemia should be systematically screened for ACTH deficiency. Studies of the natural history of pituitary dysfunction after TBI suggest a dynamic process, with many acute abnormalities recovering within 3–6 months of TBI, while new deficiencies may manifest in this period. New deficiencies are rare after 6 months. Most authorities therefore recommend formal dynamic testing at 3–6 months following TBI. Clinicians should be aware of occasional late recovery of function. Prospective studies are needed to better identify those at greatest risk of hypopituitarism, in order to improve the logistics of post-TBI pituitary hormone assessment.

S1.2

Morbidity and mortality

BA Bengtsson
Sweden.

Abstract unavailable

S1.3

Hypocorticotropism

Stylianos Tsagarakis
Department of Endocrinology, Athens Polyclinic, Athens, Greece.

Hypocorticotropism refers to ACTH insufficiency, which may be partial or complete, isolated or combined, genetic or acquired, pituitary or hypothalamic in origin. As a result it leads to secondary adrenal failure. Adrenal secretion of cortisol and of adrenal androgens is mainly affected; aldosterone secretion is normal. Symptoms of hypocorticotropism include progressive malaise and weight loss. Because aldosterone secretion is intact salt wasting, volume contraction and hypokalemia are not present. Hypoglycemia due to defective neoglucogenesis may occur and is particularly common in children. Symptoms may be more dramatic in the case of abrupt onset. Laboratory assessment includes baseline measurements of ACTH and cortisol and dynamic tests. A low morning value of cortisol (i.e. <3 μg/dl) associated with a low ACTH is diagnostic of severe hypococorticotropism. A value of 9am cortisol over 18 μg/dl excludes hypococorticotropism. For all other values dynamic tests are required. The gold standard test is the insulin stress test (IST). Alternative tests are the glucagon, the metyrapone and the standard (SST) and low Synacthen (LST) tests. The Synacthen tests because of their simplicity and safety have superseded the gold standard IST. Although the LST was considered as a more sensitive test than the SST recent data suggest that there is no difference. A cut-off of 18 μg/dl is considered as a "pass" and is safe assuming that assessment is not close to recent pituitary failure. Confirmation of hypocorticotropism requires replacement therapy. Hydrocortisone is the preferred medication. It is better given in 3 divided doses to a total daily dose of 10–20 mg. Co-administration of GH may increase the dose requirements of hydrocortisone. The dose should also be increased during stress and surgery. The question of whether adrenal androgen replacement is beneficial is still debated. Some studies showed positive effects on the quality of life of 50 mg of DHEA but others fail to confirm this. A therapeutic trial of 3–6 months in patients, particularly women, with relevant symptoms may be justified.

S1.4

Familial neurogenic diabetes insipidus

Soren Rittig
Skejby University Hospital, Aarhus, Denmark.

Although molecular research has contributed significantly to our knowledge of familial neurohypophyseal diabetes insipidus (FNDI) for more than a decade, the genetic background and the pathogenesis still is not understood fully. FNDI is, in 87 of 89 kindreds known, caused by mutations in the arginine vasopressin (AVP) gene, the pattern of which seems to be largely revealed as only few novel mutations have been identified in recent years. The mutation pattern, together with evidence from clinical, cellular, and animal studies, points toward a pathogenic cascade of events, initiated by protein misfolding, involving intracellular protein accumulation, and ending with degeneration of the AVP producing magnocellular neurons. Molecular research has also provided an important tool in the occasionally difficult differential diagnosis of DI and the opportunity to perform presymptomatic diagnosis. Although FNDI is treated readily with exogenous administration of deamino-D-arginine vasopressin (dDAVP), other treatment options such as gene therapy and enhancement of the endoplasmic reticulum protein quality control could become future treatment modalities.

Hormones and the brain – S2

S2.1

Thyroid hormone regulation of neural and oligodendrocyte precursors in the mature brain: a possibility for remyelination and neuroprotection

Laura Calzà
University of Bologna, Ozzano Emilia, Bologna, Italy.

Re-myelination in the adult CNS has been demonstrated in different experimental models of demyelinating diseases. However, there is no clear evidence that re-myelination is effective in multiple sclerosis (MS), the most diffuse demyelinating disease. Moreover, chronic disabilities in MS are believed to be due to remyelination failure and consequent neuron damage and degeneration. Due to the presence of numerous oligodendrocyte precursors inside demyelination plaques, reasons for remyelination failure are unknown. Data from embryonic development and *in vitro* studies supports the primary role of thyroid hormone in oligodendrocyte formation from neural precursors and maturation. We have obtained positive results in promoting re-myelination and neuroprotection in chronic experimental allergic encephalomyelitis (EAE), a widely used experimental model of MS, by recruiting progenitors and channelling them into oligodendroglial lineage through administration of thyroid hormone. Experiments performed in rats and confirmed in the primate Callithrix Jacchus have generated a phase 2 clinical trial that is in progress. We have also explored the role of thyroid hormone in regulating neural precursors cells in the subventricular zone of mature brain by *in vivo* and *in vitro* experiments (neurosphere assay), with regard to cell cycle and lineage regulation. Finally, we are exploring the possibility that prenatal events disturbing thyroid function, like endocrine disruptors exposure (dioxin family), might affect oligodendrocyte development and susceptibility to demyelinating agents.

S2.2

Neuroprotective actions of estrogens in the central nervous system

Luis Garcia-Segura & Iñigo Azcoitia
Instituto Cajal, CSIC, Madrid, Spain.

Sex hormones act both as endocrine signals as well as local paracrine or autocrine factors in the nervous system. In addition to target to classical endocrine and reproductive brain areas, sex hormones and its metabolites affect learning and cognition and regulate the development and plasticity of brain regions that are not directly related to reproduction. Estrogen and progesterone exert neuroprotective

effects in the central nervous system and may affect the onset and progression of several neurodegenerative and affective disorders, as well as the recovery from traumatic neurological injury. Recent studies have shown that the brain up-regulates both estradiol synthesis and estrogen receptor expression in reactive astroglia at sites of injury. Genetic or pharmacological inhibition of brain aromatase, the enzyme involved in estradiol synthesis, results in marked neuronal death after different forms of mild neurodegenerative stimuli that do not compromise neuronal survival under control conditions. This finding strongly suggest that local formation of estradiol in the CNS is neuroprotective and that the induction of aromatase and the consecutive increase in the local production of estradiol are part of the program triggered by the neural tissue to cope with neurodegenerative insults. Proteins involved in the intra-mitochondrial trafficking of cholesterol, the first step in steroidogenesis, such as the peripheral-type benzodiazepine receptor (PBR) and the steroidogenic acute regulatory protein (StAR), are also up-regulated in the brain after injury, together with the first enzyme in the steroidogenic pathway (P450scc). This suggests that brain steroidogenesis may be modified in adaptation to neurodegenerative conditions and to the brain aging process. Recent studies have shown that Ro5-4864, a PBR ligand that increases brain steroidogenesis is neuroprotective. Therefore, StAR, PBR and aromatase are attractive pharmacological targets to promote neuroprotection in the aged brain.

Supported by MEC, Spain (SAF 2005-00272) and the European Union (EWA project: LSHM-CT-2005-518245).

S2.3

Estrogen receptor signalling and cerebrovascular disease

Tommy Olsson, Magnus Strand & Ingegerd Söderström
Department of Medicine, Umeå University Hospital, Umeå, Sweden.

The transition to the postmenopausal stage is associated with an increased risk for vascular diseases, including myocardial infarction and stroke. This has been linked to a decrease in estrogen production. Estrogens mediate their effects on the brain to a major extent through binding to nuclear receptors, estrogen receptor alpha and beta. It is possible that positive and adverse effects of estrogens are related to interactions between receptor genotypes and hormones. Notably, the estrogen receptor alpha polymorphism c 454-397T/T is associated with increased risk of hemorrhagic stroke, with a synergistic relationship between this genotype and hypertension. In experimental stroke settings estrogens influence recovery of cognitive functions, possibly via induction of neurotrophic factors and specific transcription factors including NGFI-A. This may be related to increased neuroplasticity in the hippocampal formation, a key area for memory processing. Individualized treatment with estrogen receptor medulators may be beneficial for individuals with an increased risk for stroke. Estrogens may also improve recovery after stroke.

S2.4

Immunesenescence and steroid hormones

Wiebke Arlt[1] & Janet M Lord[2]
[1]Division of Medical Sciences, University of Birmingham, Birmingham, United Kingdom; [2]MRC Centre for Immune Regulation, University of Birmingham, Bimringham, United Kingdom.

Ageing is associated with a decline in immunity, also termed immunesenescence. This is paralleled by a decline in the production of several hormones as typically illustrated by the menopausal loss of ovarian oestrogen production. This lecture will give a brief overview of the physiology and pathophysiology of steroid hormones that decline with ageing. Therein a specific focus will be laid on the ageing-associated decline in adrenal dehydroepiandrosterone (DHEA) production, an event commonly termed as "adrenopause". However, this term is rather imprecise as the other major outputs of adrenal corticosteroid production, cortisol and aldosterone secretion, do not change with ageing. The regulatory processes involved in the initiation and progression of "adrenopause" still remain elusive. Current research efforts importantly aim at clarifying whether "adrenopause" contributes to immunesenescence, also addressing the issue of an altered glucocorticoid/DHEA balance that necessarily occurs if cortisol remains unchanged while DHEA steadily declines. Previous research has shown that an increased cortisol/DHEA ratio increases the likelihood of early postoperative infections requiring hospitalisation in elderly patients with hip fracture and that these changes are associated with an impairment of neutrophil function. The lecture will summarise most recent results on differential effects of DHEA and cortisol on components of the immune response, including neutrophil and natural killer cell function, including first conclusive data on underlying mechanisms. Further understanding of immune-endocrine links in the pathophysiology of immunesenescence will hopefully help to develop clinical tools for improving health in our rapidly ageing population.

Signaling and regulation of G-protein-coupled hormone receptors – S3

S3.1

Trafficking and signaling of angiotensin receptors

László Hunyady, Eszter Karip, Gábor Turu & László Szidonya
Department of Physiology, Semmelweis University, Budapest, Hungary.

The octapeptide hormone angiotensin II (Ang II) exerts its major biological effects via angiotensin AT_1 receptors (AT_1Rs). Signaling of AT_1Rs is regulated by ß-arrestins, which bind to activated AT_1Rs, uncouple them form G proteins, and initiate their internalization via clathrin-coated pits and cause G protein independent MAP kinase activation. It has been shown previously that AT_1Rs internalize via ß-arrestin-dependent and independent mechanisms, whereas angiotensin AT_2 receptors, which are unable to internalize, do not bind ß-arrestins. To study the role of G protein independent MAP kinase activation in cells, which endogenously express AT_1Rs, a mutant receptor (S109Y) was created, which is unable to bind candesartan. On the other hand, the Ang II binding and Ang II-induced functional responses of the S109Y mutant receptor are completely normal. This mutation was combined with a mutation (DRY/AAY), which can bind to ß-arrestin2, but its G protein coupling is completely impaired. The receptors were expressed in C9 cells, which express endogenous AT_1Rs. In the presence of candesartan the Ca^{2+} signal and MAP kinase activation of the endogenous AT_1R was completely eliminated. However, the Ca^{2+} signal generation and MAP-kinase activation of the S109Y mutant receptor was readily detectable. in the presence of candesartan, which inhibits the endogenous AT_1Rs, the combined S109Y and DRY/AAY mutant receptor was unable to induce Ca^{2+} signal generation, whereas it mediated Ang II-induced MAP kinase activation with a slow kinetics. These data suggest that G protein independent MAP kinase activation can occur in C9 cells.

This work was supported by OTKA T46445 and ETT 447/2006.

S3.2

Pharmacological chaperones rescue the membrane expression and function of a mutant of the vasopressin V1b/V3 receptor

Eric Clauser, Jessica Robert, Colette Auzan & Marie Ange Ventura
Institut Cochin, INSERM U567, Paris, France, CNRS UMR8104, Paris, France; University Paris V, Paris, France.

The majority of loss-of function mutations of G protein coupled receptors, leading to diseases, such as diabetes insipidus (V2 vasopressin receptor) or retinitis pigmentosa (rhodopsin) are consecutive to retention of the receptor in the endoplasmic reticulum (ER). Cell surface expression and biological function can be restored by membrane-permeable ligands called pharmacological chaperones. The V1b/V3R, one of the 3 subtypes of vasopressin receptors, is involved in the regulation of the corticotroph axis during stress. Using an original assay for cell surface expression of the receptor, we have demonstrated that a mutation of the hydropobic 341FNX2LLX3L350 motif in the C-terminus of the human pituitary V1b/V3R (MUT V3R) leads to its retention in the ER. The precise role of this motif was further investigated using SSR149415, a nonpeptide V1b/V3R antagonist.

The absence of the mutated receptor at the plasma membrane is linked to its prolonged association with the molecular chaperone, calnexine, in the ER and to its intensive degradation by the ubiquitin-proteasomal machinery. However, this ER retention is not a consequence of a lack of oligomerization of the mutant, which can be identified as dimers in the ER with BRET technique.

Treatment with SSR149415 restores expression of the mutated receptor at the cell surface and its correct maturation, resulting into the functional recovery of its signaling properties. SSR149415 acts by stabilizing the native-like conformation of the V1b/V3R, reducing its association with calnexin and favoring a secretory pathway rather than the proteasomal degradation pathway.

In conclusion, the 341FNX2LLX3L350 sequence is an important motif for the V1b/V3R conformation and the misfolfding resulting from its mutation alters the receptor export but can be reverted by SSR149415, which behaves as a pharmacological chaperone.

S3.3

Functional impact of GPCR heterodimerzation

Heike Biebermann
Institute of Experimental Pediatric Endocrinology, Charité Campus Virchow, Universitätsmedizin Berlin, Berlin, Germany.

For over two decades the hypothesis of dimerization of G protein coupled receptors (GPCR) exist. But only in 1999 it became clear by investigation of GABA B receptors that dimerization is the prerequisite for function. Since then our understanding of GPCR function is widened by the fact that nearly all GPCRs form dimers or higher order oligomers. This formation of GPCR homodimers or heterodimers influence the functional properties of a GPCR from that we know of a monomer in its ability to traffic to the cell surface, to bind one or even a variety of ligands, to initiate one or several signalling pathways, to be internalized, or not, and at least to be a therapeutical target. This receptor cross-talk seems to be crucial for fine-tuning of receptor function in controlling physiological processes of a cell. A very large number of GPCRs are known that form heterodimers/oligomers but the functional impact for a lot of these dimers still remains unclear and functional consequences of GPCR heterodimerization are nor predictable. For some GPCR dimers the functional impact is solved, e.g. for GABA B1 and GABA B2 receptors it is known that dimerization is necessary for cell surface expression and function; the taste sensation of sweet or umami is dependent on the formation of taste receptor T1R1, T1R2 and T1R3 complexes and the formation of dopamine 2 receptor/cannabinoid 1 receptor heterodimers result in activation of the Gs instead of Gi when expressed alone.

What do these data contribute to our overall understanding of physiological processes? As long as we have no other hints we have to accept that all possible interactions of GPCRs that are expressed on a given cell type are possible and therefore have to be investigated. to clarify their physiological significance. Especially this counts for the estimation of drug pharmacology targeting a GPCR.

Our group is interested in understanding the physiological processes of hypothalamic weight regulation. Therefore we set out to investigate the interaction of GPCR that are expressed on neurons of the nucleus arcuatus and nucleus paraventricularis. For example we are able to show that the MC3R forms dimers with the ghrelin receptor both are expressed on NPY/AGRP neuron of the nucleus arcuatus. The functional consequences of these dimers have to be investigated.

The determination of GPCR heterodimer function is a great challenge and will provide explanation for so far not understood cellular processes.

S3.4

Ago-allosteric effects of agonist drugs on 7TM receptors and their endogenous hormones – example from the ghrelin receptor

Thue W. Schwartz
University of Copenhagen, Copenhagen, Denmark.

Conventionally, an allosteric modulator is neutral in respect of efficacy and binds to a receptor site distant from the orthosteric site of the endogenous agonist. However, recently compounds being ago-allosteric modulators have been described i.e. compounds acting both as agonists on their own and as enhancers for the endogenous agonists in both increasing agonist potency and often providing additive efficacy - superagonism. The additive efficacy can also be observed with agonists, which are neutral or even negative modulators of the potency of the endogenous ligand. Based on the prevailing dimeric dogma for 7TM receptors, it is proposed that the ago-allosteric modulators often bind in the orthosteric binding site, but – importantly – in the "other" or allosteric protomer of the dimer. Hereby, they can act both as additive co-agonists, and through inter-molecular cooperative effects between the protomers, they may influence the potency of the endogenous agonist. It is of interest that at least some endogenous agonists can only occupy one protomer of a dimeric 7TM receptor complex at a time and thereby they leave the orthosteric binding site in the allosteric protomer free, potentially for binding of exogenous, allosteric modulators. If the allosteric modulator is an agonist, it is an ago-allosteric modulator; if it is neutral, it is a classical enhancer. Molecular mapping in hetero-dimeric class-C receptors, where the endogenous agonist clearly binds only in one protomer, supports the notion that allosteric modulators can act through binding in the "other" protomer. It is suggested that for the in vivo, clinical setting a positive ago-allosteric modulator should be the preferred agonist drug.

T.W. Schwartz & B. Holst: Ago-allosteric modulation and other types of allostery in 7TM dimeric receptors. **J. Recept. Signal. Transduct. Res.** (2006) 26: 107–128.

Gastroenteropancreatic endocrine tumors (GEP ET) – S4

S4.1

Pathological classification of GEP neuroendocrine tumors

Mauro Papotti, Luisella Righi & Marco Volante
University of Turin, Torino, Italy.

In the gastroentero-pancreatic tract, a spectrum of neuroendocrine tumors (NET) exists, including low grade tumors (carcinoid), intermediate grade malignant carcinoid, and high grade poorly differentiated carcinomas of the small and large cell types. In year 2000, the WHO presented a classification scheme for all NETs, but mostly applied to gastroentero-pancreatic tumors. This term carcinoid was replaced by (neuro)endocrine tumor, malignant carcinoid by well differentiated (neuro)endocrine carcinoma, and the term "small cell carcinoma" was confirmed for poorly differentiated NE neoplasms. The new terminology induced some confusion in the routine application and interpretation of some NETs, especially those of intermediate grade (which underwent major changes in the new classification). New diagnostic criteria pose problems to the pathologist (e.g. correct diagnosis on scarce biopsy or cytological material) and to the clinician (choice of the appropriate therapy for single histological types). The characterization of NETs includes the immunoprofiling of NE differentiation markers and hormonal products, but also the analysis of prognostic (i.e. Ki67) and therapeutic factors. The latter include somatostatin receptor expression profile (possible in surgical, biopsy or cytology specimens by immunohistochemistry), to identify possible targets of somatostatin analogs. Finally, apart from pure endocrine tumors, NE differentiation occurs also in non-endocrine tumors (*see review in Volante M, Virchows Arch 449:499, 2006*). "Mixed endocrine-exocrine carcinomas", as well as gastric, colorectal and pancreatic adenocarcinomas with foci of NE differentiation have been described. These latter tumors can account for up to 20% of cases, depending on the method used to assess the NE phenotype (eg chromogranin A immunostaining), but to date they were not found to bear any prognostic significance (as opposed to the well established prognostic role of NE differentiated prostate cancer), with the possible exception of gastric cancer, according to a recent study by Japanese authors (*Jiang SX, Am J Surg Pathol 30:945, 2006*).

S4.2

Biological, morphological work-up and screening for inherited disease

Britt Skogseid
Uppsala University, Uppsala, Sweden.

Pancreatic endocrine tumors (EPT) may occur sporadically or in association with the rare autosomal dominantly inherited tumor syndromes; multiple endocrine neoplasia type 1 (MEN1) and von Hipple Lindau (VHL). The genes causing these syndromes have been identified, and genotyping is possible which enables the laborious clinical investigations for diagnosis of lesions to be restricted to 50% of family members. For MEN1, no clinically useful genotype-phenotype correlation as been discovered and in a majority of patients the EPT will undergo malignant transformation. Timely identification and intervention by surgery before development of metastases currently represents the only cure of the disease. Thus, repeated extensive biochemical and radiological investigations for early recognition of small EPT *in situ* should be considered in asymptomatic gene carriers. Efficacies of genetic and hormonal screening programs as well as imaging will be discussed.

S4.3

Prognosis of GEP ET

E. Baudin
France.

Abstract unavailable

S4.4

Therapeutic management of GEP ET

P. Ruszniewski
France.

Abstract unavailable

Novel bioactive peptides – lessons from animals – S5

S5.1

Discovery of novel bioactive peptides: the uniquely important contribution of amphibians to mammalian neuropeptidology

Hubert Vaudry[1], Hervé Tostivint[1], Isabelle Lihrmann[1], Nicolas Chartrel[1], Alain Fournier[2], Jérôme Leprince[1], Marie-Christine Tonon[1] & J. Michael Conlon[3]
[1]INSERM U413, Laboratory of Cellular and Molecular Neuroendocrinology, IFRMP23, University of Rouen, Mont-Saint-Aignan, France; [2]INRS – Institut Armand-Frappier, Univeristy of Québec, Montreal, Canada; [3]Department Biochemistry, Faculty Medicine and Health Sciences, UAE University, Al Ain, United Arab Emirates.

The concentration of many neuropeptides in the brains of ectothermic vertebrates is several orders of magnitude higher than in the brains of mammals. We have taken advantage of this singular situation to isolate from the brain of the European green frog, *Rana esculenta*, a number of regulatory peptides that are orthologous to mammalian neuroendocrine peptides. These include α-MSH, γ-MSH, two tachykinins, two GnRH variants, CRH, PACAP, NPY, CGRP, CNP, GRP, and ODN. This peptidomics project has also led to the discovery of several novel neuroendocrine peptides that were first isolated from frog brain tissue but have subsequently been identified in mammals. In particular, we have characterized (1) the somatostatin-14 (S-14) isoform [Pro^2, Met^{13}]S-14 as well as authentic S-14, thereby providing the first evidence for the occurrence of two somatostatin variants in the brain of a single species, (2) the first tetrapod urotensin II, a peptide that had long been thought to be produced only in the caudal neurosecretory system of fish, (3) secretoneurin, a peptide derived from the post-translational processing of secretogranin II, and (4) 26RFa, a novel member of the Arg-Phe-NH_2 family of biologically active peptides. Orthologs of all these frog neuropeptides have now been identified in man and have been shown to exert important regulatory effects in mammals.

Supported by grants from INSERM (U413), the European Institute for Peptide Research (IFRMP23), the Platform for Cell Imaging of Haute-Normandie (PFRRICHN), the Conseil Régional de Haute-Normandie and the Laboratoire International Associé Samuel de Champlain.

S5.2

Comparative approaches to resolve the complexities of human appetite regulation

Dan Larhammar
Uppsala University, Uppsala, Sweden.

The regulatory processes of appetite and metabolism have turned out to be exceedingly complex and involve numerous hormones and neurotransmitters, particularly peptides. Evolutionary studies in our laboratory have shown that many genes encoding peptides and receptors were duplicated in the early stages of vertebrate evolution through chromosome duplications. Thus, many of the components have existed for 400–500 million years, for instance the various members of the families of NPY-like peptides, opioid peptides, tachykinins, glycoprotein hormone beta subunits (FSH, LH and TSH) and others. The chromosome duplications also explain the origin of many peptide receptors, for instance the NPY-family receptors, opioid receptors, oxytocin-vasopressin receptors, tachykinin receptors and CRF receptors. Also the glucocorticoid-mineralcorticoid receptors arose through a chromosome duplication. These observations of ancient chromosome duplications explain a great deal of the complexity of the vertebrate endocrine and neuronal networks. Duplication of complete genes in this manner means that the duplicates initially had identical gene regulation. This makes it particularly intriguing that some duplicates now have opposing functional roles. One striking example is the peptide hormone PYY, released from gut endocrine cells after meals, which acts as an appetite inhibitor on the Y2 receptor in the hypothalamus. In contrast, the related peptide NPY is the body's most potent stimulator of appetite, acting on receptor subtypes Y1 and Y5. Probably the switch in function occurred when the duplicated genes became expressed in different cell types. We have functionally studied the roles of the NPY-family peptides in a herbivorous species with frequent meals, the guinea-pig, and a carnivore with rare meals, the dog. The role of NPY appears to be the same in the guinea pig as in intermittent feeders like rats. We are presently evaluating the role of PYY as an appetite inhibitor in dogs. Functional studies in different species will provide a firmer basis for predicting and testing the functions of these peptides in humans as well as their possible roles in states of obesity and anorexia.

S5.3

Bioactive peptides in invertebrate model organisms

Liliane Schoofs, Inge Mertens, Geert Baggerman, Peter Verleyen & Elke Clynen
K.U.Leuven, Leuven, Belgium.

Genome sequence projects in combination with advances in mass spectrometry and bioinformatics have created several new possibilities for comparative endocrinology. In 2001 we introduced the peptidomics technology that allows the identification of the complement of native (neuro)peptides in cells, tissues, organs and organisms. Especially when genome sequence information is available (*D. melanogaster, A. mellifera, C. elegans*…), neuropeptidomes were successfully identified and compared in different physiological conditions.

Synthetic libraries of newly sequenced peptides can be used to screen orphan neuropeptide G-protein coupled receptors in cell-based assays that express the receptor. This has boosted receptor identification in insects and other invertebrates. One of the advantages of model organisms, such as *C. elegans* and *Drosophila* is their amenability for genetic manipulations and the availability of knockouts as a result of (ongoing) gene disruption programs.

In this presentation, we show how all these technological developments contributed to the discovery of novel neuropeptide signalling systems in *Drosophila* and in *C. elegans*. In the nematode worm, we will focus on the functional characterisation of neuropeptide processing enzymes and two neuropeptide GPCR signalling systems, respectively related to the mammalian GnRH receptor and the VPAC receptor in vertebrates. We will discus the implications of these findings with respect to the evolutionary conservation of these signalling systems.

S5.4

Somatostatin, cortistatin and their new and old receptors: from comparative to translational endocrinology

Justo P. Castaño, Mario Durán-Prado, Rafael Vázquez-Martínez, Antonio J. Martínez-Fuentes, Manuel D. Gahete, Raul M. Luque & Maria M. Malagón
Department Cell Biology, Physiology and Immunology. Univ. Córdoba, Cordoba, Spain.

omatostatin, originally isolated from ovine hypothalami in 1973, and cortistatin, identified a decade ago in amphibians and then in human and rodents, are two highly related peptides thought to derive from a common ancestor gene. Owing to their high structural homology, both peptides bind with similar affinity to the five so-called somatostatin receptors (sst1-sst5), and exert virtually undistinguishable effects on several physiological targets, including inhibition of endocrine secretions. Yet, each peptide also shows distinctive, specific functions, which should involve different receptors and/or signalling mechanisms still to be defined, and also display divergent patterns of expression in normal and tumoral tissues. In particular, cortistatin selectively regulates locomotion- and sleep-related processes and exerts potent antiinflammatory effects with a promising therapeutic potential. In this context, recent work from our group has aimed at characterizing the response of pituitary somatotrope cells to cortistatin and somatostatin, and to isolate sst receptors in a domestic species, the pig. This led to us to demonstrate that both peptides similarly exert a dual, inhibitory and stimulatory effect on GH release in vitro, which likely involve sst1/sst2 and sst5, respectively. Furthermore, while cloning porcine sst5, we discovered two new truncated isoforms of this receptor, termed psst5B and psst5C, which display distinct tissue distribution and, when expressed in clonal cell lines, show selective functional responses to somatostatin (psst5B) and cortistatin (psst5C). Interestingly, FRET studies revealed that these novel receptors functionally interact with their full-length counterpart psst5A, as well as with the rest of pig sst. Moreover, we recently cloned two similar human sst5 truncated isoforms (hsst5B and hsst5C) that also show selective functional response to somatostatin and cortistatin, functionally interact with and modulate hsst5A and hsst2, and are differentially distributed in normal and tumoral human tissues, suggesting a possible pathophysiological role for these novel receptors.

Support: CVI-139&P06-CTS-01705-J.Andalucia, BFU2004-03883-MEC/FEDER

Diabetes and insulin – S6

S6.1

Perspectives of islet cell transplantations

B Keymeulen
Belgium.

Abstract unavailable

S6.2

Cytokines as pathogenetic effectors in type 1 and type 2 diabetes

Thomas Mandrup-Poulsen
Steno Diabetes Center, Gentofte, Denmark; Karolinska Institute, Stockholm, Sweden.

The pro-inflammatory cytokine interleukin-1 is selectively cytotoxic to rodent and human beta-cells *in vitro*, and anti-IL-1 therapies reduce diabetes incidence in animal prevention models: (1) IL-1 alone or in combination with other inflammatory cytokines causes beta-cell destruction in rodent and human islets and in perfused pancreas via MAPK and NFkB signaling, (2) IL-1 given i.p. to non-diabetes prone animals causes transient insulinopenic diabetes (3) IL-1 is expressed early in islets of the non-obese diabetic (NOD) mouse, a model of spontaneous autoimmune diabetes (4) anti-IL-1 intervention prevents diabetes development in animal models of Type 1 diabetes and islet graft destruction and (5) transgenic mice with knock-out of the IL-1 receptor reduces diabetes incidence.

We recently completed a 13-week clinical study of IL-1 Receptor Antagonist (IL-1Ra, anakinra, Kineret^{R}, Amgen) therapy in Type 2 diabetics based on the rationale that *in vitro* glucotoxicity to human beta-cells can be prevented with IL-1Ra, and that glucose induces islet IL-1 production, which causes beta-cell apoptosis by pathways similar to those believed to operate in Type 1 diabetes. This study provided proof-of-principle that inhibition of IL-1 signalling can improve glycemia and beta-cell function in humans. Interestingly, maximal effect on glycosylated hemoglobin with anakinra was seen after 4 weeks, and fasting blood glucose was significantly reduced already after 1 week, suggesting rapid effects on beta-cell secretory capacity. These preclinical and clinical studies warrant studies to investigate the effect of IL-1 blockade in patients with recent-onset Type 1 diabetes mellitus.

S6.3

GLP-1 as a drug target

JJ Holst
Denmark.

Abstract unavailable

S6.4

Engineering beta cells to recover insulin function

Bernat Soria, A Hmadcha, JR Tejedo, FJ Bedoya & F Martin
Andalusian Center for Molecular Biology and Regenerative Medicine, Seville, Spain.

Stem cells are clonogenic cells capable of both self-renewal and multilineage differentiation. Therefore, these cells have the potential to proliferate and differentiate into any type of cell and to be genetically modified '*in vitro*', thus providing cells which can be isolated and used for transplantation. Moreover, these derived cells have proven to be useful in different animal models. Using a combination of several directed differentiation methods (nicotinamide, sonic hedgehog signalling inhibition, soluble factors from pancreatic buds] and a 'cell trapping' system, we have obtained insulin-secreting cells from undifferentiated embryonic stem cells. Lineage-trapping constructs used allows the expression of a neomycin selection system under the control of the regulatory regions of insulin gene and other B-cell genes, such as Nkx6.1. Selection of differentiated cells exclude non-differentiated cells which use to be present and are teratogenic. Transplanted animals correct hyperglycaemia within 1 week and restore body weight in four weeks. Graft removal rescued the diabetic condition. Glucose tolerance test (IPGTT) and blood glucose normalization after a challenge meal was similar in control and in transplanted mice. More recently, progenitors from peripheral human blood cells (PCMO) have been convinced to acquire an insulin-producing phenotype which normalize blood glucose of immunocompromised (SCID) diabetic mice, an option with tentative applications in regenerative medicine. This approach opens new possibilities for tissue transplantation in the treatment diabetes mellitus.

References:
1. Soria B *et al.* (2000) *Diabetes* **49**: 157–162.
2. Soria B *et al.* (2001) *Diabetologia* **44**: 407–415.
3. León-Quinto T *et al.* (2004) *Diabetologia* **47**(8): 1442–1451.
4. Runhke M *et al.* (2005) *Gastroenterology* **128**(7): 1774–1786.
5. Vaca P *et al.* (2006) *Stem Cells* **24**(2): 258–265.

Thyroid cell biology – S7

S7.1

New insights from zebrafish: the molecular and cellular base of thyroid development

Burkhard Alt, Dejan Adzic, Osama Elsalini & Klaus Rohr
University of Köln, Institute for Developmental Biology, Köln, Germany.

Due to experimental advantages such as its rapid development, transparent embryos, and accessibility for genetic analysis as well as embryonic manipulation, the zebrafish is a useful model organism for research on organogenesis. My lab has established that the basic mechanisms of thyroid development are essentially conserved on the morphological as well as on the molecular level between fish and mammals. We use zebrafish to identify as yet unknown factors involved in thyroid development. In this talk, I will give an overview about new, unique approaches to understand thyroid development in zebrafish. I will touch different aspects such as genetics of early induction, the molecular base of cellular behaviour in primordial relocalisation, and morphogenesis of the gland. Concentrating on selected molecules, I will exemplify how research on zebrafish contributes to a general understanding of thyroid development that sheds new light on the causes of congenital hypothyroidism.

S7.2

Involvement of cardiovascular development and non-cell autonomous signaling in mouse thyroid organogenesis

Henrik Fagman
Institute of Biomedicine, The Sahlgrenska Academy at Göteborg University, Göteborg, Sweden.

Thyroid dysgenesis (comprising agenesis, hemiagenesis or ectopic localization) is the major cause of congenital hypothyroidism in humans. Recent experimental observations indicate that thyroid dysgenesis may be a polygenic disease with variable penetrance depending on genetic background. Also, thyroid dysgenesis might be one manifestation of syndromic malformations. The molecular mechanisms of thyroid dysgenesis in humans are largely unknown; so far genes encoding thyroid transcription factors that are required for normal thyroid development in mouse, i.e. Titf1/Nkx2.1 (also known as TTF-1), Foxe1 (also known as TTF-2) and Pax8, have been found to be mutated only in a minority of patients. The underlying molecular mechanism is in most cases unknown, but the frequent co-incidence of cardiac anomalies (3–12%) suggests that the thyroid morphogenetic process may be linked to cardiovascular development.

I will give an overview about critical steps in murine thyroid morphogenesis. Emphasis will be put on proliferative patterns and the possible relationship between shaping of the thyroid and development of the of pharyngeal arch artery system. In this context, recent results from our laboratory providing a mechanistic explanation to thyroid dysgenesis incidentally reported to occur in children with the DiGeorge syndrome will be discussed. The role of non-cell-autonomous factors (Shh, Tbx1) in thyroid development will be put in relation to other transgenic models where thyroid dysgenesis has been described. Finally, possible clinical implications of the findings will be discussed.

S7.3

Thyroglobulin deposition and cathepsin-dependent Tg mobilization

Klaudia Brix[1], Sasa Jenko-Kokalj[2], Dusan Turk[2], Dieter Brömme[3], Nicole Kühl[1] & Silvia Jordans[1]
[1]Jacobs University Bremen, School of Engineering and Science, Bremen, Germany; [2]Jozef-Stefan Institute, Ljubljana, Slovenia; [3]University of British Columbia, Vancouver, BC, Canada.

Thyroid hormones thyroxine and triiodothyronine are essential for development, growth and metabolism. The prohormone thyroglobulin (Tg) is stored in high concentrations and in covalently cross-linked form within the lumen of thyroid follicles. Thyroid hormones are liberated from Tg in a regulated manner in that TSH triggers the secretion of lysosomal enzymes into the extracellular follicle lumen where they solubilize covalently cross-linked Tg and liberate thyroxine by partial Tg degradation. Using mice deficient in cysteine cathepsins B, K, and/or L, we showed that liberation of thyroid hormones from within Tg is based on the concerted action of a protease network. Cathepsins B and L are key players in conversion of cross-linked Tg-globules to soluble Tg. Moreover, assessment of thyroid morphology and serum thyroxine levels of cathepsin K- and L-deficient mice revealed impaired mobilization of Tg. The respective mice exhibited a phenotype reminiscent of hypothyroidism, proving the importance of cathepsins K and L for the liberation of thyroid hormones. Tg storage and Tg mobilization both occur extracellularly. Hence, the conditions for Tg processing are non-favorable for the proteolytic activity of lysosomal cysteine cathepsins. Therefore, we set-up an *in vitro* degradation assay that simulates the *in vivo* situation. Indeed, in such assays the cysteine cathepsins B, K, L and S were able to partially degrade their natural substrate Tg even at neutral pH and oxidizing conditions. Analysis of the cleavage sites of cysteine cathepsins under extracellular conditions revealed that sub-cellular and sub-follicular localization of the proteases as well as the timing of proteolysis are crucial steps in the regulation of thyroid hormone liberation from Tg. Any interference with the delicate protease network in the thyroid may result in impaired function.

S7.4

Role of the complex Megalin-RAP in thyroglobulin trafficking

M Marino
Italy.

Abstract unavailable

Advances in adrenal hypersecretory disorders – S8

S8.1

Autocrine-paracrine pathways in primary adrenal disorders

Hervé Lefebvre[1], Vincent Contesse[1], Dorthe Cartier[1], Véronique Perraudin[1], Catherine Delarue[1], Hubert Vaudry[1], Jérôme Bertherat[2], Pierre-François Plouin[3], Jean-Marc Kuhn[4] & Estelle Louiset[1]
[1]INSERM U413, IFRMP23, Laboratory of Cellular and Molecular Neuroendocrinology, University of Rouen, Mont Saint Aignan, France; [2]Department of Endocrinology, CHU Cochin & Institut Cochin, INSERM U567, CNRS UMR8104, IFR 116, University of Paris V-René Descartes, Paris, France; [3]Hypertension Unit, European Hospital Georges Pompidou, University of Paris V-René Descartes, Paris, France; [4]Department of Endocrinology, University Hospital of Rouen, Rouen, France.

It is now well demonstrated that, in the human adrenal gland, aldosterone and cortisol productions are stimulated by autocrine/paracrine factors, like serotonin (5-HT) and arginine vasopressin (AVP). Several data indicate that these signals may also be involved in the regulation of corticosteroidogenesis in adrenocortical hyperplasias and tumors. 5-HT is detected in clusters of steroidogenic cells in aldosterone-producing adrenocortical adenomas (APAs), and in both ACTH-independent macronodular adrenal hyperplasias (AIMAHs) and adenomas responsible for Cushing's syndrome. In these lesions, 5-HT stimulates steroidogenesis through activation of overexpressed eutopic 5-HT_4 and/or ectopic 5-HT_7 receptors. Immunohistochemical studies have shown the occurrence of AVP in a subpopulation of steroidogenic cells in APAs and AIMAHs. In APAs, AVP activates aldosterone production through the eutopic V_{1a} receptor whereas its stimulatory effect on cortisol secretion from AIMAH tissues is mediated by both overexpressed V_{1a} and/or ectopic V_{1b} and V_2 receptors. Interestingly, administration of V_{1a} antagonists to patients with APA induces an aldosterone response to the upright stimulation test, indicating that, in these tumors, inhibition of the vasopressinergic tone sensitizes the tissues to the action of posture-responsive hormones. Finally, the presence of ACTH has been observed in AIMAH tissues and the ACTH receptor antagonist corticostatin inhibits basal cortisol secretion from AIMAH explants, demonstrating that glucocorticoid production is dependent on the paracrine action of intraadrenal ACTH in some primary adrenal disorders causing Cushing's syndrome. In conclusion, autocrine/paracrine regulatory factors are produced within adrenocortical hyperplasias and tumors in which they play an important role in the control of steroidogenesis. These local factors may therefore represent promising targets for the treatment of primary adrenal disorders. *This work was supported by INSERM, the University Hospital of Rouen, the Conseil Régional de Haute-Normandie and the COMETE network (PHRC AOM 02068).*

S8.2

Carney complex and primary pigmented nodular adrenocortical diasease

Jérôme Bertherat
Institut Cochin, INSERM U567, Paris, France.

The Carney complex (CNC) is a dominantly inherited syndrome characterized by spotty skin pigmentation, endocrine overactivity and myxomas. The most common endocrine gland manifestations are acromegaly, thyroid tumors, testicular tumors, and ACTH-independent Cushing's syndrome due to primary pigmented nodular adrenocortical disease (PPNAD). PPNAD, a rare cause of Cushing's syndrome, is due to primary bilateral adrenal defect that can be also observed in some patients without other CNC manifestations nor familial history of the disease. Myxomas can be observed in the heart, skin and breast. Cardiac myxomas can develop in any cardiac chamber and may be multiple. One of the putative CNC genes located on 17q22-24, (*PRKAR1A*), has been identified to encode the regulatory subunit (R1A) of protein kinase A. Heterozygous inactivating mutations of *PRKAR1A* were reported initially in 45 to 65% of CNC index cases, and may be present in about 80% of the CNC families presenting mainly with Cushing's syndrome. PRKAR1A is a key component of the cAMP signaling pathway that has been implicated in endocrine tumorigenesis and could, at least partly, function as a tumor suppressor gene. More recently, germline inactivating mutations of PDE11A4 have been identified in patients with isolated primary nodular adrenocortrical disease. This underlines the importance of the cAMP signalling pathway in the pathophysiology of secreting endocrine tumors. Somatic *PRKAR1A* mutations have been observed in adrenal adenomas responsible for Cushing syndrome. *In vitro* and transgenic models have been developped to study the consequences of PRKAR1A inactivation. In these models dysregulation of the cAMP pathway, but also others signalling pathways, have been observed. The new insights coming from the genetics of CNC and these experimental models in the pathophysiology of endocrine tumorigenesis will be discussed.

S8.3

Diagnosis of primary aldosteronism

GP Rossi
Italy.

Abstract unavailable

S8.4

Adrenocortical carcinoma: current and future therapeutic options

Martin Fassnacht[1], Stefanie Hahner[1], Sarah Johanssen[1], Ann-Cathrin Koschker[1], Marcus Quinkler[2] & Bruno Allolio[1]
[1]Dept. of Medicine, University Hospital Wuerzburg, Wuerzburg, Germany; [2]Dept. of Medicine, Charite University, Berlin, Germany.

Adrenocortical carcinoma (ACC) is a rare and heterogeneous malignancy with incompletely understood pathogenesis and poor prognosis. Recent data from the German ACC Registry ($n=377$) demonstrate an overall 5-year survival of 46%. Survival is clearly stage-dependent ($P<0.01$) with a 5-year survival of

85% in stage 1, 56% in stage 2, 42% in stage 3, and 16% in stage 4, respectively.

In stages I –III open surgery by an expert surgeon aiming at a R0 resection is the treatment of choice. However even after R0 resection, only 37% of the patients are disease-free after 5 years. Therefore, adjuvant treatment options are urgently needed. In a recent series including 177 patients from Italy and Germany, adjuvant mitotane prolonged significantly disease-free survival compared to observational follow-up. In addition, adjuvant radiotherapy of the tumor bed is a promising option to prevent local recurrence.

In tumor recurrence and metastatic disease, surgery should be considered if complete resection is feasible. In patients not amenable to surgery, mitotane (alone or in combination with cytotoxic drugs) remains the treatment of choice. Monitoring of drug levels (therapeutic range 14–20 mg/l) is mandatory for optimum results. In advanced disease, the most promising therapeutic options (etoposide, doxorubicin, cisplatin plus mitotane and streptozotocin plus mitotane) are currently compared in an international phase III trial (www.firm-act.org). In 2006, we have administered EGFR inhibitors and VEGF antibody as salvage therapy in a small series of patients – so far without significant success. However, in 2007 the Collaborative group for Adrenocortical Carcinoma Therapy (CO-ACT) will initiate three new trials for salvage therapies investigating two multi-targeted tyrosine kinase inhibitors and an IGF-1 receptor antibody, respectively, leading hopefully to improved clinical outcome. Future advances in the management of ACC will depend on a better understanding of the molecular pathogenesis of ACC facilitating the use of new targeted therapies.

Imaging in endocrinology – S9

S9.1

PET in diagnostics of metabolic alterations and endocrinological tumours

Pirjo Nuutila
University of Turku, Turku, Finland.

Positron emission tomography (PET) is an imaging technique that enables direct observation of tissue radioactivity concentration over time in vivo. Unlimited number of natural substrates (e.g. glucose, fatty acid), substrate analogs can be labelled for use with PET. PET combined with tracer kinetic models measures blood flow, membrane transport, metabolism, ligand receptor interactions and recently also gene expression noninvasively and quantitatively.

An abnormal action of insulin and handling glucose and fatty acids in muscle, heart, liver, brain and visceral and subcutaneous adipose tissue have been studied *in vivo* in humans. This tissue specific assessment has increased the understanding of the pathophysiology of metabolic syndrome, obesity and diabetes and the differences in tissue specific action *in vivo*. The effects of insulin, free fatty acids, exercise and diet have been evaluated. PET is powerful tool for the assessment of tissue specific action of drugs targeted to metabolic disorders. The hybrid PET/CT scanners enable correlation of anatomic and functional information.

The clinical use of PET is rapidly expanding. In addition to (18)F-labelled deoxyglucose (FDG) which is routine used in oncology for diagnosis of cancer, many more specific tracers have been shown to improve diagnostics of neuroendocrinological tumours (NETs). The most promising of those is (18)F-fluorodihydroxyphenylalanine (FDOPA). It appears to be more useful in carcinoid tumours than scintigraphic imaging and might replace it. It is more sensitive than CT or MRI in detection of insulinomas and has quickly replaced other methods in the assessment of focal form of congenital hyperinsulinism of infancy (CHI). FDOPA-PET improves diagnostics and prediction of prognosis, and be used to assess patients' response to treatment for NETs.

S9.2

Macro-, micro-, and molecular imaging of bone

Claus-C. Glüer
Medizinische Physik, Klinik f. Diagnostische Radiologie, Universitätsklinikum Schleswig-Holstein, Campus Kiel, Germany.

The clinical diagnostic examination of patients and the research-oriented investigation of the pathophysiology of skeletal disorders require imaging techniques that allow to visualize the skeleton at different scales: from the organ level to tissue, cellular, and subcellular levels, depicting morphology and function. Progress in the field of imaging technologies resulted in methods suited for clinical investigation of patients in vivo, non-invasive methods for preclinical animal studies and sophisticated functional and molecular imaging methods for both *in vivo* and *ex vivo* characterization of bone status have been introduced.

At the macroscopic scale the mechanical function of individual bones can now be assessed by 3-D volumetric spiral CT approaches. The image-data collected can be analyzed using Finite Element Models to calculate breaking strength under simulated impacting forces. This allows more accurate identification of subjects at risk for fracture and the monitoring of progress in fracture healing.

At the microscopic scale micro-CT has seen impressive advances with ever increasing image resolution – some devices are now suited for nano-CT imaging. This technology allows studies on the effects of bone turnover in normal and diseased tissue, including metabolic bone disorders such as osteoporosis but also of arthritis and skeletal tumours and metastases. Examinations of living animals enable the non-invasive longitudinal monitoring of skeletal effects of therapeutic interventions.

Finally, molecular imaging, i.e. the visualization of molecular, biochemical or cellular processes with radiological methods: to date this method is mostly restricted to animal studies. However, the achievements seen here are impressive: localized visualization of molecular and physiological information, e.g. imaging of labelled osteoblasts and their precursors, monitoring of the effects of hormones or gene therapy, or an earlier identification of skeletal metastases. Substantial research is still required to bring these advances to the clinic but the prospects for better individualized patient care based on combined molecular imaging and therapy are most exciting.

S9.3

New tracers for neuroendocrine tumors

Marcel Stokkel
LUMC, Leiden, Netherlands.

Neuroendocrine tumors (NET) comprise a wide variety of neoplasms that have certain characteristics in common. However, they are defined not by site but by molecular characteristics. The most common forms arise in the gastrointestinal tract, but there are NET which are not directly related to this site, such as medullary cell carcinoma or small-cell lung cancer. Different secretory syndromes have also resulted in certain subtypes receiving names as carcinoid if they produce serotonin or insulinoma if they produce insulin, etc. It has to be realized however, that 50% are described as non-secretors. With respect to nuclear medicine techniques available, many reports have focussed on the use of meta-Iodobenzylguanidine (MIBG) and radiolabelled somatostatin analogs, such as Indium-111-octreotide. Especially In-111-octreotide has a reported sensitivity ranging from 65% for medullary thyroid cancer to almost 100% in small-cell lung cancers and pancreatic neuroendocriene tumors. Many other potential receptors other than the somatostatin receptors, such as GRP-R, CCK2, GLP-1-R, NK1 and VPAC1, have been developed and studied over the past years. It has been suggested that the simultaneous expression of multiple of these peptide receptors in NET provide the molecular basis for in vivo multireceptor targeting, thus improving the efficacy of radiolabelled peptides for diagnosing, staging and treating NET. Most of the peptides under study directed against the previously mentioned receptors were labelled with Indium-111 or Technetium-99m, both easily applicable in clinical practice. Despite optimal results of positron emission tomography (PET) using F18-deoxyglucose in many malignant tumors, its role in NET is still limited. In contrast, PET using F18-DOPA and Ga-68-DOTA octreotate has shown promise. C11-5-hyroxytryptophan (C11-5-HTP) has demonstrated specific and irreversibly entrapment by serotonin-producing tumors, but it has been shown that non-functioning or poorly differentiated tumors or necrotic ones cannot be detected accurately. Highly important improvements have been made by the introduction of hybrid cameras such as SPECT/CT or PET/CT. The combination of both techniques allows whole body imaging quickly providing functional and anatomic information. A close clinical relation between imaging and treatment with radiolabelled peptides has been established over the past decades. Many studies have reported good and/or promising results with respect to Lutetium-177 (Lu-177) and Yttrium-90 (Y-90) labelled peptides, such as Y-90 DOTATOC or Lu-177-lanreotide. In current presentation, an overview is given on the nuclear medicine diagnostic and therapeutic options and developments in neuroendocrine tumors.

S9.4

Echoendoscopy for the diagnosis of pancreatic endocrine tumors

Claudio De Angelis
Gastrohepatology Department, San Giovanni Battista Hospital, University of Turin, Turin, Italy.

Pancreatic neuroendocrine tumors (NET) have always represented a complex dilemma for diagnostic imaging. This is mainly due to their small size and brought during the years to a complex range of diagnostic proposals. A correct preoperative detection and staging are mandatory in order to choose management options and to optimize surgical treatment. Endoscopic Ultrasound (EUS) has been claimed to be the best technique for imaging the pancreas, it allows.

High resolution images of the main pancreatic duct and surrounding parenchyma. One of the more relevant advantages of EUS compared with US, CT and MRI was indeed the superior parenchymal resolution, that gives reason for the results of several studies that established the superior sensitivity of EUS (98%) for the diagnosis of pancreatic tumors in comparison to all the other imaging modalities. The results of EUS were even better in small tumors, less than 3 or 2 cm, where sensitivity of US and CT decreased to only 29%. However the introduction of multidetector helical CT has today revolutionized the field of pancreatic imaging. More recent data on pancreatic NETs confirmed that the distance between helical-CT and EUS has nearly been annulled. EUS remains the best method for the detection of small pancreatic insulinomas and gastrinomas, but the first imaging modality to be used today in the suspicion of a pancreatic NET must be a multislice CT. EUS is needed as a second step in the diagnostic algorithm when CT shows negative or doubtful results. So the most effective method for revealing pancreatic NET is a combined imaging protocol that consists of both CT and EUS. The endosonographic pattern of these tumors is mainly represented by small focal hypoechoic, omogeneous, round lesions, with sharp margins, often hypervascular. Several studies have shown the high sensitivity and specificity of EUS in localizing endocrine tumors of the duodeno-pancreatic area. We demonstrated a correct localisation of pancreatic tumors in 86.7% of 23 cases surgically confirmed. In conclusion EUS is highly accurate in the detection of pancreatic neuroendocrine tumors and is cost effective when used early in the preoperative localization strategy. EUS decreased the need for additional invasive tests and avoided unnecessary morbidity and resource consumption.

EUS should play a primary role in preoperative localization and staging of these tumors.

GH and prolactin at their targets – S10

S10.1

Cellular control mechanisms for GH sensitivity

Ger Strous, Peter van Kerkhof, Monique van den Eijnden & Joyce Putters
University Medical Center, Utrecht, Netherlands.

The growth hormone (GH) receptor is a key regulator of cellular metabolism. Using model cell systems we have investigated how GH-induced signaling is regulated, both in paracrine and autocrine conditions.

Three features render GHR unique: (a) an active ubiquitination system is required for both endocytosis and degradation in lysosomes; (b) uptake of receptor is a continuous process, independent of GH binding and Jak2 signal transduction; (c) only cell surface expression of *dimerised* GHRs is controlled by the ubiquitin system. Despite recent progress, molecular mechanisms underlying GHR endocytosis and degradation are unknown. Evidence from research on the interferon and prolactin receptors has identified SCF^{TrCP} as a positive factor for their degradation. This E3 is known for its regulatory role in cell division and various signal transduction pathways. Our results show that the ubiquitin ligase SCF^{TrCP} is required for GHR endocytosis: removal of its mRNA by small interference RNA results in inhibition of GH uptake. In addition, we demonstrate that interaction between GHR and beta-TrCP is independent of a canonical DSGxxS motif. These results show the involvement of a SCF E3 ligase in endocytosis, thereby regulating GH-sensitivity of cells. In cells that produce both GH and GHR, the situation is basically the same. In these cells we investigated how GH affects GHR receptor degradation, and how the Jak/Stat signaling pathway is regulated. The consequences of these studies are important for understanding autocrine-activated GHR in fetal and peri-natal, and cancer tissues.

S10.2

GH receptor signalling

Gunnar Norstedt, Petra Tollet Egnell & Amilcar Flores Morales
Karolinska Institutet, Stockholm, Sweden.

GH receptor stimulation changes intracellular protein phosphorylation and activates the JAK-STAT signalling pathway. The JAK2 - STAT5 components of this pathway seem critical for growth. Factors of essence for cellular effects of GH include the duration of GH receptor stimulation and in different species there are sex differences in GH secretion where males have an episodic and females have a more continuous mode of GH secretion. At the cellular level, these two types of GH secretion cause different gene expression patterns to emerge and this is in particular the case for GH effects on the liver. GH controls important aspects of liver metabolism and it is interesting to note that some of these seem to depend on the secretory GH pattern. Another aspect of GH signaling is that the duration of GH receptor signals is related to changes in SOCS (suppression of cytokine signaling) expression. The SOCS proteins seem to be part of an intracellular feed back loop that silence GH signals. In our studies, SOCS2 appears to be a key intracellular regulator of GH sensitivity since elimination of SOCS2 creates a situation of increased GH sensitivity. Our working hypothesis is that SOCS2 ubiqitinates the GH receptor and thereby causes its proteasomal degradation. The concept that SOCS2 is a part of an ubiquitin ligase complex is substantiated by structural and biochemical findings. Furthermore, the gene targets for GH induced signals include the SOCS2 gene. In this gene we have characterized STAT 5 DNA binding elements in proximity to another transcription factor binding site that is unique for SOCS2 the SOCS protein family. In summary our data suggest that the liver is an important tissue for GH to exert metabolic regulation and that SOCS2 is a component that determines GH sensitivity.

S10.3

Gene expression profiling of the antiangiogenic factor 16K human prolactin (hPRL) on endothelial cells underlines the key role of NF-κB and reveals novel mechanisms of action

Sébastien Tabruyn[1], Céline Sabatel[1], Ngoc-Quynh-Nhu Nguyen[1], Catherine Verhaeghe[1], Karolien Castermans[2], Ludovic Malvaux[1], Arjan Griffioen[2], Joseph Martial[1] & Ingrid Struman[1]
[1]University of Liège, GIGA-Research, B-4000 Sart Tilman, Liège, Belgium; [2]University and Hospital of Maastricht, P.O. Box 5800, NL-6202 Maastricht, The Netherlands.

The 16-kDa N-terminal fragment of human prolactin (16K hPRL) is a potent angiostatic factor described to prevent tumor growth in mouse models. Using microarray experiments, we have dissected how the endothelial-cell genome responds to 16K hPRL treatment. Of the 23.000 transcripts present on the chips, 210 are regulated by 16K hPRL. Bio-informatic analysis and experiments performed on endothelial cells with various chemical inhibitors clearly suggest that NF-κB is crucial for the direct regulation of the majority of theses genes. In addition, our results reveal that the angiogenesis inhibitor 16K hPRL regulates apoptosis and proliferation in endothelial cells by numerous non-previously identified targets. Unexpectedly, a large proportion of 16K hPRL-regulated genes turned out to be associated with the process of immunity. 16K hPRL induces expression of various chemokines and endothelial adhesion molecules. These expressions, under the control of NF-κB, result in an enhanced leukocyte-endothelial cell interaction. Furthermore, analysis of B16-F10 tumor tissues reveals a higher expression of adhesion molecules (ICAM-1, VCAM-1 or E-selectin) in endothelial cells and a significantly higher number of infiltrated leukocytes within the tumors treated with 16K hPRL than in the untreated ones. In conclusion, this study describes a new anti-tumor mechanism of 16K hPRL. Since cellular immunity against tumor cells is a crucial step in therapy, the discovery that treatment with 16K hPRL overcomes tumor-induced anergy may become important for therapeutic perspectives.

Work supported by grants from the F.R.I.A. (“Fonds pour la formation à la recherche dans l’industrie et l’agriculture”), Télévie, F.N.R.S. (“Fonds national pour la recherche scientifique”), the Fédération Belge contre le Cancer and the Université de Liège (Fonds Spéciaux).

S10.4

Development of human prolactin receptor antagonists

Vincent Goffin[1], Vincent Rouet[1], Jean-Baptiste Jomain[2], Estelle Tallet[2], Christine Kayser[1] & Paul A. Kelly[2]
[1]Inserm, Paris, France; [2]University René Descartes Paris 5, Paris, France.

Experimental, clinical and/or epidemiological evidence points to a role for prolactin (PRL) in the promotion of benign and malignant tumors of the breast

and the prostate. However, therapies reducing PRL levels (dopamine agonists) are currently not considered for the treatment of these pathologies. Dopamine agonists only target PRL secretion from the pituitary, while recent observations suggest that the involvement of autocrine PRL is perhaps even more relevant than circulating PRL in the growth of breast/prostate tumors. Therefore, alternative strategies targeting locally-produced PRL warrant investigation.

For many years, we have been working on the development of PRL receptor antagonists, by introducing mutations into appropriate regions of human PRL. Ideal antagonists should be high-affinity ligands that bind but do not activate the PRL receptor, leading to competitive inhibition of endogenous PRL actions. This presentation will describe the most representative antagonists we have designed, including their structure-function relationships based on cell and animal studies. We will also discuss the pros/cons of our lead compound, the pure antagonist del1-9-G129R-hPRL. Finally, we will address the potential therapeutic indications of this novel class of molecules.

Polycystic ovary syndrome – S11

S11.1

The CAG repeat polymorphism of the androgen receptor gene is an independent risk factor for polycystic ovary syndrome (PCOS)

Andreas Schuering[1], Andrea Jurgens[1], Jorg Gromoll[2], Michael Zitzmann[2], Barbara Sonntag[1], Eberhard Nieschlag[2], Robert Greb[1] & Ludwig Kiesel[1]
[1]Department of Obsterics and Gynecology Muenster University Hospital, Muenster, Germany; [2]Institute of Reproductive Medicine, Muenster University Hospital, Muenster, Germany.

Introduction
Polycystic ovary syndrome (PCOS) is a frequent disorder with a variable phenotype and a suspected genetic background. Androgenic effects constitute the central mechanism for the clinical, biochemical and sonographic features of PCOS. Androgenic effects are transported by the androgen receptor, whose activity can be modulated by a genetic polymorphism. We investigated the role of the CAG repeat polymorphism of the androgen receptor in PCOS.
Patients and methods
In the infertility unit of a university clinic 126 patients fulfilling the Rotterdam criteria of PCOS were compared with 184 controls undergoing a standardized diagnostic work-up prior to infertility treatment. Individuals were assessed regarding clinical, endocrine and sonographic parameters indicating the presence of PCOS. The number of CAG repeats was determined by PCR, labelling with IR-800 and PAGE. X-chromosome inactivation was assessed by a methylation-sensitive assay. CAG repeat length was compared between groups and correlated with the extent of oligomenorrhoea. In a regression analysis CAG repeat length was tested including established risk factors of PCOS.
Results
PCOS patients displayed a shorter mean CAG repeat length compared to controls ($P=0.001$). CAG repeat length correlated inversely with the extent of oligomenorrhea, a central androgen dependent feature of PCOS ($P=0.007$). In a binomial regression analysis including BMI, LH and testosterone, CAG repeat length was identified as a novel independent risk factor for PCOS ($P=0.001$).
Conclusion
The CAG repeat polymorphism was identified as a novel independent risk factor for PCOS. It could constitute a factor in the familial background, convey the phenotypic variability and transport metabolic consequences of the syndrome.

S11.2

Genetic markers of polycystic ovary syndrome (PCOS)

Joop Laven
Div Reproductive Medicine, Rotterdam, Netherlands.

Polycystic ovary syndrome (PCOS) represents the most common cause of anovulatory infertility and its etiology is still unknown. Gene expression profiles from human PCOS ovaries have identified dysregulated expression of genes encoding components of several biological pathways or systems such as Wnt signaling, extracellular matrix components, immunological factors and androgens which, seem to play a key role in the pathogenesis of PCOS.
Candidate genes have been extensively studied using Single Nucleotide Polymorphisms (SNP's). The impact of functional SNP's on Gonadotrophins, growth factors and their receptors as well as the consecutive enzymes of the steroid biosynthesis pathways have been assessed in PCOS. Up till now only two functional SNP's have been consistently associated with PCOS. An FSH receptor and an aromatase polymorphism seem to be more prevalent in PCOS and are both associated discrete changes in the endocrine environment in PCOS.
Family studies and linkage analysis is hampered by the lack of large well phenotyped family cohorts. Recently we have studied PCOS patients from an isolated population aiming to map gene(s) involved in PCOS susceptibility. The genome wide association analysis revealed only weak evidence of association for some markers scattered over the genome. Taken these findings into account it seems that PCOS constitutes a complex genetic disease with multiple genetic contributors which, might in turn be modified through different environmental factors. The individual contribution of these genetic components to the phenotype of PCOS seems to be very limited and hence, detection of genetic factors is far from easy.

S11.3

Hyperandrogenism and metabolic syndrome (MBS) in polycystic ovary syndrome (PCOS)

Andrea Dunaif
The Feinberg School of Medicine, Northwestern University, Chicago, United States.

PCOS is a complex genetic disease resulting from the interplay between susceptibility genes and environmental factors. The syndrome is characterized by hyperandrogenism, disordered gonadotropin secretion, profound insulin resistance and, frequently, obesity. It is a leading risk factor for type 2 diabetes mellitus and MBS in adolescent and young adult women. In PCOS, MBS risk increases with increasing androgen levels, independent of insulin resistance and obesity, and antagonizing androgen action ameliorates features of MBS. Obese premenarchal girls have elevated androgen levels. Hyperandrogenemia is the major reproductive phenotype in families of women with PCOS, including mothers and brothers. First-degree relatives also have metabolic phenotypes, including MBS. We have now mapped a genetic variant conferring PCOS susceptibility to an allele of a dinucleotide repeat in an intron of the fibrillin-3 gene on chromosome 19p13.2 that is both linked and associated with the reproductive phenotype. Further, the PCOS susceptibility allele is associated with metabolic phenotypes in women with PCOS and their first-degree relatives. These observations suggest that the cardinal reproductive defect in PCOS, hyperandrogenemia, itself contributes to metabolic risk. *In utero* testosterone excess can reproduce features of the PCOS reproductive and metabolic phenotypes in rodents, sheep and non-human primates. We propose that hyperandrogenemia resulting from variation in a gene(s) regulating steroidogenesis causes many of reproductive and metabolic features of PCOS by programming actions at critical periods of development as well as by ongoing actions in the adult. Additional environmental factors, such as obesity, modify these phenotypes.

S11.4

Individual pharmacological therapy for polycystic ovary syndrome: lessons from the phenotype

Renato Pasquali
Division of Endocrinology, S. Orsola-Malpighi Hospital, University of Bologna, Bologna, Italy.

Hyperandrogenism, hyperinsulinemia and insulin resistance are the cardinal features of most women with the polycystic ovary syndrome (PCOS). They contribute in different ways to its phenotypic expression, including hirsutism, menses abnormalities, oligo-anovulation, metabolic disturbances, and susceptibility to develop type 2 diabetes. From the theoretical point of view, individual pharmacotherapy of PCOS should be planned in order to counteract the main pathophysiological mechanisms, with the aim of producing an overall benefit on all clinical and biochemical aspects of the disorders, after the major complaints of each individual have been considered. Dietary-induced weight loss and life style modifications should however represent the first line therapeutic advice for every obese woman with PCOS. Whether this applies in otherwise normal weight PCOS women has not yet been demonstrated, although the scientific basis for such an

approach is appealing, particularly in those with abdominal fatness and insulin resistance.
Since almost all obese PCOS women and more than half of lean PCOS women are insulin resistant, therefore presenting some degree of hyper-insulinemia, the use of insulin sensitizers should be suggested in most patients with PCOS. Their use has been associated with a reduction in androgen levels, improvement of insulin and insulin resistance, and reversal of serum lipid abnormalities and PAI-1. This therapy has also been associated with a decrease in hirsutism and acne, although the main benefit should be expected on menses abnormalities, anovulation and infertility. In our experience, at least one third of obese PCOS women improve menses and ovulation after a short period of treatment with metformin and life style changes. Antiandrogens have been used for a long-time in the treatment of hirsutism and hyperandrogenemia, We have recently performed pilot studies to investigate potential additional effects of long-term treatment with antiandrogens, and we have found that they can selectively improve visceral fatness, lipid abnormalities and even insulin resistance, although their main effect was on hirsutism and hyperandrogenemia.
The dual approach with insulin sensitizers and/or antiandrogens may provide a rationale for targeting different therapeutical options according to the required outcomes.

Hypothalamic network controlling food intake – S12

S12.1

Processing of metabolic signals in the hypothalamus: the integrative role of the paraventricular nucleus

Zsolt Liposits[1], Ronald Lechan[2] & Csaba Fekete[1]
[1]Institute of Experimental Medicine, Budapest, Hungary; [2]Tufts-New England Medical Center, Boston, MA, United States.

The hypothalamic paraventricular nucleus (PVN) is a major regulatory centre of energy homeostasis by possessing the unique capability of simultaneously controlling endocrine axes, water balance and autonomic functions. It receives neuronal information form orexigenic and anorexigenic cell groups of the basal hypothalamus that monitor peripheral metabolic signals (leptin, insulin, ghrelin, glucose, glucocorticoids) and also from brainstem centers relaying sensory information from visceral organs. In the regulation of energy homeostasis, the hypophysiotrophic corticotropin-releasing hormone (CRH) and thyrotropin-releasing hormone (TRH) neuronal systems play a key role and both neuron populations are wired to neuronal circuits of the basal hypothalamus and the brainstem. The lecture provides information about the structural organization, functional domains and major neuronal connections of the PVN, introduces the novel glutamatergic phenotype of hypophysiotrophic CRH and TRH systems, elucidates the diverse chemical nature of their synaptic afferents and describes the structural correlates of retrograde endocannabinoid signalling acting upon inhibitory and excitatory presynaptic terminals in the nucleus. The presentation also reveals distinct hypothalamic and extrahypothalamic sources of neuronal afferents carrying orexigenic (NPY) and anorexigenic (CART) peptides to TRH and CRH neurons and demonstrates the impact of the released neuropeptides on the postsynaptic targets. In addition to rodent data, the interrelationship of NPY and α-MSH neuronal systems and the features of their projections to CRH and TRH neurons will be presented in *post-mortem* human hypothalamic samples.
Supported by grants from the National Science Foundation of Hungary (OTKA T046492, T046574), NKFP 1A/002/2004 and the Sixth EU Research Framework Programme (LSHM-CT-2003-503041).

S12.2

Neurotransmitter content of orexigenic and anorexigenic neurones

Björn Meister, Katrin Dürr & Ebba Norsted
Karolinska Institutet, Stockholm, Sweden.

During the last two decades attention has been focused on the role of different neuropeptides in hypothalamic control of feeding behavior. Se-veral hypothalamic peptides that participate in the control of ingestive behavior are produced in neuronal cell bodies of the arcuate nucleus and/or the lateral hypothalamic area. Apart from producing orexigenic or anorexigenic compounds of peptidergic nature, it has recently become apparent that these neurons also produce several classical neurotrans-mitters. The role of classical transmitters in regulating energy ba-lance has received less attention in comparison to neuropeptides. The arcuate nucleus-median eminence area, a region with a weak blood-brain barrier (BBB), contains at least two neuronal cell populations that exert oppo-sing actions on energy balance. The majority of the neurons located in the ventromedial aspect of the arcuate nucleus, which pro-duce the or-exigenic peptides neuropeptide Y (NPY) and agouti-related peptide (AGRP), in addition contain the GABA-synthesizing enzyme glutamic acid decarboxylase (GAD) and the vesicular GABA transporter (VGAT), thereby supporting their GABAergic nature. Subpopulations of anorexi-genic neurons producing proopiomelanocortin (POMC)- and cocaine- and amphetamine-regulated transcript (CART), located in the ventro-lateral division of the arcuate nucleus have recently been reported to contain the vesicular acetylcholine (ACh) transporter (VAChT) and cho-line acetyltrans-ferase (ChAT), markers for cholinergic neurons, or the vesicular glutamate transporter 2 (VGLUT2), a marker for glutamatergic neurons. In addition, two new neuropeptides in have been identified in arcuate POMC neurons. In the lateral hypothalamic area, hypocretin-/orexin neurons express VGLUT1 or VGLUT2, but not GAD, whereas some melanin-concentrating hormone (MCH) cells contain GAD. These observations support the view that ACh, GABA and glutamate, relatively neglected feeding transmitters, are present in neurons that regulate body weight and consequently may represent important orexigenic-/anorexigenic mediators that convey information from the hypo-thalamus to other brain regions that participate in regulation of energy balance.

S12.3

The picture of the hypothalamus is becoming clearer: new concept of cross-talk

Tamas Horvath, Qian Gao & Sabrina Diano
Yale University School of Medicine, New Haven, CT, United States.

Significant advancements have been made in the last century regarding the neuronal control of feeding behavior and energy expenditure. The effects and mechanism of action of various peripheral metabolic signals on the brain have become clearer. Molecular and genetic tools for visualizing and manipulating individual components of brain homeostatic systems in combination with neuroanatomical, electrophysiological, behavioral and pharmacological techniques have begun to elucidate the molecular and neuronal mechanisms of complex feeding behavior and energy expenditure. This talk will attempt to highlight some of these advancements that have lead to the current understanding of the brain's involvement in the acute and chronic regulation of energy homeostasis. The case will also be made to suggest that the hypothalamic circuitry, which governs feeding behavior, is an appropriate model to examine in order to yield the experimental proof for the causal relationship between synaptic plasticity and behavior.

S12.4

Whom is insulin in the brain speaking to?

J Bruening
Germany.

Abstract unavailable

Glucocorticosteroids – S13

S13.1

Recent developments in nuclear receptor action

JA Gustafsson
Sweden.

Abstract unavailable

S13.2

Evaluation of steroid receptor function by gene targeting in mice

Gunther Schuetz
German Cancer Research Center, Dept. of Molecular Biology of the Cell I, Heidelberg, Germany.

Germline and somatic gene targeting of genes for steroid hormone receptor allows the characterization of their functions as well as their molecular modes of action. For the glucocorticoid receptor (GR) multiple modes of action have been identified. The receptor activates expression of genes, e.g. for gluconeogenic enzymes in hepatocytes, by binding as a dimer to glucocorticoid response elements (GRE) as well as by interaction with Stat5, functioning as a coactivator for DNA-bound Stat5. This functional interdependence of GR and Stat5 is reflected by sharing one third of their target genes. The receptor is able to repress AP-1/NF kB-dependent expression of genes involved in inflammation by protein-protein interaction and inhibits proopiomelanocortian and prolactin expression via binding to negative GREs. Cre/loxP-mediated generation of somatic mutants of the mineralocorticoid receptor (MR) circumvents the early lethality observed after germline inactivation. Inactivation of MR in the forebrain leads to impaired hippocampal-dependent learning as evidenced in Morris water- and radial maze analyses. Normal circadian corticosterone levels indicate that the limbic MR is dispensable for the maintenance of basal hypothalamic-pituitary-adrenal axis activity. The mechanisms underlying the critical actions of estrogen in the secretion of the gonadotropin-releasing hormone (GnRH) are unknown. A neuron-specific ERa mutation in the forebrain leads to infertility and loss of the positive feedback effects of estrogen upon GnRH neurons. As GnRH neurons do not express ERa, these results indicate that ERa-expressing neuronal afferents to GnRH neurons are critical for the preovulatory GnRH/LH surge. These genetic approaches to evaluate steroid hormone receptor activity not only reveal novel neural functions of these regulatory molecules in gene expression, but also unprecedented modes of their activity.

S13.3

The 11β-hydroxysteroid dehydrogenase story

Jeremy Tomlinson
University of Birmingham, Birmingham, United Kingdom.

The global epidemic of obesity has heightened the need to understand the mechanisms that contribute to its pathogenesis and also to design and trial novel treatments. Patients with glucocorticoid (GC) excess, 'Cushing's syndrome' share many phenotypic similarities to patients with simple obesity. GC availability to bind and activate the glucocorticoid receptor (GR) is controlled by the type 1 isoform of 11β-hydroxysteroid dehydrogenase (11β-HSD1) that converts inactive cortisone to cortisol and therefore amplifies local GC action. We have previously shown that expression of 11β-HSD1 is crucially important in both adipocyte differentiation and proliferation; Furthermore, over-expression specifically within adipose tissue leads to obesity and insulin resistance in rodent models. In addition, we have recently been able to show that inhibition of 11β-HSD1 in human adipose tissue can limit GC induced lipolysis. Selective 11β-HSD1 inhibitors (selective in that they block the activity of 11β-HSD1 and not 11β-HSD2 which inactivates cortisone to cortisol in mineralocorticoid target tissues) are currently in development although not yet available for use in clinical studies. Rodent studies utilizing these compounds have shown dramatic improvements in insulin sensitivity as well as improvements in lipid profiles and atherogenesis. The most fundamental question is whether these observations in rodents will translate to the clinical setting. It is likely that within the very near future, data from the first human studies will be available. If these compounds prove to be as efficacious in humans, then they may well represent an entirely novel, additional therapeutic strategy in the treatment of obesity, insulin resistance and type 2 diabetes.

S13.4

Glucocorticoid sensitivity: consequences for the clinic?

Jan W Koper
Internal Medicine / Endocrinology, Erasmus MC, Rotterdam, Netherlands.

Glucocorticoids (GCs) exert a wide variety of functions throughout the human body, including mediation of the stress response, regulation of lipid and glucose metabolism, immunosuppressive and anti-inflammatory actions, vascular effects, increase of bone resorption, as well as effects on the development and function of numerous organs. The immuno-suppressive effects of GCs are routinely used in the treatment of chronic inflammatory or immune diseases (e.g. inflammatory bowel disease, asthma). However, severe side effects (including diabetes and osteoporosis) are associated with GC-treatment, limiting its therapeutic usefulness.

Within the normal population, there exists a considerable inter-individual variation in GC sensitivity. Whereas some patients develop side effects on relatively low doses of topically administered GCs, others appear to be less sensitive to GCs, as they do not show an adequate improvement in response to treatment even on high doses. Some patients are even resistant to the anti-inflammatory effects of GCs while at the same time showing side effects known to reflect normal sensitivity to GCs, including suppression of the hypothalamic-pituitary-adrenal axis. Variability in GC sensitivity can be divided into GC resistance and GC hypersensitivity.

The signaling pathway of GCs is a complex process, in which distinct pathways are involved that can influence GC sensitivity. Also, other mechanisms such as the transport, local conversion and degradation of GCs play a role in the intracellular bioavailability of GCs.

Here we will discuss the possible consequences for the clinic of genetic variation in genes involved in the GC signalling pathway, and resulting in inter-individual differences in glucocorticoid sensitivity.

Trojan horses for hormones – S14

S14.1

Alpha-fetoprotein protects the developing female brain from estrogens

Julie Bakker[1], Christelle De Mees[2], Jacques Balthazart[1] & Claude Szpirer[2]
[1]University of Liège, Liège, Belgium; [2]Université Libre de Bruxelles, Gosselies, Belgium.

The classic view of sexual differentiation in mammalian species holds that sex differences in the brain and behavior develop under the influence of estrogens derived from the neural aromatization of testosterone: the brain develops as male in the presence of estrogens and as female in their absence. In agreement with this view, it has been proposed that the female brain needs to be protected from estrogens produced by the placenta and that alpha-fetoprotein (AFP) - a major fetal plasma protein present in many developing vertebrate species and produced transiently in great quantities by the hepatocytes of the fetal liver– is the most likely candidate to achieve this protection because of its estrogen-binding capacity. However, the idea that the female brain develops in the absence of estrogens and the role of AFP in protecting the brain against the differentiating action of estrogens have been challenged. First, there is accumulating evidence that the normal development of the female brain might actually require the presence of estrogens. Second, the presence of AFP within neurons in the absence of any evidence for local AFP synthesis suggests that AFP is transported from the periphery into the brain. It was thus proposed as well that AFP acts as a carrier, which actively transports estrogens into target brain cells and, by doing so, has an active role in the development of the female brain. The availability of AFP mutant mice (AFP-KO) now finally allowed us to resolve this longstanding controversy concerning the role of AFP in brain sexual differentiation, and thus to determine whether prenatal estrogens contribute to the development of the female brain. We showed that the brain and behavior of female AFP-KO mice were masculinized and defeminized. However, when estrogen production was blocked by fetal treatment with an aromatase inhibitor, the feminine phenotype of these mice was rescued. These results clearly demonstrate that the principal action of prenatal estrogen exposure is to defeminize the brain and that AFP normally binds estradiol circulating in the female fetus and thereby protects the developing brain from defeminization.

S14.2

Role of endocytic receptors in cellular uptake of steroid hormones

Thomas Willnow[1], Annette Hammes[1], Thomas Andreassen[2] & Anders Nykjaer[2]
[1]Max-Delbrueck-Center for Molecular Medicine, Berlin, Germany; [2]University of Aarhus, Aarhus, Denmark.

Androgens and estrogens are transported bound to the sex hormone binding globulin (SHBG). SHBG is believed to keep sex steroids inactive and to control the amount of free hormones that enter cells by passive diffusion. Contrary to the free hormone hypothesis, we demonstrate that megalin, an endocytic receptor in reproductive tissues acts as a pathway for cellular uptake of biologically active androgens and estrogens bound to SHBG. In line with this function, lack of receptor expression in megalin knockout mice results in impaired descent of the testes into the scrotum in males and in blockade of vaginal opening in females. Both processes are critically dependent on sex steroid signaling and similar defects are seen in animals treated with androgen or estrogen receptor antagonists. Thus, our findings uncover the existence of endocytic pathways for protein-bound androgens and estrogens, and their crucial role in development of the reproductive organs.

S14.3

Hepatic deiodinase activity is dispensable for the maintenance of normal thyroid hormone levels in mice

Ulrich Schweizer
Charité Universitätsmedizin Berlin, Berlin, Germany.

The main product of the thyroid is thyroxine (T4). However, the physiological ligand of nuclear thyroid hormone receptors is triiodothyronine, T3. Deiodination of T4 to yield T3 is achieved by 5′-deiodinase activity. Type I-deiodinase (Dio1) was the first deiodinase cloned and its strong expression in liver and kidny, together with the size of these organs, suggested a role for Dio1 in peripheral conversion of T4 to T3. Later, Dio2 and Dio3 were cloned, enzymes with a more restricted pattern of expression that mediate 5′- and 5-deiodination, respectively. A model emerged in which activation and inactivation of thyroid hormones is governed by the concerted action of tissue-specific deiodinase expression. One aspect of this familiar textbook model, a central role of hepatic Dio1 in T3 production, was recently challenged. Since all deiodinase enzymes are selenoproteins, targeted removal of the gene encoding selenocysteine tRNA (Trsp) allowed the liver-specific inactivation of Dio1 activity. Using Albumin-Cre; Trsp fl/fl mice we showed that loss of hepatic deiodinase did not disturb circulating thyroid hormone levels. Moreover, deiodinase activities in other organs did not show compensatory up-regulation. Data derived from the conventional Dio1 knockout mice suggest that hepatic Dio1 is involved in the re-cycling of iodine from iodothyroines. Since the targeted inactivation of Dio2 perturbed pituitary feedback regulation, but did not reduce serum T3 levels, the question remains which deiodinase provides circulating T3. We have taken these investigations further and will present data regarding the effects of thyroid-specific Trsp inactivation in transgenic mice.

S14.4

IGF-independent actions of IGFBPs

Jean-Marc Ricort
[1]INSERM U515, Paris, France; [2]ENS de CACHAN, Paris, France.

The discovery that IGF binding proteins (IGFBPs) are capable of action independently of ligand binding opened up a broad scope of investigation into the mechanisms by which the IGFBPs elicit their intrinsic cellular effects. Numerous studies have demonstrated the special role of IGFBPs in as diverse processes as cell proliferation, migration and survival/apoptosis. However, the pathways by which these actions occur have not been completely defined but interactions of IGFBPs with other proteins or biomolecules must be involved.

IGFBPs can bind to many partners other than IGFs, although the relationship between most of these binding interactions and IGFBP actions remains uncertain. Several studies have identified membrane proteins that bind IGFBPs with relatively high affinity. These include proteins known to be involved in other signalling pathways (such as integrin receptor and TGFβ receptor) and putative receptors, the precise nature of which remains to be determined. Moreover, IGFBPs can also bind to intracellular (even nuclear) proteins.

Therefore, an exciting challenge in identifying the signalling pathways modulated by such interactions between IGFBPs and their partners is currently open.

Novel bone hormones and regulators – S15

S15.1

Sclerostin, an osteocyte-produced regulator of bone formation

Socrates Papapoulos
Leiden University Medical Center, Leiden, Netherlands.

Sclerosteosis and van Buchem disease are closely related, rare sclerosing disorders characterized by substantial increase in bone mass of good quality which is due to increased bone formation. Both diseases have been linked to deficiency of the *SOST* gene product sclerostin, which in the adult is localized exclusively in osteocytes, the most abundant bone cell. In particular sclerostin is localized in mature osteocytes in mineralised cortical and cancellous bone, it inhibits the activity of osteoblasts and prevents them from promoting excessive bone formation. It is, thus, a negative regulator of bone formation. Sclerostin may be transported by the canaliculi to the bone surface where it inhibits the bone-forming activity of osteoblasts.. In this respect it serves the function of the unknown inhibitory factor proposed by Marotti and Martin that is secreted by mature osteocytes and communicates with osteoblasts at a forming surface causing the adjacent osteoblast to slow osteoid formation.

Because of its structural similarity to the DAN family of glycoproteins, it was originally thought that sclerostin is a BMP antagonist. Whilst sclerostin inhibits BMP-stimulated bone formation, it does not affect BMP signaling and is distinct from classical BMP antagonists. Instead it antagonizes Wnt signaling in osteoblastic cells.

The human high bone mass (HBM) phenotype is an autosomal dominant condition that, like sclerosteosis and van Buchem disease, is characterized by increased bone mass due to enhanced bone formation in the presence of normal bone resorption. It is due to mutations of the *LRP5* gene that make it resistant to the inhibitory action of Dkk1, thereby increasing Wnt signalling. The observations that sclerostin antagonizes Wnt signaling rather than BMP signaling raises the possibility that these skeletal diseases are due to increased activity of the same signaling pathway: LRP5-mediated canonical Wnt signaling.

The restricted expression pattern of sclerostin and the exclusive bone phenotype of good quality of patients with sclerosteosis and van Buchem diseases provide a basis for the design of therapeutics that specifically stimulate bone formation, an action of primary importance for the management of patients with osteoporosis. As sclerostin is a secreted protein, one approach to achieve this is to develop humanized monoclonal antibodies capable of inhibiting the biological activity of sclerostin, mimicking, thus, the absence of sclerostin in sclerosteosis. Preliminary results of such approaches in animal models have been very encouraging.

S15.2

Hormonal regulation of periosteal bone growth

Dirk Vanderschueren & Katrien Venken
Unversity Hospitals, Leuven, Belgium.

In light of the gender differences in bone geometry, sex steroids have been proposed as key regulators of pubertal periosteal bone formation. Sex steroids may affect periosteal bone apposition following activation of sex steroid receptors [androgen receptor (AR), estrogen receptor alpha (ERα) or beta (ERβ)]. Traditionally, it has been assumed that AR-mediated androgen action stimulates periosteal bone formation and thereby determines the larger bone size in males, whereas estrogens suppress periosteal bone formation resulting in a smaller bone size in females. However, optimal periosteal growth in the male is only obtained in the presence of both AR and ER activation as demonstrated in mice with a disruption of the AR gene and in an adolescent man with a mutation in the gene encoding the aromatase enzyme. Moreover, the bone phenotypes of ERα, ERβ and double knock-out mice indicate that the presence of ERα and ERβ increase and decrease periosteal bone expansion, respectively (the former is observed in males and females, the latter only in females). Furthermore, administration of an aromatase inhibitor that blocks the conversion of androgens into estrogens also limits periosteal bone expansion in growing male mice and rats. Beside sex steroids, growth hormone (GH) and insulin-like growth factor-I (IGF-I) are also major determinants of radial skeletal growth. Moreover, sex steroids and GH-IGF-I closely interact in pubertal life in order to obtain optimal stimulation of periosteal bone formation. In this context, targeted disruption of ERα in mice or pharmacological inhibition of aromatization of androgens in mice and rats reduce serum IGF. Such finding raises the question to what extent sex steroids are able to affect periosteal bone formation independently from the GH-IGF-I axis. We therefore studied periosteal bone formation following androgen or estrogen administration in orchidectomized male mice with disrupted growth hormone receptor (GHR). GHR activation appears the main determinant of radial bone expansion, but both GHR signaling and androgen action are independently and cooperatively needed for optimal stimulation of periosteal growth in the male during puberty. Interestingly, estrogen treatment rescued periosteal bone formation in mice with disrupted growth hormone receptor which was explained by a stimulation of IGF-I synthesis in the liver independently from GHR activation.

In conclusion, optimal periosteal bone formation in the male during puberty primarily depends on a functional GH-IGF-I axis, followed by activation of the AR. However, both GH/IGF-I and androgens are independently needed for optimal stimulation of radial bone growth. Moreover, part of the androgen action on periosteal bone may be explained by aromatization and subsequent ERα activation. The latter may interact with GH/IGF-I and may influence periosteal growth by estrogen-related changes in serum IGF-I.

S15.3

Wnt signaling and LRP 5/6 regulation of bone mass

Peter Bodine
Wyeth Research, Collegeville, PA, United States.

Wnts are a large family of carbohydrate- and lipid-modified growth factors that mediate essential biological processes such as embryogenesis, morphogenesis and organogenesis. These proteins bind to a membrane receptor complex comprised of a

frizzled (FZD) G-protein-coupled receptor and a low-density lipoprotein (LDL) receptor-related protein (LRP). The formation of this ligand-receptor complex initiates a number of signaling cascades that includes the canonical/beta-catenin pathway as well as several noncanonical pathways. In recent years, canonical Wnt signaling has been reported to play a significant role in the control of bone formation and remodeling. Clinical studies have found that mutations in LRP-5 are associated with bone mineral density and fractures. Investigations of knockout and transgenic mouse models of Wnt pathway components including Wnt-10b, LRP-5 and -6, secreted frizzled-related protein-1 and -4, dickkopf-1 and -2, Sclerostin, axin-2, beta-catenin and T-cell factor-1 have shown that canonical signaling modulates almost all aspects of osteoblast physiology including proliferation, differentiation, function, mineralization, apoptosis and mechanosensory perception as well as coupling to osteoclasts. In addition, preclinical studies with pharmacologic compounds such as those that inhibit glycogen synthase kinase-3beta support the importance of the canonical pathway in modulation bone formation. Moreover, well-established bone forming agents like bone morphogenetic proteins and parathyroid hormone have been demonstrated to intersect and utilize components of Wnt signaling pathways. Future research in this swiftly expanding area of skeletal biology should focus on understanding Wnt/FZD specificity in the control of bone cell physiology, the role of noncanonical pathways in bone remodeling, the interplay between Wnt signaling and other bone metabolic pathways and direct actions of Wnts on cells of the osteoclast lineage.

S15.4

Thyroid hormones/TR and bone

Graham R Williams
Molecular Endocrinology Group, MRC CSC, Hammersmith Hospital, Imperial College London, London, United Kingdom.

Childhood hypothyroidism results in severely delayed skeletal development whereas adult thyrotoxicosis is associated with a 3–4 fold increase in osteoporotic fracture. To investigate molecular mechanisms underlying these abnormalities we characterized the skeletal phenotypes of mice harboring dominant negative mutations (TRα1PV/+, TRα1R384C/+, TRβPV/PV) or deletions (TRα0/0, TRβ−/−) of the genes encoding TRα and TRβ. Endochondral ossification, linear growth and bone mineralization were retarded in TRα0/0 mice and more severely delayed in TRα1 dominant-negative mutants. In contrast, these parameters were all advanced in TRβ knockout and PV-mutant mice. In adults, 3D bone micro-architecture and micro-mineralization densities were analyzed by quantitative backscattered electron scanning electron microscopy. TRα mice displayed increased cortical bone width, and an 8–9 fold increase in trabecular bone volume with increased thickness of individual trabeculae and greater micro-architectural complexity. In contrast, analysis of all these parameters including quantitation of bone micro-mineralization density revealed TRβ mutants were markedly osteoporotic. Studies of T3-target gene expression revealed phenotypes of skeletal hypothyroidism in TRα mutant mice but skeletal thyrotoxicosis in TRβ mutants. We further demonstrated that TRα is expressed at 15-fold higher levels in bone than TRβ, whereas TRβ is predominantly expressed in hypothalamus and pituitary and controls negative feedback regulation of TRH and TSH. Accordingly, TRα mutant mice were euthyroid whereas TRβPV/PV and TRβ−/− displayed pituitary resistance to thyroid hormone with elevated circulating thyroid hormone levels. This analysis of a series of TR mutant mice with differing genetic backgrounds unequivocally demonstrates that TRα is the predominant TR isoform in bone, and shows that skeletal responses to disrupted TRβ signaling result from effects of the mutation on systemic thyroid status.

Immune-endocrine turmoil of pregnancy – S16

S16.1

Endocrine diseases during pregnancy

Risto Kaaja
Helsinki University Hospital, Helsinki, Finland.

Successful pregnancy depends on the ability of the maternal immune system to tolerate a genetically incompatible feto-placental unit. One of the important adaptations leading to this immuno-tolerance is the shift, at implantation, of Th 1 dominance to Th 2 dominance. Successful pregnancy is a Th2 dominant immune state, therefore, it is not surprising that women with a Th 1 dominant immune disease such as rheumatoid arthritis, thyroiditis or multiple sclerosis improve during pregnancy, while patients suffering from Th 2 dependent immune disease, such as SLE, fare worse during pregnancy.

Interestingly, three autoimmune diseases, rheumatoid arthritis, multiple sclerosis and thyroiditis, that are reported to ameliorate or stabilize during pregnancy in the majority of women, are more likely to relapse during the year after delivery. The postpartum period can be regarded as a time of ongoing heightened inflammatory activity. The onset of rheumatoid arthritis is five times more likely in the puerperal period than at any other time. Multiple sclerosis is known to ameliorate during the last trimester of pregnancy. After delivery, the relapse rate is higher than that before pregnancy. Importantly, the decrease in the relapse rate during pregnancy was more marked than any drug mediated therapeutic effect reported to date. Of the acute endocrine emergencies an acute form of Sheehan's may go unrecognized, leading to unnecessary maternal deaths. Cushing's syndrome has very bad consequences for the fetus and must be diagnosed and treated urgently, if not emergently. Pheochromocytomas are always endocrine emergencies requiring urgent and sometimes emergent treatment. Hyperparathyroidism is usually mild, but severe hypercalcemia can be a true endocrine emergency.

Recognition of the interactions of these endocrine conditions and their specific treatments with the complicated maternal-fetal unit makes their diagnosis and treatment simultaneously both difficult and extremely rewarding.

S16.2

Estetrol (E4), the forgotten fetal steroid

Herjan Coelingh Bennink & Monique Visser
Pantarhei Bioscience B.V., Holland, Netherlands.

Estetrol (E4) is a natural human steroid, produced exclusively during pregnancy by the fetal liver. Estetrol has been discovered in 1965 by Diczfalusy at the Karolinska Institute in Stockholm.

The role of E4 in embryonic physiology and/or human pregnancy is not known. During human pregnancy E4 is detectable from 9 weeks pregnancy onwards. Estetrol reaches the maternal blood circulation via the placenta. Maternal and fetal E4 concentrations increase exponentially during pregnancy and peak at high levels at term with fetal levels about 10–20 times higher than maternal levels as confirmed by data obtained by Pantarhei Bioscience. Based on low receptor binding compared to estradiol (E2), E4 was thought to be a weak estrogen. Since the early eighties the molecule has been neglected.

The pharmacological properties of oral E4 as investigated by Pantarhei Bioscience can be summarised as follows. Estetrol is orally bioavailable in the rat and acts as an estrogen on bone, brain, vagina and endometrium. Estetrol suppresses hot flushes and inhibits ovulation. Surprisingly, E4 acts as an estrogen antagonist on the breast since it was shown to prevent development of breast tumors and to remove pre-existing breast tumors in the DMBA rat model.

In phase I studies in early postmenopausal women E4 showed high oral absorption, full dose linearity, high bioavailability, low inter-subject variability and a long elimination half-life with a mean of 28 hours. Estetrol appeared to be efficacious, safe and without side-effects up to a dose of 20 mg per day for 28 days.

Estetrol will be developed further for the treatment of breast cancer, prostate cancer, osteoporosis and for those Th2-mediated auto-immune diseases, that are known to improve during pregnancy.

S16.3

Regulatory T cells in pregnancy

Ana Claudia Zenclussen[1], Juliane Rau[1], Catharina Thuere[1], Anne Schumacher[1], Gerolf Zimmermann[2], Hans-Dieter Volk[1] & Henry Alexander[2]
[1]Charite, Institute of Medical Immunology, Berlin, Germany; [2]University of Leipzig, Leipzig, Germany.

The survival of the semiallogeneic fetus within the mother is thought to be due to mechanisms of immunological tolerance. Regulatory T cells (Treg) are believed to have a crucial role in maintaining pregnancy by creating a transient tolerant microenvironment within the maternal uterus as former studies confirmed. We have evidences that Treg expand in lymph nodes from normal pregnant mice already on day 2 of pregnancy. Abortion-prone mice present diminished numbers of Treg in immune organs throughout pregnancy. As both pregnancy combinations (normal pregnant and abortion-prone mice) present similar levels of progesterone, estriol and estrone, hormones do not seem to be involved in Treg expansion. However, they may be involved in their recruitment into the vaginal lumen.

An enormous augmentation in the number of $TCR\alpha\beta^+CD4^-CD8^-foxp3^+$ cells in vaginal mucus from normal pregnant animals already on day 0.5 after conception, followed by an increase in Treg numbers in lymph nodes, suggest that Treg need to be activated by male antigens for being protective. The antigen presentation would take place in the periphery e.g. in vaginal mucus, the first site

of contact with paternal antigens, directly after insemination as we could confirm by identifying paternal antigens and paternal APCs at this site. This explains previous observations on Treg transfer being effective in preventing abortion if done on days 0–2 of pregnancy but not later. Interestingly, mating CBA/J females with vasectomized BALB/c males generated foxp3$^+$ cells in lymph nodes draining the uterus, while pseudopregnancy induced by mechanical stimulation did not. Treg-induced tolerance is transient, as Treg came back to the normal levels after the disappearance of the paternal/fetal antigens, 14 days post-partum.

The molecules responsible for Treg recruitment immediately after copulation are being currently studied in our laboratory. Besides, running clinical studies will help us clarifying whether similar pathways are taking place in humans.

S16.4

The effect of pregnancy on immune disease
M. Hazes
The Netherlands.

Abstract Unavailable

Somatostatin receptors in health and disease – S17

S17.1

Pro and contra of SRIF analogue therapy in pituitary tumors
AJ van der Lely
Erasmus MC, Rotterdam, Netherlands.

Long-acting somatostatin analogues normalize serum IGF-I levels in about 65% of acromegalic patients. Somatostatin analogs reduce GH secretion but also induce GH resistance of the liver because of low portal insulin levels; i.e. patients have a relative high GH level and a GH resistance of the liver which results in a relative low IGF-I action because of high IGFBP1 levels, but the other tissues still have normal GH sensitivity. One might predict that long-term follow-up of treated acromegalic patients is mandatory for find out the potential differential effects of the various medical treatment modalities. Especially as nowadays, the combination of somatostatin analogues and GH-R antagonists will be used by clinicians more frequently in order to decrease administration interval of the GH-R antagonist, as well as reduce its dose that is necessary to control disease activity in those acromegalic patients that do not respond to long-acting somatostatin monotherapy. The novel multiligand analogue SOM230 might increase the number of patients that can be biochemically controlled. SOM230 inhibits free IGF-I in a more sustained fashion compared to octreotide, implying longer duration of action. The superior action of octreotide compared with SOM230 in stimulating IGFBP-1 levels in acromegalic patients, suggests direct regulation of IGFBP-1 by somatostatin analogues *via* the somatostatin subtype 2 receptor. In summary, somatostatin analogs are the only compounds of which, at least in acromegaly, it has been shown that they reduce tumor size in those subjects that express sst on their pituitary tumors. However, the expression of sst on other tissues, involved in glucose metabolism, might have a negative influence on glucose metabolism on some patients

S17.2

Somatostatin receptors in neuroendocrine tumors
Kjell Oberg
Department of Endocrine Oncology, Uppsala University Hospital, Upppsala, Sweden.

A unique feature of neuroendocrine tumors is that they express peptide hormone receptors. All five subtypes of somatostatin receptors are expressed in neuroendocrine tumors with dominance for receptor type 2 (SST2). Stimulation of SST2 can not only inhibit hormone release from the tumor, but also tumor cell growth. Both SST2 and 3 are involved in apoptosis of neuroendocrine tumor cells. SST's in intratumoral blood vessels might implicate a role of anti-angiogenesis of somatostatin and somatostatin analogues. Midgut carcinoids express about 80% of the tumors SST2. The same is true for most endocrine pancreatic tumors, except for benign insulin producing tumors that has a lower expression (50%). Signaling through SST2 inhibit hormone release and causes antiproliferation, whereas stimulation of SST2 and 3 causes apoptosis. ^{111}Indium-DTPA-octreotide (Octreoscan®) can be applied for localisation and staging of neuroendocrine tumors. Labelling of octreotide with either ^{177}Lutetium or ^{90}Yttrium is used for tumor targeted radioactive treatment (PRRT). The use of somatostatin analogues, Octreotide and Lanreotide, has been a real break-through in the management of functioning neuroendocrine tumors. Symptomatic and biochemical improvement has been noticed in 50-60% of the patients and tumor reduction in 5–10%. A new somatostatin analogue – SOM230 – has been applied in phase-2-trials. This analogue is binding with high affinity to receptor 1, 2, 3 and 5, but not 4. It has already demonstrated significant symptomatic effects in patients with functioning neuroendocrine tumors, resistant to octreotide treatment. In the future analysis of the expression pattern of different somatostatin receptors in neuroendocrine tumors will be important, particularly if new somatostatin analogues will be developed.

S17.3

Peptide receptor therapy
DJ Kwekkeboom
The Netherlands.

Abstract unavailable

S17.4

Cortistatin, a multi-functional somatostatin receptor analog
Luis de Lecea
Stanford University, Palo Alto, CA, United States.

Cortistatin is a neuropeptide that belongs to the somatostatin family, and shares 11 of its 14 amino acid residues with somatostatin. Studies in the central nervous system have shown that cortistatin has activities different from somatostatin, including enhancing slow wave sleep and selective conductances. However, in the periphery cortistatin appears to act as a somatostatin receptor analog. We have generated cortistatin ko mice and have analyzed the molecular, behavioral and immunological consequences of cortistatin deficiency. Our data suggest that cortistatin is a parallel system to somatostatin in the central nervous system, and may have specific and relevant functions in the immune system.

Puberty and hypogonadism – S18

S18.1

Endocrine disorders of puberty
Marek Niedziela
Poznan University of Medical Sciences, Poznan, Poland.

Puberty is a process in humans that leads to the development of secondary sexual characteristics and reproductive capabilities. The physical changes of puberty result from two separate and independent but overlapping processes: gonadarche and adrenarche. The activation of hypothalamic-pituitary-gonadal (HPG) axis plays a key role in gonadarche whereas body weight and body mass index are postulated as triggering the adrenarche. The impairment of this cascade will result in temporary or permanent disorders of reproductive endocrine function. This primarily endocrine process can be disrupted by genetic and environmental factors. The timing of pubertal onset is defined as normal if occurs between the ages of 8 and 13 years in girls and 9 and 14 years in boys. However the controversies concerning the age limit of onset of puberty have been raised. Precocity can be central (GnRH-dependent) or peripheral (GnRH-independent) in its etiology and iso- or heterosexual (consistent or inconsistent with gender). Central precocious puberty in girls is rather idiopathic whereas in boys has predominantly pathologic cause. Peripheral precocious puberty occurs rarely. The most common cause of delayed puberty is constitutional delay of growth and puberty, especially in

boys. However the other common etiologies should be considered: 1. Functional hypogonadotropic hypogonadism; 2. Permanent hypogonadotrophic hypogonadism and 3. Permanent hypergonadotrophic hypogonadism. The treatment strategy is highly specific for each single disorder. Genetic studies on newly detected factors regulating HPG axis (eg. KiSS-1 and GPR54 as gatekeepers of gonadotropin-releasing hormone release neurons or FGFR1) may improve understanding of normal variation in pubertal timing and provide further directions for treatment.

S18.2

Role of sex steroids and nitric oxide in male sexual function

Vincenzo Rochira
Department of Medicine, Endocrinology and Metabolism, Geriatrics, University of Modena and Reggio Emilia, Modena, Italy.

Nitric oxide (NO) is the main final effector for penile erection achievement and maintenance in men and it constitutes a crucial target for therapeutical strategies addressed to the treatment of erectile dysfunction. The role of sex steroids penile NO pathway is still unclear, but some data suggest a positive role of androgens. In order to study the effects of sildenafil on human sleep-related erections according to the state of androgenization, we recently evaluated the effects of sildenafil (S) or placebo (P) on sleep-related erections in hypogonadal (H) men with very low testosterone levels: <200 ng/dl (6.93 nmol/L), before (H−T) and during (H+T) testosterone replacement treatment (T) and in control (C) subjects. Sleep-related erections were impaired in hypogonadal men before testosterone treatment (H−T+P) when compared with control subjects taking placebo (C+P). Testosterone alone (H+T+P) and sildenafil alone (H−T+S) restored normal sleep related erections, however, the combined treatment (sildenafil + testosterone) resulted in the maximum positive effect on sleep-related erections parameters. The effects of testosterone plus sildenafil resulted higher than the sum of the effects of both drugs used alone. Sildenafil administered at bedtime improves sleep-related erections in hypogonadal men, suggesting that the nitric oxide pathway may be pharmacologically enrolled and enhanced despite low serum testosterone. Furthermore, these data strongly support the idea of a synergic effect of sildenafil and testosterone on sleep-related erections. In clinical practice this concept is supported by the evidence that testosterone treatment restores sildenafil efficacy in subjects with erectile dysfunction and low to low-normal serum testosterone, who were non-responder to sildenafil alone. The combined treatment seems to be efficacious also in subjects with metabolic diseases such as diabetes mellitus. Whether or not estrogens are able to modulate NO pathway within the penile tissue remains to be ascertained in detail, but an androgen-estrogen cross-talk seems to be involved in the pathophysiology of male penile erection, but concerning estrogens dose-response and in vivo studies are lacking.

S18.3

Clinical management of premature ovarian failure

S Christin-Maitre
France.

Abstract unavailable

S18.4

Gonadal function in ageing men

Jean M Kaufman
Ghent University Hospital, Ghent, Belgium.

Involutional changes of gonadal function in healthy ageing men are progressive and mostly of modest amplitude with considerable between-subject variability. Albeit some men may remain relatively spared, the occurrence of age-related changes are nevertheless well documented at the population level in both cross-sectional and longitudinal observational studies. These changes affect Sertoli cell function and spermatogenesis as well as Leydig cell function and testosterone (T) production and they are the consequence of both primary testicular changes and alterations in neuro-endocrine regulation of gonadotropin secretion. Men retain fertility until old age, but there are age-related reductions of testicular size, Sertoli cell mass and spermatogenic activity with moderate decline in semen volume, sperm motility and morphology with maintained sperm concentration. This is reflected in a progressive increase of FSH levels and marked decrease of the ratio of serum levels of inhibin over FSH. More than 20% healthy men over 60 yr present with serum T levels below the range for young men. This is the consequence of decreased Leydig cell mass and altered hypothalamic regulation of LH secretion. Together with a progressive age-related increase in serum SHBG levels, this results in a 50% reduction of the serum bioavailable fractions of serum T (i.e. free and non SHBG-bound T) between age 20 and 75 yr. Although the clinical changes of ageing in men are reminiscent of signs and symptoms of hypogonadism in young men, clinical relevancy of the decline in sex steroid levels in ageing men has not been unequivocally established and minimal androgen requirement for elderly men remain poorly defined and are likely to vary between individuals. Therefore, borderline androgen deficiency cannot be reliably diagnosed in the elderly and differentiation between "substitutive" and "pharmacological" androgen administration is not possible. Awaiting documentation of long-term risk-benefit ratio, a conservative approach to androgen treatment in elderly men seems appropriate.

Pituitary cell biology – S19

S19.1

Role of folliculo-stellate cells in the anterior pituitary: a historical review

Wilfried Allaerts
Nijverdal, Netherlands.

Cell developmental studies have frequently used the hypophysis as a model for complex differentiation pathways. Nevertheless, many of this work has been focused on the hormone-producing cell types of the anterior pituitary (AP), whereas the so-called folliculo-stellate cells (FS cells) have often been ignored in these studies. FS cells form an enigmatic, non-hormone-secreting cell group. Initially designated as supportive cells, they were soon found to be the putative source of many, newly discovered peptides and growth factors. They were also shown to be involved in paracrine communication with other pituitary cell types and in communication through electrically coupled syncytia. Moreover, several authors have provided evidence for their possible role in pituitary cell regeneration and processes of cell transdifferentiation.

So far, little is known about the precise embryological origin of the mature FS cells. Since the discovery of adult stem cell populations in various organs, several authors have indicated a possible role of FS cells in this respect too. Also new evidence relating FS cells to the production of cytokines, their involvement in nitric oxide signaling and an *in vitro* immune accessory function were added to the list of physiological roles of the FS cells. The question however is whether these multiple functions can be ascribed to one, homogeneous but pluripotent cell type, or whether the pituitary FS cells represent a heterogeneous cell group consisting of various subtypes (unrelated or related to a common ancestor cell type).

We previously demonstrated the partial overlap between immunocompetent MHC-class II-positive dendritic cells (DC) and S100 protein-positive FS cells. In a transgenic mouse model for conditional DC ablation, we showed that early macrophages could be prevented from colonizing the AP. Also, around embryonic day 12 of chick development, early macrophages were detected in the anterior pituitary before pituitary cell differentiation was completed and well before FS cells obtained their mature phenotype.

The present historical review of FS cell research highlights the importance of conceptual frameworks in cell lineage studies. Cell biological systems from the past, like the reticulo-endothelial system or the more recent mononuclear phagocyte system, nowadays are considered obsolete and incomplete. Still there is a need for theoretical frameworks in new annotation studies and for the clinical applications of contemporary research. The FS cell model not only is very interesting for the study of development of organs with two or more embryonic Anlagen. Also, questions related to the therapeutic usefulness of pituitary cell regeneration are envisaged in cases of pituitary dysfunctioning or hypopituitarism.

S19.2

Signalling in pituitary tumours: the roles of Akt, BRAF, AIP and other novel agents

Márta Korbonits
Department of Endocrinology, Barts and the London Medical School, London, United Kingdom.

Numerous growth factors, oncogenes, tumour suppressor genes and hormonal influences have been implicated in pituitary tumorigenesis. We have demonstrated that the PI3K-Akt pathway is upregulated in pituitary tumours and since Akt is a major downstream signalling molecule of growth factor-liganded tyrosine kinase receptors it is possible that an abnormality at this level could be the primary driver of pituitary tumorigenesis. The serine/threonine kinase B-Raf functions as a downstream effector of Ras, interpolated between the tyrosine kinase receptor and the mitogen-activated protein kinase (MAPK) pathway and acting in parallel to the Akt pathway. We have found significant over-expression of B-Raf mRNA in pituitary adenomas, specifically NFPAs and a positive correlation between mRNA and protein expression. B-Raf overexpression could lead to increased activation of the MAPK pathway. Using microarray we have found that the Bcl-associated athanogene (BAG1) mRNA is overexpressed in somatotroph adenomas and NFPAs; this oncogene binds to and activates Raf-1, which can potentiate B-Raf activity by heterodimerisation. In a pituitary protein array we have identified several over- and underexpressed proteins and one of the prominent differentially expressed proteins with potential importance in tumorigenesis was the heat shock protein 110 (HSP110). This showed significant overexpression in NFPAs and prolactinomas. Interestingly, another molecular chaperone, the aryl hydrocarbon receptor interactive protein (AIP) has been recently identified as a cause for pituitary adenomas in families with isolated pituitary tumours. We have identified 5 different mutations in 19 familial acromegalic families causing autosomal dominant disease with incomplete penetrance. We have also observed prominent differences in AIP mRNA and protein expression between normal pituitary cells and sporadic pituitary tumours. Previous data suggest that AIP acts as a tumour suppressor gene but the exact mechanism leading to pituitary tumorigenesis when AIP is lacking remains to be identified.

S19.3

Oncogene gsp and Gsα overexpression in pituitary cell biology

Anne Barlier[2], Corinne Gérard[1] & Enjalbert Alain[1]
[1]Laboratory ICNE UMR6544 CNRS Université de la Méditérranée, Marseille, France; [2]Laboratory of Biochemistry anf Molecular Biology, CHU Conception, Marseille, France.

Somatic mutations of the αs subunit of G proteins were initially reported by Landis and collaborators in 1989 in somatotroph tumors characterized by markedly high cAMP levels. These mutations are localized at two critical sites concerning the intrinsic guanosine triphosphatase activity of the protein leading to a constitutive activation of the adenylyl cyclase. The mutated protein has been named the *gsp* oncogene. On the other hand, Gsα mRNA level varied among human somatotroph adenomas, the highest expression being observed in *gsp*- tumors (not bearing the active Gsα mutant). We previously showed that *gsp* oncogene impacted tumoral phenotype, *gsp*+ tumors being smaller and more sensitive to octreotide treatment. We have recently showed that high Gsα expression impacted also tumoral phenotype of *gsp*- tumors.

Gsα is coded from GNAS gene which is imprinted in a tissue-specific manner. Gsα is paternally silenced in normal pituitary, but a Gsα imprinting relaxation is found in some tumoral tissue. Unexpectedly, we found that the loss of Gsα imprinting did not induce the expected Gsα overexpression and was not associated with a modification of methylation status of exon1A DMR (a differentially methylated region controling the Gsα imprinting) in human pituitary tumors.

To explore the impact on transduction pathways of mutated or overexpressed Gsα protein, we obtained somatolactotroph GH4C1 cell lines by performing doxycycline-dependent conditional overexpression of the wild type Gsa protein and expression of the gsp oncogene. Although the resulting adenylyl cyclase and cAMP levels were ten-fold lower in the wild type Gsa overexpressing cell line, a sustained MAPKinase ERK1/2 activation was observed in both cell lines. Overexpression of the wild type Gsα protein as the *gsp* oncogene initiated chronic activation of endogenous PRL synthesis and secretion, as well as chronic activation of ERK1/2-sensitive human PRL and GH promoters.

S19.4

Adipocytokines and pituitary function

Maria M. Malagon[1], Francisca Rodríguez-Pacheco[1], Antonio J. Martínez-Fuentes[1], Rafael Vázquez-Martínez[1], Manuel Tena-Sempere[1], Carlos Diéguez[2] & Justo P. Castaño[1]
[1]Dept. Cell Biology, Physiology and Immunology. Univ. Córdoba, Cordoba, Spain; [2]Dept. Physiology, Univ. Santiago de Compostela, Santiago de Compostela, Spain.

It is widely accepted that, in addition to serving as a repository for energy reserves, adipose tissue is an active endocrine organ that secretes a variety of signalling molecules, the adipokines, which play important roles in the regulation of metabolism, energy balance, feeding behaviour, vascular homeostasis and immunity. In particular, leptin, resistin and adiponectin have been implicated in energy and glucose homeostasis. Additional neuroendocrine functions have also been recognized for leptin as it regulates the secretion of pituitary GH and LH. In order to elucidate whether adiponectin, as leptin, may be involved in the regulation of pituitary cell function, we investigated the effect of this adipokine on somatotrophs and gonadotrophs and analyzed its interaction with major stimulatory regulators of these cells (ghrelin, GHRH, GnRH), as well as with their corresponding receptors (GHS-R, GHRH-R, and GnRH-R, respectively). Results show that adiponectin inhibits GH and LH secretion as well as both ghrelin-induced GH release and GnRH-stimulated LH secretion in rat pituitary cell cultures, wherein the adipokine also increases GHRH-R and GHS-R mRNA content while decreasing that of GnRH-R. Additionally, we have demonstrated that the pituitary expresses both adiponectin and the adiponectin receptors, AdipoR1 and AdipoR2, under the regulation of the adipokine. Taken together, these data indicate that adiponectin, either locally produced or from other sources, may play a neuroendocrine role in the control of both somatotrophs and gonadotrophs. These results will be further discussed on the context of adiponectin expression in pituitary tumoral cells and its interaction with other adipokines present in the pituitary.

Financial-support: CVI-139-J.Andalucia, BFU2004-03883-MEC/FEDER, and CIBER Obesity&Nutrition-ISCIII. Spain.

Thyroid – S20

S20.1

Updated guidelines for the follow-up of thyroid cancer.

Martin Schlumberger
Institut Gustave Roussy, 94805b Villejuif, France.

Follow-up of thyroid cancer patients is aimed at controlling the adequacy of thyroid hormone treatment and at the early diagnosis of recurrent disease.

Long term thyroxine treatment is given at suppressive doses only in the few patients with persistent or recurrent disease. When cure has been assessed, serum TSH should be maintained in the normal range (around 1 μU/ml).

The absence of disease is first controlled by the total body scan (TBS) performed 3 to 5 days after the post-operative administration of radioiodine. When the TBS is informative and does not show any focus of uptake outside the thyroid bed, a subsequent routine diagnostic TBS is usually not necessary.

Cure is assessed at 9–12 months with a neck ultrasonography and a serum Tg determination obtained 3 days after rhTSH stimulation (0.9 mg im, on 2 consecutive days). The quality of life of thyroid cancer patients is improved with the use of recombinant human TSH (rhTSH) that avoids hypothyroidism, provides an effective stimulation of any thyroid tissue and does not increase the global cost of follow-up.

Low risk patients with a normal neck US and an undetectable rhTSH stimulated serum Tg are considered cured. This reliable assessment of cure permits reassurance of patients, the subsequent use of replacement doses of thyroxine and the simplicity of the subsequent yearly follow-up with serum TSH and Tg determinations. There is a close relationship between basal and TSH-stimulated serum Tg levels, and the benefits of TSH stimulation may decrease with Tg methods with an improved functional sensitivity. At the present time, there is however no firm evidence that TSH-stimulated Tg determination can be obviated.

When serum Tg is detectable at a low level following TSH stimulation, another TSH stimulation should be performed 1 or 2 years later, and the trend will indicate either irradiated thyroid cells (with decreasing Tg level) or neoplastic cells (with an increasing Tg level).

S20.2

Congenital hypothyroidism with gland *in situ*

Luca Persani
Dept of Medical Sciences, University of Milan and IRCCS Ist Auxologico Italiano, Milan, Italy.

Congenital hypothyroidism (CH) is the most frequent endocrine congenital defect affecting about 1:3000 newborns. In economically/ socially advanced counties, CH is routinely screened by means of TSH (and/or T4) measurement on dry blood spot (dbs) since more than 20 years. Neonatal screening allows early recognition and treatment of affected newborns. Upon data collected in years <2000 by the Italian CH Registry, the newborns with confirmed CH and gland-in-situ constituted about 20% of total CH cases. However, in more recent years the technical improvements in TSH determination in the Center for Neonatal Screening of Milan region have lead to a progressive lowering of dbs TSH cutoff value for newborn recall down to 10 mU/l. This has resulted in a significant increase of the recall rate for CH (CH incidence 2003: 80/91,948 newborns), with gland-in-situ cases nowadays accounting for more than 55% of total CH cases. This phenomenon has several important implications concerning the correct diagnosis and adequate management of these babies. One of the most important questions raised by this new picture concerns the necessity to treat babies with mild TSH elevations. The possibility to give correct answers to these questions is complicated by the extreme heterogeneity of this CH category, highlighted by the variable thyroid phenotype as well as by the multiple possibilities of association with non-thyroid malformations/disorders. Relevant advancements have been done in recent years with the discovery of new genetic causes and the description of their underlying molecular mechanisms and related phenotypic presentation. Nevertheless, the cause of several gland-in-situ CH cases remains still unsolved justifying further efforts in this research field. These efforts will contribute to reach a more complete pathogenic classification of CH with gland-in-situ which represents one of the major steps toward an improved and evidence-based clinical management of CH patients.

S20.3

Thyroid and ageing

Istvan Szabolcs
Semmelweis University, Budaoest, Hungary.

In the healthy elderly there seems to be an age dependent decrease of TSH and FT3 but not FT4. The prevalence of TPOAb positivity increases with age but surprisingly it has been found to be decreased in centenarians. Antibody positivity is not predictive for future thyroid dysfunction in old age. The upper range of normal TSH for the healthy elderly living in sufficient iodine intake areas is higher than in case of iodine deficiency. In iodine deficient areas there is a high prevalence of nodular goiter and hyperthyroidism is mainly caused by toxic nodules. Radioiodine should be prefered for therapy of Graves' disease in old age, long term thyrostatic therapy is not safe. TAO is more severe in old age and there is a less favourable outcome of the therapeutical options. In an elderly subject subclinical hyperthyroidism with suppressed TSH is a risk factor for progression to overt disease, for atrial fibrillation, osteoporosis and may be associated with increased cardiovascular and all-cause mortality, thus we believe that it should be treated. The clinical significance of subnormal but measurable TSH is less clear, but in old age treatment may be considered in case of heart disease or osteoporosis. Subclinical hypothyroidism is a risk factor for atherosclerosis but slightly elevated TSH in old age should not be treated: it may even be favourable to have a longer life. In any case, TSH levels outside the reference intervals should first be controlled before considering treatment. The cancer risk in cold thyroid nodules increases with advanced age. According to most but not all studies, in older differentiated thyroid cancer-patients poor prognostic features are more frequent, total thyroidectomy and radioablation are recommended and additional treatment of progressive disease should not be denied because of advanced age.

S20.4

Thyroid autoimmunity: genes and environment

Pia Skov Hansen
[1]Odense University Hospital, Odense, Denmark; [2]University of Southern Denmark, Odense, Denmark.

Autoimmune thyroid diseases (AITD) comprise two clinical phenotypes, Graves' disease and Hashimoto's thyroiditis. These conditions share distinct immunological features such as autoreactivity against the key thyroid autoantigens thyroglobulin and thyroid peroxidase. Considering Graves' disease as well as Hashimoto's thyroiditis, twin studies have revealed a higher concordance rate among monozygotic (MZ) as compared to dizygotic (DZ) twins, suggesting a relative strong genetic influence in the aetiology. According to the endophenotypic approach, it might be useful to subdivide a clinical phenotype into a set of variables thought to represent more basic processes. The presence of thyroid autoantibodies in euthyroid individuals can be regarded as a central phenotypic anchor point and, using the twin design, the relative contributions of genetic as well as environmental effects in the aetiology of AITD, at this early stage of the disease process, has been clarified as well.

The genetic contribution to autoimmune disease (AID) has been intensely investigated, and a slow progress towards identification of AITD susceptibility genes is seen. There is evidence of association and, in some cases, even linkage between AITD and several genetic loci. However, one problem is often the very pronounced discrepancy between the initial and subsequent reports. On the other hand, epidemiological studies aim at identifying specific measurable environmental exposures of importance for the development of AITD. So far only a few environmental factors (e.g. iodine intake and smoking habits), with a clear detectable effect on the disease, have been characterized. The underlying challenges in trying to understand a complex phenotype, such as AITD, will be discussed.

Pheromones, odorant and taste receptors – S21

S21.1

Odorant receptors and reproduction

Hanns Hatt
Ruhr-University Bochum, Bochum, Germany.

Fertilization is still one of the nature's best-kept secrets. Despite a century of research we still lack a comprehensive understanding how mammalian sperm cells navigate inside the female body, locate, and finally fertilize the egg. More than a decade ago, the unexpected finding of olfactory receptor expression in human testicular tissue led to speculation about a potential role of these chemoreceptors in various aspects of mature sperm behavior, especially sperm chemotaxis. We could obtain first evidence in favor of this hypothesis by the identification of hOR17-4, a testicular olfactory receptor that mediates human sperm chemotaxis. We showed that *in vitro* activation of the receptor hOR17-4 by a variety of floral odorants (e.g. bourgeonal, cyclamal) mediates both chemotaxis and chemokinesis in human sperm cells. A detailed characterization of the receptor's molecular receptive range as well as the first description of a potent receptor antagonist could provide the basis for future applications in fertility treatment with important consequences in contraception. Very recently we reported cloning, recombinant expression and functional characterization of another human testicular olfactory receptor (hOR17-2). Using a combination of imaging behavioral assays, we showed activation of sperm by cognate receptor ligands and described a specific receptor-mediated motility pattern. Comparative analysis of different OR-induced signaling pathways as well as cell-specific receptor expression profiles are subject of current research. Given an estimated number of up to 40 different testiculary expressed odorant receptors, an identification of the stimulatory ligands of further members of this "unconventional" group of ORs is critical to gain new insight in their role in reproduction.

S21.2

Molecular architecture of pheromone sensing in mammals

Catherine Dulac
HHMI, Cambridge, MA, United States, Harvard University, Cambridge, MA, United States.

The neuronal processing of pheromone signals within distinctive brain structures leads to marked changes in animal behaviour and endocrine status. The highly reproducible and species-specific character of the response to pheromones offers a unique opportunity to uncover the neural basis of genetically pre-programmed behaviours. Molecular and genetic investigation of the mechanisms underlying pheromone pheromone-evoked responses in the mouse nose and brain have revealed a neural strategy that is strikingly different from that used in other chemosensory modalities such as taste and olfaction. Our studies have provided novel insights into the sensory coding of pheromone signals leading to gender identification and aggressive behaviour, and into the developmental mechanisms leading to the emergence of distinct olfactory pathways. Our most recent

experiments using conditional and GFP-expressing viral vectors are aimed at visualizing entire brain circuits responsible for innate behaviours.

S21.3

Endocrine and behavioural responses to pheromones

Peter Brennan
University of Bristol, Bristol, United Kingdom.

According to the original definition, pheromones are substances released by an individual that have definite behavioural or physiological effects on another individual of the same species. For example, male mouse urine contains a complex mixture of chemosignals, some of which, such as brevicomin and thiazole are testosterone-dependent and signal the presence of a reproductively active male. These have powerful effects as releaser pheromones to elicit aggression from other males, as well as having effects as primer pheromones on female reproductive state, such as puberty acceleration and induction of oestrus. However, as the complexities of vertebrate chemosensory communication have become evident, the original definition of pheromones has begun to appear too restrictive. For instance, peptide chemosignals related to the major histocompatibility complex convey information about individual identity, which as signaller pheromones can influence behaviour or physiology without eliciting a definite response.

In addition to mediating individual recognition in social contexts, these individuality chemosignals enable female mice to recognise the urinary pheromones of their mate, to which they are exposed at mating. This chemosensory memory is vital for their reproductive success, as it prevents the pre-implantation pregnancy failure that is induced by exposure to urinary pheromones from an unfamiliar male. This pregnancy block effect (Bruce effect) is mediated by the vomeronasal system, via the dopaminergic suppression of prolactin production by the pituitary. A range of evidence suggests that memory formation to the mating male's pheromones involves synaptic changes in the accessory olfactory bulb at the first stage of the vomeronasal pathway. This results in a selective inhibition of the mate's pheromonal signal, preventing it from activating neural circuits in the corticomedial amygdala and hypothalamus that mediate the endocrine changes responsible for pregnancy block. This is just one example of the way that learning can reinforce or inhibit innate pheromonal responses.

S21.4

Bitter taste receptors and food intake

Wolfgang Meyerhof, Maik Behrens, Bernd Bufe, Anne Brockhoff, Susann Förster, Claudia Reichling, Christina Kuhn & Marcel Winnig
German Institute of Human Nutrition, Potsdam-Rehbruecke, Germany.

Taste is an overriding determinant of food choice and leads to the development of preferences impacting on nutrition and eventually health. To investigate the molecular basis of gustation and its link to nutritional behaviour, we isolated all 25 members of the human bitter taste receptor gene family, TAS2Rs, and established their expression profile on the tongue. Using functional assays we identified the cognate bitter compounds for ~half of the encoded receptors. Our data suggest that TAS2Rs appear to be broadly tuned to detect compounds with common structural motifs, explaining how humans are capable of perceiving thousands of bitter substances with a small set of receptors. This broad tuning is likely caused by the presence of multiple binding sites for various bitter compounds on the TAS2Rs. Our experiments also revealed that the biochemical properties of the receptors define perceptual sensitivity of individuals. Moreover, frequently occurring polymorphisms in TAS2R genes determine numerous receptor variants, which can differ in the sensitivities for their cognate bitter compounds up to 1000 fold, thereby generating perceptual variability in the population. How far receptor mechanisms determine tasting is shown for saccharin, a compound that taste sweet through activation of the sweet taste receptor at low and moderate concentration, with an off-taste caused by its ability to activate two TAS2R bitter taste receptors simultaneously and to block the sweet taste receptor at higher concentrations.

To date direct evidence is still missing that convincingly proves or disproves the impact of gustation on intake behaviour. However, strong circumstantial evidence comes from the phylogenetic analysis of human TAS2R genes and from the analysis of TAS2R polymorphisms and taster phenotypes that evolved independently in chimanzees and humans as well as from an association study identifying a TAS2R16 allele as a risk factor of alcohol dependence. Taken together, our data strongly suggest that genetics and peripheral taste receptor mechanisms govern gustatory perception and perceptual variability in the population with a probable impact on nutrition and health.

Bone – S22

S22.1

Bisphosphonates: molecular mode of action and adverse effects

Michael J Rogers
University of Aberdeen, Aberdeen, United Kingdom.

Bisphosphonates are the mainstay of treatment for metabolic bone diseases such as post-menopausal osteoporosis and Paget's disease. Enormous progress has been made over the last few years in understanding how these drugs act at the molecular level. After targeting bone and selective internalisation by osteoclasts, simple bisphosphonates are incorporated into cytotoxic, non-hydrolysable analogues of ATP. By contrast, the more potent nitrogen-containing bisphosphonates inhibit FPP synthase (an enzyme of the mevalonate pathway), which disrupts the synthesis of the isoprenoid lipids FPP and GGPP. These lipids are required for the carboxy-terminal modification (prenylation) of small GTP-binding proteins such as Ras, Rho, Rac and Rabs. Prenylated small GTPases act as molecular switches, regulating processes fundamental to osteoclast function, including membrane ruffling, vesicular trafficking, cytoskeletal organisation and cell survival. Inhibition of FPP synthase by bisphosphonates prevents the prenylation of small GTPases and causes the accumulation of the unprenylated (and, in some cases, inappropriately activated) forms of the proteins, thus disrupting osteoclast function and causing osteoclast apoptosis.

The most common adverse effect of intravenous bisphosphonate therapy is a brief, 'flu-like acute-phase reaction. We have recently demonstrated that this effect appears to be due to inhibition of FPP synthase in peripheral blood mononuclear cells, which causes an accumulation of the upstream isoprenoid lipid IPP. The latter is known to stimulate the Vgamma9Vdelta2 subset of gamma,delta-T cells, causing the release of TNFalpha and IFNgamma and hence the rapid onset of 'flu-like symptoms. Esophageal irritation by oral bisphosphonates may also be caused by inhibition of FPP synthase in GI epithelial cells, however the exact cause of recently-described, rare cases of osteonecrosis of the jaw remains unclear.

Thus, the ability of nitrogen-containing bisphosphonates to inhibit the mevalonate pathway explains their well-known, potent inhibitory effects on bone-destroying osteoclasts as well some of their adverse effects.

S22.2

Calcimimetics in the management of hyperparathyroidism

Munro Peacock
Indiana Universtity, Indianapolis, United States.

The cell surface calcium receptor (CaR) in the parathyroid gland plays a central role in the regulation of serum calcium homeostasis. Activation and inactivation mutations in the CaR lead to chronic hypocalcemia and hypercalcemia states (Brown, EM. Mutations in the calcium-sensing receptor and their clinical implications. *Horm Res* 1997 **48** 199–208). Type 11 calcimimetics are a novel class of compounds that directly reduce PTH secretion from the parathyroid cell by binding to the CaR and increasing its sensitivity to extracellular ionized calcium, thus causing a left-shift in the Ca-PTH setpoint (Nemeth, EF *et al*. Calcimimetics with potent and selective activity on the parathyroid calcium receptor. *PNAS USA* 1998 **95** 4040–4045). Cinacalcet is an oral calcimimetic that has been shown to reduce serum PTH and calcium in secondary hyperparathyroidism of renal failure (Block, GA *et al*. Cinacalcet for secondary hyperparathyroidism in patients receiving hemodialysis. *N Engl J Med* 2004 **350** 1516–1525), in primary hyperparathyroidism (Peacock *et al*. Cinacalcet Hydrochloride maintains long-term normocalcemia in patients with hyperparathyroidism. *J Clin Endocrinol* 2005), and in parathyroid cancer (Silverberg, SJ *et al.* Cinacalcet reduces hypercalcemia in patients with parathyroid carcinoma. *J Bone Min Res* 2006 **21** Suppl. 1 S440).

Cinacalcet therapy is well tolerated long-term, and current studies indicate that it may play a valuable role in the medical management of diseases of hyperparathyroidism.

S22.3

Primary hyperparathyroidism: surgical approach and benefits

Svatopluk Adamek
3rd Dpt of Surgery, First Fac of Medicine, Charles University, Prague, and Teaching Hospital Motol, Czech Republic, Prague, Czech Republic.

Surgical therapy of the primary hyperparathyroidism (PHP) offers a definite and curative treatment. The cooperation with an experienced endocrinologist is necessary, because the confidence that the patient has a PHP, is the primary presumption for the proper surgical therapy of the PHP. The result of a parathyroidectomy depends mainly on preoperative localization of hyperfunctional tissue and the experience of the surgeon. The parathyroidectomy remains curative approach in 97% of patients if provided by an experienced surgeon. The neck ultrasonography and MIBI scintigraphy of parathyroid glands remain the gold standards in preoperative imaging. The surgeon must but be able to perform a parathyroidectomy in case where preoperative localizing methods are not successful. In addition, we require an indication to surgical approach in patient with concomitant thyreopathy. The basic technique of a parathyroidectomy is the bilateral exploration of the neck with the examination of all locations of the parathyroid glands, including ectopic ones, usually from the collar skin incision above the jugulum.

In terms of a minimalization of surgical approach, unilateral, radionavigated and miniinvasive approaches were developed. In case of intrathoracic-mediastinal localization of parathyroid glands, the partial median sternotomy is the basic approach. In 3.5% of 680 our patients, the neck approach was not sufficient. The complications of parathyroidectomy are not common. They include the hypoparathyroidism and the recurrent laryngeal nerve injury with following vocal cord paralysis. Benefits To date, the parathyroidectomy is a short, one-day surgery operation in surgical centers. The improvement of surgical technique offers a surgical treatment to "asymptomatic" patients. In case of a clear localization of parathyroid adenoma by sonography or MIBI scintigraphy, the operation is short, safe and does not stress the patient. In these patients, the so-called small symptoms (fatigue, musculoskeletal pain, weakness, dyspepsia, polydypsia, constipation, polyuria, pruritus, depression) are ameliorated.

Supported by IGA MZ ČR č. 8308-5/2005

S22.4

Absolute risk prediction for fracture

H Pols

Abstract unavailable

Reproductive endocrinology/andrology – S23

S23.1

Androgen regulation of spermatogenesis

Philippa Saunders[1], Lee Smtih[1], Robert Hooley[1], Karel De Gendt[2], Evi Denolet[2], Guido Verhoeven[2] & Richard Sharpe[1]
[1]MRC Human Reproductive Sciences Unit, Edinburgh, United Kingdom; [2]Catholic University of Leuven, Leuven, Belgium.

Spermatogenesis is a complex process involving interactions between the somatic cells (Sertoli, Leydig, peritubular) and germ cells within the adult testis. Androgens are key regulators of spermatogenesis and intra-testicular concentrations of testosterone (T), produced by the Leydig cells, are higher than that in blood. Androgen action is mediated by the androgen receptor (AR), an X-chromosome-encoded, ligand-activated, transcription factor. The mechanisms by which androgens regulate testis function have been explored by determining the pattern of expression of AR, by manipulating androgen concentrations, by performing studies *in vitro* on isolated tubules/cells and most recently by studying mice with cell-specific deletion of the *Ar* gene.

In adult testes AR have been immunolocalised to the nuclei of Sertoli, Leydig and peritubular myoid cells as well as the cells lining blood vessels. Expression in adult Sertoli cells is stage-dependent and *in vitro* studies have demonstrated that it is T-regulated. In rats, ablation of Leydig cells with ethane dimethane sulphonate results in an acute reduction in intra-testicular T and germ cell loss; germ cell demise is first observed in the stages of spermatogenesis in which AR expression in Sc is highest. The impact of Sertoli cell-specific ablation of Ar on testicular function has been investigated in three independent laboratories. In all cases Ar ablation resulted in a reduction in testicular size, germ cell loss and infertility. Expression of rHox5, a Sertoli cell protein previously shown to be T-regulated, was reduced as was expression of proteins involved in formation of junctional complexes. Leydig cell function was altered even though expression of Ar was maintained in these cells confirming the existence of paracrine interactions between the seminiferous and interstitial compartments. In conclusion, testicular function and male fertility are androgen dependent; expression of AR in Sertoli cells is essential for normal germ cell maturation and fertility.

S23.2

The experimental mouse model for men with Klinefelter syndrome

YanHe Lue, Christina Wang, David Jentsch, Krista Erkkila, Peter Liu, Monica Schwarcz & Ronald Swerdloff
Dept of Medicine, Division of Endocrinology, LABiomed at Harbor-UCLA Med Ctr, Torrance, CA, USA.

Klinefelter syndrome (XXY males) is the most common sex chromosome aneuploidy, occurring in about 1 per 500 men. To study the underlying molecular mechanisms caused by the extra X chromosome, we have developed an experimental mouse model for men with Klinefelter's syndrome. We have demonstrated that adult XXY mice have absence of germ cells, decreased serum testosterone levels, and elevated gonadotropin levels. Testicular failure begins early as a result of massive germ cell loss that precedes the initiation of meiosis. Loss of germ cells is mediated through apoptosis. Gene microarray with testicular RNA samples from 1-day-old mice showed inactive X specific transcripts (Xist) expression increased 4.14-fold, indicating the extra X chromosome is inactivated in XXY testes. Proapoptotic Bcl2-interacting killer-like and caspase 7 have 1.59- and 1.68-fold increase, and antiapoptotic transcripts IAP and Bcl2-like-10 have 3.73- and 2.08-fold decrease respectively in XXY mice. By immunohistochemistry, we found c-kit expression in gonocytes occurred earlier in XXY than XY siblings, suggesting early differentiation of gonocytes may contribute to germ cell loss in XXY mice. In addition to germ cell defect, androgen receptor expression in Sertoli cells is nearly depleted in adult XXY mice, suggestive of Sertoli cell dysfunction. By transplantation of XY germ cells into adult XXY testes, we found a few donor XY spermatogonia were able to survive for 10 weeks without further differentiation. Leydig cells in adult XXY mouse testes are both hypertrophic and hyperplastic. Testosterone production from XXY Leydig cells is impaired. Besides reproductive dysfunction, we have demonstrated that XXY mice have impaired learning, memory, and social interaction. By giving testosterone implants to adult XXY mice, we demonstrated that testosterone treatment significantly improves the learning ability of adult XXY mice.

S23.3

Genes involved in male infertility: sorting facts from fiction

Ewa Rajpert-De Meyts
Copenhagen University Hospital (Rigshospitalet), Copenhagen, Denmark.

Male infertility is a common disorder and a growing health problem. A large proportion of unexplained cases have been summarily categorised as idiopathic infertility. The majority of idiopathic cases, especially those with severely impaired spermatogenesis incl. azoospermia, are presumably caused by genetic defects. Genetics of male infertility has been a largely unexplored area, until quite recently, when new molecular tools unabled discovery of a growing number of genes involved in spermatogenesis and gamete maturation, e.g. genes mapped to the AZF region of the Y-chromosome and some genes on the X-chromosome. In addition, several pathways related to hormonal regulation of reproductive function contain polymorphic genes, which may affect the function of a given gene in a discrete manner, such as the CAG and GGN repeats on androgen receptor, or polymorphisms in *CYP*, *INSL3* genes. Finally, polymorphisms of genes seemingly unrelated to the reproductive function, have been associated with male infertility, e.g. mitochondrial gene polymerase, *POLG*. A rush to analyse polymorphic genes in various populations, often with poorly characterised cases and controls, created a lot of confusion in the literature as to the real pathogenetical involvement of the studied genes in male infertility. There is a need for large and well-controlled studies, underpinned by basic functional studies of the investigated genes. A great care must be taken to use proper control groups, which must be selected with fertility, ethnicity, and age of the subjects in mind. A very important point is having in mind that environmental exposures and/or lifestyle factors frequently exert their influence primarily in genetically predisposed individuals. A good description of the reproductive parameters (outcomes), preferably with the analysis of the reproductive function on children, is also essential for the analysis of the consequences of studied polymorphism/gene aberration, and for an early prognosis as to the future fertility problems.

S23.4

Genetic basis of testicular tumors

Leendert Looijenga
Erasmus MC, Rotterdam, Netherlands.

Various types of human germ cell tumors (GCTs) can be found, referred to as type I, II and III. The type I are the teratomas and yolk sac tumors of neonates and infants. No genomic aberrations have been identified in teratomas, while yolk sac tumors show chromosomal imbalances related to chromosomes 1, 6 and 20. Type II GCTs are the seminomas and nonseminomas, derived from carcinoma *in situ* (CIS)/intratubular germ cell neoplasia unclassified (ITGCNU). CIS/ITGCNU and seminoma cells mimic primordial germ cells/gonocytes, amongst others characterized by expression of the diagnostic marker OCT3/4-POU5F1. All invasive tumors show gain of the short arm of chromosome 12. The type III GCTs, i.e. spermatocytic seminomas, occur predominantly in elderly, and only in the testis. They originate from primary spermatocytes, and show consistent gain of chromosome 9, of which DMRT1 is a candidate. GCTs show specific patterns of mRNA and microRNA expression, of possible diagnostic and prognostic value. Besides familial predisposition and infertility, disorders of sex differentiation (DSD) is a risk factor for type II GCTs. This specifically forms of hypovirilization and gonadal dysgenesis, in the presence of part of the GBY region. Besides CIS/ITGCNU, gonadoblastoma can be the precursor in DSD patients. Gonadoblastoma is the earliest developmental stage in the genesis of GCTs. TSPY (testis specific protein on the Y chromosome) is a likely candidate to explain the requirement of the GBY region for malignant transformation of germ cells. A significant limiting diagnostic factor in DSD is lack of specific markers for CIS/ITGCNU in case of maturation delay of germ cells. The type II GCTs are in fact an embryonic cancer in adult patients. This explains a number of specific characteristics, like their histology (totipotency), overall sensitivity to DNA-damaging agents, as well as their chromosomal and genetic constitution.

Obesity – S24

S24.1

Altering adipocyte metabolism as a way to counteract obesity and insulin Resistance

Sven Enerbäck
Medical Genetics, Dept of Medical Biochemistry, Göteborg University, Medicinareg, 9A, Box 440, SE 405 30 Göteborg, Sweden.

Advances over the last two decades in our understanding of the adipocyte have clarified its role as a key regulator of both energy balance and intermediary metabolism. It is now known that in addition to being an insulator and energy depot, the adipocyte is a highly active cell, secreting a wealth of factors, including leptin, that play a part in CNS and appetite regulation. There is also a much greater understanding of how fat cells themselves develop from precursor cells FOXC2, pRb, PGC-1and RIP140 has been discussed as genes influencing adipocyte cell fate. By increasing the already existing pool of brown adipocyte in human adipose tissue, as a way to dissipate excess energy through uncoupling, this would help conserve ample triglyceridestorage capacity in white adipocyte and hence counteract ectopic lipid depositions in tissues like liver and muscles. Since ectopic lipid deposition is intimately connected to the development of insulin resistance and the metabolic syndrome, factors affecting white versus brown fat partitioning constitutes an interesting approach to this health problem.

S24.2

Triglyceride-lowering effect of metabolic switch in white adipose tissue

Jan Kopecky
Institute of Physiology, Academy of Sciences of the Czech Republic, Prague, Czech Republic.

High level of triglycerides (TG) in plasma is a risk factor for cardiovascular disease. Various treatment strategies aimed at decreasing plasma TG concentrations affect synthesis of lipoproteins in the liver and/or increase clearance of TG by peripheral tissues. Lipid-lowering effects of fibrates reflects modulation of the liver metabolism. Antidiabetic agents thiazolidinediones (TZD) lower plasma TG by enhancing lipoprotein lipase activity in white adipose tissue (WAT). Long-chain polyunsaturated fatty acids of *n*-3 series, namely eicosapentaenoic (EPA; 20:5 *n*-3) and docosahexaenoic (DHA; 22:6 *n*-3) acids, that are abundant in sea fish, act as hypolipidemics, while decreasing the production of lipoproteins. EPA and DHA may also affect the TG clearance. Most of the above mentioned treatments induce expression of mitochondrial uncoupling proteins (UCPs) in WAT. The aims of our studies were to characterize: (i) the potency of WAT to decrease plasma TG levels; and (ii) the involvement of WAT in the hypolipidemic effects of EPA and DHA. A large potency of WAT to decrease plasma TG was demonstrated using transgenic mice with ectopic expression of UCP1 in WAT (aP2-*Ucp1* mice). The ectopic UCP1 induces respiratory uncoupling in WAT, hence stimulating *in situ* lipid oxidation and mitochondrial biogenesis, and clearance of plasma TG. Moreover, aP2-*Ucp1* mice were resistant to high-fat diet induced obesity and showed higher whole body lipid oxidation. The obesity in wild type mice was also prevented by replacing only 9% of the dietary lipids by EPA and DHA. This dietary treatment lowered plasma TG, while inducing lipid oxidation and mitochondrial biogenesis in WAT. These results supported a possibility to induce a metabolic switch in WAT, which may change whole body phenotype, including the lowering of plasma TG. Further studies are required to assess the importance of this switch for the effectiveness of the lipid-lowering treatments.

S24.3

Adipokines and insulin sensitivity in humans

José Manuel Fernández-Real
Hospital of Girona, Girona, Spain.

Decreased insulin action has been proposed as the common factor that is in the background of the different components of the metabolic syndrome. Insulin resistance is also associated with a chronic activation of the innate immune system. The innate immune system constitutes the first line of body's defence and it is constituted by different barriers (epithelia, adipose tissue), and different blood and tissue components as macrophages, and neutrophils. Once activated, the acute phase response is activated, with generation of different acute phase proteins and cytokines that are produced in order to struggle against different aggresions, as infections and traumatisms. The aim of this response is to eradicate these agents, to repair the harmed tissues, and, through increased insulin resistance, to optimize the energetic substrates, which will be drained to vital tissues and organs (i.e. brain and the immune system). Evolution pressures have led to survival of the fittest individuals, those with genetics that allows the best defence against infection and periods of famine. The initial evolutive advantages of increased inflammatory responses, hypersecretion of proinflammatory cytokines (TNF-α, interleukin 6, interleukin 18), counterbalanced by antiinflammatory molecules (adiponectin, sCD14, BPI, MBL), turn into chronic inflammation conditions, such as obesity and type 2 diabetes. Increasing evidence is reported according to which chronic inflammation precedes these conditions. The knowledge of how these metabolic pathways interact with the inflammatory cascade will facilitate new therapeutic approaches.

S24.4

Lipodistrophy and abdominal fat accumulation: new therapeutic alternatives

LF Van Gaal
Department of Diabetology, Metabolism and Clinical Nutrition, University Hospital Antwerp, Antwerp, Belgium.

Lipodystrophy (LD) is a well-recognised clinical syndrome of peripheral fat atrophy and central adiposity, often associated with laboratory abnormalities such as dyslipidemia and glucose intolerance, and probably linked to insulin resistance. The long-term consequences of LD and its potential association with cardiovascular disease remain unknown. The visceral fat accumulation is characterised by the increased, abundant secretion of a number of peptides such as leptin, insulin-like growth factor (IGF), adiponectin and the recently reported resistin and visfatin hormones. Elevated resistin and tumour necrosis factor (TNF-alpha) levels and low levels of adiponectin secretion may have implications for the risk of development of type 2 diabetes and cardiovascular disease. LD is observed not only in rare autosomal syndromes, but also in patients positive for the human immunodeficiency virus (HIV) who have been treated with protease inhibitors. Both the origin of LD and its treatment deserve more attention and further research in clinical settings.

Potential treatment options with leptin and human growth hormone can be considered to reduce the burden and cardiovascular risk of lipodystrophy.

Novel hormones – S25

S25.1

Hormones help you live longer - the threat of Klotho

M Kuro-o
The University of Texas Southwestern Medical Center, Dallas, Texas, USA.

A defect in *klotho* gene expression in mice leads to a syndrome resembling aging, including a shortened life span, hypogonadism, growth arrest, hypoactivity, skin atrophy, muscle atrophy, hearing loss, premature thymic involution, cognition impairment, motor neuron degeneration, arteriosclerosis, osteopenia, soft tissue calcification, and pulmonary emphysema among others. In contrast, overexpression of the *klotho* gene extends life span in the mouse. Thus, the *klotho* gene functions as an aging suppressor gene. The *klotho* gene encodes a single-pass transmembrane protein and is expressed in limited tissues, notably in the kidney and brain. The extracellular domain of Klotho is shed and secreted in the blood, raising the possibility that Klotho protein itself may function as a humoral factor.

Extended life span in transgenic mice that overexpress Klotho is associated with increased resistance to insulin/IGF1 and oxidative stress, mechanisms for the suppression of aging evolutionarily conserved from worms to mammals. Klotho may affect aging processes partly through its ability to inhibit insulin/IGF1 signaling and to reduce oxidative stress.

Mice defective in fibroblast growth factor-23 (FGF23) exhibit aging-like phenotypes similar to those observed in Klotho-deficient mice, suggesting that Klotho and FGF23 may function in a common signal transduction pathway(s). My laboratory has shown that Klotho binds to multiple FGF receptors (FGFRs) and enhances the ability of FGF23 to activate FGF signaling. FGF23 was originally identified as a hormone that inhibited phosphate reabsorption in the kidney. In fact, both Klotho-deficient mice and FGF23-deficient mice exhibit elevated serum phosphate levels. In addition, many aging-like phenotypes in these mice are rescued by restriction of dietary phosphate or ablation of vitamin D activity. These findings imply a novel concept that FGF signaling and phosphate metabolism may participate in the regulation of aging in mammals.

S25.2

Phosphatonins and the regulation of renal phosphate transport

P Kumar
USA.

Abstract unavailable

S25.3

Correlation of desoxypyridinolin and c– terminal telopeptide of collagen type I within different patient collectives

Walter Fassbender, M Goedde, Vincent Brandenburg, Klaus-Henning Usadel & Ulla Stumpf
Hospital z. Hl. Geist, Kempen, Germany; University Hospital, Frankfurt/Main, Germany; University Hospital, Aachen, Germany; Endokrinologikum, Frankfurt/Main, Germany; University Hospital, Duesseldorf, Germany.

Bone metabolism can be measured indirectly with specific biochemical markers. Desoxypyridinolin (DPD) is a derivate of hydroxypyridinium, which is discharged by bone resorption and is totally excreted urinary. A further marker of collagen resorption is the c-terminal telopeptide of collagen type I, which is liberated to blood circulation within the bone's degradation and undergoes renal elimination. The aim of our investigation was to look after a correlation of these parameters in healthy subjects ($n=28$), patients with type 1 diabetes mellitus (DM) ($n=65$), and female patients with diagnosed postmenopausal osteoporosis (PMO). For the labaratory analysis of DPD we used a solid phase chemiluminescence enzymimmunoassay and for assessment of c-terminal telopeptide of type I – collagen a quantitative ELISA was used. We found correlations of both parameters within the main group ($n=181$), and all the other subgroups. The strongest correlation could be found in the group with DM type 1 ($r=0.79$, $P<0.05$) followed by the group of healthy subjects ($r=0.75$, $P<0.05$). In the group of female patients (PMO) a weaker, but significant positive correlation could be verified ($r=0.58$, $P<0.05$). The arithmatic average of DPD was in the group of healthy subjects about 15,4 nM DPD/mM Krea (95%KI: 11.1–19.72), in the group of type 1 DM patients 21.02 (11.23–30.82) and about 38.51 (28.32–48.7) nM DPD/mM Krea in the group of the female patients (PMO). Both parameters reflect the diverse amount of bone turnover and correlated significantly positive to each other. In comparison to the healthy subjects an enhanced bone turnover could be measured consistently in the group of type 1 DM patients. The hihgest values but concurrent the widest statistic spread with weaker correlation was measured in the group of female patients (PMO). This may indicate, that the results found before therapy are of limited diagnostic value, unlike in the course of antiresorptive therapy the observed significant alterations of bone resorption parameters are of specific diagnostic value.

S25.4

Hormonal regulation of iron homeostasis by hepcidin

Sophie Vaulont
Institut Cochin, Paris, France.

Hepcidin is a small circulating 25-amino-acid cysteine-rich peptide first identified in human blood and urine. The hepcidin gene is expressed mainly in the hepatocytes, secreted in the circulation and cleared by the kidney. In mammals, convincing evidence indicates that hepcidin constitutes the master regulator of iron homeostasis; the circulating peptide acts to limit gastrointestinal iron absorption and serum iron by inhibiting dietary intestinal iron absorption and iron recycling by the macrophages. To limit iron egress, hepcidin binds to ferroportin, a transmembrane iron exporter, thereby inducing its internalization and subsequent degradation, leading to decreased export of cellular iron.

As befits an iron-regulatory hormone, hepcidin synthesis is induced by iron stores and inflammation and inhibited by anemia and hypoxia. The mechanisms regulating hepcidin expression are only beginning to be understood. Recent studies have highlighted two regulatory cascades: BMP/Smad signaling of hemojuvelin (a transmembrane protein whose mutation is leading to juvenile hemochromatosis) and IL-6/STAT3 signaling of inflammation.

Dysregulation of hepcidin is involved in the pathogenesis of a spectrum of iron disorders. Most of the iron overload syndromes known to date (Hereditary Hemochromatosis and secondary iron overloads) imply a reduction of hepcidin secretion. In contrast, excessive cytokine-induced hepcidin expression causes hypoferremia and contributes to the anemia of inflammation.

The emergence of hepcidin as the pathogenic factor in most systemic iron disorders should provide important opportunities for improving their diagnosis and treatment.

Oral Communications

Thyroid clinical - OC1

OC1.1 – ESE Young Investigator Award

Prevalence of inactivating TSH receptor (TSHR) mutations in a large series of pediatric subjects with non-autoimmune mild hyper-thyrotropinemia (hyperTSH)

Daniela Cordella[1], Alessandro De Marco[2], Davide Calebiro[1], Tiziana de Filippis[2], Giorgio Radetti[3], Giovanna Weber[5], Maria Cristina Vigone[5], Marco Cappa[4], Alessandro Sartorio[2], Marta Busnelli[2], Marco Bonomi[2], Bice Chini[6], Paolo Beck-Peccoz[7] & Luca Persani[1]
[1]Dept of Medical Sciences, University of Milan and Ist. Auxologico Italiano, Milan, Italy; [2]Lab of Experimental Endocrinology, IRCCS Ist Auxologico Italiano, Milan, Italy; [3]Pediatric Unit, Bolzano, Italy; [4]Pediatric Unit, Bambin Gesù Hosp, Rome, Italy; [5]Pediatric Unit, HSR, Milan, Italy; [6]CNR, Milan, Italy; [7]Endocrine Unit, Fondazione Policlinico IRCCS, Milan, Italy.

Mild hypothyroidism is a heterogeneous and frequent disorder in the general population that is due to autoimmune disease in most of the cases. TSH resistance is considered a rare genetic disease due to germline loss-of-function TSHR mutations. However, TSHR mutations have been mainly searched in patients with large TSH elevations and their actual prevalence among patients with mild TSH elevations (as those found in mild hypothyroidism) is so far unknown. In this study, we evaluated the involvement of TSHR mutations in a large pediatric series of unrelated cases of hyperTSH ($n=48$, 26 W and 22 M; age 0–12 yrs) selected in various collaborating centers. All subjects had high TSH (4–15 mU/ml), normal freeT4 concentrations, no antithyroid antibodies and normal thyroid volume and structure at ultrasound. Through dHPLC (WAVE apparatus, Transgenomic) and direct sequencing of abnormal PCR products (ABI Prism), we analyzed TSHR coding sequence, proximal promoter and intron-exon boundaries. These investigations lead to the disclosure of 11 carriers of heterozygous TSHR mutations among the 48 patients with hyperTSH (frequency: 22.9%). Seven of these 11 carriers had at least another first-degree relative with known hyperTSH and 4/11 were positive at neonatal TSH screening. Three TSHR mutations are novel (P162L, T607I, R609Q), never found in other patients with TSH resistance and in 150 internal control alleles, and 4 mutations had been previously reported (C41S, P162A, L467P, 655delAC). The mutations C41S, P162A, T607I, 655delAC have been found in 2 unrelated cases. In conclusion, the prevalence of heterozygous TSHR mutations in a pediatric series of hyperTSH is surprisingly elevated. The diagnosis of TSH resistance by means of TSHR gene analysis retains a primary role for appropriate clinical management of subjects with hyperTSH and genetic counseling of their families.

OC1.2

Expression gene profile may be useful for the diagnosis of thyroid malignancies

Pamela Piampiani[1], Cristina Romei[1], Barbara Cosci[1], Agnese Vivaldi[1], Raffaele Ciampi[1], David Viola[1], Valeria Bottici[1], Riccardo Giannini[2], Fulvio Basolo[2], Aldo Pinchera[1] & Rossella Elisei[1]
[1]Department of Endocrinology, University of Pisa, Pisa, Italy; [2]Department of Oncology, University of Pisa, Pisa, Italy.

Although 20% of follicular neoplasms are papillary thyroid carcinoma (PTC), their cytological diagnosis is not diagnostic. A different profile of gene expression between malignant and benign thyroid tumors has been reported. Aim of this study was to identify a gene expression profile to be used in distinguishing malignant from benign thyroid neoplasms. By real-time RT-PCR we analyzed mRNA expression of 6 thyroid differentiation genes (TTF-1, PAX8, TPO, TSHr, NIS and Tg) and 5 genes known to be involved in thyroid tumorigenesis [PPARγ, Gal3, EGFR, MET and oncofibronectin (onfFN)] in 174 human thyroid tissues (87 tumor samples and 87 corresponding normal tissues) belonging to 72 patients affected with PTC and 15 patients affected with benign nodular disease (BND). Our results indicate that thyroid differentiation genes and PPARγ were significantly less expressed in PTC samples than in normal tissue (TPO, 61/72 cases, $P<0.0001$; NIS, 64/72 cases, $P<0.0001$; Tg, 59/72 cases, $P=0.0002$; TSHr, 57/72 cases, $P=0.0169$; TTF1, 47/72 cases, $P=0.002$; PAX8, 55/72 cases, $P=0.0001$; PPARγ, 57/72 case, $P<0.0001$). On the contrary, 3 genes were more expressed in the tumor than in normal tissue (onfFN, 64/72 cases, $P<0.0001$; MET, 55/72 cases, $P=0.0018$; Gal3, 53/72, $P<0.0001$). No statistically significant difference was observed for the mRNA expression of EGFR between tumoral and normal tissues. In BND a statistically significant difference between mRNA expression in tumoral and normal tissue was observed only for PPARγ as observed in PTC specimen. Summarising, our data show that 10/11 selected genes are differentially expressed in the tumor tissue with respect to normal. On the contrary only 1/11 was differentially expressed in BND with respect to its normal tissue. In conclusion, 9/11 of these genes are characterized by a gene expression profile that was specific for the malignant neoplasms. The analysis of the levels of expression of these genes in Fine Needle Aspiration material might represent a helpful and innovative method for the presurgical diagnosis of citologically indeterminate thyroid nodules.

OC1.3

Persistence of decreased peripheral B-lymphocytes after Rituximab treatment is associated to inactive disease in patients with thyroid-associated ophthalmopathy

Irene Campi[1], Guia Vannucchi[1], Danila Covelli[1], Paola Bonara[2], Nicola Currò[3], Davide Dazzi[4], Giacinta Pirola[3], Stefania Rossi[5], Claudio Guastella[6], Paolo Beck-Peccoz[1] & Mario Salvi[1]
[1]Endocrine Unit Department of Medical Science; [2]Internal Medicine; [3]Ophthalmology; Fondazione Policlinico IRCC, University of Milan, Milan, Italy; [4]Internal Medicine, Ospedale di Fidenza, Fidenza, Italy; [5]Pathology Unit, Department of Medicine, Surgery and Dentistry, University of Milan, Ospedale S. Paolo and Fondazione Policlinico IRCCS, Milan, Italy; [6]Otolaryngology, Fondazione Policlinico IRCC, University of Milan, Milan, Italy.

The anti-CD20 antibody Rituximab (RTX) induces peripheral B cells depletion. Aim of the present study was to evaluate changes of lymphocytes after RTX therapy, administered at the dosage of 1000 mg twice at 2-week interval, in 10 patients with Graves' disease, 8 of whom had associated ophthalmopathy (TAO). In all patients, we studied the standard immunophenotypic panel before therapy and monthly for up to 2 years. Total CD20+ (and CD19+) cell depletion was observed after the first infusion in 9 patients while one patient had persistence of <5% CD19+CD5+ lymphocytes. 8/10 patients were depleted for 4–6 months after RTX, while 1 and 1 patients after 2 and 10 months respectively. A reduction of CD20+ cells of about 50% from baseline was observed in 6 patients at 18 months and in 3 at 26 months. While after RTX there was no significant change of serum thyroid autoantibodies levels, nor correlation with CD20+ depletion, we observed a stable improvement of TAO with a significant decrease of the clinical activity score. Although progression to inactive TAO did not correlate with CD20+ cells, since at 5 months they began repopulating, we did not observe relapse of active TAO even after B cell return. In contrast, in the patient with persistence of CD19+5+, severe TAO relapsed at the time of CD20+ cells return. Another cycle of RTX (1000 mg) was then administered but again we observed persistence of <7% CD19+5+ with no definite improvement of the clinical signs of TAO. At subsequent orbital decompression we were able to detect CD19+5+ in the orbital tissues. In conclusion, in patients with TAO a reduction of CD20+ of about 50% from the baseline is still present at 18–24 months after RTX treatment. This may explain the consistent improvement of TAO and the lack of relapse, in patients after total B-cells depletion. Persistence of CD19+5+ lymphocytes in the peripheral blood and, perhaps, in the orbit, may associate to a not completely satisfactory therapeutic response.

OC1.4

A novel tyrosine-kinases selective inhibitor with anti-tumoral efficacy (Sunitinib) induces a block in iodine uptake and transient hypothyroidism

Deborah Mannavola[1], Guia Vannucchi[1], Marco Carletto[2], Virgilio Longari[2], Rossella Bertuelli[3], Paola Coco[3], Paolo Casali[3], Paolo Beck-Peccoz[1] & Laura Fugazzola[1]
[1]Endocrine Unit, Dept. of Medical Sciences, University of Milan, Fondazione Policlinico IRCCS, Milan, Italy; [2]Nuclear Medicine Unit, Fondazione Policlinico IRCCS, Milan, Italy; [3]Dept. of Oncology, Istituto Nazionale dei Tumori, Milan, Italy.

Sunitinib (SU11248) is a multitarget inhibitor of tyrosine-kinases (RTK) recently tested in clinical trials for the treatment of some human cancers. Side effects are mostly represented by asthenia and appear in a dose and time

correlated manner. After the unexpected observation of a myxedematous coma in a patient affected with GIST and treated with Sunitinib, we evaluated the effect of this drug on thyroid function in 24 patients treated for GISTs Imatinib resistant. Patients received the following cycles of therapy: 4 weeks of daily treatment at the dose of 50 mg/day orally (ON) and 2 weeks of withdrawal (OFF). On days 1 and 28 of each cycle TSH, FT3, FT4, thyroglobulin, anti-Tg and anti-TPO autoantibodies were measured. Eleven patients (46%) treated with SU11248 developed a transient hypothyroidism between the 1st and the 6th cycle of treatment (median 3rd cycle). Hypothyroidism was subclinical in 10 cases and overt in 1 patient. During the OFF periods TSH normalized, but a progressive increase of TSH levels was observed. After a variable number of cycles, the lack of normalization during the OFF periods was observed. In order to elucidate the possible mechanism underlying Sunitinib-induced hypothyroidism, *in vivo* morpho-functional examinations were performed. Neither ultra-sonographic alterations (in particular destructive-like), nor variations in thyroglobulin and anti-thyroid autoantibodies, were observed during the ON and OFF phases even after several cycles. On the contrary, ^{123}I uptake was normal in basal conditions and largely reduced after the 4 weeks of treatment, with partial or total normalization after the 2 weeks of withdrawal. In conclusion, SU11248 determines hypothyroidism in the 46% of patients. The absence of anti-thyroid autoantibodies and the normal echographic pattern allow to exclude autoimmune and/or destructive mechanisms. Interestingly, hypothyroidism seems to be correlated with a defect in the uptake of iodine. The possibility to perform a selective and temporary block of thyroid function could be useful in the treatment of some thyroid diseases.

OC1.5

CTLA- 4 gene polymorphisms and autoimmune thyroid diseases: meta-analyses of published and individual-level data

Fotini Kavvoura[1], Takashi Akamizu[2], Takuya Awata[3], Yoshiyuki Ban[4], Dimitry Chistiakov[5], Irena Frydecka[6], Abbas Ghaderi[7], Stephen Gough[8], Yuji Hiromatsu[9], Rafal Ploski[10], Pei-Wen Wang[11], Yoshio Ban[4], Tomasz Bednarczuk[12], Emma Chistiakova[13], Marcin Chojm[10], Joanne Heward[8], Hitomi Hiratani[2], Suh-Hang Hank Juo[14], Lidia Karabon[18], Shigehiro Katayama[3], Susumu Kurihara[3], Rue-Tsuan Liu[11], Ikuyo Miyake[9], Gholam-Hossein Omrani[15], Edyta Pawlak[18], Matsuo Taniyama[16], Teruaki Tozaki[17] & John PA Ioannidis[1]

[1]Department of Hygiene and Epidemiology, University of Ioannina School of Medicine, Ioannina, Greece; [2]Translational Research Center, Kyoto University Hospital, Kyoto University School of Medicine, Kyoto, Japan; [3]Department of Medicine, Saitama Medical University, Saitama, Japan; [4]Third Department of Internal Medicine, Showa University School of Medicine, Tokyo, Japan; [5]Department of Pathology, University of Pittsburgh Medical Center, Pittsburgh, PA, United States; [6]Department of Hematology, Medical Academy, Wroclaw, Poland; [7]Shiraz Institute for Cancer Research, Medical School, Shiraz University of Medical Sciences, Shiraz, Iran; [8]Institute of Biomedical Research, The Medical School, University of Birmingham, Birmingham, United Kingdom; [9]Kurume University School of Medicine, Kurume, Fukuoka, Japan; [10]Human Molecular Genetics Laboratory of the Department of Forensic Medicine, Department of Medical Genetics, Medical University of War, Warsaw, Poland; [11]Chang Gung Memorial Hospital-Kaohsiung Medical Center, Chang Gung University College of Medicine, Kaohsiung, Taiwan; [12]Department of Endocrinology, Medical Research Center, Polish Academy of Science, Warsaw, Poland; [13]Department of Molecular Diagnostics, National Research Center GosNIIgenetika, Moscow, Russia; [14]Graduate Institute of Medical Genetics, Kaohsiung Medical University Hospital, Kaohsiung, Taiwan; [15]Endocrine and Metabolism Research Center, Namazee Hospital, Shiraz, Iran; [16]Division of Endocrinology and Metabolism, Showa University Fujigaoka Hospital, Yokohama, Kanagawa-ken, Japan; [17]Department of Medical Information, Showa University School of Pharmaceutical Science, Showa, Japan; [18]Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wroclaw, Poland.

Background

CTLA-4 polymorphisms have been widely examined for their associations with autoimmune thyroid diseases (Graves' disease [GD] and Hashimoto thyroiditis [HT]) but their relative population effect remains unclear.

Methodology/Principal findings

Meta-analyses of group-level data from 32 ($n=11.019$ subjects) and 12 ($n=4.479$) published and unpublished studies were performed for the association of the A49G polymorphism with GD and HT, respectively. Fifteen ($n=7.246$) and 6 ($n=3.086$) studies were available for the CT60 polymorphism, respectively. Meta-analyses of individual-level data from 10 ($n=4.906$ subjects) and 5 ($n=2.386$) collaborating teams for GD and HT, respectively, using haplotypes of both polymorphisms were also performed. Group-level data suggested significant associations with GD and HT for both A49G (odds ratio 1.49, $P=6\times10^{-14}$ and 1.29 [$P=0.001$] per G allele, respectively) and CT60 (OR 1.45, [$P=2\times10^{-9}$] and 1.64 [$P=0.003$] per G allele, respectively). Results were consistent between Asian and Caucasian descent subjects. Individual-level data showed that compared with the AA haplotype the risk conferred by the GG haplotype was 1.49 (95% CI: 1.31–1.70) and 1.36 (95% CI: 1.16–1.59) for GD and HT, respectively. The AG haplotype also increased the risk of GD (1.35, 95% CI: 1.16–1.55) but not of HT (1.02, 95% CI: 0.71–1.47). The results for the GA haplotype were inconclusive. Data were consistent with a dose-response effect for the G-allele of CT60.

Conclusions/Interpretation

The CT60 polymorphism of CTLA-4 maps an important genetic determinant for the risk of both GD and HT across diverse populations.

OC1.6

Sensitization against Soybean may induce an increase in the levels of anti-thyroid peroxidase antibodies in thyroid autoimmunity

Ildikó Molnár

Kenézy County and Teaching Hospital, Debrecen, Hungary.

Soybean diet could involve in the development of goitre according to antithyroid effects of isoflavones. Isoflavones from Soybean could induce not only inhibition of thyroid peroxidase (TPO) catalyzed reactions but they are allergens for patients suffering from atopic or autoimmune diseases. Two hundred-sixty patients with thyroid autoimmunity (150 with Graves' disease, 110 with Hashimoto's thyroiditis) were investigated for the sensitization against Soybean. Allergen-specific IgE levels were measured by Western blot Allergy Screen panels and the levels of thyroid hormones (TSH, FT_4, FT_3) and anti-TPO, anti-Htg (thyroglobulin), TSH receptor (TRAK) antibodies were detected by immunoassay. The data were presented as mean $\pm$ SE.

Allergic sensitization against Soybean was as follows: 24 cases in Graves' disease and 16 cases in Hashimoto's thyroiditis. Graves' patients with Soybean allergy showed increased anti-TPO levels compared to patients who were negative for allergen (567.33 ± 82.88 IU/ml vs 264.88 ± 30.77 IU/ml, $P<0.001$). However, in patients with Soybean allergy, the elevation in anti-TPO levels was higher in hyperthyroid cases than in those without allergy (736.6 ± 138.87 IU/ml ($n=7$) vs 296.15 ± 50.81 IU/ml ($n=41$), $P<0.011$). Surprisingly, higher FT_3 (and FT_4) levels were demonstrated in sensitized hyperthyroid cases compared to nonsensitized ones (15.92 ± 4.7 pg/ml vs 5.44 ± 0.56 pg/ml, $P<0.001$ for FT_3 (and $P<0.049$ for FT_4)). The increase in anti-TPO levels for sensitized euthyroid Graves' patients strongly associated with ophthalmopathy in comparison with nonsenzitized ones (669.98 ± 162.38 IU/ml ($n=6$) vs 156.81 ± 48.46 IU/ml ($n=29$), $P<0.003$).

In conclusion, the presence of Soybean allergen-specific IgE levels in thyroid autoimmunity could contribute to the elevation in anti-TPO levels for Th2 dominant Graves' disease. The sensitization against Soybean may induce thyroid autoimmunity due to increased anti-TPO levels in disease susceptible patients.

OC1.7

Pregnant women on thyroxine substitution are often dysregulated in early pregnancy

Bengt Hallengren[1], Mikael Lantz[1], Bengt Andreasson[2] & Lars Grennert[3]

[1]Department of Endocrinology, Malmö University Hospital, Malmö, Sweden; [2]Department of Pediatrics, Malmö University Hospital, Malmö, Sweden; [3]Department of Obstetrics and Gynecology, Malmö University Hospital, Malmö, Sweden.

Background

Thyroid hormones are important for normal fetal development. The aim of this prospective study was to explore whether thyroxine treated pregnant women with hypothyroidism are adequately thyroxine substituted during pregnancy.

Material and method
During the years 1997–2002 119 pregnancies in 101 females with thyroid diseases were followed at the Department of Endocrinology. The diagnoses were autoimmune thyroiditis (AIT) with or without hypothyroidism $n=46$, hypothyroidism (non AIT) $n=9$, status post Graves' thyrotoxicosis (GD) $n=33$, active GD $n=8$, multinodular toxic goitre (MNTG) $n=2$, atoxic goitre with or without autonomous function $n=20$, operated thyroid cancer $n=1$ ($+1$ in the group status post GD).
Results
64 patients were on thyroxine due to hypothyroidism at the first visit: 50% (32/64) had serum TSH values within the reference range (0.4–4.0 mIE/l) at first laboratory control. 20% (13/64) had TSH <0.40 mIE/l, 14% (9/64) ≤ 0.1 mIE/l, 30% (19/64) had TSH >4.0 mIE/l, 14% (9/64) >10 mIE/l. 67% (44/66) had to increase the dose during pregnancy, 2/66 could stop thyroxine medication when finishing antithyroid drugs, 30% (20/66) did not have to change the dose.16 miscarriages, 1 late miscarriage, 1 intrauterine fetal death occurred. Of these 18/119 (15%) patients 78% (14/18) had TSH outside the reference range at first control. 44% (8/18) had TSH <0.40 mIE/l, 33% (6/18) had TSH >4.0 mIE/l.
Summary
In 50% of pregnant women on thyroxine substitution the serum TSH values were outside the reference range at first control. A majority had to increase the thyroxine substitution during pregnancy. In pregnant women with miscarriage a great majority had TSH values outside the reference range at first control. The study demonstrates that pregnant women with thyroxine substitution should be carefully checked and the thyroxine dose increased early in pregnancy to avoid hypothyroidism.

OC2.1 Bone and calcium – OC2

Effect of once-yearly infusion of zoledronic acid 5 mg in postmenopausal women with osteoporosis

Richard Eastell[1], Peter Lakatos[2], Dennis Black[3], Steven Boonen[4], Felicia Cosman[5], Steven Cumings[3], Pierre Delmas[6], Erik Fink Eriksen[7], Ping Chung Leung[8], Zulema Man[9], Peter Mesenbrink[10], Ian Reid[11] & Jane Cauley[12]
[1]University of Sheffield, Sheffield, United Kingdom; [2]Semmelweis University, Budapest, Hungary; [3]University of California, San Francisco, San Francisco, CA, United States; [4]Leuven University Center for Metabolic Bone Diseases, Leuven, Belgium; [5]Helen Hayes Hospital, West Haverstraw, NY, United States; [6]INSERM Unit 403, Hopital Edouard Herriot, Lyon, France; [7]Novartis Pharma AG, Basel, Switzerland; [8]The Chinese University of Hong Kong, Hong Kong, China; [9]Centro TIEMPO, Buenos Aires, Argentina; [10]Novartis Pharmaceuticals Corporation, East Hanover, NJ, United States; [11]University of Auckland, Auckland, New Zealand; [12]University of Pittsburgh, Pittsburgh, PA, United States.

Background and methods
The HORIZON-PFT is a multinational, 3-year, randomized, double-blind, placebo-controlled trial evaluating the potential of once-yearly zoledronic acid (ZOL) 5 mg, infused over 15 minutes, to decrease risk of fracture in 7736 postmenopausal osteoporotic women 65–89 years of age.
Results
Treatment with ZOL 5 mg resulted in significant relative risk reductions in morphometric vertebral fracture of 70% vs PBO (3.8% vs 12.8%; 95% CI [62%, 76%]) and in hip fracture of 41% vs PBO (1.4% vs 2.5%; 95% CI [17%, 58%]). Secondary endpoints, non-vertebral (excluding finger, toe, and facial), clinical vertebral, and any clinical fracture (including non-vertebral, hip, and clinical vertebral), were significantly reduced by 25%, 77%, and 33% (all $P<.0001$), respectively. Bone mineral density increased significantly in ZOL vs PBO at total hip (6.0%), lumbar spine (6.9%), and femoral neck (5.0%) ($P<.0001$). While transient increases in serum creatinine ≥ 0.5 mg/dl over pre-infusion levels were seen in a small fraction (1.3%) of patients in the ZOL 5 mg group, no cumulative impact on renal function was demonstrable. Hypocalcemia (serum calcium $<$ 2.075 mmol/l) was observed in 2.3% of patients. Virtually all events occurred after the first infusion of ZOL and all were asymptomatic and transient. Adverse events occurring ≤ 3 days after infusion were more frequent after first infusion (44.7% ZOL vs 14.7% PBO) but declined markedly on subsequent infusions. There were more atrial fibrillation serious adverse events in ZOL vs PBO (1.3% vs 0.5%). Two cases of osteonecrosis of the jaw (1 in PBO, 1 in ZOL) were identified on adjudication; both resolved with antibiotic therapy and limited debridement.
Conclusion
Once-yearly infusion of ZOL 5 mg over 3 years achieves a highly significant decrease in vertebral, hip, and other fracture risk and is generally safe and well tolerated.

OC2.2

Role of IGF system on the regulation of osteoblast aromatase activity in vitro

Claudia Palermo, Marica Arvigo & Francesco Minuto
Department of Endocrinological & Metabolic Sciences and Center of Excellence for Biomedical Research, University of Genoa, Genoa, Italy.

Several studies demonstrated that IGFs stimulate aromatase activity in ovary but no data on bone are available. In the present study the role of IGF system components on aromatase action has been characterized during osteogenic differentiation in a model of rat tibial osteoblasts. At confluence (day 0) cells have been transferred to differentiating medium supplemented with 1% FCS and delta4androstenedion with or without IGF-II 3 nM, IGFBP-2 1 nM and IGFBP-3 1 nM. Cells have been treated with test substances continuously for 9 days or at intervals corresponding to the stages of osteogenesis: Stage 1 = proliferation; Stage 2 = extracellular matrix deposition; Stage 3 = mineralization of extracellular matrix. The aromatase activity has been evaluated by measuring in the conditioned medium the concentration of estradiol by a competitive chemiluminescent enzyme immunoassay. The differentiating effect has been evaluated by the measurement of alkaline phosphatase, which is an early marker of osteogenesis and of calcium incorporation, which is a late marker of osteogenesis. The secretion of the metalloproteinases by means of zymogram has been evaluated in different stages. The results showed that the continous treatment for 9 days with IGF-II and IGFBP-2 alone or in combination, inhibits the physiologic decrease of aromatase and stimulates the differentiation markers, including the metalloproteinase activation. Conversely, treatment with IGFBP-3 inhibits both aromatase activity and cellular differentiation. IGF-II, IGFBP-2 and IGFBP-3 exerted their action on aromatase activity and cellular differentiation also when added in S1 stage. IGF-II resulted ineffective when added alone in S2 or S3 but in S2 addition of IGFBP-2 restored the effect. IGFBP-3 and IGFBP-2 exerted their action also in S2 but not in S3.

In conclusion, these preliminary data suggest that in our cell system the aromatase activity is related to the osteogenic differentiation stages. Moreover, the IGF system plays an important role in the regulation of both bone aromatase activity and osteogenesis.

OC2.3

Clinical and biochemical differences in patients affected with sporadic and type 1 multiple endocrine neoplasia (MEN) related primary hyperparathyroidism

Cristina Eller-Vainicher[1], Sara Massironi[2], Maddalena Peracchi[2], Luca Persani[3], Paolo Beck-Peccoz[1], Anna Spada[1] & Sabrina Corbetta[4]
[1]Endocrine Unit, Medical Sciences Department, University of Milan, Fondazione Ospedale Maggiore, Mangiagalli, Regina Elena IRCCS, Milan, Italy; [2]Gastroenterology Unit, Department of Medical Sciences, University of Milan, Fondazione Ospedale Maggiore, Mangiagalli, Regina Elena IRCCS, Milan, Italy; [3]Lab of experimental endocrinology, Department of Medical Sciences, University of Milan, Istituto Auxologico Italiano IRCCS, Cusano Milanino, Milan, Italy; [4]Endocrinology and Diabetology Unit, Department of Medical-Surgical Sciences, University of Milan, Policlinico San Donato IRCCS, San Donato, Milan, Italy.

Primary hyperparathyroidism (PHPT) may occur sporadically or within MEN syndromes. It is classically thought that PHPT in MEN occurs at earlier ages than sporadic PHPT without significant differences in clinical and biochemical presentation. The aim of the study was to compare clinical and biochemical parameters between sporadic PHPT and MEN1 patients. The study included 41 genetically diagnosed MEN1 patients (14M, 27F) and 88 sporadic PHPT patients (24M, 64F) matched for age at diagnosis. All PHPT patients were studied for calcium metabolism parameters and renal and bone complications and evaluated

for familial history and the presence of signs or symptoms possibly related to MEN1. Young (<50 ys) MEN1 patients showed significantly lower serum PTH (71.23±50.89 *vs* 224.42±220.20 mg/dl, mean±SD, $P=0.019$), total (11.05±0.56 *vs* 12.02±1.22 mg/dl, $P=0.015$) and ionized calcium levels (1.48±0.07 *vs* 1.62±0.19 mmol/l, $P=0.021$) compared with age-matched sporadic PHPT patients, while such differences were not detected in old (51–70 ys) MEN1 *vs* sporadic PHPT patients. Despite the low PTH and calcium levels in MEN1, the prevalence of nephrolithiasis and osteoporosis was similar in the two PHPT forms. A female to male ratio of 1:1 was observed both in MEN1, as expected, and young sporadic PHPT patients. Moreover, young sporadic PHPT patients showed significantly higher serum calcium levels than the old patients (12.0±1.2 *vs* 11.2±0.9 mg/dl, $P=0.008$), in contrast to the pattern observed in MEN1. Our data suggested that milder hypercalcemia and PTH levels within the normal range were not uncommon in young MEN1 with respect to young sporadic PHPT patients, though both groups of patients did not differ for renal and bone complications. In conclusion, young symptomatic hyperparathyroid patients with slightly elevated serum calcium and PTH levels should be carefully screened for MEN1 diagnosis.

OC2.4

Assessment of prevalent vertebral deformities in morphometric X-ray absorptiometry

Elzbieta Skowronska-Jozwiak[1], Pawel Pludowski[2], Elzbieta Karczmarewicz[2], Andrzej Lewinski[1] & Roman Lorenc[2]
[1]Department of Endocrinology & Metabolic Diseases, The Medical University of Lodz, Lodz, Poland; [2]Department of Biochemistry and Experimental Medicine, The Children's Memorial Health Institute, Warsaw, Poland.

Vertebral morphometric X-ray absorptiometry (MXA) is a new tool developed to evaluate the presence of vertebral deformities. Low dose of radiation, fan-beam and the centerline scan technique are believes more advantageous than the classic morphometry using conventional lateral radiograms. We assessed the prevalence of vertebral fractures by MXA in adult population of Łódź region as a part of Polish population studied in EPOLOS epidemiological study.

Patients and methods

362 subjects without history of osteoporosis in anamnesis were examined [244 women, mean age 53±16 years (x±SD) and 97 men, mean age 53±14 years]. MXA lateral scans were performed using DXA system Expert-XL. Six point digitization were used to calculate the anterior (Ha), central (Hc), and posterior (Hp) height of the vertebral bodies Th_4-L_4. Vertebra were defined as having prevalent deformities when at least one ratio value (Ha/Hp, Hc/Hp, Hp/Hp up, or Hp/Hp low) fell 3 SD below or even more than the reference mean of that ratio at any vertebral level.

Results

3969 vertebrae were analyzed. 126 (3.17%) vertebrae in 863 subjects (22.7% of examined individuals) were classified as deformed. In 56 subjects (69.13%) one deformity and in 25 subjects multiple deformities were detected. In 89% of fractures, mild deformities (grade 1) were observed. The prevalence of vertebral fractures was higher in women and increased with age. Th_8 and Th_{12} were the most frequently deformed.

Conclusions

Bone studies indicated that, as in other regions of Poland, also in Łódź region vertebral osteoporotic fractures are common. Thus, the morphometric X-ray absorptiometry (MXA) seems to be a useful and safe tool in the diagnostics of vertebral fractures.

OC2.5

Effect of gonadal status on baseline and after rhGH treatment prevalence of spinal deformities in adult patients with growth hormone deficiency (GHD)

Gherardo Mazziotti[1], Antonio Bianchi[2], Stefania Bonadonna[1], Monica Nuzzo[1], Vincenzo Cimino[2], Alessandra Fusco[2], Laura De Marinis[2] & Andrea Giustina[1]
[1]Department of Internal Medicine, University of Brescia, Brescia, Italy; [2]Department of Endocrinology, Catholic University, Rome, Italy.

Adult GHD patients may have reduced BMD, which is thought to be reverted by long-term rhGH replacement therapy. We have recently reported high prevalence of vertebral osteoporotic deformities in untreated adult GHD patients. Gonadal status is the main determinant of bone loss in patients with primary form of osteoporosis.

In this cross-sectional study, we investigated whether the prevalence and degree of spinal deformities in adults with treated or untreated GHD was in relation to the gonadal status of the patients. Seventy-six adult hypopituitary patients (46 males and 30 females; mean age 46.8 years, range: 16–81) with severe GHD were evaluated for BMD (dual-energy X-ray absorptiometry) and vertebral deformities (T4-L5 quantitative morphometric analysis according to Genant score). At the study entry, 41 patients were eugonadic (21 patients with preserved gonadal function and 20 patients in adequate replacement therapy), whereas 35 patients were hypogonadic.

Vertebral deformities (>20%) were found in 48 patients (63.2%), with higher prevalence in untreated (42 cases) vs. treated patients (24 cases) [76.9% vs. 33.3%; $P<0.001$]. Eugonadic and hypogonadic patients with untreated GHD showed comparable fracture rate (78.6% vs. 75.0%; $P=0.8$). rhGH replacement therapy was accompanied by a significant decrease in fracture rate as compared to untreated patients [eugonadic: 35.3% vs. 75.0%, $P=0.01$; hypogonadic: 28.6% vs. 78.6%, $P=0.01$]. Eugonadic patients had slightly but significantly higher BMD than hypogonadic patients. Multivariate logistic regression analysis demonstrated that no treatment with rhGH was the only factor significantly influencing the occurrence of spinal deformities in adult GHD patients (odds ratio: 5.8, CI 95% 1.9–18.1) whereas no significant correlation was found with gonadal status, BMD, sex and age.

Gonadal status of adult patients with GHD may be not critical for the prevalence of vertebral radiological deformities which is instead mainly affected by the replacement treatment with rhGH.

OC2.6

Sunlight exposure and vitamin D supplementation at the institutionalized elderly – effects on calcium and bone metabolism

Peter Bottermann[2], Dumitru Branisteanu[1], Voichita Mogos[1] & Eusebie Zbranca[1]
[1]University of Medicine and Pharmacy "Gr.T.Popa", Iasi, Romania; [2]Technical University, Munchen, Germany.

We investigated calcium and bone metabolism in a group of 123 institutionalized volunteers between 60 and 98 years old, 73 females and 50 males. 25OH-D_3 was measured by an indoor RIA technique. 1.25$(OH)_2D_3$ was measured by HPLC, serum calcium by photocolorimetry, bone alkaline phosphatase by immunoenzymatic technique, whereas serum PTH and urinary deoxypyridinoline (DPD) were measured by IRMA. Almost all volunteers (92.6%) had low 25OH-D_3 values, but normal or even increased levels of the active hormone, 1.25$(OH)_2D_3$. High PTH was found in 40 cases (32.5%), of which three were primary hyperparathyroidism, whereas the others had low or low-normal calcium levels (secondary hyperparathyroidism). PTH-induced 1α hydroxylation in the elderly with undamaged kidney function seems to compensate the paucity of vitamin D substrate. More than half of the cases had high DPD levels, suggesting high bone turnover. Bone turnover parameters were higher in females than in males ($P<0.05$). A positive correlation between PTH and urinary DPD was noticed ($R^2=0.351$), suggesting the role of secondary hyperparathyroidism in high turnover bone loss. We further supplemented the vitamin D intake in 42 volunteers with a daily dose of 2000 IU of 25-OHD_3 for three months in the summer period, whereas other 42 volunteers received placebo (vitamin B). Normalization of 25-OHD_3 levels was seen in both groups, suggesting that even mild sun exposure increases skin resources of vitamin D. A more significant increase in both 25OH-D_3 and 1.25$(OH)_2D_3$ was however observed in the vitamin D-treated group. Normalization of serum PTH, but not of turnover parameters was observed in both groups. Mild hypercalcemia and increase in serum creatinine were noticed in the vitamin D-treated group. Vitamin D supplementation might therefore be accompanied by hypercalcemic and nephrotoxic effects at doses higher than 2000 IU/day. Sunlight exposure seems efficient to replenish vitamin D reserves at institutionalized patients.

OC2.7

Vitamin K2 induces phosphorylation of protein kinase A and expression of novel target genes in osteoblastic cells

Kuniko Horie-Inoue[1], Tomoe Ichikawa[1] & Satoshi Inoue[2]
[1]Research Center for Genomic Medicine, Saitama Medical University, Hidaka-shi, Saitama, Japan; [2]Graduate School of Medicine, the University of Tokyo, Bunkyo-ku, Tokyo, Japan.

Vitamin K is known as a critical cofactor in blood coagulation and bone homeostasis by helping the function of vitamin K-dependent gamma-carboxylase. We have recently shown that vitamin K2, one of the natural vitamin Ks, has a novel function to regulate the transcription of extracellular matrix-related genes in osteoblastic cells and increase collagen accumulation by activating the steroid and xenobiotic receptor, SXR. In the present study, we searched for novel vitamin K target genes up-regulated specifically by menaquinone-4 (MK-4), a potent vitamin K2 isoform, using oligonucleotide microarray analysis in human osteoblastic MG63 cells. Among these genes, growth differentiation factor (GDF15) and stanniocalcin 2 (STC2) were characterized as MK-4-specific targets, as their mRNA expression was not induced by vitamin K1, another vitamin K2 isoform MK-7, or the MK-4 side chain structure geranylgeraniol. The MK-4-specific induction of GDF15 and STC2 was also observed in murine MC3T3-E1 cells and shown to be independent of either gamma-carboxylation or SXR signaling. As a possible mechanism for MK-4-specific gene regulation, we investigated the contribution of protein kinase A (PKA), one of the key regulators of transcription in osteoblasts. We found that MK-4 enhanced PKA phosphorylation, and the MK-4-specific induction of GDF15 and STC2 genes was reduced by treatment with the PKA inhibitor H89 or siRNA against PKA alpha-catalytic subunit. In conclusion, vitamin K2 has novel functions beside its activity as a coenzyme and plays a significant role in regulating various gene expression and modulating collagen production in osteoblastic cells.

Endocrine tumors and neoplasia – OC3

OC3.1

Multiple somatostatin receptor subtypes activation reduces cell viability in non-functioning pituitary adenomas by inhibiting Vascular Endothelial Growth Factor secretion

Maria Chiara Zatelli[1], Daniela Piccin[1], Federico Tagliati[1], Maria Rosaria Ambrosio[1], Antonio Bianchi[2], Marta Bondanelli[1], Herbert A Schmid[3], Massimo Scanarini[4], Laura De Marinis[2], Giulio Maira[5] & Ettore C degli Uberti[1]
[1]Section of Endocrinology, Department of Biomedical Sciences and Advanced Therapies – University of Ferrara, Ferrara, Italy; [2]Institute of Endocrinology, Catholic University of Rome, Rome, Italy; [3]Novartis Institutes for Biomedical Research, Oncology, Basel, Switzerland; [4]Division of Neurosurgery – Hospital of Padova, Padova, Italy; [5]Department of Neurosurgery, Catholic University of Rome, Rome, Italy.

Somatostatin (SRIF) analogs have been employed in medical therapy of non-functioning pituitary adenomas (NFA), with contrasting results. Previous evidence showed that SRIF can exert its antiproliferative effects by reducing Vascular Endothelial Growth Factor (VEGF) secretion and action, and that VEGF expression may be related to pituitary tumor growth. The aim of our study was to clarify the possible effects of a multireceptor SRIF ligand on VEGF secretion and cell proliferation in human NFA primary cultures, we assessed SRIF receptors (SSTR1-5) expression and the *in vitro* effects on VEGF secretion and on cell viability of SRIF and of the stable SRIF analogue pasireotide (SOM230) which activates SSTR1, 2, 3 and 5. Twenty-five NFA were examined by RT-PCR for expression of α-subunit, SSTR, VEGF, and VEGF receptors 1 (VEGF-R1) and 2 (VEGF-R2). Primary cultures were tested with SRIF and with pasidreotide. All NFA samples expressed α-sub, VEGF and VEGFR-1 and 2, while SSTR expression pattern was highly variable. Two different groups were identified according to VEGF secretion inhibition by SRIF. VEGF secretion and cell viability were reduced by SRIF and pasireotide in the "responder" group, but not in the "non responder" group, including NFA expressing SSTR5. SRIF and pasireotide completely blocked Forskolin-induced VEGF secretion. In addition, SRIF and pasireotide completely abrogated the promoting effects of VEGF on NFA cell viability. Our data demonstrate that pasireotide can inhibit NFA cell viability by inhibiting VEGF secretion, and suggest that the multireceptor-SSTR agonist pasireotide might be useful in medical therapy of selected NFA.

OC3.2 – ESE Young Investigator Award

Adrenal lesions in multiple endocrine neoplasia type 1: data from the French Group for the Study of Endocrine Tumors (GTE)

Blandine Gatta[1], Maud Monsaingeon[1], Pierre Goudet[2], Arnaud Murat[3], Patricia Niccoli-Sire[7], Alain Calender[5], Vincent Rohmer[6], Olivier Chabre[4] & Antoine Tabarin[1]
[1]Dpt Endocrinology, Bordeaux, France; [2]Dpt Surgery, Dijon, France; [3]Dpt Endocrinology, Nantes, France; [4]Dpt Endocrinology, Grenoble, France; [5]Lab Genetics, Lyon, France; [6]Dpt Endocrinology, Angers, France; [7]Dpt Endocrinology, Marseille, France.

The characteristics of adrenal involvement in Multiple Endocrine Neoplasia type 1 (MEN1) have been defined from studies involving a limited number of patients. We have assessed retrospectively the prevalence, characteristics and evolution of adrenal involvement from the French group for the study of endocrine tumours (GTE) registry, involving 688 patients with MEN1. In our series, adrenal tumours identified at abdominal imaging occurred in 130 patients (18.9%). The mean age of patients at the discovery of the adrenal lesion was 46.1 yr (range, 2–78 yr). Adrenal lesions were bilateral in 32% of cases and the mean tumor diameter was 27.6 mm (range, 7–70 mm). Hormonal hypersecretion was found in 16% of patients with adrenal involvement (10 cases of Cushing's syndrome, 7 cases of primary hyperaldosteronism, 2 cases of hyperandrogenism and 1 pheochromocytoma). Among adrenal lesions that were removed, histopathologic examination revealed benign lesions (adenoma and hyperplasia) in 87.5% of cases, adrenal carcinoma in 7.5% and adrenal metastasis in 5%. Overall, malignancy of adrenal lesions was documented in 3.8% of the whole series. Adrenal lesions were associated with enteropancreatic tumors in 66.4% of cases. In patients in whom follow-up imaging was available (mean 6.6 years), 15% demonstrated significant tumoral progression and 13% developed controlateral lesions. No case of adrenal malignancy was found during the follow-up. No correlation was found between genotypic lesions of the *menin* gene and the presence or the type of adrenal lesion. In our series, adrenal tumours are a less frequent than previously reported. Most of adrenal lesions are small in size benign and not responsible for hormonal hypersecretion. Our series do not support the hypothesis of a physiopathologic link between pancreatic tumours and adrenal lesions in MEN1.

OC3.3

BMP dependent effects on adrenal tumorigenesis and function

Inga Johnsen[1], Sarah Kaufmann[1], Lilia Spady[1] & Felix Beuschlein[2]
[1]Institute of Molecular Medicine and Cell Research, Albert-Ludwigs-University Freiburg, Freiburg, Germany; [2]Medical Clinic, University Hospital Innenstadt, Ludwig Maximilians University, Munich, Germany.

Members of the TGFb family of ligands - including bone morphogenic proteins (BMPs) - have been demonstrated to profoundly impact tumorigenesis in a variety of tumor entities. As for the adrenal cortex, BMP6 has been implicated as an important modulator of aldosterone secretion. To screen for alterations of TGFß dependent pathways in adrenal tumorigenesis we performed gene profiling experiments. By comparing human adrenal carcinoma (ACC) against normal adrenal tissue samples (Co) we detected a down-regulation of various BMPs (e.g. BMP2 and BMP5) which was further validated by Real Time analysis (BMP5, ACC vs. Co $6.1 \pm 1.4\%$ vs. $100 \pm 29.7\%$, $P < 0.01$; BMP2, ACC vs. Co $35.1 \pm 1.2\%$ vs. $100 \pm 17\%$, $P < 0.01$). As similar expression pattern with loss of BMP5 expression was evident in NCIh295 cells, this cell line was used as an *in vitro* model to assess potential impact of BMP dependent pathways. Incubation with recombinant hBMP5 induced phosphorylation of SMAD 1/5/8 and subsequent increase of ID protein expression levels in a dose dependent manner, while co-incubation with the physiological BMP antagonist Noggin neutralized these effects. Thus, these findings demonstrated the integrity of the pathway in NCIh295 cells. Notably, BMP5 treatment resulted in a decrease in cellular viability ($68.3 \pm 1.1\%$ vs. $100 \pm 2.7\%$, $P < 0.01$) but increase in the expression levels of steroidogenic enzymes such as StAR ($225 \pm 9.6\%$ vs. $100 \pm 2.3\%$, $P < 0.01$) and SCC ($460.3 \pm 58.8\%$ vs. $100 \pm 0.53\%$, $P < 0.01$). The BMP5 dependent reduced viability was accompanied by concomitant changes in the cell cycle possibly through an increased rate in apoptosis. Taken together, we demonstrate that loss of BMP expression is a common finding in ACC. Moreover, we provide first evidence that BMP dependent pathways might be involved in modulation of the malignant phenotype of adrenocortical cancer.

OC3.4

RET mutation – Tyr791Phe – the genetic cause of different diseases derived from neural crest

Eliska Vaclavikova[1], Sarka Dvorakova[1], Petr Vlcek[2], Richard Skaba[3], Radovan Bilek[1] & Bela Bendlova[1]
[1]Dept. of Molecular Endocrinology, Institute of Endocrinology, Prague, Czech Republic; [2]Dept. of Nuclear Medicine and Endocrinology, 2nd Faculty of Medicine, Charles University and Hospital Motol, Prague, Czech Republic; [3]Dept. of Pediatric Surgery, 2nd Faculty of Medicine, Charles University and Hospital Motol, Prague, Czech Republic.

Familial medullary thyroid carcinoma (MTC), multiple endocrine neoplasia types 2A and 2B (MEN2A, 2B) and Hirschsprung disease (HSCR) are inherited neurocristopathies linked to germline mutations in the RET proto-oncogene. Activating germline RET mutations are presented in patients with FMTC, MEN2A and MEN2B, on the other hand, inactivating germline mutations in patients with HSCR. Nevertheless, there is an overlay in specific mutations in the exon 10 of the RET proto-oncogene. The aim of this study was to screen 6 exons (10,11,13,14,15 and 16) of the RET proto-oncogene by fluorescent sequencing method in three different groups of patients - 174 families with MTC (including MEN2A, 2B), 73 families with HSCR and 20 patients with only pheochromocytoma. In this report, we show that the point mutation Tyr791Phe in exon 13 of the RET proto-oncogene can cause different diseases derived from neural crest. We found Tyr791Phe mutation in 5 families with MTC (3%), 2 families with HSCR (3%) and 1 family with pheochromocytoma (5%). All these patients with the mutation have also a silent polymorphism Leu769 (T/G) in exon 13. In addition, in 2 families with MEN2 double germline mutations were detected: MEN2A family Tyr791Phe + Cys620Phe (exon 10) and MEN2B family Tyr791Phe + Met918Thr (exon 16). Tyr791Phe mutation had not been previously observed in HSCR patients. Detection of Tyr791Phe mutation in MEN2/MTC and HSCR families leads to a question whether this mutation has dual "Janus" character (gain-of-function as well as loss-of-function) as mutations described in exon 10 in HSCR/MEN2A patients. This study shows other character of this strange and frequently discussed Tyr791Phe mutation. On the basis of our genetic finding total thyroidectomy was recommended for all patients with Tyr791Phe mutation.

The work was supported by IGA MZ CR NR/7806-3, GACR 301/06/P425 and IGA MH CR NR/8519-3 and was approved by local Ethical committee.

OC3.5 – ESE Young Investigator Award

[^{123}I]Iodometomidate as a radiotracer for adrenal scintigraphy – first clinical experience

Stefanie Hahner[1], Andrea Stuermer[1], Martin Fassnacht[1], Michael Kreissl[2], Christoph Reiners[2], Felix Beuschlein[3], Martina Zink[1], Ilse Zolle[4], Andreas Schirbel[2] & Bruno Allolio[1]
[1]University of Wuerzburg, Dept. of Endocrinology and Diabetology, Wuerzburg, Germany; [2]University of Wuerzburg, Dept. of Nuclear Medicine, Wuerzburg, Germany; [3]University of Munich, LMU, Division of Endocrine Research, Munich, Germany; [4]University of Vienna, Ludwig-Boltzmann-Institute of Nuclear Medicine, Vienna, Austria.

Adrenal masses are highly prevalent tumours comprising of a variety of entities. Therefore, therapeutic consequences also vary considerably. The CYP11B-specific PET-tracer [^{11}C]metomidate has been shown to be suitable to characterize adrenal lesions. However, its availability is restricted to PET-centers with an on-site cyclotron. Also imaging is hindered by the short tracer half-life (20 min). Therefore, we have developed [^{123}I]iodometomidate as a tracer for adrenal imaging. Pharmakokinetics and biodistribution after i.v.-injection of 40 MBq of [^{123}I]iodometomidate were analyzed in mice using small animal single photon emission computed tomography (SPECT). A 49 year old woman with bilateral adrenal tumors (hounsfield units >10 suggesting a non-adenoma lesion) and borderline urinary catecholamines (patient 1) and a 22 year old man after adrenalectomy for adrenocortical carcinoma with a lesion suspicious for metastasis in the os sacrum (patient 2) were investigated with [^{123}I]iodometomidate-SPECT. Adrenals were excellently visualized in mice with high tracer uptake and little background activity. In patients, adrenals were first detected 60 min p.i. with a maximum uptake in the adrenals after 5–6 hours indicating slow pharmacokinetics of the tracer. At 24 h.p.i. high uptake was detected exclusively in the adrenals. In patient 1 both tumours exhibited high tracer uptake confirming the adrenocortical origin of the lesions. In patient 2 the remaining hyperplastic adrenal was clearly visible. However, no uptake was detected in the os sacrum lesion. Subsequent biopsy revealed a periostal chondroma. For both patients calculated whole body radiation exposure was 3.2 mSv. This is the first description of [^{123}I]Iodometomidate as a radiotracer in patients. Iodometomidate is a highly suitable tracer combining specific uptake in adrenocortical tissue with far lower radiation exposure compared to norcholesterol scintigraphy. Availability and pharmacokinetics are superior to [^{11}C]metomidate-PET. Furthermore, radiotherapy of adrenocortical carcinoma using [^{131}I]iodometomidate appears to be feasible.

OC3.6

ERß-specific transcriptional profile in colon cancer

Valentina Martineti[1], Massimiliano Mascherini[4], Carmelo Mavilia[1], Silvia Carbonell Sala[1], Isabella Tognarini[1], Chiara Azzari[3], Federico Mattia Stefanini[4], Francesco Tonelli[2] & Maria Luisa Brandi[1]
[1]Department of Internal Medicine, School of Medicine, University of Florence, Florence, Italy; [2]Department of Clinical Physiopathology, School of Medicine, University of Florence, Florence, Italy; [3]Department of Pediatrics, School of Medicine, University of Florence, Florence, Italy; [4]Department of Statistics, University of Florence, Florence, Italy.

Epidemiological data clearly evidence a protective role of estrogens against the development of colon cancer and ERβ has been identified as the predominant ER subtype in human colon. More recently it has been identified as a favourable prognostic marker in this disease, possibly explaining the protective effect of estrogens against colon cancer development. To understand the specific role and mechanism of action of ERβ in colon tumorigenesis we developed an *in vitro* engineered cell model through transfection and cloning of HCT8 human colon cancer cell line for stable over-expression of wild type human ERβ (HCT8β8), providing the first direct evidence that ERβ plays an important role in colon cancer as a regulator of cell proliferation through induction of G1-S phase transition arrest. To investigate the molecular events underlying growth arrest we analyzed specific ERβ-regulated genes by comparing expression profile of HCT8β8 cells versus its non-engineered counterpart using Agilent's Human 1A Oligo Microarray (V2) chips harbouring over 22,000 human genes and ESTs. A list of 189 reproducibly ERβ-regulated targets, comprising 64 up-regulated and 125 down-regulated genes, emerged indicating that ERβ over-expression heavily affects different aspects of HCT8 cell function regarding both its intracellular metabolism and relationship with the extracellular milieu. According to their function, ERβ-modulated genes have been grouped into 16 categories, our interest for further validation (by quantitative real time RT-PCR and Western blotting) focused on cell cycle and mitosis genes category and this technique confirmed 50% of gene modulations. On the whole a trend to the slowing down of the cell cycle is demonstrated and one of the up-regulated genes is E4F transcription factor 1 (E4F1), which is already known to be an estrogen-modulated transcription factor. Two of their downstream targets are $p21^{waf1}$ and cyclin E whose altered expression has already been documented in our cell model. We hypothesize that E4F1- $p21^{WAF1}$-cyclin E is an ERβ specific pathway in colon cancer cells.

OC3.7

Fasting insulin levels are predictors of colonic lesions in patients with acromegaly: an observational, open, prospective study in 189 patients

Annamaria Colao[1], Rosario Pivonello[1], Mariano Galdiero[1], Renata S. Auriemma[1], Diego Ferone[2], Paolo Marzullo[3] & Gaetano Lombardi[1]
[1]Department of Molecular and Clinical Endocrinology and Oncology, section of Endocrinology, University "Federico II" of Naples, Naples, Italy; [2]Department of Endocrine and Metabolic Diseases, University of Genova, Genova, Italy; [3]Section of Endocrinology, Auxologic Institute of Verbania, Verbania, Italy.

Elevated insulin levels are correlated with colonic adenomas and carcinomas in the general population. Patients with acromegaly are considered at high risk to develop colonic lesions and have a high insulin levels. To evaluate the role of insulin levels on colonic polyps (hyperplastic, adenomatous, single or synchronous) or adenocarcinoma in acromegaly we designed this analytical, observational, open, prospective, study enrolling 189 patients (100 women, 89 men, age 20–82 yrs) undergoing pan-colonoscopy at diagnosis. Age, gender, estimated disease duration, body mass index, GH and IGF-I levels, fasting glucose and insulin

levels, HOMA-index [R (resistance) and β (β-cell function)] were considered as predictors. Colonic lesions were found in 74 patients (39.1%): hyperplastic polyps in 31 (16.4%), adenomatous polyps in 24 (12.7%), both hyperplastic and adenomatous polyps in 14 (7.4%) and adenocarcinoma in 6 patients (3.2%); polyps were single in 22 patients (29.8%) and synchronous in 52 (70.3%). Colonic lesions were positively correlated with patients' age, insulin levels, HOMA-R and HOMA-β ($P<0.0001$), negatively with GH levels ($P=0.006$) but not with estimated disease duration, IGF-I levels, BMI or glucose levels. Compared to patients with normal glucose tolerance, patients with impaired glucose tolerance had a prospective risk (RR) to develop colonic lesions 2 times higher (95% CI 1.2–3.3) while those with diabetes 2.9 times higher (95% CI 1.8–4.6). Serum fasting insulin levels were the strongest predictor of the presence of colonic lesions. The best cut-off of insulin levels to predict the presence of colonic lesions was 20.6 mUI/liter [sensitivity = 73.8% (61.5–84%); specificity = 81.1% (72.5–87.9%); positive predictive value = 69.6%, negative predictive value = 84.1]. The patients with fasting insulin levels > 20.6 mU/liter at the diagnosis of acromegaly had a RR to develop colonic lesions 5.1 times higher than those with levels ≤ 20.6 mU/liter (95% CI 3.1–8.5). In conclusion, high fasting insulin levels predict the presence of adenomas and adenocarcinomas.

Neuroendocrinology basic – OC4

OC4.1

Organismal, cellular and molecular evolution of water balance regulation in vertebrates: the amphibian hinge

Roger Acher, Jacqueline Chauvet & Yves Rouillé
University Paris VI, Paris, France.

Amphibia, through metamophosis, recapitulate the evolution of water homeostasis from aquatic life to terrestrial one. Whereas the tadpole has the status of a freshwater fish, the adult has developed a three osmoregulatory organ system, including kidney, bladder and skin, for facing terrestrial dehydration. Amphibia have differentiated epithelial hydroosmotic cells in each organ: principal cells in nephron collecting duct, granular cells in urinary bladder, principal cells in ventral skin. These cells, equipped with hormone receptors and effectors (aquaporins, ion channels, urea transporter) are largely controled by neurohypophysial hormones. Each vertebrate possesses two similar neurohypophysial nonapeptides. From the 13 peptides chemically characterized in the laboratory, we have traced two main evolutionary paralog lines: vasotocin (nonmammalian vertebrates) – vasopressin (mammals) involved in osmoregulation, and isotocin (bony fish) – mesotocin (nonmammalian tetrapods) – oxytocin (mammals) possibly implicated in reproduction.

Twelve amphibian species originating from Europa, North- and South-America, Africa and Asia have been investigated. Neurohypophysial secretory granules have been isolated from the neurointermediate pituitary by sucrose gradient centrifugation and their components, purified by HPLC, identified by aminoacid sequencing and/or coelution with synthetic peptides. Along with vasotocin ([Ile^3]-vasopressin) and mesotocin (([Ile^8]-oxytocin), vasotocinyl-Gly (hydrin2) has been identified in all species. This peptide results from a limited processing of the 141-residue provasotocin. A 4-enzyme cascade operating in secretory granules on vasotocinyl-Gly-Lys-Arg sequence leads usually to the alpha-amidated vasotocin but down-regulation of the last amidating enzyme gives, in amphibians only, vasotocinyl-Gly. Vasotocin and hydrin2 have different conformations and act on distinct receptors. Whereas vasotocin shows a water (re)absorption activity in kidney, bladder and skin, hydrin2 is devoid of antidiuretic activity and is more active than vasotocin on the skin. Hydrin2 is twice more abundant in species living in arid countries. Evolution has synchronized a new osmoregulatory organ (skin) with a new specific hormone, making two hormones from a single precursor.

OC4.2

Growth Hormone-Releasing Hormone (GHRH) exerts protective effects on adult rat hippocampal progenitor cells

Silvia Destefanis[1], Inger Johansson[2], David Aberg[2], Riccarda Granata[1], Ezio Ghigo[1] & Jorgen Isgaard[2]
[1]University of Turin, Turin, Italy; [2]Sahlgrenska University, Goteborg, Sweden.

Growth hormone releasing hormone (GHRH) is a neuropeptide mainly synthesised in the hypothalamus, known to exert a stimulatory effect on the synthesis and release of growth hormone (GH) from the pituitary via the activation of specific receptors. New data indicate that GHRH is also produced in both extrahypothalamic brain areas and in peripheral tissues. GHRH-receptor splice variants (SVs) have been found in several peripheral normal and neoplastic human tissues and mediate effects on cell proliferation and differentiation. At present, central non-endocrine effects of GHRH in extra-pituitary brain tissues have not yet been characterised. The aim of the present study was to investigate the effects of GHRH on cell survival in rat adult hippocampal progenitor cells (AHP) and to study the intracellular pathway involved. Cell viability was assessed by the Alamar blue assay. RT-PCR was performed to detect the presence of GHRH receptor mRNA. The results showed that GHRH receptor is expressed in AHP cells. GHRH dose dependently increased cell survival on AHP cells compared to control. After GHRH administration a significant increase of cAMP levels analyzed by ELISA was observed, suggesting a GHRH-induced activation of cAMP pathway. Consistently, western blot analysis showed a significant activation of Akt and ERK 1/2 survival pathway after GHRH administration. Activation of these signalling pathways preceded CREB phosphorylation, which plays an important role in the differentiation and maturation of newborn neurons in hippocampus. In conclusion, this study shows that GHRH has a protective effect on AHP cells. Moreover, in these cells GHRH is able to activate the cAMP-CREB pathway. Akt and ERK1/2 seem to be involved in this survival signalling. Thus, GHRH and its receptor may play an important role for hippocampal progenitor cells survival.

OC4.3

Absence of germline AIP mutations in early onset sporadic somatotropinomas

Leonor Gomes[1], Hugo Prazeres[2], Isabel Paiva[1], Cristina Ribeiro[1], Olinda Rebelo[3], Teresa Martins[2], Manuela Lacerda[2] & Manuela Carvalheiro[1]
[1]Serviço de Endocrinologia, Diabetes e Metabolismo dos Hospitais da Universidade de Coimbra, Coimbra, Portugal; [2]Laboratório de Patologia Molecular do Serviço de Anatomia Patológica do Centro Regional de Oncologia de Coimbra do Instituto Português de Oncologia, EPE, Coimbra, Portugal; [3]Serviço de Anatomia Patológica dos Hospitais da Universidade de Coimbra, Coimbra, Portugal.

Objective
The pathogenesis of pituitary tumours is still incompletely understood. Somatotropinomas occur both sporadically and in the context of familial syndromes, such as multiple endocrine neoplasia type 1 (MEN1), Carney complex (CNC) and isolated familial somatotropinoma (IFS). Recently, germline mutations were reported in *AIP* (aryl hydrocarbon receptor interacting protein) gene in Finish and Italian families and in Finish patients with apparently sporadic pituitary tumours. The aim of this study was to determine if *AIP* gene mutations influence individual susceptibility to develop sporadic pituitary somatotropinomas in a group of young patients originating from the central region of Portugal.
Methods
Blood samples were obtained from 20 patients (8 males and 12 females) with sporadic somatotrophinomas, including 6 plurihormonal for GH and PRL, who were diagnosed when they were younger than 35 years of age (mean age 25.7±4.97, 16-33 years). Detection of the *AIP* germline mutations was carried out by PCR amplification of genomic DNA, followed by direct sequencing of the entire gene coding sequence and intron-exon boundaries as previously described.
Results
In this series of patients, with early onset sporadic oversecreting-GH pituitary adenomas, no *AIP* germline mutations were found. A heterozygous synonymous C→T polymorphism (Asp45Asp) was found in a single patient.
Conclusions
Our results provide evidence that *AIP* germline mutations are not associated with sporadic pituitary tumours. We studied patients diagnosed at young ages, with a hypothetically higher probability of harbouring occult germline mutations. The absence of germline mutations in this group of patients suggests that *AIP* germline mutations probably do not play an important role in the pathogenesis of sporadic pituitary somatotropinomas. Similar observations have been made by other groups. Further studies are needed in order to identify other genetic factors underlying early onset sporadic pituitary tumours.

OC4.4

Is there a role for dopamine D_2 receptor gene polymorphisms in determining cabergoline sensitivity in prolactin-secreting pituitary adenomas?

Marcello Filopanti, Anna Maria Barbieri, Rita Angioni, Andrea Lania, Giovanna Mantovani, Anna Spada & Paolo Beck-Peccoz
Endocrine Unit, Dept. Medical Sciences, University of Milan; Fondazione Ospedale Maggiore Policlinico IRCCS, Milano, Italy.

Dopamine agonist cabergoline (CB) is the first-choice treatment in prolactin-secreting adenomas (PRL-omas). It is effective in reducing PRL secretion and tumour size in about 90% of patients by binding dopamine D_2 receptor (DRD2). Although no mutations in DRD2 were found, it has been reported that several polymorphisms of this locus associate with alcoholism and schizophrenia, diseases in which dopaminergic system plays an important role. To assess the possible association of DRD2 gene polymorphisms (i.e. TaqIB, HphIG/T, NcoIC/T and TaqIA) with the sensitivity to CB, a multicentric retrospective study was carried out including 252 patients with PRL-oma and 211 healthy controls. Genotyping was carried out by restriction fragment length polymorphism analysis (RFLP) on blood DNA. Pituitary MRI and PRL assay were performed at diagnosis and during CB therapy follow-up (median 17 months, range 5–49). Patients were defined as resistant when they failed to normalize PRL levels and/or to reduce tumor size with a CB dosage higher than 3 mg/week. According to this definition, in our series the overall prevalence of resistant patients was 8% and 3.4%, respectively. As far as DRD2 genotypes were concerned, no differences in allele frequencies between patients and normal subjects were observed. Moreover, any polymorphism correlated with clinical presentation, biochemical data and tumor size. Conversely, we observed an higher frequency of NcoIT+ allele in subjects defined as resistant to CB in term of both normalization of PRL levels [$(\chi^2)P=0.038$] and tumor size reduction [$(\chi^2)P=0.006$]. Finally, [A1-/B1-/T-/T-] haplotype was found to be associated with a greater sensitivity to CB in term of PRL normalization. In fact, this haplotype was found in 34% of patients taking less of 3 mg/week of CB vs 11% of resistant patients [$(\chi^2)P=0.021$]. In conclusion, further studies are required to assess the mechanisms underlying the involvement of DRD2 gene polymorphisms in determining the CB sensitivity.

OC4.5

Lack of nuclear Hes1 expression coincides with transformation of endocrine pancreatic cells in Men1 knock out mice

Térèse Johansson, Margareta Halin Lejonklou & Britt Skogseid
Institution of Medical Sciences, Uppsala, Sweden.

Background
Homozygous inactivation of the *MEN1* tumor suppressor gene frequently occurs in endocrine pancreatic tumors (EPT); however, a heterozygous germ line inactivation of the gene seems to lead to development of an increase amount of endocrine pancreatic cells. The Notch signaling cascade plays a vital role in sustaining the balance between cell proliferation, differentiation and apoptosis during pancreatic development. Whether Notch signaling is *MEN1* dependent is unknown.
Aim
To explore the Notch pathway by means of the transcription factors *Hes1*, *Hey1* and *Mash1* expression pattern and their role in endocrine tumour progression by in $Men1^{+/-}$ mice.
Methods
Notch1, *Hes1*, *Hey1*, *Mash1*, and *Men1* mRNA expression were investigated by qPCR. Fifteen mice (10 $Men1^{+/-}$, five Wt, 12 or 18 month,) were used; the endocrine tissue was divided according to size: small islets, islets, small tumors and larger tumors. Protein expression were assessed by immunohistochemistry (13 $Men1^{+/-}$ and 12 Wt, 9–22 month)
Results
Men1, *Notch1*, *Hes1,* and *Hey1* mRNA expression was found in endocrine tissue of all sizes; *Mash1* was found in 28/55 samples. Variable degree of loss of menin (the *Men1* protein) expression was observed in tumors of $Men1^{+/-}$ mice age 14–22 month. $Men1^{+/-}$ and Wt mice showed no difference in *Notch1*, *Hey1*, and *Mash*1 immunoreactivity. Wild type mice of all ages expressed nuclear *Hes1*, whereas only the younger $Men1^{+/-}$ mice displayed nuclear *Hes1* immunoreactivity. The tumors of the heterozygous mice age 14–22 month had lost nuclear *Hes1* expression.
Conclusions
Mash1 immunoreactivity was invariably and abundantly displayed. The lack of *Hes1* in tumor cell nuclei in elderly $Men1^{+/-}$ mice indicates that *Hes1* might be of importance in endocrine pancreatic tumorigenesis.

OC4.6

Metabolic abnormalities in patients with adrenal adenomas may be associated with BclI polymorphism in the glucocorticoid receptor (GR) gene and expression of tumor-specific hsp70 isoforms

Svetozar Damjanovic, Jadranka Antic, Bojana Beleslin, Sanja Ognjanovic, Jovana Vignjevic, Milan Petakov, Djuro Macut, Ivan Paunovic, Tatjana Isailovic, Bojana Popovic & Ivana Bozic
Institute for Endocrinology, Diabetes and Metabolic Diseases, Belgrade, Serbia.

Introduction
Intronic *Bcl*I polymorphism of glucocorticoid receptor (GR) gene and adrenal adenomas of incidental discovery are frequently associated with metabolic syndrome. We studied in these patients metabolic and hormonal parameters, the sequence alteration in *Bcl*I polymorphism of the GR gene from constitutive DNA and the expression of Hsp70 within the tumor and in tissue adjacently to tumor.
Patients and methods
We assessed 114 healthy subjects (79 men; age range 21–68 years) and 30 patients operated due to non-functioning adrenal mass (5 men; age range 36–76 years). Besides clinical and anthropometrical assessment, morning cortisol and fasting insulin levels were determined. DNA was obtained from leucocytes and after amplification, PCR fragments were digested with *Bcl*I enzyme. Subsequently, the sequence of the fragments was analyzed. Equal amounts of protein obtained from total cell lysates of adenomatous and adjacent normal adrenal tissue was resolved by 9% SDS-PAGE and transferred to nitrocellulose membranes (Western blot analysis).
Results
We found a G-to-C transition in the second intron of GR gene in 24 of 26 (92%) patients that is significantly higher frequency of the larger allele within patients than in normal population (42% vs 4.2%; $P<0.001$). Patients and controls had similar BMI and morning cortisol levels. However, the frequency of diabetes type 2, and hypertension were significantly higher in patients with adrenal tumor ($P=0.002$ for both) and these patients had significantly higher HOMA index than controls (6.8 ± 1.9 vs 2.9 ± 0.1; $P<0.001$). In all tumor tissues two isoforms of Hsp70 co-expressed while only higher molecular weight isoform was detected in adjacent normal tissue.
Conclusion
Increased sensitivity to glucocorticoids associated with specific *Bcl*I polymorphism of GR gene and altered trafficking of GR by the Hsp90/Hsp70-based chaperone machinery within adrenal adenoma seems to play role in the development of insulin resistance in these patients.

OC4.7

Function and evolution of GHRH, PACAP and PRP in vertebrates

Billy KC Chow, Francis KY Siu, Janice KV Tam & Leo TO Lee
University of Hong Kong, Pokfulam, HK, SAR, China.

In mammals, GHRH is the most important neuroendocrine factor that stimulates the release of GH from the anterior pituitary. In non-mammalian vertebrates, however, the previously named GHRH-like peptides were unable to demonstrate robust GH-releasing activities. In this report, we provide evidence that these GHRH-like peptides are homologues of mammalian PACAP-related peptides (PRP). Instead, GHRH peptides encoded in cDNAs isolated from goldfish, zebrafish and African clawed frog were identified. Moreover, receptors specific for these GHRHs were characterized from goldfish and zebrafish. These GHRHs and GHRH-Rs are phylogenetically and structurally more similar to their mammalian counterparts than the previously named GHRH-like peptides and GHRH-like receptors. Information regarding their chromosomal locations and organization of neighbouring genes confirmed also that they share the same origins as the mammalian genes. Functionally, the goldfish GHRH activates cAMP production in receptor-transfected CHO cells as well as GH release from goldfish pituitary cells. Tissue distribution studies by real-time PCR showed that the goldfish GHRH is expressed almost exclusively in the brain, while the goldfish GHRH-R is actively expressed in brain and pituitary. In addition, specific receptors for PRPs (formerly GHRH-like peptides) were cloned from goldfish, zebrafish and Xenopus, clearly suggesting a function of PRP in these species. By phylogenetic and chromosomal syntenic studies, we found PRP receptors only in non-mammalian vertebrates but not in mammals, indicating that the receptor was lost in the mammalian lineage. Based on these data, a comprehensive evolutionary scheme for GHRH, PRP-PACAP, PHI-VIP genes in relation to 3 rounds of genome duplication early on in vertebrate evolution is proposed. Finally, the newly discovered GHRHs, also found in flounder, Fugu, medaka, stickleback, Tetraodon and rainbow trout, provide new research directions regarding the neuroendocrine control of growth in vertebrates.

Thyroid basic – OC5

OC5.1

Structure-function of glycoprotein hormones using site-directed mutagenesis and gene transfer: designing new agonists and antagonists

Fuad Fares, Naiel Azam, Rinat Bar-Shalom & Zaki Kraiem
Carmel Medical Center and the Faculty of Medicine, Technion-israel Institute of Trechnology, Haifa, Israel.

Objectives
The main goal of the present study is to investigate the role of N-linked oligosaccharides on the structure and function of human thyrotropin (hTSH). A second aim of the present study is to design new analogs of hTSH.
Methods
Overlapping PCR technique was used to convert hTSH heterodimer to a biologically active single chain by fusing the α subunit to the carboxyl terminal end of hTSHβ subunit in the absence (hTSH$\beta\alpha$) or presence of a $\sim$30 aminoacid peptide from hCGβ (CTP) as linker (hTSHβCTPα). hTSH mutants lacking the sequence site of N-linked oligosaccharides were prepared using site-directed mutagenesis. hTSH variants were expressed in CHO cells. The TSH receptor binding activities of the variants were determined by radioligand receptor assay using CHO cells stably transfected with hTSH receptor. *In vitro* bioactivity was tested using cultured human thyroid follicle cells and *in vivo* longevity and bioactivity were tested in mice animal model.
Results
The single-peptide variants of hTSH were biologic active *in vitro* and *in vivo* with a longer half-life. Variants lacking the N-linked oligosaccharides were expressed and secreted from CHO cells. Interestingly, the deglycoselated variants were significantly less potent than TSH wild type. Moreover, the deglycoselated variants blocked cAMP formation and T_3 secretion stimulated by hTSH or by hTSI in the receptor level. The variants were found to bind the hTSH receptor with high affinity. In addition, deglycoselated hTSH variants had a partial activity *in vivo* and significantly inhibited TSH bioactivity
Conclusions
Human TSH single peptides are biologically active. Deglycosylated variants inhibit the activity of hTSH and hTSI. These variants may offer novel therapeutic strategies in the treatment of Thyroid diseases.

OC5.2

Tyroglobulin (Tg) depletion in receptor associated protein (RAP) KO mice is due to a reduction of Tg aggregates

Simonetta Lisi, Roberta Botta, Aldo Pinchera, Claudio Marcocci & Michele Marinò
Department of Endocrinology, University of Pisa, Pisa, Italy.

RAP KO mice have a reduction of colloidal Tg resulting in subclinical hypothyroidism and histological signs of goiter. The difference in colloidal Tg between RAP KO and WT mice was striking by immunohistochemistry, but could not be detected in thyroid extracts. To explain this discrepancy, we hypothesized that the reduction of Tg reflected a reduction of Tg aggregates discarded during tissue extraction. To investigate this possibility, pellets obtained by thyroid homogenization were solubilized with 6M guanidine and analyzed by Western blotting. Tg resolved into two bands at 660 and 330 kDa, which were found in WT, but not in RAP KO mice, supporting a reduction of Tg aggregates in the latter. We then investigated the effects of detergents, denaturation and pH on homogenates separated into membrane-associated and cytoplasmic fractions. The Tg bands were detected in all samples from RAP KO and WT mice. Detergents and high pH increased the intensity of the bands in the cytoplasmic fractions from WT mice, suggesting the presence of Tg aggregates of high molecular mass. Under denaturing conditions the Tg bands were less intense, probably due to Tg degradation. In RAP KO mice, cytoplasmic Tg was less sensitive to detergents and pH, possibly because of a reduced number of Tg aggregates compared with WT mice. Higher amounts of Tg were found in the membrane-associated than in the cytoplasmic fractions, regardless of the extraction procedure and the genotype, representing Tg-containing vesicles within the colloid, Tg within intracellular organelles, and cell membrane-bound Tg. In RAP KO mice the amounts of membrane-associated Tg were greater than in WT mice, in agreement with immunohistochemical findings. In conclusion, the absence of RAP in the thyroid gland results in a reduction of colloidal Tg aggregates, which are known to represent the major storage form of thyroid hormones.

OC5.3

Polarized plasma membrane targeting of the Na^+/I^- symporter (NIS) is regulated by its carboxy terminus

Orsolya Dohan[2], Carla Portulano[1], Chris Ginter[1] & Nancy Carrasco[1]
[1]Albert Einstein College of Medicine, Bronx, New York, United States; [2]Institute of Experimental Medicine Hungarian Academy of Sciences and Szent Rokus Hospital and Institutions, Budapest, Hungary.

The Na^+/I^- symporter (NIS), a glycoprotein expressed at the basolateral plasma membrane of thyroid epithelial cells, mediates active I^- uptake for the biosynthesis of thyroid hormones and radioiodide transport for diagnosis and treatment in thyroid cancer. Our cloning of the NIS cDNA and generation of anti-NIS antibodies provided the basis to investigate the decrease in I^- transport in thyroid cancer relative to healthy thyroid cells. Instead of finding only the expected lower NIS expression, we have reported that in the majority of thyroid cancers, NIS is surprisingly overexpressed as compared to the surrounding tissue but retained intracellularly. Therefore, it is of considerable interest to elucidate the mechanisms underlying NIS plasma membrane targeting, a pursuit that could lead to new therapeutic interventions to increase the sensitivity of radioiodide diagnostic imaging and the effectiveness of radioiodide therapy. We report that the NIS carboxy terminus contains crucial information for NIS trafficking and that the length of the carboxy terminus correlates linearly with functional cell surface expression of the transporter. We also demonstrate that whereas the last four amino acids ($E_{615}TNL_{618}$) are not necessary for NIS trafficking, even though they comprise a PDZ binding motif, the 602–614 sequence carries essential determinants for NIS basolateral targeting.

OC5.4

$BRAF^{V600E}$ mutations but not RET/PTC rearrangements are correlated with a lower expression of NIS mRNA expression in papillary thyroid cancer (PTC)

Cristina Romei[1], Pamela Piampiani[1], Pinuccia Faviana[3], Fulvio Basolo[3], Valeria Bottici[1], Mariangela Sculli[1], Piero Berti[2], Gabriele Materazzi[2], Aldo Pinchera[1] & Rossella Elisei[1]
[1]Department of Endocrinology, University of Pisa, Pisa, Italy; [2]Department of Surgery, University of Pisa, Pisa, Italy; [3]Department of Oncology, University of Pisa, Pisa, Italy.

Several studies have identified a relationship between oncogene activation and dedifferentiation of PTC. Mutations of RAS, RET/PTC and BRAF modulate the expression of thyroid genes. An impaired NIS expression has been demonstrated in PTCs harboring the $BRAF^{V600E}$ mutation.Aim of this study was to analyze BRAF and RET/PTC1-3 alterations and their influence on the expression of thyroid differentiation genes. Seventy-one PTC samples were studied. Quantitative analysis of TPO, Tg, TSH-R, TTF1 and NIS were performed by real time RT-PCR. Our results indicate that 44/71 cases (62%) were positive for one genetic alteration and 7/71 (9.8%) showed the simultaneous presence of 2 gene mutations. In particular $BRAF^{V600E}$ and RET/PTC rearrangements were present in 32.2% and 19.7% of cases respectively. $BRAF^{V600E}$ was more frequently found in the classical than in the follicular variant ($P=0.02$). At variance no correlation was identified between RET/PTC rearrangement and clinico-pathological features of PTCs. Genetic alterations were correlated with mRNA expression (ΔCt) of Tg, TPO, TSH-R, TTF-1, NIS. mRNA expression of NIS gene was significantly lower ($P=0.0001$) in PTCs harbouring the BRAF mutation with respect to not mutated samples. By immunohistochemistry we did not find any relationship between $BRAF^{V600E}$ and NIS protein. No difference in NIS mRNA expression was found in PTC with or without RET/PTC rearrangements. We did not observe any significant difference in the expression of thyroid differentiation genes neither when compared with BRAF mutation or RET/PTC rearrangements. Furthermore no relationship was found between serum TSH and the expression of NIS mRNA in thyroid tumors. In conclusion our data indicate that (a) the frequency of $BRAF^{V600E}$ mutations and RET/PTC rearrangements was 35% and 20% respectively; (b) in our series 10% of PTC cases harbored 2 different genetic alterations; (c) NIS mRNA expression was significantly lower in PTCs harboring a BRAF mutation but not a RET/PTC rearrangement; (d) the expression levels of other thyroid differentiation genes were not correlated with the presence of gene alterations.

OC5.5

Transcriptional regulation of human type 2 deiodinase and chorionic gonadotropin genes in human placenta: emerging evidence of a common promoter code

Gianluca Canettieri, Maria Giulia Santaguida, Antonella Franchi, Michele Della Guardia, Alberto Gulino & Marco Centanni
University of Rome "La Sapienza"- Polo Pontino, Latina, Italy.

Human type 2 deiodinase (hD2) regulates T3 production in placenta during trophoblast development. hD2 mRNA and protein levels are elevated only during the first trimester of gestation then becoming barely detectable. These variations are similar to those of chorionic gonadotropin (hCG), a well-known marker of early gestation secreted by the cytotrophoblast. A peculiar promoter architecture of the gene encoding the alpha subunit of hCG allows a CRE-mediated synergism between cAMP and EGF, leading to elevated levels of hCG-mRNA only during early pregnancy. In addition, hCG promoter contains several CCAAT boxes, that are likely to confer tissue specificity to this gene. Similarly, in our previous studies we have demonstrated that *Dio2* promoter is synergistically stimulated by cAMP and mitogens. These signals are integrated and converge to the Dio2 CRE, which recruits a transcription factor complex including CREB, c-Jun and c-Fos. Here we show that CCAAT enhancer binding proteins (C/EBPs), are master regulators of Dio2 expression in JEG3 cells, a cell line similar to early trophoblast. RT-PCR studies have demonstrated that C/EBPs significantly increases hD2 mRNA levels. With functional assays of micro-deletion mutant constructs we have shown that C/EBPs robustly enhanced the transcriptional activity of hD2 gene through a highly conserved CCAAT element, located nearby the TATA box. Biochemical evidence confirmed the binding of C/EBPs to this regulatory site. Remarkably, the inducibility was dramatically increased in promoter constructs lacking the CRE or when CREB/CRE interaction was prevented by an acidic dominant negative inhibitor. This latter observation suggested that CREB and C/EBP regulates transcription of *Dio2* gene in an antagonistic fashion. In conclusion we have found that αCG and Dio2 genes seem to share a common promoter code, represented by CCAAT, CREs, TATA/TSS units, that imparts tissue specificity and inducibility to both genes in early trophoblast.

OC5.6

A crucial role of interleukin-6 in the pathogenisis of thyrotoxicosis-related disturbances of bone turnover in mice

Janusz Mysliwiec[1], Robert Zbucki[2], Maria Malgorzata Winnicka[2], Boguslaw Sawicki[3], Agnieszka Nikolajuk[1], Karol Kaminski[4], Wlodzimierz Musial[4], Piotr Mysliwiec[5], Zofia Bondyra[6], Jerzy Walecki[6] & Maria Gorska[1]
[1]Dept. of Endocrinology, Diabetology and Internal Diseases, Bialystok, Poland; [2]Dept. of General and Experimental Pathology, Bialystok, Poland; [3]Dept. of Histology and Embryology, Bialystok, Poland; [4]Dept. of Cardiology, Bialystok, Poland; [5]II Dept. of General Surgery, Bialystok, Poland; [6]Dept. of Radiology, Bialystok, Poland.

Interleukin-6 (IL-6) has been shown to be involved in the pathogenesis of several bone diseases characterized by a negative balance between bone resorption and formation.

The aim of the study was to estimate serum markers of bone turnover: osteoclast-derived tartrate-resistant acid phosphatase form 5a (TRACP 5b) and osteocalcin in IL-6 knock-out mice to assess the role of IL-6 in the pathogenesis of thyrotoxicosis-related disturbances of bone metabolism.

Material and methods

C57BL/6J (wild-type; WT) and C57BL/6J$^{IL6-/-Kopf}$ (IL-6 knock-out; IL6KO) mice randomly divided into 4 groups with 10 in each one: 1/WT mice in thyrotoxicosis (WT-thx), 2/WT controls (WT-ctrl), 3/IL6KO mice with thyrotoxicosis (IL6KO-thx) and 4/ IL6KO controls. Experimental model of hyperthyroidism was induced by intraperitoneal injection of levothyroxine. The serum levels of TRACP 5b and osteocalcin were determined by ELISA.

Results

Serum concentration of TRACP 5b (median and interquartile ranges) were significantly increased in both groups of mice with thyrotoxicosis: WT (28.2 (18.8–41.6) U/l) and IL6KO (26.4 (23.0–31.2) U/l) as compared to the respective controls. Osteocalcin serum levels in IL6KO-thx mice (111.9 (103.1–175.6) ng/ml) were significantly elevated in comparison to WT-thx animals (46.1 (32.5–58.9) ng/ml).

Conclusions

The results of the present study suggest that IL-6 plays a crucial role in thyrotoxicosis-related disturbances of bone turnover in mice, determining the imbalance between bone resorption and bone formation caused by excess of thyroid hormones predominantly by inhibition of bone formation.

Acknowledgements

This work was supported by AMB Grant 3-50693L (to JM) and KBN Grant 2P05B01826 (to KK).

OC5.7

The 1188A/C polymorphism of IL-12 gene in Graves' disease

Natalia Wawrusiewicz-Kurylonek, Maria Gorska, Janusz Mysliwiec & Adam Jacek Kretowski
Department of Endocrinology, Diabetology and Internal Medicine, Medical University of Bialystok, Bialystok, Poland.

Background and aims

Interleukin-12 (IL-12) is a pro inflammatory cytokine, which was suggested to play a key role in the pathogenesis of Th1-cell-mediated autoimmune diseases. The aim of our study was to estimate the association of 1188A/C polymorphism of IL12B gene with the predisposition to Graves' disease (GD) in Polish population.

Materials and methods

The study was performed in the group consisting of 245 individuals with GD sequentially recruited from the endocrinology outpatient clinic. GD was confirmed on the basis of clinical observation, biochemical criteria of thyrotoxicosis and the presence of TSH receptor antibodies. Two hundred and one healthy volunteers served as the control group. In all subjects A1188C polymorphism in the 3′-UTR region of the IL-12B gene was determined by direct sequencing of the appropriate fragment of IL-12B gene.

Results

In our study the frequencies of 1188C allele and 1188CC genotype were significantly higher in patients with GD in comparison to healthy subject (respectively, 22.1% vs. 16.2%, $P=0.027$ and 7.7% vs. 1.5%, $P=0.003$). There were no differences in the distribution of 1188AA and 1188AC genotype IL-12B gene between the studied groups. Furthermore we also observed that frequency of 1188CC genotype was higher in patient with ophtalmopathy in comparison to subject without ophtalmopathy and healthy controls. The frequency of 1188CC IL-12 genotype was also higher among patients, who developed GD before the age of 40 years, when compared to subjects with Graves' disease onset before age of 40.

Conclusions

We observed that the frequency of 1188CC genotype of IL-12B gene is higher in patients with GD and with ophtalmopathy in comparison to subject without ophtalmopathy and healthy controls. This suggests that 1188A/C polymorphism in IL-12B gene could have a role in predisposition to Graves' ophthalmopathy.

Cardiovascular endocrinology – OC6

OC6.1

Growth hormone-releasing hormone prevents cardiomyocyte apoptosis and activated PI3K/AKT, ERK1/2 and CREB signaling pathways

Riccarda Granata, Letizia Trovato, Silvia Destefanis, Fabio Settanni, Marta Annunziata, Davide Gallo & Ezio Ghigo
Dept. Internal Medicine, Div. Endcocrinology and Metabolism, University of Turin, Turin, Italy.

The hypothalamic hormone growth hormone-releasing hormone (GHRH), has been shown to function via its receptor splice variants as an autocrine/paracrine growth factor in normal and malignant cell lines and tissues, besides positively regulating growth hormone (GH) synthesis and secretion from the pituitary. Moreover, GHRH antagonists are known to suppress the proliferation of a wide variety of cancer cells through mechanisms yet to be fully elucidated. Aim of this study was to investigate the effect of GHRH on cell death and apoptosis induced by either serum deprivation or by the β adrenergic agonist isoproterenol (ISO) in rat H9c2 cardiomyocytes and in isolated adult rat cardiac myocytes. H9c2 cells and cardiac myocytes were cultured in serum-deprived medium for 48 h in the presence or absence of either ISO (100 μM) or GHRH (0.5 μM). RT-PCR analysis revealed the presence of GHRH receptor (GHRH-R) mRNA in both H9c2 cells and rat cardiac myocytes. GHRH (0.5 μM) significantly counteracted serum starvation- and ISO-induced cell death and apoptosis in both cell models. Further, either GHRH or isoproterenol induced ERK1/2 phosphorylation, whereas only GHRH activated Akt survival signaling pathway. Interestingly, both GHRH and ISO induced cAMP increase and phosphorylation of its down-stream transcription factor cyclic AMP response element-binding protein (CREB) in H9c2 cells. Finally, the GHRH-R antagonist JV-1-36 completely abolished the survival effects of GHRH in H9c2 cells, under both serum starvation- and ISO-induced cell death and apoptosis.

These results indicate that GHRH is a survival factor for cardiac myocytes. Moreover, they suggest that this molecule may play a role in the prevention of cardiac cell loss in pathological conditions that ultimately lead to the development of heart failure.

OC6.2

Testosterone replacement attenuates fatty streak formation and improves the HDLC profile in the Tfm mouse: an effect which is independent of the classical androgen receptor

Joanne E Nettleship[1], Richard D Jones[1], Kevin S Channer[2] & Hugh T Jones[1]
[1]Hormone and Vascular Biology Group, Academic Unit of Diabetes, Endocrinology & Metabolism, Division of Genomic Medicine, University of Sheffield Medical School, Beech Hill Road, Sheffield, S10 2RX, United Kingdom; [2]Department of Cardiology, Royal Hallamshire Hospital, Sheffield Teaching Hospitals, NHS Trust, Glossop Road, Sheffield, S10 2JF, United Kingdom.

Research indicates that low testosterone is associated with CAD in men. Evidence suggests that men with hypotestosteronemia and concomitant CAD may benefit from physiological testosterone replacement therapy (PTRT). The mechanism by which testosterone produces these cardio-protective effects and the role of the androgen receptor remains largely unknown. The aim of this study was to determine whether testosterone modulates atheroma formation via its classical signalling pathway, via conversion to 17β-estradiol or via an alternative-signalling pathway. Group 1: 8-week-old Tfm (exhibiting a dysfunctional androgen receptor and testosterone deficiency) and control mice were castrated or sham-operated. Group 2: 9-week-old Tfm and controls were administered either placebo, PTRT, PTRT in conjunction with ERα-antagonist or Anastrazole. At 10-weeks both groups were administered a cholesterol-enriched-diet. Mice were sacrificed at 28-weeks. Sections through the aortic sinus were stained using oil-red-O, and lipid-stained areas quantified via digital analysis, and expressed as percentage of medial area. Total cholesterol, HDLC, testosterone and 17β-estradiol were quantified via ELISA. Low endogenous testosterone was associated with fatty-streak formation following feeding on cholesterol-enriched-diet. PTRT prevented aortic fatty streak formation in the Tfm mouse, and increased levels of HDLC. Fatty-streak formation was less marked in PTRT-treated mice, in conjunction with ERα-antagonist or Anastrazole, although this was still significantly lower than that of placebo-treated Tfm mice. Improvement in HDLC was completely attenuated by co-treatment with these agents. PTRT in the Tfm mouse is associated with a reduction in aortic fatty-streak formation. The majority of this action is due to a direct non-genomic action of testosterone, with a component of the response being medicated via conversion to 17β-estradiol and subsequent activation of ERα. The beneficial effect of PTRT upon HDLC appears to be solely mediated by conversion of testosterone into 17β-estradiol, via modulation of genomic ERα-dependent pathways.

OC6.3

Plasma brain natriuretic peptide (BNP) levels predict acute right ventricular dysfunction in pulmonary embolism – prospective study on 70 patients

Alina Mihaela Pascu[1], Mariana Rădoi[2] & Mihail Coculescu[3]
[1]"Transilvania" University, Faculty of Medicine, Department of Patho-physiology, Cardiology Clinic, Brasov, Romania; [2]"Transilvania" University, Faculty of Medicine, Department of Internal Medicine, Cardiology Clinic, Brasov, Romania; [3]"Carol Davila" University of Medicine and Pharmacy, Department of Endocrinology, "C.I. Parhon" Institute of Endocrinology, Bucuresti, Romania.

Background
Acute right ventricular dysfunction (RVD) on echocardiography (ECHO) is critical for risk stratification in pulmonary embolism (PE). Plasma BNP, a consecrated marker of left ventricular dysfunction, could represent a valuable biomarker of RVD in PE.
Aim and objective
Assessment of plasma BNP levels in patients with PE in relationship with right ventricular (RV) function evaluated by ECHO.
Methods
Prospective study of 70 patients with confirmed PE, 42 men (60%), mean age 52.5 ± 8.8. Plasma BNP levels were measured on admission using a quantitative fluorescence immunoassay (Triage BNP). ECHO evaluation of the RV function was performed in the first hour after admission. Study protocol was approved by local Ethical Committee. Patients were divided in two groups: group 1 – with acute RVD on ECHO, $n = 24$ patients (34.3%); group 2 – without acute RVD on ECHO, $n = 46$ patients (65.7%).
Statistics
SPSS 14.0; MedCalc 8.1.
Results
Plasma BNP levels were significantly higher in patients with acute RVD on ECHO (group 1), median value (25th, 75th percentiles) = 79.75 (45.77, 329.75) pg/mL vs. 7.85 (6.22, 16.07) pg/mL in patients without acute RVD on ECHO (group 2), $P < 0.0001$. BNP proved good in discriminating between patients with and without acute RVD – area under the receiver operating characteristic curve = 0.86 (95% Confidence Interval C.I. 0.77–0.94), $P < 0.0001$. The cut-off level of plasma BNP = 50 pg/mL had the best sensitivity = 0.84 (95% C.I. 0.79–0.88) and specificity = 0.80 (95% C.I. 0.75–0.85) in the same time in identifying acute RVD. Plasma BNP correlated significantly with RV end-diastolic diameter ($R = 0.74$, $P < 0.0001$), RV systolic pressure ($R = 0.77$, $P < 0.0001$). Logistic regression analysis showed that plasma BN $P > 50$ pg/mL was the best acute RVD predictor, odds ratio 21.0 (95% C.I. 5.5–79.5).
Conclusions
Plasma BNP higher than a cut-off level of 50 pg/mL could predict acute right ventricular dysfunction in patients with pulmonary embolism with a good sensitivity and specificity.

OC6.4

Carotid IMT and stiffness, aortic stiffness and pulse pressure: association with hormone therapy in postmenopausal women: baseline findings from the CASHMERE trial

T Simon[1], P Boutouyrie[2], S Christin-Maitre[1], A Gompel[3], R Joanides[4], A Kearny-Schwartz[5], Ch Kuntz[6], S Laurent[2], B Pannier[7], B Pornel[8], HH Struijker-Boudier[9], Ch Thuilliez[4], L Van Bortel[10], F Zannad[5], I Pithois-Merli[11] & P Jaillon[1]
[1]Saint Antoine-UPMC ParisVI university-Hospital, Paris, France; [2]HEGP-Paris V-University Hospital, Paris, France; [3]Hotel Dieu-Paris V-University Hospital, Paris, France; [4]CHU Rouen, Rouen, France; [5]CHU Nancy, Nancy, France; [6]CHU Strasbourg, Strasbourg, France; [7]Service de Cardiologie, Fleury-Mérogis, France; [8]Centre de Ménopause, Bruxelles, Belgium; [9]Cardiovascular Department, Maastricht, Netherlands; [10]Cardiovascular Department, Ghent, Belgium; [11]Pfizer, Paris, France.

Common carotid artery intima media thickness (CCA-IMT), aortic stiffness (carotid-femoral pulse wave velocity-PWV) and central pulse pressure (PP) are early markers of atherosclerosis. The influence of hormonal replacement treatment (HRT) on arterial parameters in menopausal women remains to be investigated.

We used baseline data of 665 menopausal women with hypercholesterolemia, screened for the CASHMERE study, a 12-month double-blind randomized trial comparing the effects of atorvastatin (80 mg/day) versus placebo, ± HRT, on the progression of CCA-IMT. CCA-IMT, PP, PWV were measured by using a high-definition echotracking device (Esaot®), aplanation tonometry (Sphygmocor®), and Complior® respectively. Mean age was 58 ± 6 years with a mean duration of menopause (M) of 8 ± 7 years. Age at M was 50 ± 5 years. Among them, 17% were smokers, 23% had hypertension and 28% were HRTusers.

	CCA-IMT (μm)			Central PP (mmHg)			PWV (m/s)		
Independent determinants	β coef.	R^2 increment	*P*	β coef.	R^2 increment	*P*	β coef.	R^2 increment	*P*
Age at M (5 yrs)	25	2.9	<0.001	3.0	4.0	<0.001	0.4	4.7	<0.001
M duration (5 yrs)	25	4.8	<0.001	3.5	7.2	<0.001	0.6	12.4	<0.001
Current use of HT (yes)	−37	2.3	0.002	−2.7	0.9	0.003	−0.3	0.9	0.01
Mean BP (10 mmHg)		–	–	7.0	32.4	<0.001	0.4	8.8	<0.001
Central PP (10 mmHg)	9	1.3	0.004	–	–	–	–	–	–
Total R^2		13.2			48.3			24.0	

R^2: % of explained variance, β coef: slope of the multivariate correlation.

Conclusions
Duration and age at menopause were associated with thickening and stiffening of large arteries. Current users of HRT had significantly thinner and more distensible arteries than non users.

OC6.5

Effects of ezetimibe and/or simvastatin on LDL receptor protein expression and on LDL receptor and HMG-CoA reductase gene expression in mononuclear blood cells: a randomized trial in healthy men

Ioanna Gouni-Berthold, Heiner Berthold, Helena Gylling, Yon Ko, Jogchum Plat & Wilhelm Krone
Department of Internal Medicine II, University of Cologne, Cologne, Germany; Drug Comission of the German Medical Association, Berlin, Germany; Department of Clinical Nutrition, University of Kuopio, Kuopio, Finland; Medical Poliklink, University of Bonn, Bonn, Germany; Department of Human Biology, University of Maastricht, Maastricht, Netherlands.

Context
Ezetimibe and simvastatin are often used in combination to lower blood lipid levels. The consequences of this combination at the molecular level are unknown.
Objective
To examine their effects on the LDL receptor (LDLR) protein expression and on the LDLR and HMG-CoA reductase gene expression in peripheral blood mononuclear cells (PBMC).
Design, setting and participants
Prospective, randomized, parallel 3-group trial. Twenty-four healthy men (mean age 32 ± 9 years) received a 14-day treatment with either ezetimibe (10 mg/day), or simvastatin (40 mg/day) or their combination. Blood was drawn before and after treatment.
Main outcome measures
LDLR protein expression, and LDLR and HMG-CoA reductase gene expression, lipid levels, non-cholesterol sterols and the ratio of precursor sterols over cholesterol concentrations, a valid marker of cholesterol synthesis and HMG-CoA reductase activity.
Results
LDL-C decreased by $22 \pm 10\%$, $41 \pm 12\%$, and $60 \pm 10\%$ in the ezetimibe, simvastatin and combination groups, respectively (all $P < 0.0001$). The HMG-CoA reductase gene expression increased significantly in the simvastatin ($+33\%$; $P = 0.032$) and combination groups ($+36\%$; $P = 0.0056$) and remained unchanged in the ezetimibe group ($+14\%$; $P = 0.27$). Similarly, the LDLR gene expression increased significantly in the simvastatin ($+72\%$; $P = 0.024$) and combination groups ($+56\%$; $P = 0.0012$), but not in the ezetimibe group ($+14\%$; $P = 0.49$). The LDLR protein expression, however, remained unchanged in all groups.
Conclusions
Unlike simvastatin, the lipid-lowering effects of ezetimibe do not involve an upregulation of the HMG-CoA reductase or LDLR gene expression. The simvastatin-induced upregulation of the LDLR gene expression did not lead to an increase in the LDLR protein. Further studies are necessary to fully clarify the posttranscriptional mechanisms regulating LDLR protein abundance.

OC6.6

The importance of the TAAAA(n) alleles at the SHBG gene promoter for the severity of cardiovascular disease in women

Katerina Saltiki[2], Nectaria Xita[3], Adriana Cimponeriu[1], Ioannis Kanakakis[2], Emily Mantzou[1], Charalambos Doukas[2], Ioannis Georgiou[3] & Maria Alevizaki[1]
[1]Endocrine Unit, Evgenidion Hospital, Athens University School of Medicine, Athens, Greece; [2]Dept Medical Therapeutics, Alexandra Hospital, Athens University School of Medicine, Athens, Greece; [3]Medical Genetics Unit, Dept of Obstetrics and Gynaecology, Medical School, University of Ioannina, Ioannina, Greece.

Objective
Androgen may be detrimental in the development of coronary artery disease (CAD) in women. We investigated possible associations between the (TAAAA)n polymorphism of sex hormone binding globulin (SHBG) gene promoter, which influences transcriptional efficiency of the SHBG gene and the severity of CAD in women.
Methods
One hundred and twenty women (37–82 yrs), undergoing coronary angiography. CAD severity, history of angina, myocardial infarction and reproductive history were recorded and hormonal parameters measured. According to the number of SHBG gene promoter repeats polymorphisms, patients were classified as short (≤ 7), medium length ($=8$) and long repeat (≥ 9) allele groups.
Results
Significant CAD was more prevalent in the group with the long-repeat allele carriers: 75% of the patients with 3 vessels with severe stenosis belonged to the long repeat allele group while only 37% of patients with mild CAD belonged to this group ($P = 0.004$). History of angina and prevalence of hypertriglyceridemia was more frequent in the long repeat allele group ($P < 0.05$). SHBG levels correlated inversely with BMI and waist perimeter ($P < 0.05$).
Conclusions
Longer (TAAAA)n repeats in the SHBG gene promoter are associated with more severe CAD in women undergoing coronary angiography, a finding not previously reported. This association may reflect the life-long tissue exposure to higher free androgens and supports the adverse cardiovascular effect of androgenic exposure in this highly selected group of women.

OC6.7

Evaluation of tolvaptan, an oral vasopressin V2 receptor antagonist, in 'asymptomatic' hyponatremia: effects on sodium concentration and patient reported health outcomes

Peter Gross[1], Joseph Verbalis[2], Guy Decaux[4], John Ouyang[3], Mary Hobart[3], Cesare Orlandi[3] & Frank Czerwiec[3]
[1]Universitätsklinikum Carl Gustav Carus, Dresden, Germany; [2]Georgetown University, Washington, DC, United States; [3]Otsuka Maryland Research Institute, Rockville, MD, United States; [4]Erasme University Hospital, Brussels, Belgium; [5]For the SALT Study Investigators, in Europe, Canada and the, United States.

Background
Hyponatremia ($Na + \leq 134$ mmol/L), the most common electrolyte derangement, is caused by inappropriate vasopressin-mediated water resorption in the kidney. Treating symptomatic hyponatremia is difficult and risky; as difficult as maintaining normal sodium levels. We tested if tolvaptan, an oral vasopressin V2 receptor antagonist, improves hyponatremia and self-reported health outcomes.
Methods
Two multicenter, randomized, double-blind, placebo-controlled trials evaluated tolvaptan in asymptomatic, non-hypovolemic hyponatremia patients. Upon obtaining local Ethics Committee approval and patients' consent, oral placebo ($n = 223$) or tolvaptan ($n = 225$) was given for 30 days. The first single daily dose (15 mg) was monitored in-hospital with optional fluid restriction. Patients were discharged and fluid intake and study drug (30 or 60 mg) were titrated as clinically indicated. Co-primary endpoints were the average daily area under the curve of serum sodium concentration change from baseline to day 4 and 30. Overall SF-12 Physical (PCS) and Mental Component Summary (MCS) score changes from baseline to day 30 were secondary endpoints. A hyponatremia disease-specific survey (HDS) was also tested.
Results
Serum sodium increased more with tolvaptan than placebo over the first 4 days ($P < 0.001$) and the entire 30-days ($P < 0.001$). On stopping tolvaptan therapy, sodium concentrations fell to placebo levels. The day 30 PCS was unchanged, however the MCS was significantly improved in the tolvaptan group ($P = 0.02$). MCS improvements correlated positively with rise in serum sodium ($r = 0.2$, $P = 0.001$). Tolvaptan differed from placebo in the HDS survey in the moderately severe hyponatremia subjects (< 130 mmol/L) in mental concentration, calculation and memory ($P < 0.05$ or better). Side effects associated with tolvaptan included increased thirst, dry mouth, and increased urination.
Conclusions
Tolvaptan, an oral V2 receptor antagonist, effectively increased and maintained serum sodium concentrations in hyponatremic patients. These changes were associated with improved perception of mental/cognitive health.

Reproduction 1 – OC7

OC7.1

Kallmann syndrome: mutations in the genes encoding prokineticin-2 (PROK2) and prokineticin receptor-2 (PROKR2)

Catherine Dodé & Jean-Pierre Hardelin
Institut Cochin, Paris, France.

Kallmann syndrome (KS) combines hypogonadotropic hypogona dism and anosmia. Anosmia is related to the hypoplasia of the olfactory bulbs and tracts. Hypogonadism is due to deficiency in gonadotropin-releasing hormone (GnRH), and probably results from a failure of the embryonic migration of GnRH-synthesizing neurons. This is a genetically heterogeneous disease, which affects 1:8000 males and five times less females. Loss-of-function mutations in *KAL1* and *FGFR1* account for the X-chromosome linked form and an autosomal dominant form of the disease, respectively. *KAL1* encodes anosmin-1, a locally restricted glycoprotein of embryonic extracellular matrices, which is likely to be

involved in FGF-signaling through FGFR1. Nearly 80% of the KS patients, however, do not carry a mutation in either of these genes.

We considered the genes, encoding the PROKR2 and PROK2, most relevant candidates because olfactory bulbs do not develop normally in *prokr2*$^{-/-}$ or in *prok2*$^{-/-}$ mice. *Prokr2*$^{-/-}$ mice have a severe atrophia of the reproductive system related to the absence of GnRH-synthesizing neurons in the hypothalamus. We sought mutations in *PROKR2* and *PROK2* in a cohort of 192 unrelated individuals affected by KS. Ten different *PROKR2* mutations were detected in 14 patients in heterozygous, homozygous, or compound heterozygous state, and heterozygous *PROK2* mutations were found in 4 KS patients. Notably, *PROKR2* and *PROK2* mutations were also present in some clinically unaffected individuals. These results shed new light on the complex genetics of KS.

OC7.2 – ESE Young Investigator Award

Neuropilin-2 and its ligands are involved in the migration of GnRH-secreting neurons

Anna Cariboni[2], Sonja Rakic[1], Jason Hickok[3], William Andrews[1], Shelley Tischkau[3], Roberto Maggi[2] & John Parnavelas[1]
[1]University College London, London, United Kingdom; [2]University of Milan, Milan, Italy; [3]University of Illinois at Urbana-Champaign, Urbana-Champaign, United States.

Reproduction in mammals is centrally regulated by neuroendocrine neurons scattered in the hypothalamus and secreting the decapeptide GnRH (gonadotropin releasing hormone). During development, GnRH-secreting neurons originate in the olfactory placode – at least in rodents – and migrate along olfactory nerves (the vomeronasal and the terminalis) to gain access to the forebrain and reach their final destinations in the hypothalamus. Defects in the migration of these neurons in humans result in infertility. The mechanisms underlying the establishment of the migration route and the movement of GnRH neurons are not very well understood and are thought to involve different classes of molecules. Candidates comprise semaphorins and their receptors (neuropilins) because of their high levels of expression in the developing olfactory system, which is intimately related with the development of the GnRH neurons. Moreover, reproductive problems and defects in the fasciculation of the vomeronasal nerves have been reported in the mutant mice for Neuropilin-2 (Npn-2), one of the class III semaphorins receptors, leading to investigate the role of these molecules in the migration of GnRH neurons. Analysis of newborn Npn-2$^{-/-}$ mice showed a significant reduction in number of GnRH neurons within the brain but an abnormal presence of such neurons stacked in the nasal regions. Expression studies performed on RNA derived from GFP-GnRH FACS-sorted cells showed presence of Npn-1, 2 and their ligands (Sema3A, 3F), suggesting the importance of these molecules in this system. *In vitro* experiments using immortalized GnRH neurons (GN11) showed that semaphorins 3A and 3F inhibit their migration, whereas VEGF, another Npn-2 ligand, reverted this effect, suggesting the possibility that *in vivo* the migration of the GnRH neurons might be influenced by a balance between positive (VEGFs) and negative (semaphorins) cues, acting through common receptors (neuropilins). These findings provide new insights into the molecular mechanisms of the migration of GnRH neurons and propose new candidate genes, likely involved in the pathogenesis of hypogonadotropic hypogonadisms.

OC7.3

Gonadotrophins regulate germ cell survival, not proliferation, in normal adult men

Saleela Ruwanpura, Robert McLchlan & Sarah Meachem
Prince Henry's Institute, Clayton, VIC, Australia; Monash University, Clayton, VIC, Australia.

Men with suppressed gonadotrophins, as induced by androgen-based contraceptive treatment, exhibit a 70% reduction in germ cell numbers (1). The mechanisms by which the germ cell populations are decreased are unknown. This study aimed to quantify the amount of germ cell apoptosis and proliferation and to identify the pathway(s) involved in gonadotrophin-induced germ cell loss in men. Testicular tissues from normal fertile men that received no treatment or testosterone (200 mg i.m. weekly) plus depot medroxyprogesterone acetate (300 mg i.m. once) for 2 or 6 weeks ($n = 5/10$ per group) to suppress gonadotrophins and consequently spermatogenesis were used (1). Apoptosis and proliferation were identified by TUNEL (a DNA fragmentation marker) and PCNA (a cell cycle marker) labelling methods, respectively. Intrinsic and extrinsic apoptotic pathways were identified by co-localisation of TUNEL-labelled germ cells with the pathway-specific proteins: activated caspase (aCaspase) 9 and 8 by confocal microscopy. The proportion of cells labelled and co-labelled by each method was quantified using stereological techniques. By 2 and 6 weeks of gonadotrophin suppression, the proportion of TUNEL-labelled spermatogonia was increased to 354% and 268% of control ($P < 0.001$), respectively. The proportion of TUNEL-labelled spermatocytes was increased (139% and 303% of control, respectively, not significant (NS)), with no TUNEL-labelled spermatids being observed. No difference in the number of PCNA-labelled cells was observed in gonadotrophin-suppressed men compared to control. By 2 and 6 weeks of gonadotrophin suppression, there was a trend that aCaspase 9 activity was increased to 130% of controls (NS), with no changes in aCaspase 8 activities. This study demonstrates for the first time that gonadotrophins act as survival factors for the spermatogonial (and possibly spermatocyte) population, possibly by regulating the intrinsic pathway of apoptosis. Understanding the mechanisms by which germ cells progress may provide important clues in infertile men where germ cells fail to progress due to hormonal pertubations.

(1) McLachlan *et al. Journal of Clinical Endocrinology and Metabolism* 2002 **87**: 546.

OC7.4

Capacitation and acrosome reaction in human ejaculated spermatozoa involve activation of a novel SRC tyrosine kinase

Gabriele Varano, Adriana Lombardi, Gianni Forti, Elisabetta Baldi & Michaela Luconi
University of Florence, Dept. Clinical Physiopathology, Florence, Italy.

Tyrosine phosphorylation of proteins is one of the main processes associated with the development of some specific functions of ejaculated human spermatozoa. Although this process, as well as the identity of the phosphorylated targets, has been well characterized, only few tyrosine kinases (TKs) have been identified so far. Moreover, their roles in regulating sperm functions are still unknown.

In the present work, we report the presence and localization of Src kinase in ejaculated human spermatozoa and investigate the role played by this TK during capacitation. Immunoprecipitation and western blot analysis of protein lysates from human spermatozoa using specific anti-p60src antibodies identified a single band of about 70 kDa molecular weight. Immunofluorescence analysis of fixed and permeabilized sperm localized positivity mainly in the post-acrosomal region of sperm head and midpiece in over 80% of the sperm population. By both immunoprecipitation and immunofluorescence techniques with antibodies recognizing tyrosine phosphorylation of Src at 416 or at 527 position, which identify the active or inactive kinase respectively, we showed an increased phosphorylation in Y416 during sperm capacitation. Blocking Src activity with its inhibitor SU6656 resulted in a significant reduction in tyrosine phosphorylation of sperm proteins, in particular in the 80–115 kDa molecular weight range. Moreover, such inhibitor completely blocked progesterone-induced acrosome reaction and interfered with calcium response to progesterone evaluated in fura-2 loaded sperm. No effects on sperm motility and hyperactivation parameters resulted from incubation of sperm with SU6656. Finally, by the use of TK and PKA inhibitors (erbstatin A and H89, respectively), we demonstrated that Src activation during capacitation is dependent on tyrosine kinase but not on protein kinase A activity. In conclusion we identified a novel Src isoform in human spermatozoa and demonstrated its involvement in capacitation and acrosome reaction.

OC7.5

Estrogens regulate epididymal contractility through RhoA/Rho-kinase signaling

Sandra Filippi[3], Annamaria Morelli[1], Linda Vignozzi[1], Rosa Mancina[1], Sara Mungai[1], Stefano Ambrosini[4], Gabriella Barbara Vannelli[4], Gianni Forti[2] & Mario Maggi[1]
[1]University of Florence, Department of Clinical Physiopathology, Andrology Unit, Florence, Italy; [2]University of Florence, Department of Clinical Physiopathology, Endocrinology Unit, Florence, Italy; [3]University of Florence, Interdepartmental Laboratory of Functional and Cellular Pharmacology of Reproduction, Dept. of Pharmacology and Clinical Physiopathology, Florence, Italy; [4]University of Florence, Department of Anatomy, Histology and Forensic Medicine, Florence, Italy.

Epididymis (epi) is a sex steroid-sensitive duct provided with spontaneous motility, allowing sperm transport. We previously demonstrated that human epi expresses a high abundance of mRNA for ER-alpha and ER-beta. We demonstrated that in epi estrogens up-regulate either oxytocin (OT) responsiveness, acting at the receptor level, and responsiveness to endothelin-1 (ET-1), another well-known stimulator of epididymal motility. However, we did not find any significant change either at gene or protein level in ET-1 and its receptors. Hence, other molecular effectors should

mediate the increased sensitivity to ET-1. In particular we hypothesized that estrogens up-regulate some contractile effectors, such as RhoA/Rho-kinase pathway, downstream to the ET-1 receptors. To investigate the effect of changing endocrine milieu on RhoA/Rho-kinase pathway, we induced hypogonadism (hypo) in rabbits with a single administration of a long-acting GnRH analog, triptorelin, and we replaced weekly hypo rabbits with different sex steroids (Testosterone, T or estradiol valerate, E2). After 8 weeks from GnRH analog administration, T plasma levels were decreased and the relaxant effect of the Rho-kinase inhibitor, Y-27632 on ET-1 pre-contracted epididymal strips, was significantly decreased. T administration restored T plasma levels, but not Y-27632 sensitivity in the epididymal strips. E2 not only completely restored Y-27632 responsiveness but even amplified it, as indicating that the RhoA/Rho-kinase calcium sensitizing pathway is up-regulated by E2. Accordingly, real time RT-PCR studies, western blot and immunohistochemistry analysis indicate that Rho kinase gene and protein was induced by E2 but not by T. To verify whether endogenous estradiol is involved in the regulation of Y-27632 responsiveness, we treated intact rabbits with an aromatase inhibitor, letrozole. Blocking aromatase activity abolished Y-27632 responsiveness in epi. In conclusion, our results support the hypothesis that epi is a male target for E2, which regulates its motility tuning up contractile hormones and local peptides responsiveness by increasing RhoA/Rho-kinase signalling and therefore calcium sensitivity.

OC7.6

Serum anti-Müllerian hormone levels in men with normo- and oligozoospermia

Frank Tüttelmann[1], Nina Dykstra[1], Axel PN Themmen[2], Jenny Visser[2], Eberhard Nieschlag[1] & Manuela Simoni[1]
[1]Institute of Reproductive Medicine of the University, Münster, Germany; [2]Department of Internal Medicine, Erasmus MC, Rotterdam, Netherlands.

Objective
Anti-Müllerian hormone (AMH) has recently been evaluated as a marker for follicle reserve and as a new marker for ovarian function in women. In the male, it is produced in Sertoli cells (SC) in the testis. We evaluated serum levels of AMH as a marker of SC function and male fertility by comparing normo- and oligozoospermic men.
Materials and methods
Serum levels of AMH were determined by enzyme immunoassay in two groups of men with normal ($n=105$) and reduced ($n=79$) sperm concentration (above or below 20×10^6/ml). These data were retrieved from the institute's database Androbase©.
Results
Significant differences ($P<0.001$) between the two groups were observed in sperm concentration (58.6 ± 37.9 in normo- vs. $9.1\pm10.6\times10^6$/ml in oligozoospermic, mean±S.D.) and count (202.6 ± 147.4 vs. $33.8\pm40.2\times10^6$) as well as in the percentage of progressively motile sperm ($50.6\pm7.0\%$ vs. $40.8\pm13.9\%$), percentage of normal morphology ($12.3\pm5.1\%$ vs. $7.2\pm4.7\%$) and testicular volume (55.8 ± 14.6 ml vs. 44.0 ± 13.8 ml), which were all lower in the oligozoospermic men as expected. Follicle-stimulating hormone (FSH) was higher in this group (4.1 ± 3.0 U/l vs. 7.0 ± 7.2 U/l), AMH showed a trend towards lower levels (7.7 ± 4.8 ng/ml vs. 6.7 ± 4.8 ng/ml, $P=0.06$), but neither LH (3.6 ± 1.9 U/l vs. 4.0 ± 2.2 U/l) nor testosterone (T, 15.2 ± 5.1 nmol/l vs. 14.2 ± 4.3 nmol/l) was different between the groups. We found a significant ($P<0.01$) negative correlation between AMH and FSH ($r=-0.48$), and relatively weak positive correlations with sperm concentration/count ($r=0.44$ and 0.39) and sperm motility ($r=0.35$). By contrast, in the normozoospermic men AMH correlated only very weakly with T and free T ($P<0.05$, $r=0.21$ and 0.22) but with no other hormone or semen parameters.
Conclusions
In contrast to normozoospermic men, AMH correlates with FSH and sperm parameters in oligozoospermic men and might serve as a new marker for reduced SC function.

OC7.7

Use of atorvastatin, but not simvastatin in men with Type 2 diabetes is associated with lower total testosterone levels with no effect on bioavailable or free testosterone

Roger D Stanworth[1], Dheeraj Kapoor[2], Kevin S Channer[3] & T Hugh Jones[4]
[1]Academic Unit of Endocrinology, University of Sheffield, Sheffield, United Kingdom; [2]Department of Diabetes and Endocrinology, Barnsley Hospital NHS Foundation Trust, Barnsley, United Kingdom; [3]Faculty for Health and Wellbeing, Sheffield Hallam University, Sheffield, United Kingdom; [4]Department of Cardiology, Sheffield Teaching Hospitals NHS Foundation Trust, Sheffield, United Kingdom.

There is a high prevalence of low testosterone levels in men with type 2 diabetes (DM2) and low testosterone predates the onset of DM2. Testosterone replacement therapy for hypogonadal men with DM2 improves insulin sensitivity and glycaemic control as well as reducing central obesity. This may lead to an increase in biochemical assessment of hypogonadism in men with DM2. Androgens and other steroid hormones are produced from cholesterol and it has been postulated that treatment with HMG-Co-enzyme A reductase inhibitors (statins) could decrease testosterone levels by reducing the availability of cholesterol and/or inhibiting steroidogenesis. Low testosterone levels in men with DM2, and the widespread use of statins in DM2 mean that any such effect would be particularly important in this group.

We compared androgen status with statin use in a group of 355 Caucasian men with DM2. Data was collected in year 2002–2003. In our group, 168 patients were treated with statins (mainly simvastatin and atorvastatin) and 187 men were untreated. There were no significant differences between treated and untreated men in terms of glycaemic control, blood pressure or obesity. Statin use was associated with lower total testosterone (TT) ($P=0.009$) and SHBG ($P=0.005$) levels but bioavailable (BioT) and calculated free testosterone (cFT) were not significantly reduced. ADAM hypogonadal symptom score was not affected.

Atorvastatin was associated with reduced TT ($P=0.006$) and SHBG ($P=0.005$) compared with no treatment and there was an apparent dose response effect with the lowest levels of testosterone seen in men treated with higher doses of atorvastatin. Simvastatin did not cause a significant reduction in testosterone or SHBG levels. Our study illustrates the importance of using measured or calculated bioavailable or free testosterone in the assessment of hypogonadism in men with DM2 treated with statins, particularly atorvastatin.

Neuroendocrinology clinical – OC8

OC8.1

Growth hormone response during OGTT: the impact of assay method, gender and BMI on the estimation of reference values in patients with acromegaly and in healthy controls

Ayman M Arafat[1], Mathias Möhlig[1], Martin O Weickert[1], Frank H Perschel[2], Johannes Purschwitz[1], Joachim Spranger[1], Christian J Strasburger[3], Christof Schöfl[1] & Andreas FH Pfeiffer[1]
[1]Department of Endocrinology, Diabetes and Nutrition, Charité-University Medicine Berlin, Campus Benjamin Franklin and the German Institute of Human Nutrition, Berlin, Germany; [2]Department of Clinical Chemistry and Pathobiochemistry, Charité-University Medicine Berlin, Campus Benjamin Franklin, Berlin, Germany; [3]Department of Clinical Endocrinology, Charité-University Medicine Berlin, Campus Mitte, Berlin, Germany.

Objectives
Besides the measurement of IGF-1, GH suppression during OGTT to assess the biochemical status in acromegaly is recommended. However, as a consequence of the development of highly sensitive and specific GH assays a critical re-evaluation of the criteria for the diagnosis and follow-up management of acromegaly is mandatory. The aim of our study was to evaluate the between-method discrepancies in GH determinations by different immunoassays considering further confounders like age, gender, and BMI.
Methods
GH was measured during a 75-g OGTT in 10 controlled and 22 uncontrolled acromegalics (12 men; age 31–62 years; BMI 21–30 kg/m^2) and in 213 apparently healthy subjects (66 men; age 20–76 years; BMI 19–62 kg/m^2) using 3 different assays (DPC Immulite 2000, Nichols and DSL-10-19100) that are calibrated against recommended standard (IS 98/574). Ethical Committee approval was obtained.
Results
There was a strong correlation between all assays ($r=0.72$–0.994, $P<0.0001$). However, the results obtained with DPC were, on average, 2.4-fold higher than those obtained with Nichols and 11-fold higher than those obtained with DSL. GH-nadir in controlled acromegalics was 0.98 ± 0.26 μg/l (DPC) and 0.5 ± 0.15 μg/l (Nichols), whereas in those with an active disease was 7.98 ± 1.7 and 4.5 ± 1.2, respectively. In controls, GH-nadir was 0.13 ± 0.01 μg/l (DPC), 0.06 ± 0.01 μg/l (Nichols) and 0.018 ± 0.004 μg/l (DSL). Both basal and nadir-GH were significantly higher in females than in males (DPC: 2.2 ± 0.28 vs. 0.73 ± 0.15 μg/L and 0.16 ± 0.013 vs. 0.08 ± 0.01 μg/L, $P<0.001$, respectively). Age, BMI and waist/hip ratio correlated negatively with both basal and nadir-GH ($r=-0.2$, -0.32 and -0.48, $P<0.01$). In multiple regression analysis age, BMI and waist/hip ratio were independent predictors for both the basal and the nadir-GH (β-values ranging from -0.2 to -0.3 and -0.14 to -0.3, respectively).
Conclusions
Post-glucose GH-nadir values are assay-, gender-, age- and BMI-specific indicating the need of individual cut-off limits for each assay.

OC8.2 – ESE Young Investigator Award

Effect of GH receptor antagonist pegvisomant on cardiovascular risk and atherosclerosis in acromegalic patients resistant to somatostatin analogues

Maria Cristina De Martino[1], Renata S Auriemma[1], Gregorio Brevetti[2], Mariano Galdiero[1], Monica De Leo[1], Gaetano Lombardi[1], Annamaria Colao[1] & Rosario Pivonello[1]
[1]Departments of Molecular and Clinical Endocrinology and Oncology, Federico II University, Naples, Italy; [2]Department of Internal Medicine, Division of Cardiovascular and Immunological Sciences, Federico II University, Naples, Italy.

Acromegaly is known to be associated to an increased cardiovascular risk, due to the increased prevalence of glucose intolerance and dyslipidemia and pre-atherosclerotic lesions. The aim of this study was to evaluate the effect of treatment with the GH receptor antagonist pegvisomant on cardiovascular risk and atherosclerosis in patients with acromegaly resistant to somatostatin analogues. Twelve patients (4 m, 8 f, 28–58 yrs) and 24 sex-, age- and BMI-matched controls entered the study. The patients were evaluated before and after 18 months of treatment with pegvisomant at the dose of 10–40 mg/day. In all patients and controls, serum total, LDL and HDL cholesterol, triglycerides, glucose, insulin and fibrinogen levels, total/HDL cholesterol ratio and HOMA index, as well as common carotid intima-media thickness (IMT) were measured and correlated with serum GH and IGF-I levels. At baseline, increased GH and IGF-I levels were confirmed in all patients. HDL-cholesterol were significantly lower ($P<0.05$) whereas total/HDL-cholesterol ratio ($P<0.001$), glucose levels ($P<0.05$), HOMA index ($P<0.001$) and fibrinogen levels ($P<0.001$) were significantly higher in patients than controls. Moreover, maximal IMT were significantly higher in patients than in controls (1.13 ± 0.55 *vs* 0.69 ± 0.1 mm; $P<0.001$). At 18-month follow-up, serum IGF-I levels were normalized in 9 (75%) patients and significantly reduced in the remaining patients. Both serum glucose levels (5.62 ± 1.33 *vs* 4.86 ± 0.73; $P<0.05$) and HOMA index (3.31 ± 2.24 *vs* 1.10 ± 0.22; $P<0.05$) were significantly decreased after treatment. A trend to a decrease in maximal IMT (1.13 ± 0.55 *vs* 0.96 ± 0.16 mm) was also found after 18 months of treatment with pegvisomant. A significant correlation was found between the changes in serum IGF-I levels and maximal IMT ($P<0.05$). The results of the current study demonstrated that the treatment with pegvisomant is able to improve the cardiovascular risk, especially through the improvement of glucose tolerance, and prevent the progression of atherosclerosis in patients with acromegaly resistant to somatostatin analogues.

OC8.3

Pituitary imaging abnormalities in patients with and without hypopituitarism after traumatic brain injury

Harald J. Schneider[1], Chiara G. Croce[2], Ginevra Corneli[2], Ezio Ghigo[2], Günter K. Stalla[1] & Gianluca Aimaretti[2]
[1]Internal Medicine/Endocrinology and Clinical Chemistry, Max Planck Institute of Psychiatry, Munich, Germany; [2]Division of Endocrinology and Metabolism, Department of Internal Medicine, Turin, Italy.

Recent evidence shows that patients with traumatic brain injury (TBI) are at substantial risk of hypopituitarism. However, the pathomechanisms are still not completely understood. Little is known about the association of morphological changes in the sella region with pituitary function in TBI. In this study, we assessed morphological abormalities of the sella region in patients with TBI and their relation to endocrine function.

We have studied MR or CT scans of 22 patients with TBI (17 men, 5 women, age [mean +/− SD] 43.5+/− 10.6 years). Of these, 15 patients had some degree of hypopituitarism.

We found abnormalities of the sella region in 80% of the patients with hypopituitarism and 29% of those without hypopituitarism ($P=0.03$). The most common abnormality was loss of volume or empty sella, followed by inhomogeneities, perfusion deficits, and lack of neurohypophyseal signal.

This is the first study to investigate the association of morphological alteration and pituitary function in TBI. Our results indicate that pituitary imaging abnormalities are more common in TBI patients with than without hypopituitarism. Possibly, necrosis and/or hemorrhage play a potential role in posttraumatic hypopituitarism.

OC8.4

Idiopathic central hypothyroidism: report of a human natural model of congenital TRH receptor (TRHR) absence

Marco Bonomi[1], Marta Busnelli[1], Alba Pilotta[2], Elena Prandi[2], Mario Maggi[3], Fabio Buzi[2], Paolo Beck-Peccoz[1] & Luca Persani[1]
[1]Dept. of Medical Sciences, University of Milan; Istituto Auxologico Italiano IRCCS and Fondazione Ospedale Policlinico IRCCS, Milan, Italy; [2]Growth and Development Unit, Children's Hospital, Brescia, Italy; [3]Dept. of Physiopathology, University of Florence, Florence, Italy.

Central Hypothyroidism (CeH) is a rare thyroid hormone production defect due to an insufficient stimulation of a normal thyroid gland. Candidate genes for isolated CeH include TSHβ (several cases reported) and TRHR (only one case reported so far). Here, we report the clinical and genetic studies in 2 males and 3 females affected with isolated CeH with normal/low TSH levels (0.05–0.95 mU/L) and low FT4 levels (3.6–4.6 pM). None of the patients was detected at neonatal screening, but came to medical attention during childhood or even adulthood (3–42 years). MRI alterations were detected only in one case (empty sella). Ultrasound showed hypoplastic/normal thyroids. None of the patients presented thyroid autoimmunity. In 3 subjects, TRH test showed absent TSH but normal PRL responses but TSHβ gene analysis was negative. The fourth patient presented CeH associated with severe obesity and type 2 diabetes mellitus and a normal TSH response to TRH. No mutations were identified in TRH as well as in Leptin and LeptinR genes. The last case presented with growth delay at 11 years. Absent TSH/PRL responses after TRH stimulation suggested TRH resistance. We identified a C to T homozygous nonsense mutation in TRHR gene resulting in a premature stop codon (R17X) and the production of a truncated receptor lacking the 7 transmembrane domains. This is the 2nd patient with TRHR mutations and represents a natural model of TRHR congenital absence associated with CeH and absent/poor neonatal manifestations. Since TRH is considered to play an essential role in postnatal adaptation to extrauterine life and maturation of thyroid axis, our findings may challenge this view or uncover the possible existence of other TRHR isoforms also in humans. The lack of mutations in 4/5 cases suggests the existence of still unknown candidate genes for CeH.

OC8.5

Inoperable pituitary tumours treated with ^{90}Y-DOTA-TATE – initial results

Grzegorz Kaminski[1], Norbert Szalus[1], Grzegorz Zielinski[1], Zbigniew Podgajny[1], Wojciech Zgliczynski[2], Anna Kasperlik-Zaluska[2], Joanna Cyperling-Kaminska[3] & Eugeniusz Dziuk[1]
[1]Military Institute of Health Services, Warsaw, Poland; [2]Medical Center of Postgraduate Education, Warsaw, Poland; [3]LIM Medical Center, Warsaw, Poland.

Introduction
The patients with inoperable hormone - secreting pituitary tumours are treated with cold somatostatin analogues, but it is not always effective. DOTA-TATE preparation is a somatostatin analogue coupled with β (−) emitter ^{90} Y. The efficacy of the treatment is based on excessive expression of somatostatin receptors (SSTR) in these tumours.
The aim of the study
To assess the feasibility of treatment of pituitary tumours with ^{90}Y-DOTA-TATE preparation.
Material and methods
^{90}Y-DOTA-TATE preparation was used in 4 patients with inoperable tumour: 3 patients with acromegaly and 1 with the Nelson's syndrome. The presence of SSTR was confirmed in scintigraphy with $^{99\,m}$Tc-HYNIC-TATE preparation earlier. Both radiopharmaceuticals are produced by POLATOM – Swierk/Poland. In 2 pts with acromegaly the dose was repeated twice. 1 pt with acromegaly and 1 pt with Nelson's syndrome were treated with the ^{90}Y-DOTA-TATE four times (3.7 GBq per dose). The renal protection was provided by 10 hours infusion of 1000 ml 10% amino acids preparation with max. speed of 120 ml/h. The local Ethical Committee approval has been obtained before the study.
Results
There were no serious adverse events observed after ^{90}Y-DOTA-TATE treatment. An insignificant, transient decrease of thrombocytes and lymphocytes was noted. In patients with the Nelson's syndrome the ACTH serum concentration decreased by 31%, in patients with acromegaly GH serum concentration decreased by about 30–40%, and clinical improvement was obtained.
Conclusions
^{90}Y-DOTA-TATE radiopharmaceutic is feasible and promising in treatment of inoperable pituitary tumours.

OC8.6

Improved glucocorticoid replacement therapy by a novel oral hydrocortisone modified-release tablet

Stanko Skrtic[2], Hans Lennernäs[3], Anna Nilsson[1], Ragnhildur Bergthorsdottir[1], Thomas Hedner[2] & Gudmundur Johannsson[1]
[1]Dept of Endocrinology, Sahlgrenska University Hospital, Goteborg, Sweden; [2]Dept of Clinical Pharmacology, Sahlgrenska Academy, Goteborg University, Goteborg, Sweden; [3]Dept of Biopharmacy and Pharmacokinetics, Uppsala University, Uppsala, Sweden.

Background
Mortality rate in patients with primary and secondary adrenal insufficiency is increased. A contributing factor could be the dose and the pattern of glucocorticoid replacement therapy. Hydrocortisone administered twice or thrice daily produces high serum peaks and low trough values in-between. A novel, once daily, hydrocortisone modified release tablet with combined immediate and extended release characteristics was developed.
Purpose
The aim was to determine single-dose pharmacokinetics and dose-proportionality of oral 5 and 20 mg modified-release hydrocortisone tablets in healthy volunteers.
Material and methods
Studies were performed with betamethasone suppression. The two first study days were blinded and randomized between the 5 and 20 mg tablet in a fasting state and the third was open with the 20 mg tablet taken 30 min after a high calorie, high fat meal. The plasma samples were assayed using a validated (GLP) LC-MS/MS method. The plasma pharmacokinetic variables were calculated using non-compartmental data analysis.
Results and discussion
The time to reach a clinically significant serum concentration of cortisol (>200 nmol/L) was within 25 minutes and a peak of 400–450 was obtained within 50 min after the 20 mg tablet. Serum cortisol levels remained above 200 nmol/L for around 6 h thereafter whereas all serum concentrations 18–24 h after intake were below 50 nmol/L. In the fed state the time to 200 nmol/L was delayed by 45–50 minutes. The 5 mg and 20 mg tablets produced almost superimposable profiles.
Conclusion
This modified-release tablet allows for a once-daily administration producing a near physiological serum cortisol profile. The time to clinically significant cortisol concentrations was short and after the peak level a slow decline occurred throughout the day allowing for a cortisol-free interval 18–24 hour after intake. This new tablet for once-daily administration may help to improve compliance and outcome in patients with adrenal insufficiency.

OC8.7

A single intravenous bolus of dexamethasone for the diagnosis of Cushing's syndrome

Gabriel Munter[1], Moriah Kirshner[2], Rosler Ariel[2], Shilo Shmuel[1], Leibowitz Gil[2] & Glaser Benjamin[2]
[1]Shaare Zedek Medical Center, Jerusalem, Israel; [2]Endocrinology Service, Hadassah-Hebrew University Medical Center, Jerusalem, Israel.

The diagnosis of Cushing's syndrome (CS) is based primarily on diagnostic tests evaluating the cortisol response to dexamethasone suppression. Tests based on oral administration of dexamethasone may be compromised by poor compliance. We evaluated the diagnostic accuracy of a novel intravenous dexamethasone suppression test (IDST). The test is performed by intravenous (iv) bolus injection of 8 mg dexamethasone, with blood cortisol determinations made before injection, then hourly during the first 6 h and finally at 24 h. ACTH is measured prior to dexamethasone injection and at 6 and 24 h following injection. We performed a retrospective analysis of patients studied for suspected CS in Hadassah, between 1994–2004. The study included 101 patients: 54 patients with pituitary CS, 22 with adrenal CS, 4 with ectopic ACTH CS (EAS) and 24 in whom the diagnosis of CS was excluded. Patients without CS showed rapid suppression of cortisol and ACTH that persisted for 24 hours. Patients with pituitary CS showed suppression of cortisol and ACTH levels at 6 hours with subsequent escape at 24 hours. Patients with adrenal CS or with EAS failed to suppress cortisol or ACTH levels. Using 60% suppression of blood cortisol at 24 h as the cutoff for the diagnosis of CS, IDST had 94% sensitivity, 95% specificity and 98% positive predictive value (PPV) for the diagnosis of CS. Similar results were obtained by using a cortisol level of 200 nmol/l at 24 hours as the cutoff for the diagnosis of CS. Adding the criteria of ACTH levels >4 pmol/l at 24 hours, the PPV of the IDST increased to 100%. Conclusions: IDST is a reliable, simple and accurate test for diagnosing hypercortisolism. Measuring cortisol levels before and 24 h after 8 mg i.v. dexamethasone administration is required to adequately diagnose patients with CS. ACTH levels at 24 h may increase the test's PPV. The sensitivity, specificity and PPV of the IDST are equal or higher than those reported for other commonly used non-invasive tests. Further studies are required to determine if IDST can discriminate effectively between pituitary disease and EAS.

Signal transduction – OC9

OC9.1 – ESE Young Investigator Award

Investigation of the role of MRAP in the functional expression of the melanocortin 2 receptor

Sadani Cooray, Lou Metherell, Mike Cheetham & Adrian Clark
William Harvey Research Institite, London, United Kingdom; Institute of Ophthalmology, London, United Kingdom.

Mutations in the ACTH receptor (Melanocortin 2 receptor/MC2R) are associated with Familial Glucocorticoid Deficiency/FGD. FGD is an autosomal recessive disorder that results from ACTH insensitivity at the adrenal cortex. However, only about 25% of FGD are caused by mutations in the MC2R suggesting the genetically heterogeneous nature of the disease. The transfection-mediated functional expression of the MC2R can only be achieved in cell lines of adrenal origin implying that the receptor may require an adrenal specific accessory factor/factors for functional expression. The causative gene for FGD type 2 (normal MC2R) was identified in our lab. It encoded a novel single transmembrane domain protein of unknown function that we subsequently named MRAP (melanocortin receptor accessory protein). We demonstrated that MRAP assists the MC2R to the cell surface as determined by confocal microscopy on CHO and SKN-SH cells. MRAP was also shown to play a role in the production of a functional MC2R in these cell lines as was indicated by the enhanced cAMP response to ACTH when co-transfected with MC2R and MRAP (Metherell L.A., *et al.*, *Nature Genetics* 2005 **37** 166–170). The knockdown of MRAP expression by transient transfection of MRAP siRNA (small interfering RNA) duplexes in Y1 mouse adrenocortical cells resulted in a reduction in MC2R signalling as determined by the significant decrease in cAMP when stimulated with ACTH. The expression and function of MRAP was restored in the clonal cell lines expressing mouse MRAP shRNAs by the transfection of the human MRAP sequence. Co-immunoprecipitation studies showed an interaction between MRAP and MC2R but not the other four melanocortin receptors. The production of cAMP through MC1R, MC3R, MC4R and MC5R was not enhanced in the presence of MRAP. In summary MRAP was found to be essential for the functional expression of the MC2R.

OC9.2

The human orexin receptor type 2 gene: Alternative promoters determining tissue-specific expression and identification of alternate splice variants and altered translational activities

Jing Chen & Harpal Randeva
University of Warwick, Medical School, Coventry, United Kingdom.

Orexins, acting via their receptors, are involved in the control of feeding, sleep-wakefulness, arousal, neuroendocrine homeostasis and autonomic regulation. However, the 5′ structure and regulation of human orexin type 2 receptor (OX2R) gene remains is not known. We present original findings regarding the 5′ structural organization of the human OX2R gene and identify four OX2R mRNA transcripts that differ in their 5′-untranslated region (UTR). The four transcripts revealed that the three alternative exons arise from alternative splicing. These exon 1 variants, arising from a single OX2R gene, were distributed over a region of 29504 bp and designated as exons 1A, 1B and 1C on the basis of their 5′ to 3′ order. In transfection studies, different transcripts exerted cell-specific effects on mRNA, but consistently reduced protein expression. Tissue-specific expression of these transcripts in human tissues has been demonstrated by RT-PCR. We show those 5′ -flanking regions to exon 1A and exon 2, but not exon 1C, drive alternative promoter activity in HEK-293 and SH-5YSY cells. Using progressive deletion analysis, a proximal promoter region between −456 and −123 (relative to the translation start site) was shown to exhibit the higher activities in HEK-293, SH-5YSY and NT2 cells. One CRE, GATA-2

and Oct-1 motif was identified within this region, which was responsible for the stimulation both by Dibutyryil-cAMP (db cAMP) and phorbol-12-myristate-13-acetate (PMA). Mutational studies demonstrated that these motifs functioned co-operatively to stimulate hOX2R gene transcription. Using the chromatin immunoprecipitation assay, we demonstrated that three motifs bind to the region of hOX2R proximal promoter. These novel data suggest that usage of alternate promoters, 5′-UTR and alternative splicing may contribute regulatory mechanisms for tissue-specific expression of the hOX2R gene.

OC9.3

Orexin-A inhibits glucagon secretion and proglucagon gene expression

Eva Göncz[1], Carsten Grötzinger[1], Marily Theodoropoulou[2], Bertram Wiedenmann[1], Mathias Strowski[1] & Ursula Plöckinger[1]
[1]Charite, Medizinische Klinik mit Schwerpunkt Hepatologie, Gastroenterologie & Interdisziplinäres Stoffwechsel-Centrum/Endokrinologie und Diabetes Mellitus, Campus Virchow-Klinikum, Berlin, Germany; [2]Max Planck Institut für Psychiatrie, München, Germany.

Background and aim
Orexin-A (OXA) increases insulin secretion and inhibits glucagon secretion, suggesting a role in regulating glucose homeostasis. The effects of OXA on pancreatic A-cells on the cellular level have not yet been demonstrated. Aim of our study was therefore to characterise the underlying signal transduction pathways and to study the OXA effects on proglucagon gene transcription.
Methods
The effects of OXA on glucagon secretion were evaluated using an *in situ* perfused rat pancreas model and clonal pancreatic A-cells (InR1-G9). OXR-1 expression in InR1-G9 cells was detected by western blot and immunofluorescence. The effects of OXA on intracellular cyclic AMP, AKT, PDK-1, CREB and EGR-1 were measured by ELISA and western blots, intracellular calcium (Ca^{2+}) by Fura-2. Proglucagon and Foxo1 mRNA levels were quantified by real-time PCR. Foxo1 was silenced using short interfering RNA (siRNA).
Results
Pancreatic A-cells express OX1R. OXA reduced glucagon secretion and proglucagon gene expression. OXA decreased intracellular cyclic AMP and Ca^{2+} concentrations, and increased the phosphorylation of AKT und PDK-1. PI-3 kinase inhibitor blocked the effects of OXA on proglucagon gene expression. OXA reduced the expression and phosphorylation of CREB, and EGR-1. Silencing of Foxo1 had no effects on basal proglucagon gene expression; however the inhibitory effect of OXA on glucagon gene expression was reversed.
Conclusions
We demonstrate for the first time the direct interaction of OXA with pancreatic A-cells and identify cAMP/AKT/PDK-1 and Ca^{2+} as intracellular target molecules for OXA action. We identify transcription factors Foxo1, CREB and EGR-1 as downstream targets for OXA signalling, suggesting a role in mediating the inhibitory effects of OXA on glucagon gene expression. We have now increasing evidence that OXA affects glucagon homeostasis.
Inhibition of glucagon secretion by OXA may have potential implication at lowering hyperglucagonemia frequently encountered in type 2 diabetes.

OC9.4

Signalling and internalisation properties of corticotrophin-releasing hormone (CRH) receptor type 2

Danijela Markovic, Hendrik Lehnert & Dimitris Grammatopoulos
Warwick Medical School, University of Warwick, Coventry, United Kingdom.

The family of urocortins (UCNs) exert important pathophysiological actions in the control of peripheral homeostatic mechanisms, through activation of the type 2-corticotropin releasing hormone receptor (CRH-R2). This G-protein coupled receptor preferentially binds urocortins (UCN, UCNII and UCNIII) than CRH. In most tissues, CRH-R2 activation leads to increased cAMP production. In this study we used HEK293 cells stably overexpressing recombinant CRH-R2β receptors to investigate intracellular events controlling receptor functional activity and their potential link to activation of distinct signalling cascades. Our results showed that agonist-induced CRH-R2β activation is followed by receptor endocytosis. Interestingly, we identified important agonist-specific temporal differences in receptor internalization kinetics; UCNII (a CRH-R2 specific agonist) induced CRH-R2β internalization within 15 min whereas the weaker agonist, CRH, induced CRH-R2β internalization only after 30–45 min of treatment. The role of intracellular molecules involved in GPCR internalization was also investigated. Confocal microscopy studies revealed that β-arrestin and clathrin were recruited to the plasma membrane as early as 2 min following UCNII treatment, and 5 min following CRH treatment. Furthermore, clathrin, but not β-arrestin, co-localize with the internalized receptor in the cytoplasm. We also investigated agonist induced ERK1/2 activation; both UCNII and CRH induced a transient ERK1/2 activation that returned to basal within 30 min. Confocal microscopy studies showed that activated ERK1/2 was uniformly distributed in the cytoplasm and nucleus. Receptor internalization inhibitors (conconavalin A and MDC) as well as expression of a dominant negative β-arrestin (319–418) markedly reduced UCNII and CRH induced ERK1/2 phosphorylation. In conclusion, we provide novel evidence of agonist-specific differences in the internalization characteristics of CRH-R2β which involve recruitment to clathrin coated-pits and β-arrestin to the plasma membrane. Receptor transport to the cytoplasm involves association with clathrin but not β-arrestin. This mechanism appears to be crucial for activation of distinct signaling cascades such as ERK1/2.

OC9.5

The third intracellular loop of human SST5 is crucial for receptor internalization after SS28 stimulation

Erika Peverelli[1], Giovanna Mantovani[1], Andrea G Lania[1], Davide Calebiro[1], Andrea1 Doni[2], Sara Bondioni[1], Paolo Beck-Peccoz[1] & Anna Spada[1]
[1]Endocrine Unit, Dpt. of Medical Sciences, Fondazione Ospedale Maggiore Policlinico Mangiagalli e Regina Elena IRCCS, University of Milan, Milan, Italy; [2]Istituto Clinico Humanitas, Rozzano, Milan, Italy.

Somatostatin (SS) is a widely distributed polypeptide that exerts inhibitory effects on hormone secretion and cell proliferation by interacting with five different receptors (SST1-SST5), that display important differences in tissue distribution, coupling to second messengers, affinity for SS and intracellular trafficking. SS analogues currently used in the treatment of acromegaly inhibit hormone secretion and cell proliferation by binding to SST2 and 5. Beta-arrestins have been implicated in regulating SST internalization but the structural domains mediating this effect are largely unknown. The aim of this study was to characterize the intracellular mechanisms responsible for internalization of human SST5 in the rat pituitary cell line GH3. To this purpose we evaluated by fluorescence microscopy SS28-mediated trafficking of receptor fused to DsRed and beta-arrestin2 fused to GFP. To identify the SST5 structural domains involved in these processes, we evaluated progressive C-terminal truncated proteins, SST5 mutants in which serine or threonine residues within the third cytoplasmic domain were mutated (S242A, T247A) and a naturally occurring R240W mutant in the third loop previously found in one acromegalic patient resistant to somatostatin analogues. We tested the ability of these mutants to associate with beta-arrestin2 and to internalize under agonist stimulation. The truncated mutants are comparable to the wild-type receptor with respect to beta-arrestin recruitment and internalization, whereas third cytoplasmic loop mutants show a significantly reduced internalization and arrestin translocation upon SS28 stimulation. Surprisingly, SST5 with both C-terminal truncation and third loop mutation exhibits normal internalization and beta-arrestin recruitment. Our results indicate SST5 third intracellular loop as an important mediator of beta-arrestin/receptor interaction and receptor internalization, while the role of the C-terminal tail would be to sterically prevent beta-arrestin/receptor interaction in basal conditions. Further elucidation of the molecular signals underlying SST5 intracellular trafficking will provide a better understanding of its function during prolonged agonist treatment.

OC9.6

Somatostatin receptor subtype-2 and -3 – selective agonists inhibit insulin secretion from INS-1 cells through modulation of the R-type Ca^{2+} channel

Stefan Mergler, Vandana Singh, Sylvia Zacharias, Bertram Wiedenmann, Ursula Plöckinger & Mathias Strowski
Hepatology & Gastroenterology, Berlin, Germany.

Objectives
Somatostatin (SST) inhibits insulin secretion from pancreatic B-cells through a reduction of intracellular free calcium ($[Ca^{2+}]_i$). The influx of Ca^{2+} is mediated by voltage-operated Ca^{2+} channels (VOCCs). The role of VOCCs of the R-type ($Ca_V2.3$) in SST-mediated processes is unknown. Therefore, we designed a study to identify SST-receptor subtypes (SSTR) in insulinoma cells (INS-1) and characterize the role of the $Ca_V2.3$ in mediating the effects of SST in these cells.
Methods
The expression of SSTRs in INS-1 cells was detected by RT-PCR. The effects of highly SSTR-selective agonists (SSTR-Ag) on cyclic AMP, insulin secretion and $[Ca^{2+}]_i$ were measured by ELISA, RIA and cell fluorescence imaging. VOCCs were characterized by patch-clamp technique.
Results
INS-1 cells express SSTR2 and SSTR3. SSTR2-selective agonist (SSTR2-Ag) more potently reduced cyclic AMP production than SSTR3-Ag. SSTR2-Ag transiently increased $[Ca^{2+}]_i$ which then rapidly decreased below the basal. Blockade of L- and R-type channels modulated $[Ca^{2+}]_i$ changes in response to SSTR2-Ag treatment. In contrast, SSTR3-Ag lowered $[Ca^{2+}]_i$ after 30 min, only. Blockade of R-type channels of cells treated with SSTR3-Ag less potently influenced $[Ca^{2+}]_i$ than SST or SSTR2-Ag. SST (EC50: 0.04 nM) and SSTR2-Ag (EC50: 0.06 nM) more potently inhibited 20 mM glucose/10 nM exendin-4-stimulated insulin secretion than SSTR3-Ag. The specific R-type channel blocker SNX-482 more potently reduced the inhibition of insulin secretion by SST and SSTR2-Ag as compared to SSTR3-Ag.
Conclusions
INS-1 cells express SSTR2 and SSTR3. SSTR2-Ag more effectively reduces intracellular cyclic AMP-accumulation and insulin secretion than SSTR2-Ag. Blockade of R-type Ca^{2+} channels prevents SSTR2- and SSTR3-induced inhibition of insulin secretion, suggesting that these agonists inhibit insulin secretion through modulation of R-type channel activity.

OC9.7

Seven transmembrane receptors mediated actin cytoskeleton rearrangement: comparison with constitutively active mutants of G protein alpha-subunits

Alenka Hrovat, Robert Frangez, Azra Pogacnik & Milka Vrecl
Veterinary Faculty, University of Ljubljana, Ljubljana, Slovenia.

Reorganization of the actin cytoskeleton could coincide with the activation of several seven transmembrane receptors (7TM receptors) (1). Stimulation of Rho family members leads to rapid remodeling of the actin cytoskeleton and subsequent stress fiber formation and certain 7TM receptors were shown to induce Rho-dependent responses via heterotrimeric G-proteins. $G\alpha_{12}$, $G\alpha_{13}$ as well as $G\alpha_{q/11}$ can link 7TM receptors to RhoA activation. However, some controversy exists over the exact role of $G\alpha_{q/11}$ (2).

The study's aim was to examine whether activation of the $G\alpha_{q/11}$- and $G\alpha_s$-coupled 7TM receptors involves changes in cell morphology and reorganization of the actin cytoskeleton. Actin cytoskeletal organization was also monitored in cells transfected with constitutively active mutants of Gprotein α-subunits and compared with the receptor-mediated redistribution pattern. Autofluorescently-tagged β-actin (pEYFP-actin) was co-expressed together with receptor constructs (neurokinin type 1 receptor (NK1-R) and $\beta_2$adrenergic receptor, β_2-AR)) or constitutively active mutants of $G\alpha_q$, $G\alpha_{12}$, and $G\alpha_s$ in the HEK 293 cells. Evaluation of the autofluorescently-labeled actin filaments was performed with the use of confocal microscope.

The acquired data shows that the $G\alpha_{q/11}$-coupled NK1-R activation caused changes in cell morphology, enhancement in the cortical actin signal and stress fiber formation. After the activation of other $G\alpha_{q/11}$-coupled receptors comparable results were also observed. Furthermore, the presence of over-expressed constitutively active $G\alpha_q$ and $G\alpha_{12}$ also lead to noticeable stress fiber formation. In contrast, neither the β_2-AR activation nor constitutively active mutant of $G\alpha_s$ caused any apparent changes in actin cytoskeleton status in the HEK-293 cells. Based on these findings it could be assumed that only $G\alpha_{q/11}$-coupled receptors activation coincides with the robust changes in the actin cytoskeleton organization.

References
1. Merrifield CJ. *Trends in Cell Biology* 2004 **14** 352–358.
2. Barnes WG, Reiter E, Violin JD, Ren XR, Milligan G, Lefkowitz RJ. *Journal of Biological Chemistry* 2005 **280** 8041–8050

Obesity and metabolism – OC10

OC10.1

The selective neuronal deletion of cannabinoid type 1 receptor is still able to provide resistance to diet-induced obesity

Cristina Cervino[1], Daniela Cota[2], Luigi Bellocchio[1], Giacomo Mancini[3], Beat Lutz[3], Giovanni Marsicano[4], Renato Pasquali[1] & Uberto Pagotto[1]
[1]Endocrine Unit and CRBA Hospital S.Orsola-Malpighi, Bologna, Italy; [2]Psychiatry Department, Cincinnati, United States; [3]Physiological Chemistry Department, Mainz, Germany; [4]Avenir INSERM, Bordeaux, France.

It is well known that cannabinoid type 1 receptor (CB1) antagonist drugs may reduce body weight and improve metabolic profiles in obese animals and humans by a double mechanism: at first, targeting mesolimbic and hypothalamic nuclei and, thereafter, peripheral organs involved in energy storage and expenditure. However, it is still unknown which of these sites of action may have a predominant role in the endocannabinoid effect on energy balance regulation. To solve this question we generated a mouse line in which the CB1 coding region is flanked by two loxP sites ($CB1^{f/f}$). By crossing this with mice that express Cre recombinase under the control of the regulatory sequences of the Ca^{2+}/calmodulin-dependent Kinase IIa gene ($CB1^{CaMKIIaCre}$ mice), we obtained $CB1^{f/f;CaMKIIaCre}$ mice in which CB1 receptor is deleted in all principal neurons of the forebrains, including those at mesolimbic and hypothalamic level modulating the positive incentive to palatable food and the orexigenic signals, respectively. Here we show that adult male $CB1^{CaMKIIaCre}$ (*n*. 15 each group, age 16–21 weeks for each diet) were still statistically significant leaner than the wild type littermates either undergoing standard diet or with high fat diet (40% kcal given by fat). However, when cumulative food intake was investigated, adult male $CB1^{CaMKIIaCre}$ mice did not show any statistically significant difference in caloric intake as compared to wild types with both diets. These data seem to indicate that other neuronal pathways may overcome the lack of the central CB1 orexigenic drive; on the other hand, it may suggest that CB1 may still play a crucial role at cerebral level as a sensor of yet unknown peripheral signals involved in energy homeostasis.

OC10.2

11beta-hydroxysteroid dehydrogenase type 1 (11beta-HSD1) mRNA expression in liver of patients with non-alcoholic steatohepatitis

Sarah Konopelska[1], Tina Kienitz[1], Matthias Pirlich[1], Jürgen Bauditz[1], Paul M. Stewart[2], Herbert Lochs[1], Christian J. Strasburger[1] & Marcus Quinkler[1]
[1]Internal Medicine, Center for Gastroenterology, Hepatology and Endocrinology, Charite Campus Mitte, Charite Universitaetsmedizin Berlin, Berlin, Germany; [2]Division of Medical Sciences, Institute of Biomedical Research, University of Birmingham, Birmingham, United Kingdom.

Background
Non-alcoholic fatty liver disease (NAFLD) is recognized as common liver disorder that represents the hepatic manifestation of the metabolic syndrome including visceral obesity, type 2 diabetes, insulin resistance and hyperlipidemia. Non-alcoholic steatohepatitis (NASH) is the progressive form of liver injury with the risk for progressive fibrosis, cirrhosis and end-stage liver disease. The pathophysiology that leads to NAFLD and NASH is not well understood. We hypothesize that an altered cortisol metabolism in the liver may be a pathogenetic factor. Hepatic 11beta-HSD1 regenerates cortisol from its inactive metabolite cortisone and requires NADPH as cosubstrate, which is supplied by hexose-6-phosphate-dehydrogenase (H6PDH).
Methods
76 patients (29 men, 48 women) underwent liver biopsy due to elevated liver enzymes. We quantified 11beta-HSD1 and H6PDH mRNA expression by real-time PCR with 18S as housekeeping gene using a BioRad iCycler. In addition, anthropometric measurements and analysis of 24 hour excretion rates of glucocorticoids using gas chromatographic-mass spectrometric (GC-MS) analysis were performed.
Results
11beta-HSD1 mRNA expression correlated significantly ($r^2=0.803$; $P<0.001$) with H6PDH mRNA expression. We detected a significant correlation between 11beta-HSD1 mRNA expression and waist-to-hip ratio ($r^2=0.211$; $P<0.05$), but not to urinary (THF+5alphaTHF)/THE ratio, total cortisol metabolite excretion, age or BMI. No gender specific differences were seen in mRNA gene expression.

Discussion
Our data suggest that 11beta-HSD1 gene expression highly depends on H6PDH gene expression. Surprisingly, 11beta-HSD1 gene expression did not correlate with any urinary glucocorticoid ratio showing the limitation of urinary analysis. In our patient's cohort a higher waist-to-hip-ratio (abdominal obesity) was associated with a lower 11beta-HSD1 mRNA expression in the liver.

OC10.3

Selective leptin resistance within the brainstem of histamine deficient mice

Anna Földes, Veronika Márkus & Krisztina Kovács
Institute of Experimental Medicine, Budapest, Hungary.

Histamine is an important anorexic factor that suppresses food intake via hypothalamic H1 receptors and increases energy expenditure by stimulating lipolysis. Mice with targeted deletion of the key enzyme of histamine biosynthesis, histidine decarboxylase (HDC-KO), are unable to synthesize histamine. These animals display a metabolic phenotype with adult onset obesity, selective increase in visceral fat depots, impaired glucose tolerance and hyperleptinemia. To test the possibility that changes in the leptin-induced signal transduction pathways are responsible for leptin resistance in histamine deficient mice, we have analyzed phosphorylation of signal transducer and activator of transcription (STAT-3) a key component of leptin action in target cells. Adult male, wild type and HDC-KO animals were injected ip with leptin and phosphoSTAT-3 (Tyr 705) immunoreactivity was revealed 30 min after injection by conventional avidin-biotin-peroxydase histochemical reaction and the number of phosphoSTAT-3 cell nuclei was counted. Wild type mice display leptin-induced phosphoSTAT-3-ir in the arcuate-, dorsomedial- and ventromedial nuclei in the hypothalamus, in the midbrain as well as in the dorsal vagal complex (DVC) of the brainstem. In histamine deficient mice, the distribution of leptin-responsive neurons and the number of pSTAT-3 ir profiles within the hypothalamus was similar to those seen in wild type animals. In contrast, cells in the dorsal vagal complex of HDC-KO mice display significantly less phospho-STAT-3 -immunoreactivity than the wild type controls in response to exogenous leptin. These data suggest that leptin action in the brainstem, but not in hypothalamus, is specifically impaired in histamine-deficient mice. Defects in leptin signaling in neurons within the DVC may contribute in the pathogenesis of leptin-resistant obesity as well as in the inability of HDC-KO animals to mobilize their energy stores.

OC10.4

Restoration of signalling capabilities in total loss of function MC4R mutations

Harald Brumm[1], Florian Bolze[2], Susann Friedel[3], Anke Hinney[3], Martin Klingenspor[2], Johannes Hebebrand[3], Annette Grüters[1] & Heike Biebermann[1]
[1]Charité Universitätsmedizin Berlin, Inst. Exp. Ped. Endocrinology, Berlin, Germany; [2]Phillipps-Universität, Inst. Biologie/Tierphysiologie, Marburg, Germany; [3]Rheinische Kliniken Essen, Universität Duisburg Essen, Klinik für Psychiatrie und Psychotherapie des Kinder- und Jugendalters, Essen, Germany.

Objectives
The melanocortin 4 receptor (MC4R) belonging to the large superfamily of G-protein coupled receptors plays a crucial role in hypothalamic weight regulation. In approximately 3–5% of investigated obese patients inactivating MC4R mutations are the underlying molecular cause for early onset obesity. Functional characterisation revealed for specific partial loss of function MC4R mutations that restoration of receptor function is possible by usage of highly potent MC4R analogs. The analogue NDP-α-MSH is capable to restore wild type signalling in some cases of partial loss of function. However, for total loss of function receptors this procedure is insufficient.
Methods
To prove functional restoration cell surface expression was determined by cell a surface ELISA approach with N-terminal HA-tagged mutant MC4R. Signalling was determined by cAMP measurement with radioisotope labelled adenine.
Results
In the present study we set out to investigate the restoration of specific total loss of function mutations by usage of bioactive agents. We are able to show that in dependence of the location and the kind of the mutation a functional rescue is possible to different degrees.
Conclusion
This study is the first to show that *in vitro* restoration of signalling properties in total loss of function MC4R is possible.

OC10.5

Mice lacking CRF receptor type 1 (CRFR1) have reduced vulnerability to diet-induced obesity

Yolanda Diz-Chaves[1], Maud Monsaingeon[1], Luc Penicaud[2], Daniel Ricquier[3], Angelo Contarino[1] & Antoine Tabarin[1]
[1]Lab Nutrition et Neurosciences, Bordeaux, France; [2]Lab Neurobiologie et Metabolisme, Toulouse, France; [3]Lab Metabolisme Mitochondrial, Paris, France.

Evidence has accumulated about the involvement of the CRF system in the regulation of energy balance. The effects of CRF are mediated by two receptors: CRFR1 and CRFR2. The role of the CRFR1 in the regulation of energy balance is not well defined. To address this issue, adult male CRFR1 KO mice and WT littermates were given low fat (LFD) or high-fat (HFD) diets for 4 months. Under LFD no differences between genotypes were seen on body weight (BW) and caloric intake. KO mice had lower fat mass ($13.6 \pm 0.6\%$ vs $19.1\% \pm 1.7$; $P < 0.01$) and increased lean mass (26.0 ± 0.4 g vs 23.9 ± 0.6 g, $P < 0.01$). During a HFD, KO mice had similar intake of calories but gained only 10% of the fat mass that the WT mice did, indicating a reduced feeding efficiency. 24-h locomotor activity was similar between genotypes. Plasma FFA and Betahydroxybutyrate levels in KO mice suggested increased fat oxidation and KO mice had a increased expression of UCP 1 in BAT. Since CRFR1 deletion impairs the HPA axis activity, KO mice were given 5 μg/ml of Cort (KO-Cort) or vehicule (KO-Veh) in drinking water. After two weeks on HFD, BW increases in KO-Cort mice and reached that of WT mice after 16 weeks. Cort supplementation decreased biological markers of fat oxidation in KO-cort mice to the levels of WT mice. No difference in muscle expression of enzymes involved in FFA oxidation was found between groups. Conclusion: CRFR1 have constitutively reduced fat mass, increased fat oxidation and BAT thermogenic activity resulting in a reduced vulnerability to diet-induced obesity. The decreased vulnerability to HFD-induced obesity in CRFR1 KO mice seems to depend mainly of their constitutively low corticosterone secretion.

Supported by Grants from ATC Nutrition, FRM and Conseil Régional d'Aquitaine

OC10.6

3-Iodothyronamine (T1AM) is a novel modulator of metabolic rate and glucose homeostasis

David K. Grandy, Katherine L. Suchland, Thomas M. Keck, Feifei Yan, Show-Ling Shyng & Thomas S. Scanlan
Oregon Health & Science University, Portland, Oregon 97239, United States.

3-Iodothyronamine (T1AM) is a novel endogenous derivative of thyroid hormone (TH), recently described by Scanlan *et al.* (*Nat. Med.* **10**: 638, 2004). *In vitro*, T1AM can stimulate the production of cAMP via activation of a heterologously expressed G protein-coupled receptor (GPCR) now referred to as trace amine-associated receptor 1 (TAAR1; Lindemann et al. *Genomics* **85**: 372, 2005). In adult, unanesthetized C57Bl6/J mice, T1AM produces profound and long-lasting anergia, bradycardia, hypophagia, and hypothermia ($-10\,^{\circ}C$ @ $T_{ambi} = 24\,^{\circ}C$). In an effort to better understand these manifestations of T1AM, we evaluated its effect on metabolic rate. In addition, experiments were performed to characterize T1AM's effect on blood sugar and the pancreatic hormones glucagon and insulin. Finally, the effect of T1AM on an *in vitro* cellular model of glucose-stimulated insulin release was investigated. Within minutes of its injection (i.p.) into male mice housed at $T_{amb} = 22\,^{\circ}C$, and prior to the development of hypothermia, T1AM (25 mg/kg) reversibly depressed metabolic rate ~50% of vehicle-injected controls, as measured by oxygen consumption (ml/g/min). Also within minutes, T1AM dose-dependently elevated blood sugar, reaching a maximum of ~320 mg/dL, almost 3 times normal, by 3.5 hrs post injection. By 2 hrs post-injection, T1AM had produced a dose-dependent increase in circulating glucagon (~400 pg/ml) that was nearly twice the vehicle controls. Furthermore, T1AM (50 mg/kg) administered to fasted mice (26 hrs) prior to their receiving a bolus of D-glucose (3 g/kg, i.p.) blocked the sugar's ability to stimulate circulating insulin levels compared to vehicle-treated mice. Finally, *in vitro* studies revealed T1AM could dose-dependently prevent glucose-stimulated insulin release from cultures of rat INS1823/13 insulinoma cells. Taken together, these results support the thesis that T1AM is a rapid-acting novel modulator of metabolism with actions opposite in direction to those of TH. As such, T1AM and its related compounds may signal via one or more GPCRs to fine-tune TH's effects and thereby help the organism efficiently meet its metabolic needs minute-to-minute.

OC10.7

Serum level of retinol binding protein 4 in obese individuals with insulin resistance and with type 2 diabetes mellitus treated by metformin

Mária Tajtáková[1], Zuzana Semanová[1], Gabriela Ivancová[2], Jozefína Petrovicová[3], Viera Donicová[2] & Eva Zemberová[4]
[1]1st Department of Internal Medicine, University of P.I.Šafárik, Košice, Slovakia; [2]Department of Diabetes, Košice, Slovakia; [3]Institute of Medical Informatics, University of P.I. Šafárik, Košice, Slovakia; [4]RIA Laboratory, Košice, Slovakia.

Objective
To reveal whether there are differences in serum level of retinol binding protein 4 (RBP4) in obese individuals with insulin resistance (IR) and without diabetes in comparison to those with 2 type diabetes mellitus (2 DM) treated by metformin and not obese controls.
Methodology
The serum level of retinol binding protein 4 was examined by RIA method in 28 obese individuals with insulin resistance, 11 patients with 2 type diabetes mellitus treated by metformin and 17 controls. The results were compared within groups. RBP4 in the group with IR and in controls was correlated with insulin.
Results
The highest level of RBP4 (561.6 ± 209 ng/ml) was found in obese individuals with IR (IRHOMA 3.9) and the lowest level in patients with 2 DM treated by metformin (391.1 ± 133.5 ng/ml, $P < 0.01$). The controls had significantly lower level of RBP4 in comparison to obese individuals with IR (452.8 ± 104.6 ng/ml $P < 0.05$), however, RBP4 was not significantly higher in comparison to obese individuals with 2 DM treated by metformin (391.1 ± 133.5 ng/ml). RBP4 correlated with insulin ($r = 0.46$, $P < 0.03$).
Conclusions
The increase of RBP4 in obese individuals through a back regulation GLUT4 in adipocytes contributes to the development and worsening of IR. Thus, metformin by influencing the expression of RBP4 in adipocytes can improve the overall insulin sensitivity in obese individuals (also with MS) and slower the manifestation of 2 DM. RBP4 could be considered as a marker of the worsening tolerance of glocose in obese individuals.

Reproductive Endocrinology 2 – OC11

OC11.1

Hypogonadotrophic hypogonadism in mice lacking a functional Kiss-1 gene

Xavier d'Anglemont de Tassigny[1], Mark Carlton[2] & William Colledge[1]
[1]PDN, University of Cambridge, Cambridge, United Kingdom, [2]Paradigm Therapeutics Ltd, Cambridge, United Kingdom.

Activation of the G-protein coupled receptor GPR54 (AXOR12, OT7T175) by peptide ligands (kisspeptins) encoded by the *Kiss-1*gene is central to acquisition of reproductive competency in mammals. Administration of exogenous kisspeptins stimulates GnRH release from hypothalamic neurons in several species including humans. To confirm that kisspeptins are the natural agonist of GPR54 *in vivo* and to determine if these ligands have additional physiological functions, we have generated mice with a targetted disruption of the *Kiss-1*gene. *Kiss-1* null mice are viable but fail to undergo sexual maturation at puberty. Mutant female mice do not progress through the oestrus cycle, have thread-like uteri, small ovaries and do not produce mature Graffian follicles. Mutant males have small testes and spermatogenesis arrests mainly at the early haploid spermatid stage. Both sexes have low circulating gonadotrophin (LH and FSH) and sex steroid (β-estradiol or testosterone) hormone levels. Migration of GnRH neurons into the hypothalamus appears normal with appropriate axonal connections to the median eminence and total GnRH content. The hypothalamic-pituitary axis is functional in these mice as shown by robust LH secretion after peripheral administration of kisspeptin-10. These data provide the first direct proof that kisspeptins are the true physiological ligand for the GPR54 receptor *in vivo* and that loss of *Kiss1* cannot be overcome by compensatory mechanisms.

OC11.2

Leukemia inhibitory factor promotes the chemomigration of immature GnRH neurons

Elena Dozio[1], Massimiliano Ruscica[1], Anna Cariboni[1], Hajime Watanobe[2], Marcella Motta[1], Roberto Maggi[1] & Paolo Magni[1]
[1]University of Milan, Milano, Italy; [2]University of Health and Welfare, Otawara, Japan.

Leukemia inhibitory factor (LIF), a pleiotropic cytokine of the interleukine-6 superfamily, is involved in several functions including the control of reproduction at the embrionic-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 β (LIFRβ) subunit and the gp130 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 LIFRβ and gp130 genes and proteins. Exposure to 100 ng/mL LIF activated the Janus kinases (Jak)-signal transducer and activator of transcription 3 (STAT3), the mitogen-activated protein kinase (MAPK)-extracellular regulated kinase 1/2 (ERK1/2) and the 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 Jak involved in LIF signalling. LIF stimulated chemotaxis at a concentration-dependent manner, with a plateau at 100 ng/mL after both 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 may modulate the development of the reproductive axis by directly influencing the migration of GnRH neurons to the hypothalamus.

OC11.3

Sex steroid and leptin regulation of KISS1/GPR54 system, a new regulator of the neuroendocrine reproductive axis, in human fetal GnRH-secreting neurons

Annamaria Morelli[1], Mirca Marini[2], Rosa Mancina[1], Linda Vignozzi[1], Anna Pezzatini[3], Michaela Luconi[3], Sara Mungai[1], Benedetta Fibbi[1], Sandra Filippi[4], Gabriella Barbara Vannelli[3], Gianni Forti[3] & Mario Maggi[1]
[1]University of Florence, Dept. Clinical Physiopathology, Andrology Unit, Florence, Italy; [2]University of Florence, Anatomy, Histology and Forensic Medicine, Florence, Italy; [3]University of Florence, Dept. Clinical Physiopathology, Endocrinology Unit, Florence, Italy; [4]University of Florence, 2Interdep. Lab of Functional and Cellular Pharmacology of Reproductio, Florence, Italy.

The molecular mechanisms underlying the reawakening of hypothalamic GnRH neurons at puberty remain to be elucidated. Recently, the G protein-coupled receptor 54 (GPR54) and its endogenous ligand kisspeptin, encoded by the KISS1 gene, have been involved. In fact, GPR54 mutations cause idiopathic hypogonadotropic hypogonadism in human and mice. We used the previously characterized primary culture of human fetal olfactory GnRH-secreting neurons, FNC-B4, to study *in vitro* the KISS-1/GPR54 regulation. Kisspeptin and GPR54 were immunolocalized in fetal olfactory mucosa, and in FNC-B4. Using confocal microscopy, co-expression of GnRH and GPR54 or GnRH and kisspeptin was found in fetal olfactory mucosa and FNC-B4. The 24 h exposure to sex steroids regulated both gene (qRT-PCR) and protein (western blot and immunocitochemistry) expression of KISS1/GPR54 in FNC-B4. Increasing doses of 17beta-estradiol (0.01–1 nM) significantly and dose-dependently decreased KISS1/GPR54 mRNA. Conversely, androgens (DHT, 0.01–1 nM) significantly stimulated KISS1/GPR54 mRNA. Immunofluorescence with anti-kisspeptin confirmed that 1 nM 17beta-estradiol significantly reduced, whereas 1 nM DHT significantly increased, the % of kisspeptin-positive FNC-B4 cells. Testosterone treatment showed no effect, but, blocking its aromatization with letrozole, it mimicked DHT stimulatory activity. In addition, 24 h exposure to leptin (1 nM), an adypocyte-derived hormone acting on the hypothalamus to influence puberty, significantly increased KISS1/GPR54 gene and protein expression. Leptin treatment in FNC-B4 significantly increased also the androgen receptor (AR) mRNA, as well as the mRNA of its own receptor (LEPR), which resulted induced also by 1nM DHT. These data suggest a synergistic action between AR and LEPR to finally up-regulate KISS1/GPR54 system, which, in contrast, was inhibited by estrogen. In conclusion, our results revealed for the first time that sex steroids and leptin regulate KISS-1/GPR54 system in human GnRH neurons, providing new insights into the comprehension of those permissive signals for pulsatile GnRH secretion and puberty onset.

OC11.4

EGFR ligands mediate key events of female reproduction: reduced litter size due to impaired fertilization in a transgenic mouse model

Marlon R. Schneider[1], Ana A. Gratao[1], Maik Dahlhoff[1], Fred Sinowatz[2] & Eckhard Wolf[1]
[1]Institute of Molecular Animal Breeding and Biotechnology, University of Munich, Munich, Germany; [2]Institute of Veterinary Anatomy, University of Munich, Munich, Germany.

EGFR ligands, a family of seven related peptide growth factors, are emerging as key factors regulating different aspects of female reproduction including oocyte maturation and ovulation, and implantation. Betacellulin (BTC) is a rather neglected EGFR ligand whose biological activities have been mostly associated with the endocrine pancreas. During the routine breeding of recently established BTC transgenic mouse lines (Schneider et al., Endocrinology 146, 5237–5246, 2005), reduced female fertility became evident. Thus, a systematic study of different aspects of female reproduction was carried out. While puberty onset and estrous cyclicity were not affected in the transgenic animals, controlled matings revealed reduced litter size as the major reproductive deficit of BTC transgenic females (5.3 ± 0.7 *vs.* 9.9 ± 0.3 pups/litter in non-transgenic controls). Embryo implantation (visualized by injection of blue dye) was shown to be delayed. However, the number of embryos implanted or recovered from the uterus was already reduced by about 50% in the transgenic group, indicating that delayed implantation was not the cause of reduced litter size. Collection of oocytes from transgenic and control females mated to non-transgenic males revealed that the number of ovulated oocytes was not different between the groups (10.4 *vs* 10.7, respectively). However, the proportion of fertilized oocytes recovered from transgenic females was significantly reduced (54% *vs.* 81.7%). Next, *in vitro* maturation (IVM) and fertilization (IVF) were carried out to study these aspects more closely. While IVM rate was only slightly affected, the proportion of fertilized oocytes obtained from transgenic females was strongly reduced as compared to the rate observed in oocytes derived from the control group (57.5% *vs* 84.6% cleavage rate). Localization of strong transgene-derived BTC levels in the cumulus and granulosa cells of transgenic follicles supports this observation. In summary, excess of BTC perturbs oocyte maturation and fertilization. Implantation is delayed but appears to have no consequence for the overall reproductive performance of transgenic females.

OC11.5

Integration of the EGF network with early LH signal in preovulatory follicles

Sara Panigone[1], Minnie Hsieh[2], Luca Persani[1] & Marco Conti[2]
[1]Lab Experimental Endocrinology, IRCCS Istituto Auxologico Italiano, University of Milan, Cusano Milanino, MI, Italy; [2]Division of Reproductive Biology, Department of Obstetrics and Gynecology, Stanford, University School of Medicine, Stanford, CA, United States.

Recent studies demonstrate an essential role of the EGF network in propagating the LH signal within ovarian preovulatory follicles. However, the molecular bases for the integration are poorly characterized. Here, we propose that the early LH signal leading to ovulation is amplified through activation of the EGF network.

For this study, preovulatory follicles from euthanized gonadotropin-primed mice were isolated and cultured with or without recombinant LH (rLH) and/or specific inhibitors. Primary granulosa cells were used in additional experiments. Analysis of EGF receptor (EGFR) and MAPK activation was performed by immunoprecipitation, western blot and immunohistochemistry (IHC). An increase in EGFR phosphorylation was detected as early as 30 minutes after LH stimulation. This activation is most likely cAMP dependent and sensitive to AG1478, an EGFR kinase inhibitor, as well as to inhibitors of matrix-metalloproteases (GM6001 and TAPI-1), suggesting the involvement of shedding of EGF-like factors in LH-induced EGFR transactivation. A target of EGFR signaling is the MAPK pathway. In IHC assays, signal for phosphorylated MAPK was observed in mural granulosa cells of preovulatory follicles within 15–30 minutes of hCG stimulation, and in both granulosa and cumulus cells after 1 h. In cultured follicles, LH-induced MAPK activation is partially inhibited by AG1478 and GM6001, indicating that this pathway is regulated in part by the EGF network. Furthermore, treatment of granulosa cells with a combination of neutralizing antibodies against amphiregulin, epiregulin and betacellulin (EGF-like factors described as regulators of ovulation) significantly inhibits EGFR phosphorylation and MAPK activation, supporting a role for these ligands in the LH-induced EGFR signaling in mural granulosa cells.

In conclusion, we provide evidence of early activation of EGF network following LH stimulation, involving rapid shedding of EGF-like ligands and EGFR transactivation. This mechanism partecipates in the rapid amplification and propagation of the LH signal within preovulatory follicles.

OC11.6

Visceral fat amount as predictor for subclinical cardiovascular disease in women with polycystic ovary syndrome

Teresa Cascella[1], Stefano Palomba[2], Ilario De Sio[3], Francesco Manguso[4], Laura Vuolo[1], Francesco Giallauria[5], Alessandra Grieco[1], Gaetano Lombardi[1], Annamaria Colao[1], Domenico Tafuri[6] & Francesco Orio[7]
[1]Department of Molecular & Clinical Endocrinology and Oncology, "Federico II" University of Naples, Naples, Italy; [2]Department of Obstetrics and Gynecology, University "Magna Graecia" of Catanzaro, Catanzaro, Italy; [3]Department of Internal Medicine, Hepatology, and Gastroenterology, Second University of Naples, Naples, Italy; [4]Gastroenterology Unit, "Cardarelli" Hospital, Naples;, Naples, Italy, [5]Department of Clinical Medicine, Cardiovascular and Immunological Sciences, Cardiac Rehabilitation Unit, Institute of Internal Medicine and Metabolic Disease, "Federico II" University of Naples, Naples, Italy; [6]Teaching and Methods of Sportive Activity, "Parthenope" University of Naples, Naples, Italy; [7]Endocrinology, Faculty of Motor Science, "Parthenope" University of Naples and Department of Molecular & Clinical Endocrinology and Oncology, "Federico II" University of Naples, Naples, Italy.

Introduction and aim
Polycystic Ovary Syndrome (PCOS) is a common disorder associated with a wide range of endocrine and metabolic abnormalities. Obesity is present in about 45–50% of PCOS women. Increased cardiovascular risk factors and evidence of subclinical cardiovascular disease (CVD) have been reported in PCOS. The aim of the present study was to evaluate whether visceral fat amount may be considered as predictor for early CVD in PCOS women.
Patients and methods
The procedures used were in accordance with the guidelines of the Helsinki Declaration on human experimentation. The study was approved by the local Ethical Committee. Two-hundred PCOS women and 100 healthy age- and body mass index-matched women were enrolled in this prospective baseline-controlled clinical study. Non-invasive markers of early CVD [carotid intima-media thickness (IMT), brachial arterial flow-mediated dilation (FMD)] and visceral fat amount [using abdominal ultrasonography] were evaluated. Inflammatory biomarkers [C-reactive protein (CRP), fibrinogen, white blood cells (WBC) count, plasminogen activated inhibitor (PAI)-1], hormonal and metabolic parameters were also investigated.
Results
Subjects with PCOS had significantly ($P<0.001$) higher visceral fat compared to healthy women [$31.4+7.3$ *vs.* $28.0+6.1$, mm + SD), respectively] which were directly related to HOMA ($r=0.918$, $P<0.001$), AUC_{INS} ($r=0.879$, $P<0.001$) and WC ($r=0.358$; $P<0.001$). Stepwise linear regression model showed that visceral fat amount was an independent predictor of IMT, FMD and CRP.
Conclusions
The early impairment of endothelial structure and function, the increase of low-grade chronic inflammation and insulin resistance in women with PCOS are associated with increased central fat excess. Visceral fat amount could be an important predictor of subclinical CVD in PCOS.

OC11.7

The current definitions of the metabolic syndrome underestimate the prevalence of nascent metabolic abnormality in adolescents with PCOS

Didier Dewailly, Sophie Catteau-Jonard & Anne-Céline Reyss
Lille University Hospital, Lille, France.

Background
The prevalence of the metabolic syndrome (MS) is notably higher in patients with PCOS than in the general population. Presumably, this prevalence increases as a function of age but the subgroups of patients less than 20 yr. old studied so far are small.
Design
In order to further document this issue, we have selected for this study 498 patients with PCOS aged 12.5–38 yr (Rotterdam definition) and 188 control women aged 15.5–38.5 yr, that have been consecutively included in a database and in whom the required clinical, hormonal and ultrasound data were available. A metabolic score has been calculated according to the ATP-III classification and defined the MS when ≥ 3.
Results
The prevalence of the MS was significantly higher in the PCOS than in the control group (15.2% *vs* 4.8%, $P<0.0001$). It did not differ significantly ($P=0.063$) between the 3 subgroups of patients with PCOS according to age, i.e., 12.8% in patients aged $\leq$ 0 yr ($n=47$), 13.9% in patients aged 21–30 yr ($n=301$) and 18.7% in patients aged 31–40 yr ($n=150$). However, we observed that a metabolic score of 1 or 2 tended to be more frequent in the adolescent group than in the groups of older patients

(cumulated rate 1+2: 66.0% *vs* 51.8% and 48.7%, respectively, $P=0.09$). In the patients having a score=1, a HDL-Cholesterol (HDL-C) <0.5 g/L was found in 57.1% adolescents *vs* 26.3% and 27% patients aged 21–30 and 31–40 yr, respectively ($P=0.068$). In the same group, a waist circumference (WC) >80 cm was found in 35.7% adolescents *vs* 69.7% and 70.3% patients aged 21–30 and 31–40 yr, respectively ($P=0.047$). In patients with a score=2, a HDL-C <0.5 g/L and a WC>80 cm were found in 100% of cases in every subgroup.

Conclusions

By requiring some items that reflect relatively late complications of insulin resistance (hypertriglyceridemia, hypertension, glucose intolerance or diabetes), the current definitions of the MS in adults underestimate the prevalence of nascent metabolic abnormality in adolescents. Our data indicate that a HDL-C <0.5 g/L is the most sensitive marker of such abnormality, while a WC >80 cm seems to be less sensitive than in adults.

Diabetes – OC12

OC12.1

Hypoglycemia and cerebral ATP synthesis in Type 1 Diabetes

Martina Mandl[1], Stefan Zbyn[1], Vladimir Mlynarik[2], Miriam Promintzer[1], Anton Luger[1], Michael Krebs[1], Christian Anderwald[1] & Martin G. Bischof[1]
[1]Division of Endocrinology and Metabolism, Department of Internal Medicine III, Medical University of Vienna, Vienna, Austria; [2]MR Center of Excellence, Medical University of Vienna, Vienna, Austria.

The mechanisms responsible for the progressive failure of hypoglycemia counter-regulation in type-1 diabetes (T1DM) are poorly understood. Alterations of brain energy metabolism could influence glucose sensing by the brain and, thus contribute to hypoglycemia associated autonomic failure. Thus, we measured intraneuronal kinetics of total ATP-synthesis from PCr (k_{for}) in T1DM patients and effects of hypo/hyperglycemia on this brain energy metabolism. Healthy nondiabetic humans (CON; 5 m/1f, BMI=23.5±1.0 kg/m^2, age=25±1 yr, HbA1c=5.1±0.1%), T1DM patients with good ($T1DM_{good}$; 5 m/1f, BMI=25.5±0.4 kg/m^2, age=24±2 yr, HbA1c=6.8±0,1%) and poor ($T1DM_{poor}$; 5 m/1f, BMI=24.9±1.6 kg/m^2, age=25±2 yr, HbA1c=8.9±0.3%) glycemic control were examined before, during and after hyperinsulinemic-(1.5 mU·kg^{-1}·min^{-1})-hypoglycemic-(~50 mg/dl) or –hyperglycemic-(~250 mg/dl)-clamp tests. k_{for} in the occipital lobe was measured by ^{31}P-nuclear-magnetic-resoance spectroscopy (3T) using saturation transfer, and calculated with *McConnell* equations. In $T1DM_{poor}$, k_{for} was increased during hypoglycemia (0.58±0.07 s^{-1}), when compared to CON (0.36±0.03 s^{-1}; $P=0.006$), $T1DM_{good}$ (0.41±0.02 s^{-1}; $P=0.03$), and baseline (0.43±0.05 s^{-1}; $P=0.03$). During post-hypoglycemic recovery, $T1DM_{poor}$ showed higher k_{for} (0.57±0.07 s^{-1}), when compared to CON (0.40±0.05 s^{-1}, $P<0.05$), and $T1DM_{good}$ (0.37±0.01 s^{-1}, $P=0.03$). HbA1c-levels were positively correlated with k_{for} during hypoglycemia ($r=0.47$, $P=0.02$), but not at baseline ($r=0.20$, $P=0.37$) or during recovery ($r=0.39$, $P=0.07$).

Conclusion

^{31}P NMRS with saturation transfer can be used for non-invasively measurement of cerebral ATP-synthesis during hypoglycemia *in vivo*. The positive correlation of HbA1c levels and k_{for} during hypoglycemia hints at an involvement of the CK system in the pathogenesis of hypoglycemia associated autonomic failure.

OC12.2

Uncoupling protein 2 mutations – a new explanation for congenital hyperinsulinism?

Henrik Thybo Christesen[1], Khalid Hussain[3], Lone Svargo[2], Bendt Brock Jacobsen[1] & Klaus Brusgaard[2]
[1]HC Andersen Children's Hospital, Odense, Denmark; [2]Dept. of Clinical Genetics, Odense University Hospital, Odense, Denmark; [3]London Center for Paediatric Endocrinology and Metabolism, Great Ormond Street Hospital, London, United Kingdom.

Background

Congenital Hyperinsulinism (CHI) is genetically unexplained today in up to 50% of the patients with persistent or recurrent disease. The uncoupling protein 2 (UCP2) gene is a candidate gene for medical-responsive CHI, since knock out studies have shown that UCP2 deficiency leads to increased glucose-stimulated insulin secretion.

Patients and methods

In a large series of 142 patients with transient, persistent or recurrent CHI, we examined for mutations using DHPLC and direct sequencing, or cutting with restriction enzyme for specific variations, in the known disease-causing genes *ABCC8* ($n=141$), *KCNJ11* ($n=140$), *Gck* ($n=21$), *GLUD1* ($n=27$), *SCHAD* ($n=10$), and *UCP2* ($n=46$), (number of investigated patients in brackets).

Results

In 53 of all patients (37%), a genetic explanation was found, while 90 patients had no mutations detected. Of these, 46 had persistent or recurrent medical-responsive hyperinsulinaemic hypoglycaemia and available DNA for *UCP2* analysis. No mutations were found in *UCP2*. The well-known polymorphism A55V was seen in 29 patients.

Conclusion

UCP2 mutations are rarely – if ever – found in CHI patients with persistent or recurrent CHI. Other genetic explanations should be considered.

OC12.3

Adhesion molecules two years after gestational diabetes

Thomas Prikoszovich[1], Giovanni Pacini[2], Christine Winzer[1], Anton Luger[1], Oswald Wagner[3] & Alexandra Kautzky-Willer[1]
[1]Medical University Vienna, Department of Internal Medicine III, Division of Endocrinology and Metabolism, Vienna, Austria; [2]Institute of Biomedical Engineering, CNR, Padova, Italy; [3]Medical University Vienna, Department of Medical and Chemical Laboratory Diagnostics, Vienna, Austria.

Objectives

We investigated in women with prior GD (pGD) at risk of diabetes and premature atherosclerosis in comparison to women with normal glucose tolerance during and after pregnancy (C) parameters of inflammation, endothelial dysfunction and glucose tolerance in a follow-up study.

Methods

119 pGD and 41 C underwent an oral glucose tolerance test 3 months, 1 and 2 years after delivery with measurements of plasma concentrations of circulating adhesion molecules (cAMs: VCAM, ICAM-1, ELAM), endothelin, leptin, sCRP, IL-6, fibrinogen, PAI-1 and ADMA. Intima-media-thickness (IMT) of the common carotid artery was measured by ultrasound and insulin sensitivity (S_I) was calculated from insulin-modified FSIGTs at baseline.

Results

At baseline ICAM ($P<0.0001$), VCAM ($P<0.005$), ADMA ($P=0.0005$), sCRP ($P=0.04$) and PAI-1 ($P=0.01$) were higher and S_I ($P=0.001$) was lower in pGD than in C. S_I inversely related to all cAMs ($r=-0.20$; $P<0.02$), sCRP ($r=-0.52$; $P<0.0001$), IL-6 ($r=-0.25$; $P=0.01$), and fibrinogen ($r=-0.22$; $P=0.006$). All cAMs also related to leptin ($r=0.17$; $P<0.04$) and BMI ($r=0.18$; $P<0.03$). IMT was associated with S_I ($r=-0.32$; $P=0.03$), BMI ($r=0.31$; $P=0.02$) and PAI-1($r=0.30$; $P=0.03$). After two years ELAM ($P<0.02$), ADMA ($P<0.0007$), PAI-1 ($P<0.001$), vWF ($P<0.04$), blood pressure ($P<0.001$) decreased, while ICAM-1, VCAM and BMI remained unchanged. Leptin ($P=0.01$), TNFα ($P<0.001$) and endothelin ($P<0.04$) increased compared to baseline. Higher age ($P<0.05$) and BMI ($P<0.0001$), increased levels of ELAM ($P<0.003$), Leptin ($P<0.0005$) and a lower insulin sensitivity (OGIS;$P=0.01$) at baseline characterised those pGD with deterioration of their initial normal glucose tolerance ($n=15$) in comparison to those who retained normal glucose tolerance ($n=65$) within 2 years. Logistic regression revealed BMI (OR[CI]: 1.313[1.03-1.67]) and ELAM (OR[CI]: 1.064[1.01-1.12]) as independent predictors of a deterioration of glucose tolerance.

Conclusion

Women with pGD are characterised by higher plasma ICAM and VCAM relating to insulin-resistance and inflammatory parameters. Moreover the degree of obesity and ELAM at baseline predicted deterioration of glucose tolerance within 2 years after delivery.

OC12.4

Polymorphisms of PSMA6 gene and its adjacent genomic sites and their association with type II diabetes mellitus in the Latvian population

Ilva Poudziunas, Tatjana Sjakste, Martins Kalis, Maris Lagzdins, Valdis Pirags, Nikolajs Sjakste & Jelizaveta Sokolovska
University of Latvia, Riga, Latvia.

Introduction

A possible involvement of proteasomes in the pathogenesis of type II diabetes mellitus has been recently reported. Therefore, association of polymorphism of proteosomal genes with type II diabetes mellitus is of particular interest. In this study, molecular markers of the proteasomal alpha subunit 6 gene PSMA6 and its adjacent genomic sites have been analyzed.

The goal of this study was to characterize polymorphisms of the HSMS801, HSMS702, HSMS701 and HSMS602 HSMS006 HSMS602 microsatellite

repeats and SNPs at positions –110 and –8 from the translation start of PSMA6 gene and to investigate their eventual association with type II diabetes mellitus.
Methods
In this study, 250 DNA samples of type II diabetes and healthy controls were used. Genotyping was performed using allele-specific PCR and restriction fragment analysis.
Results
For the HSMS006 marker, the 193 bp allele was more common the group of cases rather than controls (0.154 and 0.085 respectively, $P=4.64\%$). HSMS801 allele of 155 bp was found more often in the control group, as the HSMS602 marker allele of 169 bp. HSMS801 genotype of 148 bp/152 bp was more frequent in the control group (0.000 and 0.041 respectively, $P=4.22\%$). Significant differences were observed between cases and controls in all ten haplotype distributions created by combinations of all the microsatellites by two. In these combinations linkage disequilibrium was revealed, indicating the non-random association of alleles in two or more loci on a chromosome. Genotype –8CG was significantly more frequent in type 2 diabetes patients, and haplotype C^{-110}/G^{-8}, compared to $C^{-110}/C^{-}8$ was associated with a higher risk of type II diabetes.
Conclusion
These results show association between microsatellite and SNP alleles of PSMA6 gene and its adjacent genomic sites with type II diabetes mellitus.

OC12.5

The influence of concomitant diabetes mellitus on mortality in Addison's disease

Ragnhildur Bergthorsdottir[1], Maria Leonsson-Zachrisson[1], Anders Odén[2] & Gudmundur Johannsson[1]
[1]Department of Endocrinology, Sahlgrenska University Hospital, Gothenburg, Sweden; [2]Department of Mathematical Sciences, Chalmers University of Technology, Gothenburg, Sweden.

Background
The prevalence of type 1 diabetes mellitus (DM) among patients with primary adrenal insufficiency (Addison's disease) is 3-4 times higher than the expected prevalence in the population. The mortality rate due to DM is more than 3-fold the mortality rate in the background population. The impact of DM on mortality rate in patients with Addison's disease is not known.
Objectives
To study the frequency of DM and its impact on mortality rate in patients with Addison's disease.
Study design
In a population-based retrospective observational study between the years 1987 and 2001 using the Swedish Hospital Register we followed patients from the first registered hospitalisation where the diagnosis of Addison's disease appeared until end of follow-up or death. We looked for the concomitant presence of DM at the time of detection.
Results
We identified 1675 patients, 995 women and 680 men, diagnosed with primary adrenal insufficiency. Concomitant DM was observed in 199 (12%) of the identified patients. DM had a significant influence on total mortality with the relative risk (RR) for death 1.82 (CI 1.29–2.06) for men and 1.52 (CI 1.11–2.07) for women with Addison's disease and DM compared with those patients with Addison's disease without DM.
The impact of DM on the excess mortality in the whole group of Addison's patients was limited since excluding patients with concomitant DM only decreased the RR for death by 7% in both men (2.19 *vs* 2.04) and women (2.86 *vs*. 2.68).
Conclusions
Having DM and Addison's disease significantly increased the risk of death when compared with having Addison's disease alone. However, the overall impact of concomitant DM on the total mortality in all patients with Addison's disease was minor.

OC12.6

Short-term effects of atorvastatin on endothelial functions and oxidized LDL levels in type 2 diabetic patients

Aysen Akalin[1], Gokhan Temiz[2], Nevbahar Akcar[3] & Banu Sensoy[3]
[1]Osmangazi University Medical Faculty Department of Endocrinology, Eskisehir, Turkey; [2]Osmangazi University Medical Faculty Department of Internal Medicine, Eskisehir, Turkey; [3]Osmangazi University Medical Faculty Department of Radiology, Eskisehir, Turkey.

Objective
We aimed to investigate the short term effects of atorvastatin on endothelial function and oxidized LDL (oxLDL) levels and to evaluate the association of endothelial dysfunction to oxLDL levels and inflammatory markers in type 2 diabetic patients.
Material and methods
After ethical committee approval thirty type 2 diabetic and 11 healthy subjects with LDL levels between 100–160 mg/dl. Without a history of cardiovascular event were included in the study.Both groups were matched with respect to age, gender, body mass indices, body composition and lipid levels. Flow-mediated dilatation (endothelium-dependent, FMD) and nitroglycerine-induced dilatation (endothelium- independent, NID) were measured in the brachial artery using high-resolution ultrasound in all participants. Carotid artery intima media thickness (IMT) was also evaluated. OxLDL levels, lipid parameters, blood glucose, C-peptide, HbA1c and inflammatory markers including C-reactive protein(CRP), fibrinogen, erithrocyte sedimentation rate (ESR) were studied. Type2 diabetic patients recived 10 mg. Atorvastatin for 6 weeks and FMD, NID, IMT reevaluated and ox-LDL levels and inflammatory markers were measured.
Results
Basal FMD, NID, IMT and ox-LDL levels besides inflammatory markers were not significantly different between patients and controls. No correlation was found between inflammatory markers and FMD and NID. Only IMT correlated with the NID and fibrinogen levels obtained before treatment. In nondiabetics, IMT also correlated with oxLDL levels (*P*:0.013). FMD and NID significantly improved after atorvastatin therapy (7.62 ± 7.6 *vs*. 12.65 ± 7.8, $P<0.001$ and 18.22 ± 9.57 *vs*. 21.43 ± 9.6, $P=0.007$, respectively). Atorvastatin significantly reduced ox-LDL levels (57.85 ± 10.33 *vs*. 44.36 ± 6.34, $P<0.001$) and IMT (0.627 ± 0.17 *vs*. 0.597 ± 0.16, *P* 0.02) in diabetics.
Conclusions
Atorvastatin improves endothelial functions and reduces oxLDL levels in type 2 diabetics with average lipid levels in the short term and may have beneficial effects in the prevention of early atherosclerotic changes.

OC12.7

A propensity-based comparison of haemodialysis and peritoneal dialysis among diabetic patients with end-stage renal disease in the United States

LA O'Shea & AG Stack
Department of Renal Medicine, LGH, Co. Donegal, Ireland.

Renal transplantation is the optimal treatment strategy for patients with end-stage renal disease (ESRD); few are afforded the opportunity due to limited organ supply. Of the alternatives, peritoneal dialysis (PD) and hemodialysis (HD), it is unclear which confers the greater survival advantage, as prior comparisons have demonstrated conflicting results due to lack of case-mix adjustment, limited follow-up, and failure to consider switches in modality over time.

We compared all-cause and cause-specific mortality between PD and HD in national cohort of 263,556 new ESRD patients in the U.S. who began treatment between 5/1995 and12/2000, and followed until 12/2001. A propensity analysis, predicting the probability of assignment to PD, was used to control for baseline differences through regression adjustment and matching based on 23 demographic and comorbid indicators. The C-statistic for this model was 0.75, indicating excellent discrimination between treatments. Time-dependent Cox regression, stratified by age and diabetes, compared PD and HD using an intent-to-treat and as-treated approach and patients were censored at transplantation, loss to follow-up or end of study.

There were 122,672 deaths (46.5%), 24,596 renal transplants (9.3%) and 17,432 (6.6%) patients lost to follow-up within the 6-yr period. The adjusted relative PD/HD hazards ratios [RR] with 95% Confidence Intervals for all-cause and cause-specific mortality are shown (intent-to-treat analysis).

Mortality risks were significantly greater for PD compared with HD among diabetic patients and were principally confined to older patients. The excess mortality could be accounted for, in decreasing order, by increased death risk from infection, cardiac, stroke and the other causes of death category.

In conclusion, haemodialysis should be preferentially considered over PD among older (>50 yrs) diabetics with ESRD in order to improve patient survival.

Poster Presentations

Comparative Endocrinology – presented on Sunday

P1

Human adrenal NCI-H295R cells produce more C19 steroids than NCI-H295A cells – a possible model to study regulation of androgen biosynthesis?

Elika Samandari, Petra Kempna, Gaby Hoffer, Primus E Mullia & Christa E Fluck
University Hospital, Bern, Switzerland.

The human adrenal cortex consists of three layers in which specific steroid hormones are produced. Human adrenal NCI-H295A (A) and NCI-H295R (R) cells, originate from the same adrenocortical tumor and express all genes essential for steroidogenesis. Therefore they often serve as a suitable model to study human steroidogenesis. No data are available comparing steroidogenesis of A vs. R cells. Assuming no difference, research data from these two cell lines are directly compared. To characterize A and R cells, we investigated steroidogenesis of both cell lines. We found differences in the steroid profile of A and R cells. A cells converted [^{3}H]-pregnenolone predominantly to aldosterone and cortisol while only traces of androgens were produced. R cells converted [^{3}H]-pregnenolone to aldosterone, cortisol and androgens. The observed differences may be either due to differences in gene expression and /or posttranslational modifications which may lead to different activities of specific enzymes. Having found a profound difference in androgen synthesis, we compared HSD3B2 and CYP17 gene expression performing RT and real time PCR. We observed higher HSD3B2 expression in A cells compared to R cells while no difference in the expression of CYP17 was found. Functional studies were performed for P450c17 and 3betaHSDII enzymes. To study the activities of P450c17 (17alpha-hydroxylase and 17, 20 lyase), cells were treated with trilostane (3betaHSD inhibitor) prior to [^{3}H]-pregnenolone or [^{3}H]-17alpha-hydroxypregnenolone incubations. R cells showed higher 17, 20 lyase activity. To study 3betaHSDII activity, cells were incubated with [^{3}H]-DHEA. Interestingly, lower 3betaHSDII activity was detected in R cells. In summary, we show that A and R cells differ in their steroid profile. R cells produce significantly more androgens. Further comparative studies of A vs. R cells may help to understand mechanism/s regulating human androgen production in health and disease.

P2

Effects of ethanol and blokade of synthesis of nitric oxide on level of ACTH in female rats

Dragoslava Djikic, Sanja Vranjes-Djuric, Mirela Budec & Andjelka Spasic
University of Belgrade; Institute for Medical Research, Belgrade, United States, Institute for Nuclear Sciences "Vinca", Belgrade, United States; University of Belgrade, School of Medicine, Institute for Forensic Medicine, Belgrade, United States.

We showed previously that a single dose of ethanol acts as a stressor in female rats (Milovanovic *et al.*, 2003). In order to extend this observation, we investigated whether the effect of ethanol on ACTH is dose-related and possible interactions between nitric oxide (NO) and alcohol on the level of ACTH. To this end, adult female Wistar rats showing diestrus day 1 were treated with: (a) ethanol (2 or 4 g/kg, i.p.), (b) Nω-nitro-L-arginine-methyl ester (L-NAME), which blocks the activity of all isoforms of nitric oxide synthase, (30 mg or 50 mg/kg, s.c.) followed by ethanol (2 or 4 g/kg, i.p.) 3 h later and (c) L-NAME (30 mg or 50 mg/kg, s.c.) followed by saline 3 h later. Untreated rats were used as controls. The animals were sacrificed 0.5 h after ethanol administration. Blood ethanol levels were measured using gas chromatography. Plasma concentrations of ACTH were determined by radioimmunoassay. Obtained results showed that acute ethanol treatment significantly, dose-relatedly, enhanced the level of ACTH ($P<0.01$). The same phenomenon was observed in the groups treated with different doses of L-NAME followed by ethanol ($P<0.05$). Elevated concentration of ACTH was also found in the groups injected with L-NAME followed by saline ($P<0.05$).

Our results suggest that acute ethanol treatment increases the level of ACTH in dose-dependent manner. Although endogenous NO exerts negative influence on ACTH, it seems that it is not involved in the observed effect of ethanol under these experimental conditions.

Milovanovic T. *et al.* J Stud Alcohol 64:662–668, 2003.

(Supported by grant 145087 from the Ministry of Science of Serbia)

P3

Study of the hypothalamic-pituitary-adrenal axis in patients with the antiphospholipid syndrome

Renato Carignola[1], Valeria Data[1], Rosetta Vitetta[1], Adriana Severino[1], Tiziana Bertero[2], Mario Bazzan[3], Giuseppe Reimondo[1], Fulvia Daffara[1], Alberto Angeli[1] & Massimo Terzolo[1]
[1]Dipartimento di Scienze Cliniche e Biologiche – Medicina Interna I, ASO San Luigi, Orbassano, Italy; [2]UONA Immunologia e Allergologia Clinica, Ospedale Mauriziano, Torino, Italy; [3]UOA Ematologia e Malattie Trombotiche, Humanitas Cellini, Torino, Italy.

Objective
The Antiphospholipid Syndrome (APS) is a thrombophilic disorder characterised by recurrent venous and/or arterial thromboses and increased pregnancy morbidity. There is growing evidence supporting a functional interplay between the neuroendocrine and immune system; the hypothalamic-pituitary-adreal (HPA) axis plays a pivotal role in this network. Previous studies have described normal cortisol levels in APS patients while occurrence of acute adrenal failure was reported as a manifestation of this syndrome. However, it is still unknown whether subtle alterations of the HPA axis do exist in APS patients without overt hypoadrenalism.
Method
In the present study, we performed either a low-dose (1 μg) short Synacthen test (LDSST) or a 250 μg Synacthen test (SST) in 15 subjects of both sexes with primitive APS (diagnosed according to the Sapporo Criteria) and in 11 age and sex-matched healthy subjects. In addition, the patients underwent 1 mg dexamethasone suppression test (DST). None of the evaluated subjects were receiving any drug known to affect the HPA axis. The local Ethical Committee approval has been obtained.
Results
The patients with APS showed significantly higher cortisol levels than controls either at baseline (31.2 ± 15.6 vs. 18.3 ± 9.0 μg/dl, $P<0.01$) or at $+30$ min following 250 μg ACTH (57.3 ± 14.2 vs. 39.6 ± 12.8 μg/dl, $P<0.01$). Cortisol levels after 1 μg ACTH were also significantly increased in the subjects with APS compared to controls ($P<0.01$). Moreover, in only 2 patients we observed cortisol levels lower than 1.8 μg/dl after 1 mg DST (mean, 3.4 μg/dl; range 1.4–9.2) and two patients had cortisol values above 5.0 μg/dl after suppression.
Conclusions
In conclusion, although APS may cause adrenal insufficiency in selected cases, the present data seem to suggest that the HPA axis is not suppressed in APS patients. A possible explanation might be the state of chronic stress that usually accompanies long-standing autoimmune diseases.

P4

Survey of thyroid function of Hungarian Vizsla population in Hungary

Julianna Thuroczy[1], Aniko Tibold[1] & Lajos Balogh[2]
[1]SzIU, Faculty of Veterinary Science, Budapest, Hungary; [2]FJNCH, National "FJC" Research Institute for Radiobiology and Radiohygiene, Budapest, Hungary.

The prevalence of hypothyroidism in women of childbearing age is relatively high. The incidence of hypothyroidism during pregnancy has been calculated as between 0.3% and 0.7%. Overt abnormalities in thyroid function are common endocrine disorders affecting more than 19.2% of pregnant women in certain geographic areas of Hungary. 80% of Hungarian inhabitants are living in an iodine deficient area. The aim of this study was to investigate the prevalence of thyroid dysfunction in Hungarian Vizsla, a traditional breeding dog population.

A screening study was done on 95 Hungarian Vizsla, females and males. Serum total thyroxin, free thyroxin, triiodotyronine, total cholesterol and triglyceride concentrations were measured. The owners were asked to fill in a questionnaire concerning feeding and reproductive problems. TT4, freeT4 and T3 concentrations were determined by ELISA validated for use in canine serum.

The means and standard errors of the data were calculated and subjected to ANOVA and Student's *t*-test where appropriate. Significance was set at $P\leq0.05$.

Total T4 concentration of 36 dogs was lower (15.72 ± 2.62 (mean $\pm$ S.D.)) than the reference range (20.0–45.0 nmol/l). Total T4 level of 56 dogs was in reference range 26.83 ± 4.68 and of five was higher, 92.97 ± 64.86, than range. Total T4, free T4 and K values were different in the three groups at level of significance. T3 concentrations of suspected hypothyroid dogs (0.66 ± 0.24), dogs with normal thyroid function (0.77 ± 0.45) and dogs with suspected hyperthyroidism (0.67 ± 0.06) were not different at level of significance. TT4 concentrations of 25 (26.3%) dogs with familiar relations were out of reference range.

Our approach of a clinical investigation-based screening was rather efficient in suspicion of overt thyroid dysfunction but not for detecting many cases with

subclinical dysfunction. The high incidence of TT4 values out of range indicates a suspicion that the effect of iodine deficiency on thyroid function of dogs is similar to that in human subjects.

P5

Pancreatic polypeptide (PP) radioimmunoassay in acute phase and regression of cerulein induced pancreatitis

Grazyna Orlicz-Szczesna & Jolanta Zdanowska-Filipczak
Department of Internal Diseases University School of Medicine, Lublin, Poland.

Acute pancreatitis is a real medical problem with high patients mortality. Pathogenic interdependence between pancreas follicles function and islet endocrine secretion is under research. PP cells are pancreatic polypeptide (PP) producing cells, they determine about 1% islets, but their function is not completely known yet. Vagus nerve and peptidergic stimulation regulates PP secretion.

The aim of study was to estimate cerulein induced pancreatitis effects on rat serum PP concentration and pancreas morphology characteristics.

The study was conducted on male Wistar rats. They were anaesthetized with ketamine. We measured serum PP concentration during experimental cerulein-induced acute pancreatitis and different inflammatory process regression stages. Acute pancreatitis was developed through i.v. cerulein infusion 5 μg/kg per hour. Rats were divided into several groups in dependence on infusion time – 3,6,9,12 hours. Then rats had free access to standard nourishment and water. Blood samples from rat group with 12 hours cerulein infusion were taken after 3,6,9 and 12 days of observation. Control groups received i.v. 0.9% NaCl infusion. Pancreas histological changes were analyzed. Serum amylase and PP concentrations were assessed with DRG International Inc. (USA) kit. Both rabbit serum with antibodies against PP and goat's anti-rabbit gamma-globulin in buffer were used.

After 12 hours lasting cerulein infusion we obtained full biochemical and morphological acute pancreatitis picture. These changes start to regress after cerulein infusion withdrawal. Serum PP concentration was decreased after 3 hours of cerulein infusion, still decreased until the end of infusion (0.99 pg/ml). After cerulein cessation, progressive PP increase was observed, attained control PP concentration after 9 days (2.4 pg/ml) and exceed it after 12 days (3.5 pg/ml).

Cerulein significantly influence on serum PP concentrations - decreases it during pancreatitis induction and increases in regression stage. PP determines exocrine function stimulation, correlates with tissue destruction degree and pancreas enzymes disturbances.

P6

Evaluation of neuroendocrine disfunction in hypothalamo-pituitary-adrenal axis in diagnosis of depressive and non-depressive alcohol-dependent persons

Martina Matovinovic, Darko Katalinic, Andreja Maric, Miljenko Solter, Josip Ivica, Josip Resetic, Jure Murgic & Milan Vrkljan
Sestre Milosrdnice University Hospital, Zagreb, Croatia.

Acute and chronic alcohol intake and alcohol withdrawal induce dysfunction of neuroendocrine and other regulatory systems. The aims of this study were to assess a possible hypothalamo-pituitary-adrenal (HPA) axis dysfunction in population of alcoholics, using dexamethasone suppression test (DST). The study was approved by local Ethical Committee. The serum and urinary cortisol were compared between the groups of 89 male patients (64.5% depressive and 35.5% nondepressive alcoholics) (Hamilton test), before and after DST. In nondepressive patients, 50% was nonsupressive in DST. In depressive patients 46% was suppressive in DST test (serum cortisol). Twenty-four hours urinary excretion in group of nondepressive patients was supressed in 78% of cases; depressive patients showed 50.9% nonsupressors. Basal serum cortisol secretion was significantly lower in group of nondepressive than depressive patients. Also, serum concentration at 16 hours were significantly higher in group of the depressive nonsupressive patients. Basal urinary cortisol excretion was in normal range in all patients, but after dividing the patients into suppressible and nonsuppressible groups, significantly higher ($P<0.002$) basal urinary cortisol concentrations were found in latter. We concluded on the basis of DST test, as well basal cortisol measurement, that the neuroendocrine dysfunction of alcoholic patients could be present even if the depression is pronounced.

P7

Effects of melatonin on glutathione peroxidase activity after adriamycin in normal and pinealectomized rats

Katarzyna Dabrowska[1], Michal Stuss[1], Jolanta Gromadzinska[2], Wojciech Wasowicz[2] & Ewa Sewerynek[1]
[1]Department of Bone Metabolism; Medical University of Lodz, Lodz, Poland; [2]The Nofer Institute of Occupational Medicine, Lodz, Poland.

Adriamycin (ADR) is a potent chemotherapeutic agent, effective in treatment of leukemias, lymphomas and of many solid tumours. However, its clinical usage is often limited by cardiotoxicity, induced by oxygen radical damage of membrane lipids.

Melatonin (MEL), is a well-known antioxidant. It has been shown that melatonin can scavenge free radicals, both directly or indirectly, stimulating the activity of antioxidative enzyme, e.g., glutathione peroxidase (GSH-Px).

The aim of the study was to examine the effect of Mel on the GSH-Px activity in serum, erythrocytes and the heart after adriamycin.

Materials and Methods

Wistar rats were divided into the 3 groups: pinealectomized (PX), sham-operated (Sham-PX) and control animals (Intact). Each of the groups was divided into 4 subgroups, injected with: 1 – saline, 2 – MEL, 3 – ADR and 4 – MEL + ADR. ADR was administered 2 months after PX as a single dose (15 mg/kg, i.p.), 1 hour after the fourth melatonin injection. Melatonin (5 mg/kg, i.p.) was administered for 4 days before and 4 days after ADR. After 8 days of treatment the rats were killed by decapitation. Their hearts and blood were collected for measurements.

Results

The activity of GSH-Px in the heart increased significantly in all the examined groups after ADR injections. On the contrary, in serum, GSH-Px activity decreased in all the groups after ADR. In erythrocytes, GSH-Px decreased after ADR in Px-animals. Mel did not change GSH-Px activity after ADR.

Conclusion

MEL did not influence the activity of GSH-Px, either in normal or in pinealectomized rats after ADR.

Grant No 502-11-293 of the Medical University of Łódź.

Cytokines and growth factors – presented on Sunday

P8

Is there any role for anti-inflammatory cytokine Interleukin-10 in advanced congestive heart failure?

Azza Farrag, Nouran El Gahndour & Mohab Halim
Cardiology Department, Cairo University, Cairo, Egypt; Internal Medicine Department, Cairo University, Cairo, Egypt; Biochemistry Department, Cairo, Egypt.

Background

CHF manifestations can be explained by the biologic effects of tumor necrosis factor-alpha (TNF-alpha). Interleukin-10 (IL-10) has potent deactivating properties and is considered a potent anti-inflammatory cytokine that inhibits TNF-alpha production. This study was designed to examine the role of IL-10 in patients with CHF and to test its correlation with pro-inflammatory markers.

Methodology

Fifty patients with CHF were studied. Patients were classified according to NYHA functional class into 29 (NYHA II), 11 (NYHA III) and 9 patients (NYHA IV). Serum samples for TNF-alpha, IL-10, soluble TNF receptors (sTNF-R1 and sTNF-R2), transforming growth factor-beta (TGF-beta) as well as high sensitivity C-reactive protein (hs-CRP) were taken from all patients and also from healthy, age and sex matched 50 controls.

Results

CHF patients had a significantly lower level of IL-10 compared to controls (2.28 ± 1.1 vs 5.39 ± 1.4 pg/ml, $P<0.0001$). Patients with NYHA class IV had the lowest serum levels of IL-10 and TGF-alpha which were statistically significant when compared to patients with NYHA class III (0.67 ± 0.4 vs 1.9 ± 0.5 pg/ml, $P<0.001$) and (1348 ± 92 vs 1653 ± 111 pg/ml, $P<0.05$) respectively, but they had the highest serum level of TNF-alpha, sTNF-R1, sTNF-R2 and hs-CRP when compared to the same group (8.6 ± 1.9 vs 7.1 ± 0.8 pg/ml, $P<0.01$), (2380 ± 141 vs 1831 ± 185 pg/ml, $P<0.01$), (3410 ± 174 vs 2841 ± 191 pg/ml, $P<0.01$) and (26.4 ± 2.7 vs 14.4 ± 3.9 mg/L, $P<0.01$) respectively.

Conclusion

Patients with CHF had a significant decrease in their serum level of IL-10 and increase in TNF-alpha, sTNF-R1, sTNF-R2 and hs-CRP when compared to normal subjects and these levels change significantly with advanced NYHA class.

P9

Regulation of GAGEC1, a cancer-testis associated antigen family member, by sex steroid hormones and TGF-beta: implications for prostatic disease

Natalie Sampson[1], Gerlinde Schuhfried[1], Eugen Plas[2] & Peter Berger[1]
[1]Institute of Biomedical Aging Research, Austrian Academy of Sciences, Innsbruck, Austria; [2]Ludwig Boltzmann Institute for Andrology and Urology, Hospital Lainz, Vienna, Austria.

Prostate homeostasis and function are regulated by complex interactions between the fibromuscular stroma and secretory epithelium via locally-derived and systemic paracrine- and autocrine-acting growth factors and sex steroid hormones. Stromal tissue remodelling due to alterations in transforming growth factor beta (TGF-β) and sex steroid hormone signalling are associated with benign prostatic hyperplasia (BPH) and prostate cancer (PCa), two of the most common proliferative disorders affecting elderly men.

We previously demonstrated that GAGEC1, a member of cancer-testis associated antigens, is up-regulated in response to TGF-β in *in vitro* models of age-associated prostatic stromal remodelling. GAGEC1 expression is restricted to male and female reproductive tissues and is up-regulated in the prostates of patients with symptomatic BPH and PCa. Consistent with its restricted expression profile to classical steroidogenic tissues, *GAGEC1* is induced by sex steroid hormones, particularly oestradiol and dihydrotestosterone. Transiently expressed recombinant GAGEC1 undergoes constant shuttling between cytoplasmic and nuclear cell compartments, a process that may be regulated via post-translational phosphorylation.

Our data suggest that age/disease-associated changes in TGF-β1 and sex steroid hormones may account for the reported increase in *GAGEC1* expression in BPH and PCa. Functional analyses indicate that the biological activity of GAGEC1 is regulated via phosphorylation-dependent nucleo-cytoplasmic trafficking raising the possibility that GAGEC1 is involved in signal transduction mechanisms. Given that its expression is restricted in males to the prostate and testis, *GAGEC1* represents a promising target for therapeutic intervention of BPH and PCa.

P10

Normalization of serum testosterone level alters local GnRH-II and IL-2R mRNA expression in peripheral lymphocytes in patients with idiopathic hypogonadotrophic hypogonadism (IHH)

Fatih Tanriverdi[1], Hilal Akalin[2], Okay Caglayan[2], Umit Demirkoparan[1], Yusuf Ozkul[2], Munis Dundar[2], Fahri Bayram[1] & Fahrettin Kelestimur[1]
[1]Erciyes University Medical School Department of Endocrinology, Kayseri, Turkey; [2]Erciyes University Medical School Department of Genetics, Kayseri, Turkey.

Although the existence of the interaction between sex steroids and immune system is well known, the mechanisms of this interaction are still unclear. Recently a second form of GnRH (GnRH-II) has been described in human, which is significantly expressed in immune tissues suggesting a potential function. In a recent *in-vitro* study it has been demonstrated that GnRH-II decreases local expression of IL-2R in peripheral lymphocytes (1). However *in-vivo* interactions of testosterone, IL-2R and GnRH-II expression at lymphocyte level have not been investigated yet. Therefore in the present study we investigated the effects of conventional gonadotrophin therapy on local GnRH-II and IL-2R expression in peripheral lymphocytes in patients with IHH.

Fourteen males with IHH (24.5 ± 6.3) and 15 age-sex matched controls were investigated. Patients were treated with hCG and hMG for 12 months. Quantitative Real-Time RT-PCR (2 independent repeats) was used to determine the expression of GnRH-II (target gene), IL-2R (target gene) and beta-actin (reference gene) in peripheral lymphocytes derived from patients before and after treatment, and the controls.

Serum testosterone level before treatment in patient group was significantly low when compared to controls. After gonadotrophin treatment testosterone level significantly increased. Baseline GnRH-II and IL-2R mRNA levels (% of the control) were % 1451 ± 300 and % 285 ± 46 in the patient group, respectively. Significant decrease in GnRH-II and IL-2R mRNA levels were found after treatment**.**

In-vivo interactions between testosterone, IL-2R and GnRH-II at lymphocyte level were shown first time in the literature. Present findings clearly suggest that some immune effects of the sex steroids may occur via regulating the local GnRH-II and IL-2R expression.

P11

Blocking undesired leptin action *in vivo* with leptin antagonists prepared by site-directed mutagenesis of human, ovine, rat and mouse leptin site III

Leonora Niv-Spector, Dana Gonen - Berger, Gili Salomon, Isabelle Callebaut, Jean Djiane & Arieh Gertler
Institute of Biochemistry, Food Science and Nutrition, Faculty of Agricultural, Food and Environmental Quality Sciences, The Hebrew University of Jerusalem, Rehovot 76100, Israel.

Recent reports have revealed that leptin's effects are not restricted to central control of body weight, demonstrating instead that leptin is a pleiotropic hormone with a wide variety of different biological actions. Leptin exhibits undesirable effects in autoimmune diseases, in atherosclerosis, and possibly in several types of cancer, and increases the risk of cardiovascular disease in obese people. Therefore, preparation of reagents capable of abolishing leptin's action is both valid and timely.

As no structural information on the 3D structure of leptin receptor (LEPR) is available, the model of interleukin 6 (IL6) was applied. We identified leptin's putative binding site III, which does not affect binding but is necessary for receptor activation, by modeling LEPR on the basis of its alignment with gp130, and fitting leptin on IL6 in the IL6/gp130 complex.

Six muteins of human, ovine, rat, and mouse leptins, mutated to Ala in amino acids 39–41 or 39–42, were prepared by site-directed mutagenesis of the putative site III, and purified to homogeneity. All muteins had typical cytokine secondary structure, acted as true antagonists—namely, they interacted with LEPR with an affinity similar to that of the wild-type hormone (as evidenced by SPR and RRA), were devoid of biological activity in several leptin-response bioassays, and specifically inhibited leptin action *in vitro* and *in vivo.* These muteins can be prepared in gram amounts and thus serve as a novel tool for studying leptin function *in vitro* and *in vivo.* To prolong their lives in circulation, some muteins were pegylated using 40-, 30- and 20-kDa polyethylene glycol. Although pegylation decreased their *in-vitro* activity, increasing circulation half-life can compensate for this deficit *in vivo.*

Antagonizing leptin has been suggested as a possible therapy in autoimmune diseases and heart failure. Thus, leptin antagonists not only offer a novel tool to elucidate the role of leptin in mammalian physiology but have a potential role as a therapeutic drug.

P12

Role of soluble Fas-antigen (sFas), interleukin-6 (IL-6) and vascular endothelial growth factor (VEGF) in adrenocortical carcinoma patients

Nikolai Kushlinsky, Timur Britvin, Irina Kazantseva & Arian Kalinin
Russian N.N.Blokhin Cancer Research Center of RAMS, Moscow, Russia; M.F.Vladimirsky Moscow Regional Clinical and Research Institute, Moscow, Russia.

The numerous growth factors and cytokines take part in mechanisms of tumor growth and metastasing.

The aim of this study was determination of sFas, IL-6 and VEGF serum levels in 19 patients with adrenal cortical carcinoma (11 women and 8 men aged 21–72 years). The control group comprised 40 practically healthy donors (22 women and 18 men aged 19–70 years). sFas, IL-6 and VEGF before adrenalectomy and in the control were measured by ELISA. Mean IL-6 (4.6 ng/ml) and VEGF (438.7 pg/ml) levels in adrenocortical carcinoma patients were significantly ($P = 0.004$) higher than in the control (IL-6 – 1.3 ng/ml, VEGF – 126.5 pg/ml). There was no difference in serum sFas between patients (2.0 ng/ml) and the control (0.8 ng/ml). sFas, IL-6 and VEGF were markedly elevated in patients with advanced (III-IV) stages of the disease as compared to early (I-II) stages. In patients with nonfunctional adrenal cortical carcinoma, serum level of VEGF (571.9 pg/ml) was significantly ($P = 0.046$) higher than that in patients with Cushing's syndrome (460.1 pg/ml). No differences in serum sFas and IL-6 levels were revealed between patients with nonfunctional and hormonally-active tumors. Direct correlation was found between VEGF and IL-6 ($P = 0.56$; $r = 0.009$). 5-year overall survival (100%) of patients with serum VEGF less then 300 pg/ml was significantly ($P = 0.049$) higher compared to patients with serum VEGF exceeding 300 pg/ml (34.3%). 5-year overall survival didn't depend on the pretreatment serum sFas and IL-6 levels.

We suggest that VEGF serum level in adrenal cortical carcinoma patients may be used as a factor of clinical behaviour and prognosis.

P13

Rosiglitazone interferes with the inflammatory response induced in human endothelial cells by TNFalpha and IFNgamma through a new mechanism involving extracellular signal-regulated kinases (ERKs)

Adriana Lombardi[1], Elisabetta Piscitelli[1], Michela Francalanci[1], Tommaso Mello[1], Veronica Santarlasci[2], Stefania Gelmini[1], Giulia Cantini[1], Andrea Galli[1], Lorenzo Cosmi[2], Francesco Annunziato[2], Gianni Forti[1], Mario Serio[1] & Michaela Luconi[1]
[1]Dept. Clinical Physiopathology, University of Florence, Florence, Italy; [2]Dept. Internal Medicine, University of Florence, Florence, Italy.

Microvascular endothelium is one of the main character and target involved in the inflammatory response. Upon specific activation, endothelial cells massively recruit Th1 IFNgamma secreting lymphocytes at the inflammatory site. In the present study, we investigate the intracellular signalling mediating TNFalpha (T) and IFNgamma (I) inflammatory response in a human endothelial cell line (HMEC-1) *in vitro* and the interfering effects of rosiglitazone (RGZ), a peroxisome proliferator-activated receptor (PPARgamma) agonist currently used in clinical treatment of diabetes mellitus. We show that T and I alone stimulate interferon gamma-inducible protein-10 (IP-10) secretion by HMEC-1, effect which is dramatically increased when the two cytokines are used in combination. IP-10 secretion in response to T, I and RGZ is accompanied by a re-modulation of surface expression of cell adhesion molecules (CAM), such as VCAM-1 and ICAM-1. Although these stimulatory effects of T and I are mediated by a similar rapid increase in phosphorylation/activation of ERK1/2, as demonstrated by the use of ERK inhibitors, confocal microscopy analysis suggests that the synergistic action of T and I is partly mediated by a different subcellular localization of the activated ERKs. Concomitant treatment with RGZ reverts both activation of ERKs and interferes with IP-10 secretion and CAM expression elicited by T and I through a novel rapid mechanism not involving transcriptional activity of PPARgamma, as further confirmed by the inability of BADGE, an inhibitor of such a transactivational action, to revert RGZ effects. Our findings shed new light on the molecular mechanisms underlying the inflammatory response in the endothelium and on the possible therapeutic use of RGZ in such a process.

This study has been supported by project TRESOR of Region of Tuscany

P14

Role of growth hormone/insulin like growth factor 1 system in the remodelling process of the right ventricle in top levels rowers

Giovanni Vitale[1], Maurizio Galderisi[2], Annamaria Colao[3], Pasquale Innelli[2], Germano Guerra[4], Ermelinda Guerra[3], Frank Lloyd Dini[5], Antonio Soscia[4], Oreste de Divitiis[2] & Gaetano Lombardi[3]
[1]Chair of Endocrinology, Faculty of Medicine – University of Milan, Istituto Auxologico Italiano, Milan, Italy; [2]Cardioangiology and CCU Division, Department of Clinical and Experimental Medicine, University of Naples Federico II, Naples, Italy; [3]Department of Molecular and Clinical Endocrinology & Oncology, University of Naples Federico II, Naples, Italy; [4]Department of Biomorphological and Functional Sciences, University of Naples Federico II, Naples, Italy; [5]Cardiovascular Diseases Unit 2, Ospedale Santa Chiara, Pisa, Italy.

The intensive physical activity is often associated with cardiac changes, particularly involving the right ventricular (RV) chamber. However, the molecular mechanisms involved in the RV physiologic adaptation to long-term training are not completely understood. In the present study we investigated the role of the growth hormone/insulin like growth factor 1 (GH/IGF-1) axis in the RV remodeling of athletes.

Nineteen male top levels rowers and 19 age-matched healthy sedentary male controls underwent blood determination of fasting serum GH, IGF-1, IGF binding protein 3 (IGFBP-3) and acid-labile subunit levels and standard Doppler echocardiography combined with pulsed Tissue Doppler of RV tricuspid annulus. Myocardial pre-systolic (PS_m), systolic (S_m), early diastolic (E_m) and atrial (A_m) velocities as well as myocardial time intervals adjusted for heart rate were calculated.

Rowers had serum IGF-1 levels ($P<0.05$), RV internal chamber size ($P<0.05$) and RV wall thickness ($P<0.0001$) significantly higher than controls. Additionally, rowers had improved RV systolic (higher tricuspid annular systolic excursion, higher PS_m and S_m velocities; lower myocardial pre-contraction time) and diastolic function (lower A velocity, shorter deceleration time, isovolumic relaxation time and myocardial relaxation time; higher E/A ratio, E_m and E_m/A_m ratio) compared to controls. In the rowers, IGF-1 was associated with PS_m velocity ($r=0.55$, $P=0.01$) and myocardial pre-contraction time ($r=-0.57$, $P=0.01$), GH with pre-ejection period ($r=-0.50$, $P<0.05$) and E_m ($r=0.47$, $P<0.05$). These associations remained significant after adjusting for age, heart rate and body surface area.

In conclusion, this study shows for the first time that the GH/IGF-1 axis is responsible for the RV functional remodeling in high-top rowers, improving mainly the systolic activity. This effect seems to be primarily modulated by the IGF-1 overproduction, as a physiological adaptation to prolonged training.

P15

Clinical significance of simultaneously determined serum interleukin-6, dehydroepiandrosterone and its sulphate levels in melanoma

Mariann Boldizsár, Borbála Vincze, Katalin Gilde, Teodora Bánfalvi, Zsuzsanna Fejos, Kinga Borbola, Bence Kapuvári, Gabriella Liszkay & Szabolcs Otto
National Institute of Oncology, Budapest, Hungary.

Among well-known circulating melanoma markers 5-S-cysteinyldopa (5-SCD), a precursor of pheomelanin biosynthesis and S-100 beta (S-100B) are extensively investigated. Our earlier observations confirmed that serum concentration of 5-SCD and S-100B correlates well with the stages and progression of the disease. Interleukin-6 (IL-6) is a multifunctional inflammatory cytokine implicated in advanced stage of various diseases and tumour recurrence. Malignant melanoma cells are known to secrete IL-6. According to the recent reports skin could produce DHEA and DHEA-S due to the presence of key enzymes. This study was aimed to establish the significance and the possible relationship among different serum parameters. In 124 melanoma patients with ($n=63$) or without ($n=61$) metastasis, concentrations of IL-6, DHEA, DHEA-S were simultaneously measured in comparison with the metastatic markers of 5-SCD and S-100B. The presence of metastasis was verified by conventional imaging techniques. Serum 5-SCD concentration was determined by high pressure liquid chromatography with electrochemical detection. Serum levels of IL-6, DHEA, DHEA-S and S-100B were measured by RIA/IRMA and ILMA methods. For statistical analysis MedCalc Software was used. In patients with metastases compared to the metastasis-free cases significant increase in 5-SCD, S-100B and IL-6 serum levels were observed. On the contrary, significant decrease in DHEAS and DHEA concentrations was found. Correlations between serum concentrations of 5-SCD and IL-6 ($P<0.0001$), as well as DHEA and DHEA-S ($P<0.0001$) were significant and Spearman's coefficient of rank correlation (rho) was 0.69 and 0.71, respectively. Using multiple regression analysis a negative correlation between IL-6 and DHEA or DHEAS levels was found. These results suggest that simultaneous determination of IL-6, DHEA and DHEA-S together with 5-SCD and S-100B measured in melanoma patients could be predictive factors of the disease.

This research was supported by the Hungarian Research Fund (OTKA No. T 049814).

P16

Changes in growth hormone messenger RNA (GHmRNA) expression in rats anterior pituitary after single Interferon (IFN)alpha administration.

Wojciech Romanowski, Ryszard Braczkowski, Aleksander Danikiewicz, Ewa Nowakowska-Zajdel, Malgorzata Muc-Wierzgon, Zuzanna Muryn & Barbara Zubelewicz-Szkodzinska
Dept. of Internal Medicine Bytom, Silesian Medical University, Katowice, Poland.

Introduction
Interferon alpha (IFN alpha) is a cytokine with pleiotropic effects and via different pathways influences secretion of certain cytokines and hormones. Growth Hormone (GH) secreted from the pituitary has its physiological effects on various target tissues. The question is how IFN alpha administered in various type of diseases influences GH secretion. This study investigated acute effect of IFN-alpha on GH mRNA expression in rat anterior pituitary
Objective
The aim of the study was to measure the cellular expression of GH mRNA by *in situ*hybridisation in anterior pituitary after IFN alpha single administration
Material and methods
Rats were administered intraperitoneal injection of IFN alpha or saline. Rat pituitaries were taken 2 and 4 hours after IFN/saline administration and kept frozen until *in situ*hybridisation histochemistry. 31–base^{35}S-labelled oligonucleotide probe complementary to part of the exonic mRNA sequences coding for GH mRNA was used. All control and experimental sections were hybridised in the same hybridisation reaction

Results
Interferon α acute administration increases GH mRNA expression in the anterior pituitary in 4 hours group in comparison to the control group, and there was no difference between control group and 2-hours rats.
Conclusion
The influence of single IFN alpha administration on anterior pituitary GH mRNA expression has been found. These observations may pave the way for presenting a new possible IFN alpha action.

P17

Does stress test influence Interleukin (IL)-2 and IL-8 concentration in serum patients with stable ischaemic heart disease?

Wojciech Romanowski[1], Janusz Szkodzinski[2], Andrzej Kaszuba[1], Adam Blazelonis[1], Zuzanna Muryn[1], Pietka-Rzycka Anna[1] & Barbara Zubelewicz-Szkodzinska[1]
[1]Dept. of Internal Medicine Bytom, Silesian Medical University, Katowice, Poland; [2]Silesian Center for Heart Diseases, Zabrze, Poland.

Background
There is growing evidence that adhesion molecules, proinflammatory cells and cytokines play an important role in a variety of cardiac pathophysiological conditions; Cytokines are responsible for the modulation of immune and inflammatory processes. It has been suggested that cytokines such as IL-1, IL-2, IL-6, IL-10 and TNF alpha are important modulators of atherosclerotic effects with IL-2 and Interferon γ having a proinflammatory atherogenic effect and IL-8 and IL-10 having an anti-inflammatory protective role. Atherosclerotic lesions in the coronary vessels are heavily infiltrated by cellular components associated with inflammation (macrophages/monocytes, t-lymphocytes, eosynophils and NK-cells). These cells are also a source of cytokines and that is why the **Objective** of the present study was to measure IL-2 and IL_8 concentration in serum patients with stable ishaemic heart disease (i.h.d.).
Patients and method
26 patients (11 women, 15 men) age 47–65 years with diagnosed stable ischaemic heart disease were included into study. The control group consists of 20 patients matched with age and sex. All patients from examined group fulfilled the inclusion criteria (stable i.h.d. with standard treatment, rest ECG without ischaemic changes and with coronary sufficiency belongs to I or II class of CCS-(Canadian Cardiovascular Society scale).The exclusion criteria were typical for the study concerns cytokines.
In all patients we have measured the concentration of IL-2 and IL-8 concentration in serum by ELISA using R&D System kits
Additionally, patients with diagnosed i.h.d. had IL-2 and IL-8 concentration measured after the stress test done to assume the cardiac sufficiency in that group.
Results
Concentration of IL-2 and IL-8 in patients with i.h.d. is significantly higher then in the control group ($P<0.05$). After stress test in i.h.d. patients there were no significant changes of IL -2 concentration ($P=0.054$) and increase of IL-8 ($P<0.001$) concentration observed.

P18

Concentration of inflammatory factors such as CRP (acute phase response protein) and Interleukin (IL)-6 in serum patients with stable ischemic heart disease during trimetazidine treatment

Janusz Szkodzinski[1], Marek Szewczyk[2], Wojciech Romanowski[2], Aleksander Danikiewicz[2], Malgorzata Muc-Wierzgon[2] & Barbara Zubelewicz-Szkodzinska[2]
[1]Dept. of Internal Medicine Bytom, Silesian Center for Heart Diseases, Zabrze, Poland; [2]Silesian Medical University, Katowice, Poland.

Background
The patomechanism of developing ischemic heart disease (i.h.d.) is stenosis of coronary blood vessels with plaque placed on vascular endothelium built with monocytes/macrophages, foam cells, oxidized LDL, leukocytes, platelets and collagen. Atherosclerotic lesions are heavily infiltrated by cellular components associated with inflammation and influenced by other inflammatory factors. Trimetazidine, a clinically effective antianginal agent acts by optimizing cardiac energy metabolism through inhibition of free fatty acid oxidation.

Up to now there have been no study associating trimetazidine possible anti-inflammatory effect which could be a result of trimetazidine influence on granulocytes in-flow to ischemic region and atherosclerotic plaque and in consequence influence on granulocyte products such as cytokines and other inflammatory predictors.
Objective
The aim of the study was to determine if trimetazidine treatment in stable ischemic heart disease altered the concentration of certain inflammatory factors such as CRP (acute phase response protein) and Interleukin (IL)-6.
Patients and method
26 patients (11 women, 15 men) age 47–65 years with diagnosed stable ischemic heart disease were included into study. All of them fulfilled the inclusion criteria (stable i.h.d. with standard treatment, rest ECG without ischemic changes and with cardiac sufficiency belongs to I or II class of CCS-(Canadian Cardiovascular Society scale).
All patients have measured the concentration of Il-6 and CRP at the onset of trimetazidine treatment and 3 months after. Il-6 concentration has been measured by ELISA using R&D System kits and CRP concentration by immunoturbidometric method.
Results
3-months trimetazidine treatment caused significant decrease of CRP concentration in serum of patients with stable i.h.d. ($P<0.001$) and significant increase of IL-6 concentration ($P<0.05$).
Conclusion
Decrease of CRP concentration in serum after 3 months of trimetazidine treatment could be due to trimetazidine hepatoprotective properties. An increase of Il-6 concentration after 3 months of treatment with trimetazidine is possibly a result of different mechanism of its action.

P19

Insulin decreases IGF-I bioactivity in patients with impaired glucose tolerance and in healthy subjects

Ayman M Arafat[1], Jan Frystyk[2], Martin O Weickert[1], Joachim Spranger[1], Christof Schöfl[1] & Mathias Möhlig[1]
[1]Department of Endocrinology, Diabetes and Nutrition, Charité-University Medicine Berlin, Campus Benjamin Franklin and the German Institute of Human Nutrition Potsdam-Rehbruecke, Berlin, Germany; [2]Medical Research Laboratories and Medical Department M, Aarhus University Hospital, DK-8000 Aarhus C, Denmark.

Objectives
Insulin resistance (IR) is a very common metabolic abnormality in obesity, which is often associated with reduced growth hormone (GH) secretion. GH deficiency is associated with increased in intra-abdominal fat and several parameters of the metabolic syndrome. IGF-I improves IR but the IGF-binding proteins are supposed to regulate its bioactivity although only little information exists. We postulated that the elevated insulin levels due to IR do not only suppress GH but also IGF-I bioactivity, and therefore we tested the effect of insulin on serum levels of bioactive IGF-I.
Methods
24 healthy subjects (12 men; age 21–72 years; BMI 25.9±0.9 kg/m2) and 19 patients with impaired glucose tolerance (IGT; 8 men; age 26–71; BMI 28.9±1.2) were studied using an OGTT and a hyperinsulinemic euglycemic clamp. IR was estimated by calculating the homeostatic model assessment (HOMA-IR) index and the glucose infusion rate (GIR). IGF-I bioactivity was estimated using a novel IGF-I kinase receptor activation assay (KIRA) under fasting conditions and during the steady state of the clamp. Ethical Committee approval was obtained.
Results
Insulin significantly decreased IGF-I bioactivity in IGT patients (1.8±0.2 vs. 1.5±0.2 μg/l, $P=0.004$) and in healthy controls (1.8±0.2 vs 1.6±0.2 μg/l, $P=0.001$). Age, BMI and fasting IGF-I bioactivity did not significantly differ between groups. However, patients with IGT showed a higher HOMA-IR and a lower GIR (2.3±0.4 vs. 1.3±0.2 and 2.5±0.3 vs. 4.7±0.3 mg/kg min, $P<0.05$, respectively). Moreover, inverse correlations were seen between bioactive IGF-I levels and age ($r=-0.38$, $P=0.01$), BMI ($r=-0.46$, $P=0.002$) and waist to hip ratio ($r=-0.51$, $P=0.01$).
Conclusion
Our data indicate that insulin infusion acutely decreased serum IGF-I bioactivity in humans. Hyperinsulinemia as seen in IR may per se be responsible for this reduction. Estimation of IGF-I bioavailability using the KIRA method may, therefore, have a predictive value in the diagnosis of the metabolic syndrome.

P20

Plasma free fatty acids and adipocytokines concentration in relation to insulin sensitivity in patients with anorexia nervosa.

Monika Karczewska-Kupczewska, Irina Kowalska, Marek Straczkowski, Agnieszka Adamska, Agnieszka Nikolajuk, Malgorzata Karolczuk-Zarachowicz, Elzbieta Otziomek & Maria Gorska
Department of Endocrinology, Diabetology and Internal Medicine, Medical University of Bialystok, Bialystok, Poland.

Anorexia nervosa (AN) is an eating disorder, resulting in sustained low weight. In AN, similarly to syndromes of lipodystrophy, one observes the significant loss of the adipose tissue. In lipodystrophies, despite the lack of subcutaneous adipose tissue, insulin resistance is observed. Adipose tissue is known as a source of a variety of bioactive peptides, known as adipocytokines. The aim of the present study was to examine the plasma concentration of adipocytokines in relation to insulin sensitivity in women with AN.

The study group consisted of 16 women with AN, 16 women with obesity and 18 healthy normal weight female controls. The oral glucose tolerance test and euglycemic hyperinsulinemic clamp were performed in all the patients. The plasma concentrations of adiponectin, TNF-α, soluble TNF-α receptors (sTNFR1, sTNFR2) and IL-6, soluble form of IL-6 receptor (sIL-6R) were estimated.

Insulin sensitivity index (M) was not different in AN and healthy controls, but was significantly increased in AN in comparison to obese women ($P=$ 0.002). Adiponectin plasma levels were significantly higher in AN than control subjects and obese women ($P=0.01$, $P=0.003$, respectively). There were no differences in plasma concentrations of TNF-α, sTNFR1, sTNFR2, IL-6, sIL-6R among groups, however plasma free fatty acids (FFA) were significantly lower in AN than control subjects and obese women ($P=$ 0.00003, $P=0.00001$, respectively). Adiponectin levels were negatively correlated with BMI ($r=-0.40$, $P=0{,}005$) and waist girth ($r=-0.44$, $P=$ 0.002). Fasting FFA concentrations were related negatively to insulin sensitivity ($r=-0.55$, $P=0.00007$) and to adiponectin concentrations ($r=-0.34$, $P=0.026$).

Our data show that lack of adipose tissue observed in anorectic patients has no influence on insulin sensitivity, probably due to low plasma FFA concentration. It points out that in AN the adipocytes are still capable of functioning at the level that is sufficient to prevent the metabolic consequences.

P21

Improved glucose metabolism and altered pancreatic structure in transgenic mice overexpressing betacellulin

Maik Dahlhoff[1], Nadja Herbach[2], Rüdiger Wanke[2], Eckhard Wolf[1] & Marlon R. Schneider[1]
[1]Institute of Molecular Animal Breeding and Biotechnology, University of Munich, Munich, Germany; [2]Institute of Veterinary Pathology, University of Munich, Munich, Germany.

Betacellulin, one of several peptides activating the EGFR (ErbB1) and related receptors, is a multipotent growth factor known to posses the unique ability to promote growth and differentiation of pancreatic β-cells.

We investigated the effects of betacellulin overexpression in a recently established transgenic mouse model (Schneider *et al.*, Endocrinology 146, 5237–5246, 2005). In transgenic animals, overall glucose metabolism was improved as demonstrated by reduced blood glucose levels in fasted animals and a better response after a glucose tolerance test (associated with increased serum insulin levels). Unexpectedly, the absolute and relative (proportional to body weight) pancreas weights were significantly reduced in transgenic mice. Histomorphometrical analyses revealed a reduction in the volume of the exocrine pancreas while the islet and β-cell volume remained unchanged. This resulted in an increase in the relative volume of the latter compartments. Interestingly, the proportion of β-cells within the islets remained unchanged in betacellulin transgenic mice. While betacellulin is normally expressed in the islets, immunohistochemistry revealed that the growth factor is, in addition, strongly expressed in the exocrine pancreas in transgenic mice. This uncovers a hitherto unknown negative effect of betacellulin in the exocrine compartment. Finally, we identified, by immunohistochemistry, an opposite expression pattern of ErbB1 and ErbB4, the primary receptors for betacellulin, in the pancreas. In this organ, ErbB1 is expressed predominantly in the islets, while ErbB4 expression is mostly restricted to the exocrine compartment. Thus, this particular receptor distribution may provide an explanation for the opposing effects exerted by betacellulin in the different pancreatic compartments.

Current experiments involve the study of cell proliferation and apoptosis as well as functional studies on isolated islets including insulin content and release.

P22

Screening of 120 adipokines in subcutaneous adipose tissue of patients with growth hormone deficiency reveals changed protein levels

Daniela Gasperikova[1], Jozef Ukropec[1], Adela Penesova[1], Henrike Sell[2], Martina Skopkova[1], Miroslav Vlcek[1], Zofia Radikova[1], Juraj Koska[1], Vitazoslav Belan[3], Mikulas Pura[4], Peter Vanuga[4], Juraj Payer[5], Jurgen Eckel[2] & Iwar Klimes[1]
[1]Institute of Experimental Endocrinology, Bratislava, Slovakia; [2]German Diabetes Center, Dusseldorf, Germany; [3]Department of Radiology, Faculty Hospital, Bratislava, Slovakia; [4]National Institute for Endocrinology and Diabetes, Lubochna, Slovakia; [5]V. Clinic of Internal Medicine, Faculty Hospital, Bratislava, Slovakia.

The role of adipokines and inflammatory cytokines of adipose tissue for development of the growth hormone deficiency (GHD)-related metabolic derangements has not yet been completely understood. Therefore, we screened the protein level of 120 adipokines in subcutaneous adipose tissue (ScAT) of patients with GHD in adulthood.

Subjects and methods

Sixteen GHD (10M/6F) with BMI 27 ± 1.0 kg/m^2, age 30 ± 2 yrs and sixteen controls matched for BMI, sex and age were included into the study. ScAT biopsies were performed after an overnight fast. Protein expression of adipokines was determined in tissue lysates using the RayBio®Human Cytokine Antibody Array C Series 1000.

Results

GHD subjects had higher waist circumference, circulating hsCRP levels and impaired glucose tolerance (as assessed by oGTT) ($P<0.05$). From 120 proteins, one showed to have higher (IGFBP-1) and three (BDNF, NT-3, SDF-1) lower levels in ScAT of the GDH subjects in comparison with controls ($P<0.05$). Majority of the observed changes were related to waist circumference, as became evident when we had separated individuals of both groups according to the IDF criteria (men ≥ 94 cm and female ≥ 80 cm). Interestingly, CNTF, EGF, GDNF, IL-1α, MIP3A, TGFβ1 and GCP2 were elevated, and GM-CSF lowered in parallel with increasing waist circumference selectively in the GHD individuals. On the other hand, HGF and TIMP2 were elevated while IL-7, MIP-3A, GITR, IGF1 SR, IL-17, IL-2Rα, MIP1β and Oncostatin M lowered with increasing waist circumference only in the controls.

Conclusions

Our data provide the first information on specific changes in the ScAT adipokine protein levels in GHD adults. Moreover, they implicate a different regulation of cytokine ScAT levels in a comparable inflammatory setting, i.e. in equally obese subjects who differ in their metabolic status.

Supported by APVV-51-0406/02 and Slovak Diabetes Association. The study was approved by the local Ethics Committee and conforms to the ethical guidelines of the Helsinki Declaration.

P23

Human somatotrophic (GH) adenoma cells – interleukin (IL)-1β induces production of il-6 and il-8.

Signe Diness[1], Åse Krogh Rasmussen[1], Klaus Bendtzen[2], Michael Kosteljanetz[3] & Ulla Feldt-Rasmussen[1]
[1]Department Of Medical Endocrinology, Copenhagen University Hospital, Copenhagen, Denmark; [2]Institute Of Inflammatory Research, Copenhagen University Hospital, Copenhagen, Denmark; [3]Department Of Neurosurgery, Copenhagen University Hospital, Copenhagen, Denmark.

Aim

to establish a human *in vitro* system for the study of pituitary cells in culture and subsequently to study the influence of the pro-inflammatory cytokines interleukin (IL)-1β and tumour necrosis factor (TNF)-α on the function of the somatotrophic cells inclusive the ability of the cells to produce IL-6 and IL-8.

Methods

Pituitary adenomas were obtained from hypophysectomies of patients with acromegaly. The tissue was enzymatically digested and cultured in 24-chamber polystyrene plates in medium supplemented with nutritional factors and

antibiotics and with 10^5 cells per well. GH and cytokines were measured in the harvested supernatants.
Results
GHRH (GH releasing hormone) (30,000 ng/ml) stimulated significantly 72 h GH production from the somatotrophic cells (25% (10–50), median (range), $n=12$ chambers, $P<0.05$) compared to controls (3525 mU/l (49-17450)), while somatostatin (0.1–10,000 ng/ml) inhibited the 72 h GH production from the cells compared to controls ($P<0.05$, $n=12$–18). The GH production was significantly lower in cells cultured more than 15 days compared to younger cell cultures (<15 days). IL-1β (1000 and 100 pg/ml) stimulated modestly the 72 h GH production from the cells compared to controls (20% (10–50), $n=18$) and (15% (10–60), $n=18$), while TNF-α had no influence the function of the cells. The effect of IL-1β was reversible. IL-1β (10,000, 100, 10 pg/ml) also stimulated 72 h IL-6 and IL-8 production from the cells. IL-1β (10,000 pg/ml) induced a mean 12.3 and 8.2-fold increase in IL-6 and IL-8, respectively compared to control (mean 1472 pg/ml and 1948 pg/ml, respectively) in 4 different cultures.
Conclusion
We have established a robust *in vitro* system for studying the function of GH producing pituitary cells; GH production from the cells exhibited the expected responses to GHRH and somatostatin. IL-1β further stimulated the release of IL-6 and IL-8 from the cells, an effect that has been established also in other endocrine cells such as e.g. thyrocytes. The physiological and/or pathophysiological roles of these findings remain to be shown.

Diabetes and cardiovascular – presented on Sunday

P24

Serum ferritin concentrations in an impaired fasting glucose population and their normal control group

Faranak Sharifi, Yahya Jaberi & Nuraddin Mousavinasab
Zanjan University of Medical Sciences, Zanjan, Iran.

Background
Some recent studies have revealed the relationship among excess ferritin, coronary heart disease, and insulin resistance. To assess the association between serum ferritin concentration and Impaired Fasting Glucose, a prediabetes situation with insulin resistance, this study was designed in Zanjan, Iran.
Materials & Methods
187 people including 91 impaired fasting glucose (IFG) subjects and 96 normal glucose subjects who had been recognized in a large epidemiological study in Zanjan in 2001 were enrolled. The cohorts were well matched for age, sex and BMI. Body mass index and blood pressure of the participants were measured and serum cholesterol, triglyceride and ferritin were evaluated. All the data were analyzed by t-test, x^2 test and analysis of variance.
Results
Serum ferritin was higher in the IFG cohort (85.5±6.6 μg/l vs. 49.4±3.7 μg/l, *P*: 0.001). A positive correlation was found between fasting plasma glucose and serum ferritin in this study (*r*: 0.29, *P*: 0.001). Using multiple regression analysis, we found an association between serum ferritin and BMI (0.06, *P*:0.4), blood pressure (0.15, *P*:0.01), FPG (0.29, *P*:0.001), triglyceride(0.08, *P*:0.01) and cholesterol(0.07, *P*:0.03). The odd's ratio for the association of IFG in male subjects with the high serum ferritin level was 8.3 (C.I 95%:1.2–11.9, *P*:0.01) and for females was 3.06 (C.I 95%:0.58–15, *P*:0.1).
Conclusion:
Our study, implying that hyperferritinemia occurs before elevation of plasma glucose concentration more than 126 mg/dl. If prospective and interventional studies Confirm an etiologic role of iron overload in the pathogenesis of insulin resistance and type 2 diabetes, reduced dietary iron intake, especially in men with additional risk factors for type 2 diabetes, would appear to be a logical consequence.

P25

Implications of serum resistin in overweight diabetic patients with ischemic heart disease

Azza Farrag, Sameh Salama & Amal Rizk
Cardiology Department, Cairo University, Cairo, Egypt; Cardiology Department, Cairo University, Cairo, Egypt; Critical Care Medicine, Cairo University, Cairo, Egypt.

Background
Resistin is a recently discovered adipocyte-secreted hormone that links obesity with insulin resistance and/or metabolic and cardiovascular risk. This study was designed to investigate whether serum resistin concentrations constitute a significant coronary risk factor, with a particular focus on diabetes and one of its microvascular complications; nephropathy.
Methodology
Serum resistin was measured in 86 overweight patients with acute coronary syndrome (ACS) and 16 overweight healthy controls. Patients were divided into two groups according to presence or absence of diabetes: IHD with diabetes ($n=46$), and IHD without diabetes ($n=40$). In addition, patients with diabetes were subdivided into two groups: diabetics with microalbuminuria ($n=26$) and without ($n=20$).
Results
Non-diabetic IHD patients had a significantly higher level of serum resistin when compared to control participants (15.3±13 vs 6.3±2.7 ng/ml, $P=0.008$). IHD patients with diabetes had a significantly higher level of serum cholesterol, LDL and resistin compared to IHD patients without (204±43 vs 181±31 mg/dl, $P=0.048$), (129±36 vs 111±23 mg/dl, $P=0.048$) and (41±33 vs 15.3±13 ng/ml, $P=0.002$) respectively. Working on diabetic patients, the only significant difference between patients with microalbuminuria and those without is serum resistin concentration (55±37 vs 23±14 ng/ml, $P=0.011$). Pearson correlations including all subjects showed that serum resistin concentration had a significant positive correlation with both total serum cholesterol ($r=270$, $P=0.05$) and serum LDL ($r=313$, $P=0.026$).
Conclusion
This study showed that serum resistin concentration is associated independently with coronary atherosclerosis in overweight patients. Serum resistin is increased in patients with diabetes mellitus particularly those with microalbuminuria.

P26

The protective effect of tribulus terrestris in diabetes

Amr Amin, Mohamed Lotfy & Ernest Adeghate
UAE Universiy, Al-Ain, United Arab Emirates.

Tribulus terrestris (*TT*) is used in the Arabic folk medicine to Q1 treat various diseases. The aim of this study was to investigate the protective effects of *TT* in diabetes mellitus (DM). Diabetes is known to increase reactive oxygen species (ROS) level that subsequently contributes to the pathogenesis of diabetes. Rats were divided into six groups and treated with either saline, glibenclamide (Glib), or *TT* for 30 days. Rats in group 1 were given saline after the onset of streptozotocin (STZ)-induced diabetes; the second diabetic group was administered Glib (10 mg/kg body weight). The third diabetic group was treated with the *TT* extract (2 g/kg body weight), while the first, second, and third nondiabetic groups were treated with saline solution, Glib, and *TT* extract, respectively. At the end of the experiment, serum and liver samples were collected for biochemical and morphological analysis. Levels of serum alanine aminotransferase (ALT) and creatinine were estimated. In addition, levels of malonyldialdehyde (MDA) and reduced glutathione (GSH) were assayed in the liver. The tested *TT* extract significantly decreased the levels of ALT and creatinine in the serum ($P<0.05$) in diabetic groups and lowered the MDA level in liver ($P<0.05$) in diabetic and ($P<0.01$) nondiabetic groups. On the other hand, levels of reduced GSH in liver were significantly increased ($P<0.01$) in diabetic rats treated with *TT*. Histopathological examination revealed significant recovery of liver in herb-treated rats. This investigation suggests that the protective effect of *TT* for STZ-induced diabetic rats may be mediated by inhibiting oxidative stress.

P27

Prevalence of I27L polymorphism of hepatic nuclear factor-1[alpha] in diabetic patients younger than 35 years old.

Norma Amador, Manuel López, Jose Sánchez, Juan Guízar, Alejandra Zapién & Silvia Martínez
Mexican Institute Of Social Security, Leon/Guanajuato, Mexico.

Objective
In last decades prevalence of type 2 diabetes mellitus (DM) in children and young people worldwide has been reported increase. It is necessary to know according to biochemical and genetic characteristics the frequency of DM no corresponding to type 1 in our population. The objective of this study was to determine the prevalence of mutations on hepatic nuclear factor 1 [alpha], and 4 [alpha] in

diabetic patients younger than 35 years old with features of clinical autosomal dominant inheritance.
Material and Methods
The study included 140 diabetic patients (85 children and 55 young adults). It was approved by the local Ethical Committee. Glucose, C peptide, and β-cell autoantibodies measurements were performed. Polymorphisms of HNF [1 alpha] (I27L, G319S), and HNF [4 alpha] (T130I) were determined in all patients, when one of the polymorphisms was identified in a patient, all his/her family was studied by genetic evaluation.
Results
More than 50% patients showed overweight or obesity. The presence of DM in the father, overweight, and C peptide levels were higher in adults, while obesity, hypercholesterolemia, and β-cell autoantibodies were more frequent in those patients younger than 18 years old. Forty one (29.2%) patients showed the I27L polymorphism (24-Ile^{27}Leu and 17-Leu^{27}Leu). These patients were older, had higher BMI and C peptide levels than Ile^{27}Ile patients, and only 3 of them showed β-cell autoantibodies. In 5 patients we identified Thr^{130}Ile, and in one Gly^{319}Ser polymorphisms. I27L mutation was present in 30 families and T130I in one family. Patients in these families were older and showed higher BMI and C peptide levels, but lower glucose levels.
Conclusion
I27L polymorphism was present in almost a third part of diabetic patients with clinical autosomal dominant inheritance of the disease. These patients showed clinical and biochemical characteristics of DM no corresponding to Type 1 DM.

P28

Novel mechanism of chronic exposure of oleic acid-induced insulin release impairment in rat pancreatic β-cells

Takanori Kudo[1], Yoshiji Ogawa[1], Sechiko Suga[2], Ken Tomotsune[1], Noriyuki Hasegawa[1], Toshihiro Suda[1] & Makoto Wakui[3]
[1]Third Department of Internal Medicine, Hirosaki University School of Medicine, Hirosaki, Aomori, Japan; [2]Center for Education and Research of Lifelong Learning, Hirosaki University, Hirosaki, Aomori, Japan; [3]Division of Clinical Research, Hirosaki National Hospital, Hirosaki, Aomori, Japan.

A sustained, high circulating level of free fatty acids (FFAs) is an important risk factor for the development of insulin resistance, islet beta-cell dysfunction, and pathogenesis of type 2 diabetes. Here, we report a novel mechanism of chronic exposure of oleic acid (OA)-induced rat insulin release impairment. Following a 4-day exposure to 0.1 mM OA, there was no significant difference in basal insulin release when comparing OA-treated and untreated islets in the presence of 2.8 mM glucose, whereas 16.7 mM glucose-stimulated insulin release increased 2-fold in control, but not in OA-treated, islets. Perforated patch-clamp recordings showed that untreated beta-cells exhibited a resting potential of $-62.1 +/-0.9$ mV and were electrically silent, whereas OA-treated beta-cells showed more positive resting potentials and spontaneous action potential firing. Cell-attached single-channel recordings revealed spontaneous opening of ATP-sensitive potassium (K(ATP)) channels in control, but not in OA-treated, beta-cells. Inside-out excised patch recordings showed similar activity in both OA-treated and untreated beta-cells in the absence of ATP on the inside of the cellular membrane, whereas in the presence of ATP, K(ATP) channel activity was significantly reduced in OA-treated beta-cells. Electron microscopy demonstrated that chronic exposure to OA resulted in the accumulation of triglycerides in beta-cell cytoplasm and reduced both the number of insulin-containing granules and insulin content. Collectively, chronic exposure to OA closed K(ATP) channels by increasing the sensitivity of K(ATP) channels to ATP, which in turn led to the continuous excitation of beta-cells, depletion of insulin storage, and impairment of glucose-stimulated insulin release.

P29

Quality of care in a diabetic outpatient clinic

Ema Lacerda Nobre, Paula Chambel, Andreia Domingues, Marlene Paes da Silva, Zulmira Jorge, Valentim Santos, Joaquim Raimundo & João Jácome de Castro
Hospital Militar Principal, Lisboa, Portugal.

Background and aims
Type 2 Diabetes Mellitus affects a growing number of people allover the world. It is associated with serious complications. Several studies have shown that it is possible to prevent and minimize type 2 diabetes complications if it is treated appropriately over time. In our Hospital there is, since 1998, an outpatient clinics of diabetes. This study aimed to determine the quality of care provided to diabetic patients in our institution.
Subjects and methods
We reviewed the medical records of 776 diabetic patients, receiving care at our outpatient clinics since 1998.
Results
A total of 588 patients were included in the study, 58% were men with a mean age of 66.8 ± 27.2. HbA1c levels averaged 7.2 ± 1.65. 25.3% met the target blood pressure of 130/80 mmHg; 48% met the goal LDL cholesterol level <100 and 80% <130 mg/dl. 6.8% of patients met the combined ADA goal for BP, LDL and HbA1c. Concerning therapeutic regimens: 71.5% used oral hypoglycaemic agents (OAD) alone (52.1% of these were using 2 or more agents); 28.5% were treated with insulin (16.2% in combination with OAD).
Conclusions
HbA1c values reflects a good metabolic control. We emphasise the importance of combined therapy in the achievement of optimal glycaemic levels.The percentage of patients treated to the recommended BP of 130/80 mmHg is consistent with the results of other studies. LDL cholesterol levels compares favourable to the NHANES III study and is comparable with other published data. Despite the proved benefits of CV risk factors control in diabetic patients, international recommendations are difficult to achieve in clinical practice.

P30

Cardiovascular risk factors (CVRF) as predictors of microalbuminuria (MA) in type 2 diabetes mellitus (T2DM) patients

Francisco Javier del Cañizo-Gomez[1] & Maria Natividad Moreira-Andres[2]
[1]Hospital Virgen de la Torre, Madrid, Spain; [2]Hospital Universitario 12 de Octubre, Madrid, Spain.

MA is a marker of greatly increased cardiovascular morbidity and mortality in T2DM patients.
Objective
To perform a prospective study of normoalbuminuric T2DM patients, analysing the association between CVRF at baseline and the development of MA at follow-up.
Materials and methods
The prospective observational study was performed at Montes de Barbanza public health center, a specialized secondary referral center, which provides services to the 31 urban district of Madrid, Spain, and consisted in 348 T2DM patients. The inclusion criterion at baseline in 2002 was normoalbuminuria (urine albumin <30 mg/24 h.), and the exclusion criteria were previously diagnosed micro or macroalbuminuria or nephropathy. The clinical end-point was MA (urine albumin 30–300 mg/24 h.) at follow-up in 2005. The variables at baseline in 2002 were age, gender, onset-age of T2DM, HbA_{1C}, systolic (SBP) and diastolic blood pressure (DBP), total cholesterol (TCh), HDL-Ch, LDL-Ch, triglycerides (TGs), BMI and smoking; and were obtained from our records. Diagnosis of MA was made by two consecutive quantitative test of urine collected over 24 h. Comparison of mean levels were performed with the Student's "*t*" test for unpaired samples, and proportions with the chi-square test. Logistic regression analyses were performed with MA as a dependent variable, and age, gender, diabetes duration, and other CVRF as independent variables. An odds ratio (OR) >1.0 signifying a positive association, and $P<0.05$ was considered significant (SPSS, v. 13.0).
Results
Compared to those who still had normoalbuminuria at follow-up, the ones progressing to MA were males ($P=0.000$), and more likely to have a higher SBP ($P=0.001$) and TGs ($P=0.005$), and a lower HDL-Ch ($P=0.002$). The principal independent CVRF at baseline for the development of MA at follow-up were male gender (OR:3.36; $P=0.000$), elevated TGs (OR:2.17; $P=0.005$) and increased SBP levels (OR:1.03; $P=0.001$).
Conclusions
Male gender, elevated TGs and increased SBP, were independent CVRF for the development of MA in T2DM patients of the population studied. Other CVRF, as decreased HDL-Ch, was associated to MA in T2DM patients

P31

The prevalence of metabolic syndrome and its relation to metabolic control in patients with diagnosed type 2 diabetes

Florian Toti[1], Gazmend Bejtja[2], Kliti Hoti[1], Eduart Shota[1] & Erion Thengjilli[2]
[1]UHC "Mother Theresa" Service of Endocrinology & Metabolic Diseases, Tirana, Albania; [2]Institute of Public Health, Tirana, Albania.

Background and Aims
The identification of metabolic syndrome (MS) is important so that components of this syndrome can be managed appropriately to prevent or delay progression of associated cardiovascular risk factors. The aim of our study was to determine the prevalence of the Metabolic Syndrome as the NCEP/ATP III criteria in a selected population of type 2 diabetes from the Tirana Register of Diabetes.
Materials and Methods
In Tirana district we randomly selected 300 patients from the Tirana Register of Diabetes. 220/300 (73.3%) of the patients responded. All the patients had completed anthropometric measures and lipid profile after an 8-hour fast. All the patients having three or more of the criteria were defined as having Metabolic Syndrome (MS).
Results
The prevalence of the MS was 64.5%, in men 56.8% and 75.7% for women. The prevalence increased with age, from 16% before 40 years of age to 78% after 70 years. Diabetes duration was not different in patients with MS than those without it (M: 6.7 ± 3.4 vs 6.9 ± 3.7; F: 7.2 ± 3.8 vs 6.8 ± 3.6 yrs). The number of components of the MS was related to the age (ANOVA $P<0.05$) but not to diabetes duration. Central obesity was present to 36% of men and 85.4% of women, HTA 49.6 and 60.2%, low HDL 52 and 90%, high triglycerides 70.9 and 66.7% respectively. HbA1c was higher in persons with MS (9.6 ± 2.2 vs $8.7\pm1.4\%$, $P<0.01$).
Conclusion
The results show that MS is two-fold more prevalent in type 2 diabetes, compared with the general albanian population (64.5 vs 32%). The levels of cardiovascular risk factors are increased in type 2 diabetics and urged immediate efforts directed at controlling the components (mainly obesity, physical inactivity and lipid control) of MS especially in type 2 diabetes.

P32

Effects of rosiglitazone (RGZ) and pioglitazone (PGZ) on serum androgens and urinary steroid profile in patients with type 2 diabetes: A prospective, randomised cross-over study

Bernard Chappuis, Monika Braun, Christoph Stettler, Peter Diem & Emanuel Christ
Department of Endocrinology; Diabetology and Clinical Nutrition, University Hospital of Bern, Inselspital, Bern, Switzerland.

Background
Glitazones (GZ) influence androgen biosynthesis in PCO syndrome. At present it is unknown whether a) steroid hormone metabolism is influenced by GZ in patients with type 2 diabetes b) there is a differential effect of RGZ and PGZ on steroid hormone metabolism c) this effect is sex-specific and d) this effect is mediated by changes in insulin sensitivity. Therefore, urinary steroid profiles and serum total testosterone and DHEA levels were analysed before and after therapy with RGZ and PGZ in patients with type 2 diabetes.
Methods
17 patients with type 2 diabetes (7 women, 10 men, age: 60.8 ± 9.6, years, mean $\pm$ SD; BMI: 29.2 ± 4.7, kg/m^2; $HbA1_c$: $7.6\pm0.6\%$) were included in the study and assigned to RGZ or PGZ in a randomised cross-over study design for 12 weeks with an eight-week wash-out period in-between. Identical investigations (24-h-urinary steroid profile, plasma glucose (FPG), insulin (FI), $HbA1_C$, serum total testosterone and DHEA concentrations) were performed before and after each treatment period.
Results
RGZ and PGZ therapy resulted in a similar decrease in $HbA1_C$, FPG and FI concentrations without sex-specific differences. In *men*, RGZ resulted in a significant increase in serum testosterone levels compared to PGZ (RGZ: $+2.5\pm2.1$; nmol/L; mean $\pm$ SD; PGZ: $+0.5\pm3.3$; $P<0.04$), whereas DHEA concentrations remained unchanged. In *men* changes of urinary androstentriol, an androgen precursor, were significantly different after RGZ compared to PGZ (RGZ: $+45.7\pm158.1$; mcg/24 h; PGZ: -119 ± 161.1; $P<0.05$). In *women*, RGZ therapy resulted in a significant decrease in serum testosterone concentrations after RGZ compared to PGZ (RGZ: -0.3 ± 0.3; nmol/L; PGZ: $+0.3\pm0.4$; $P<0.05$). Serum DHEA levels were unaffected by PGZ and RGZ. In *women*, there were similar effects of PGZ and RGZ on urinary androgen metabolites.
Conclusion
These data suggest that 1. GZ impact on steroid hormone synthesis, 2. there is a differential effect of RGZ and PGZ 3. this effect is sex-specific and 4. this effect is not mediated by a differential effect of RGZ

P33

Abnormal glucose challenge test reflects mild gestational diabetes

Ayse Kafkasli[2], Ayse Sertkaya Cikim[1], Engin Burak Selcuk[3], Kezban Dogan[2], Feza Burak[5] & Saim Yologlu[4]
[1]Inonu University Faculty of Medicine Department of Internal Medicine Division of Endocrinology and Metabolism, Malatya, Turkey; [2]Inonu University Faculty of Medicine Department of Obstetrics and Gynecology, Malatya, Turkey; [3]Inonu University Faculty of Medicine Department of Family Medicine, Malatya, Turkey; [4]Inonu University Faculty of Medicine Departmant of Biomedical Statistics, Malatya, Turkey; [5]Ozel Dogu Hastanesi, Malatya, Turkey.

Objective
The status of carbohydrate metabolism of pregnant women with positive glucose challenge test (GCT), but normal oral glucose tolerance test (OGTT); and their neonates have not defined clearly.
Methods
Pregnant women with normal GCT (*n*: 120), with abnormal glucose challenge test (AGCT) but normal OGTT (*n*: 67) and those with gestational diabetes (GDM) (*n*: 67) were included into the study. Local ethical committee approval was obtained. Insulin sensitivity was evaluated by fasting insulin level, homeostasis model assessment of insulin resistance index (HOMA-IR); quantitative insulin check index (QUICKI) and IS_{OGTT}. Serum insulin and glucose values during OGTT were documented. The patients with both AGCT and GDM were treated either with diet or if needed with insulin until achieving the goals for defined glucose values. Perinatal outcome and delivery modalities were also compared between these three groups.
Results
Both GDM (31.6 ± 5.9 yrs) and AGCT groups (29.0 ± 4.0 yrs) were older than control subjects (28.1 ± 4.9 yrs). Body mass index (BMI) was found to increase with a correlation to the severity of carbohydrate intolerance as the predominant factor affecting both AGCT and GDM groups (odds ratios were 3.78 and 5.97 respectively). Despite there was no significance between insulin indices; serum glucose and insulin values were similarly different than controls in both AGCT and GDM groups. Macrosomic infant and caesarean section rates were higher than control group in both GDM and AGCT groups in favor of gestational diabetics (6.6% vs. 18.9%; $P=0.0001$ and 20% vs. 27.7% $P=0.0001$ respectively).
Conclusion
Pregnant woman with abnormal glucose challenge test have impaired carbohydrate metabolism as in gestational diabetics with a lesser severe degree.

P34

Ambulatory blood pressure reduction after rosiglitazone treatment in normotensive type 2 diabetic patients

Yasemin Kemal[1], Neslihan Tutuncu[1], Nilgun Guvener[1], Asli Unal[1], Ilyas Atar[2] & Haldun Muderrisoglu[2]
[1]Baskent University Faculty of Medicine Department of Endocrinology, Ankara, Turkey; [2]Baskent University Faculty of Medicine Deoartment of Cardiology, Ankara, Turkey.

Objective
The thiazolidinediones are new and potentially useful developments in the treatment of type 2 diabetes and impaired glucose tolerance. We tested the effects of the thiazolidinedione, rosiglitazone on blood pressure in normotensive type 2 diabetes.
Methods
After receiving approval from the local ethics committee, 25 normotensive diabetic patients were were enrolled to the study. Before the rosiglitazone treatment we measured plasma glucose, HbA1c, Hb, lipid profile and BMI. Also each subject underwent ambulatory blood pressure recording. Subjects were then placed on rosiglitazone treatment (8 mg per day) for twelve weeks, and baseline tests were repeated.

Results
At the end of twelve weeks there were significant decreases in total average diastolic blood pressure (67.02±8.06 vs 62.58±5.90, $P<.009$) and daytime average diastolic blood pressure (68.64±8.51 vs 65.12±6.34, $P<.01$). In addition, there were also significant decreases in fasting plasma glucose ($P=.007$), postprandial plasma glucose ($P=.01$), HbA1c ($P=.010$), and Hb levels ($P=.005$). Correlation analysis revealed that changes in diastolic blood pressures were not correlated with the decrease in both Hb, HbA1c. Also there was no significant correlation between the improvement in fasting and postprandial blood glucose and the decline in blood pressure.
Conclusion
Our study demonstrated a significant and sustained reduction in diastolic blood pressure with rosiglitazone therapy for 12 weeks, which was independent from the blood-glucose–lowering effect of the drug. Long-term studies are needed to determine the TZD-associated effects on blood pressure and other cardiovascular risk factors.

P35

Time dependent effects of rosiglitazone on heart and fluid dynamics: a 6-month follow up study

Yasemin Kemal[1], Nilgun Demirag[1], Neslihan Tutuncu[1], Aylin Yildirir[2], Asli Atar[2] & Asli Unal[1]
[1]Baskent University Faculty of Medicine Department of Endocrinology, Ankara, Turkey; [2]Baskent University Faculty of Medicine Department of Cardiology, Ankara, Turkey.

Objective
Thiazolidinediones (TZDs) have become a powerful tool for lowering insulin resistance. The problem of cardiovascular adverse events including fluid retention and risk of heart failure, although of a low incidence, should be well known and recognized. We aimed to evaluate the effects of rosiglitazone treatment on cardiac function and show whether these effects are reversible when we continued this treatment.
Methods
Fourty-six type 2 diabetic patients -without any symptoms and findings of heart failure-were randomized to treatment with rosiglitazone, metformin and control group after receiving approval from the local Ethical Committee. There were no significant differences between the groups in the duration of diabetes, HbA1c and plasma brain natriuretic pepetide (BNP) levels, body mass index (BMI) and myocardial performance indexes (MPI) before the treatment. After three months and after six months all these parameters were repeated.
Results
After three months period with rosiglitazone treatment, plasma BNP levels increased rapidly. Except one subject we did not see any clinical adverse effect including excessive weight gain, edema, and dyspnea so we continued rosiglitazone treatment. At the end of the six months period, this rapid increase didn't continue. Similarly, lateral wall MPIs worsened after three months- although statistically nonsignificant- and then improved significantly after six months in rosiglitazone group ($P=0.001$). Also the changes in hemoglobin values were highly correlated with other results that provide evidence of these reversible findings.
Conclusion
Our study showed the stability and reversibility of the adverse effects of TZDs on cardiovascular function and fluid dynamics in type 2 diabetics.

P36

Peculiarities of heart rate control in patients with non-insulin dependent diabetes mellitus and hypertension

Andris Vitols, Daina Voita & Mara Vitola
Institute of Cardiology, University of Latvia, Paul Stradins Clinical Hospital, Riga, Latvia.

Objective
To assess the sensitivity of exercise induced heart rate (HR) and baroreceptor reflex (BR) chronotrope reaction and HR variability for an early detection of autonomic nervous system impairment in non-insulin dependent diabetes mellitus (NIDDM) patients with arterial hypertension.
Design and Methods
On 25 NIDDM pts (group A, 63±1.8 yrs. aged men, HbA_{1C} 10.2±0.9%), 17 essential hypertension (EH) pts without glucose metabolism disturbances (group B, gender and age matched) and 20 controls (C) at rest and during handgrip (with force 50% of maximal for 60 s), beat-to-beat HR and finger mean arterial pressure (MAP) were monitored and bradycardic reaction to BR activation (by neck suction −60 mmHg) was analysed. HR variability by time and frequency domain analysis of ECG 512 R-R interval files was performed in supine and upright postures.
Results
Group A comparing to B and C was characterised by increased HR (81±2 vs.72±3 vs.70±3 bpm; $P<0.05$) and decreased bradycardic reaction to BR activation (1.95±0.3 vs. 4.9±0.9 vs.10±0.6 bpm; $P<0.05$). At 60th sec of handgrip MAP increase was similar in all groups but HR increase was reduced in group A vs. B vs.C (12±2 vs. 24±2 vs.18±2 bpm; $P<0.05$), but reaction to BR activation disappeared in group A and B, whereas in C remained in 32±11% of resting value. R-R interval variability in group A and B was diminished ($P<0.01$), but its decrease in upright position was less in group A than in C (108±12 vs. 254±21 ms; $P<0.05$), whereas the difference of increase in low-high frequency band ratio (LF:HF) was not significant in group A and B.
Conclusion
In patients with non-insulin dependent diabetes mellitus and hypertension, HR reaction to exercise and BR activation has an advantage over HR variability analysis to ascertain an early impairment of autonomic control of sinus node.

P37

One injection of Detemir insulin administered before the lunch improves the metabolic control in type 1 diabetic patients

Juan Carlos Ferrer-García[1], Juan Francisco Merino-Torres[2], Raquel Segovia-Portolés[2], Vicente Campos-Alborg[2], Agustín Herrera Ballester[1], Francisco Piñón Sellés[2] & Carlos Sánchez Juan[1]
[1]University General Hospital, Valencia, Spain; [2]University Hospital La Fe, Valencia, Spain.

Objective
To compare 2 modalities of bolus-basal insulin therapy with aspart-detemir, according to the moment of administration of detemir (DET), before the lunch or bed-time, in type 1 diabetic patients with poor metabolic control.
Methods
We conducted a prospective study of 40 type 1 diabetic patients, with poor metabolic control (HbAc 7–12%), randomized to receive treatment with 1 injection of DET before the lunch or bed-time and followed-up during 24 weeks. Physician decided the addition of one second dose, administering DET every 12 hours (DET-12 h) if the objectives in glycemic control were not obtained. Insulin analog aspart was used for the post-prandial control. Weight, insulin units/Kg/day, HbA1c, score in a test of quality of life (ITQ7) and hypoglycemias were determined.
Results
19 patients in DET pre-lunch group and 16 in DET bed-time group completed the study. 10 patients of group DET pre-lunch and 12 of DET bed-time needed DET-12 h. After 24 weeks of bolus-basal insulin therapy, a reduction of HbA1c was demonstrated, and the group DET pre-lunch showed a major reduction of HbA1c. By groups of treatment: DET pre-lunch 8.5 vs 7.1% ($P<0,05$); DET bed-time 9.0 vs 7.6% ($P<0.05$.) and DET-12 h 8.8 vs 8.1% ($P<0.05$.). The ITQ7 demonstrated an improvement without differences between the groups (score baseline visit 74.5±17.3 versus 62.0±19.2; $P<0.01$). There were no differences in weight and number of non-serious hypoglycemia. Serious hypoglycemia was presented in one patient of DET bed-time group. An increase in the insulin requirements was demonstrated in the 3 groups of treatment (average: 0.78±0.2 u/kg/day in baseline visit versus 0.86±0.2; $P<0.05$).
Conclusion
after this study, we recommend to begin detemir insulin treatment with one injection administered before the lunch. However, a strict monitoring is necessary because some patients will require two injections of detemir.

P38

Oral antibodies to insulin receptor are found effective in the treatment of streptozotocin-induced diabetes in rats

Julia Dugina[1], Andrey Martyushev-Poklad[1], Irina Kheyfets[1], Alexander Spasov[2], Maria Samokhina[2], Oleg Epstein[1] & Svetlana Sergeeva[1]
[1]'Materia Medica Holding' Company, Moscow, Russia; [2]Volgograd State Medica University, Volgograd, Russia.

An experimental study was designed to test a drug candidate for the treatment of diabetes mellitus in rats with streptozotocin (STZ) diabetes.

Diabetes was induced in outbred male rats (280–300 g) by single iv injection of streptozotocin 50 mg/kg. The animals showing hyperglycemia (12–15 mM) 72 hours after injection were randomized to receive daily intragastric doses of distilled water, glibenclamid 8 mg/kg, or polyclonal antibodies to C-terminal fragment of insulin receptor, beta subunit (ultra-low doses, anti-InsR); the last group received insulin subcutaneously (12 U/kg). For 7 weeks, the animals were monitored for fasting glycemia, glycosuria, and glucose tolerance.

STZ caused a sustained hyperglycemia (12–21 mM versus 2.3–3.2 in intact rats, maximum at day 42) and glycosuria (2.7–3.7 mM versus 0.8–1.8 mM in intact rats). Glucose tolerance reduced 3.5–5.5-fold (calculated by AUC in glucose load test). The rats featured polydipsia (an 2.7–3.2-fold increase in water consumption), body weight reduced by 50%. Due to diabetes and its complications, survival rate reduced to 12.5% (from 100% in intact rats).

Glycemia reduced by 30–50% in insulin group, and by 10–42% glibenclamid group, though remained abnormal. STZ-induced glycosuria remained unaffected in both groups. Survival rate increased up to 20%. Peroral anti-InsR was much more effective in reduction of glycemia (to normal values, 5.0–3.0 mM) and glycosuria (below 0.8 mM). Anti-InsR enhanced survival to 30%. The increase in glucose tolerance was most considerable in insulin and anti-InsR groups, less marked in glibenclamid group.

The peroral anti-InsR agent is regarded as a promising candidate therapeutic for the treatment of diabetes mellitus.

P39

1 year endurance training at the level of the ventilatory threshold in type-2 diabetics reduces by 50% healh costs: a controlled randomized trial

Brun Jean-Frédéric, Jaussent Audrey, Picot Marie-Louise, Mercier Jacques & Oréfaut Christian
CERAMM Service Central de physiologie Clinique, Hôpital Lapeyronie, Montpellier, France.

This trial was undertaken in order to evaluate the effects of endurance training on health cost in type 2 diabetes. 35 diabetic patients were randomly assigned to 2 groups: After 10 drop-outs, 15 followed a training program (8 sessions followed by training at home at the level of the ventilatory threshold V_T) while 10 had only routine treatment. Both groups were followed over 1 year with evaluation at 30, 120, 240 and 365 days for health costs, blood pressure, and a standard maximal exercise test, glycemic and lipid equilibrium, 6-min walking test, and exercise (Voorips) and quality of life questionnaires. The effectiveness of training was confirmed in the trained group by an increase in the Voorips score (5.25 ± 3.3 $P < .001$) and a lack of decrease in VO_{2max} and P_{max} while in the untrained group VO_{2max} decreased slightly (-2.16 ± 2.5 $P = 0.014$). Thus trained subjects at the end of the study reached a higher percentage of the theoretical maximal power ($P = 0.041$). The 6-min walking distance (472.2 ± 98.9 vs 547.6 ± 56.7 $P = 0.020$) was also higher than in the control group. Blood pressure, lipid profile and glycemic control did not significantly improve during this period in either groups, due decreasing doses in treatments prescribed by their physicians. In the trained group there was no hospitalization, in contrast ($P = 0.047$) with controls in whom there was 1.27 ± 2.20 (ie, 0 to 5 days) of hospitalization. The total health cost over this period is lowered by 50% in the trained group ($P = 0.018$). In conclusion, endurance training at the level of the V_T significantly prevents the progressive decline in aerobic working capacity evidenced in untrained diabetics over this period of observation. It results in a marked reduction in health cost due to a decrease in treatment and fewer hospitalizations.

P40

PED levels are increased in peripheral blood leucocytes from euglycaemic subjects at-risk of type 2 diabetes

Rossella Valentino[1], Gelsy Arianna Lupoli[1], Pietro Formisano[1], Iolanda Esposito[1], Anna Teresa Alberobello[1], Anna Perfetti[1], Michele Cirillo[2], Nicola Gennarelli[2], Giovanna Gargiulo[2], Roberto Lobello[2] & Francesco Beguinot[1]
[1]Dpt. Cellular and Molecular Biology and Pathology, Naples, Italy; [2]Dpt. Endocrinology/General and Digestive Surgery, Naples, Italy.

Background
Phosphoprotein enriched in diabetes (PED) is a scaffold protein widely produced in different tissues; it is involved in multiple cellular functions, including insulin-regulated glucose transport. Previous findings showed that in individuals with type 2 diabetes (T2D) the PED gene is overexpressed in skeletal muscle (SM) and adipose tissue (AT), both target tissues for insulin activity. Our group has recently evidenced that PED protein is also expressed in peripheral blood leucocytes (PBLs) and overexpressed in about 30% of diabetics.
Aim
To investigate the presence of any correlation in PED expression between PBLs and insulin-sensitive tissues, in order to validate this method as a possible screening in at-risk subjects for T2D.
Subjects and methods
21 subjects were recruited: 14 euglycaemic (7 T2D first degree relatives (FDR) and 7 without T2D family history) and 7 T2D patients. We evaluated PED protein expression analysing lysates from AT and SM, and PBLs by immunoblotting with specific PED antibodies.
Results
A two-fold increase in PED levels in AT and SM was found both in T2D patients and in FDR, compared with euglycaemic controls. On the whole, PED levels were 30% higher in PBLs than in SM and AT ($P < 0.001$) from the same subjects. Moreover, in all subjects there were significant correlations between PED levels in the PBLs and those in AT and in SM ($P < 0.001$).
Conclusions
PED expression can be detected in PBLs and its expression is correlated with that in insulin-sensitive tissues. Therefore, this method could become a valid aid to identify at-risk individuals for diabetes in large scale studies.

P41

Effectiveness of α-lipoic acid in prevention of peripheral diabetic neuropathy

Nino Jikurauli, Ellen Giorgadze, Nino Svani, Vasil Chachibaia, Felix Bestavashvili, Tea Lominadze, Nia Chanturia & Anna Malazonia
N4 clinical hospital, Tbilisi, Georgia.

Background
The aim of our study was to assess the effectiveness of α-lipoic acid for the reducing the risk of developing peripheral diabetic neuropathy.
Subjects and methods
We have studied 38 patients with type I and II diabetes, from age 28 to 35. The mean duration of disease was 9 years. Patients were divided in two groups. In group I were included 15 patients which got α-lipoic acid in order to prevent peripheral diabetic neuropathy in dosage 600 mg-50 ml a day i/v infusion during 3 weeks, then 600 mg a day per-os, during 2 months. The treatment was provided twice a year during the 2 year period. In group II were included 23 patients, who did not got α-lipoic acid. In both groups we studied HbAIC, fasting glucose, lipid profile, sensation screening tests; knee-jerk and tendon reflexes, subjective complaints were estimated by TSS scale.
Results
In I group of the patients the mean value of HbAIC was 7.0%, fasting glucose 133 mg/dl (±20); total Chol. 208 (±30), Trig 198 (±15), HDL 76 (±10); LDL 112 (±12), vibration sensation was decreased in 4 and a temperature sensation in 2 patients. The tactile and pain sensation were preserved. Knee-jerk and tendon reflexes were not changed, subjective symptoms by TSS were – 1.00;
In group II HbAIC was 7.2%, fasting glucose 138 mg/dl (±25); total Chol. 234 (±33); Trig 265 mg/dl; HDL 71 mg/dl; (±10); LDL 116 mg/dl, vibration sensation was decreased in 10, tactile sensation - in 2 and a temperature sensation in - 6 patients. The tactile and pain sensation were preserved. Knee-jerk and tendon reflexes were not changed, subjective symptoms by TSS was – 1.33;
Conclusion
Administration of α-lipoic acid for the lowering the risk of developing of peripheral diabetic neuropathy is required.

P42

Adhesion molecules s-VCAM-1 and s-ICAM-1 in members of families with familial combined hyperlipidemia

David Karásek, Helena Vaverková, Milan Halenka, Zdenek Fryšák, Dalibor Novotný & Marie Budíková
University Hospital, Olomouc, Czech Republic.

Objective
Familial combined hyperlipidemia (FCH) is the most common familial hyperlipidemia with a high risk of the early atherosclerosis. The aim of this study was to compare levels of s-ICAM-1 and s-VCAM-1 in asymptomatic members of FCH families with healthy controls and to find out relation between s-ICAM-1, respective s-VCAM-1, and risk factors accompanying FCH. We also investigate association between adhesion molecules and intima-media thickness of common carotid artery (IMT) in FCH families.
Methods
82 members of 29 FCH families were divided into the 2 groups: HL (probands and hyperlipidemic first-degree relatives, $n=47$) and NL (normolipidemic first-degree relatives, $n=35$). The control groups – HL-C ($n=20$) and NL-C ($n=20$) – consisted of sex- and age–matched healthy individuals.
Results
Hyperlipidemic members had significantly higher concentration of s-ICAM-1 (633.7 ± 169.6 ng/ml vs 546.2 ± 155.9 ng/ml, $P<0.05$). The elevation of s-VCAM-1 was not significant (880.8 ± 202.9 ng/ml vs 826.5 ± 174.6 ng/ml, N.S.). Levels of s-ICAM-1, respectively of s-VCAM-1 in normolipidemic relatives were not significantly different compared to the control group (530.8 ± 113.9 ng/ml vs 530.0 ± 101.0 ng/ml, respectively 860.2 ± 265.7 ng/ml vs 822.1 ± 197.0 ng/ml). There was significant correlation between s-ICAM-1 and apoB ($r=0.42$; $P<0.01$) in hyperlipidemic subjects and between s-ICAM-1 and proinsulin ($r=0.54$; $P<0.01$) in normolipidemic subjects. S-ICAM-1 correlated with IMT ($r=0.32$; $P<0.05$) in all members of FCH families.
Conclusions
The increase of s-ICAM-1 in asymptomatic hyperlipidemic members of FCH families reflects their high cardiovascular risk. The positive association between s-ICAM-1 and IMT could indicate s-ICAM-1 as a potential predictor of atherosclerosis manifestation.
This work was supported by grant IGA MZCR NR/ 9068-3.

P43

Body fat distribution, lipid and adipokine levels in South African type 2 diabetic patients of African and Indian origin

Rita Waisberg, Janice Paiker, Jaya George & Nigel Crowther
NHLS, Johannesburg, Gauteng, South Africa, WITS; Johannesburg, Gauteng, South Africa.

Introduction
The increased insulin resistance seen in Type 2 diabetic patients has been shown to be associated with abdominal fat accumulation, hypertension, and dyslipidaemia. The dyslipidaemia is characterized by raised serum triglycerides and decreased high density lipoprotein cholesterol (HDL) levels. The aim of the present study was to investigate the relationship between abdominal fat, lipid and adipokine secretion in South African type 2 diabetic patients of Indian and African origin.
Methods
Plasma and serum samples were collected from 20 African and 20 Indian diabetic females. Adipokines were measured using ELISA kits. Fasting plasma glucose, serum cholesterol, HDL-cholesterol, and triglycerides were assayed on the ROCHE MODULAR System. Insulin resistance was calculated using HOMA. CT-scans were performed to measure abdominal visceral and subcutaneous fat areas.
Results
Data presented as mean values $\pm$ SEM. The results for diabetic African (DA) and diabetic Indians (DI), respectively were as follows: Leptin (ng/ml) 40.6 ± 2.49 and 43.6 ± 2.1, soluble leptin receptor (U/ml) 21.0 ± 1.71 and 20.5 ± 1.7, IL-6 (pg/ml) 3.15 ± 0.58 and 3.87 ± 1.08, TNF-alpha (pg/ml) 7.06 ± 1.38 and 2.26 ± 0.42 ($P=0.003$), CRP (mg/l) 11.4 ± 3.09 and 8.97 ± 1.58, cholesterol (mmol/l) 4.78 ± 0.22 and 5.24 ± 5.24, HDL (mmol/l) 1.17 ± 0.05 and 1.76 ± 0.4, and triglyceride (mmol/l) 1.41 ± 0.11 and 2.11 ± 0.36, respectively. HOMA results for DI were 7.54 ± 0.74 and for DA 6.56 ± 1.26 ($P=0.507$). The visceral fat area was higher in diabetic Indian 117.47 ± 9.94 compared to African diabetic patients 93.85 ± 6.22 ($P=0.044$). No difference in BMI was noted between the groups.
Conclusions
Although visceral fat area is higher in diabetic Indian than diabetic African patients this seems to have no influence on adipokine levels. However, it may influence triglyceride metabolism.

P44

Low dose cyclosporin and methotrexate administration induces remission of Type 1 diabetes mellitus

Douglas Sobel, Val Abbassi & Annette Henzke
Georgetown University, Washington, DC, United States.

Although, high doses of cyclosporine (cyclo)has been demonstrated to inhibit the development of type 1diabetes mellitus (T1D), its usefulness was limited by its toxicity. Since methotrexate (Mtx) and cyclo have been shown to synergistically act in other disease processes, we determined if low dose cyclo and Mtx therapy could inhibit the development of diabetes and reduce or eliminate the need for insulin therapy in a pilot study.
Methods
Insulin dose and glycemic control were compared in 7 children (mean age 13.7 year) with new onset T1D who were administered cyclo at 7.5 mg/kg/day for 6 weeks and then 4 mg/kg/day and Mtx 5 mg/kg/day for one year and in 10 newly diagnosed diabetic control children (mean age 12.5 year). After 6 weeks, cyclo doses were adjusted to maintain blood cyclo levels 100–200 ng/ml. All children were treated with two daily doses of NPH and fast acting insulin. Clinical and biochemical toxicity of drug therapy was assessed. The study was approved by the Institutional Review Board.
Results
There were two episodes of mild mucositis which required transient lowering of the Mtx dose and one case of transient mild elevation of bilirubin. There were no abnormalities in other liver function tests, creatinine, BUN, or CBC. Mean HbA1c levels were similar in the experimental and control groups at baseline (12.6% vs 11.5%) and at 3, 6, 9, and 12 months. Daily Insulin requirements of the groups were similar at baseline. However the mean insulin dose (u/kg) at 3, 6, 9, and 12 months were significantly ($P<0.001$) lower in the experimental group (0.14 vs 0.56 at 3 months, 0.12 vs 0.61 at 6 months, 0.16 vs 0.55 at 9 months, and 0.22 vs 0.71 at 12 months). No control subjects became non-insulin requiring. However 4 of 7 experimental drug treated subjects were entirely off insulin therapy for 2.5, 4.5, 7 and 12 months. While off insulin therapy, the HbA1c levels of 3 of the 4 subjects were normal. The other subject's HbA1c was only mildly elevated at 6.7%. In conclusion. low dose cyclosporine and MTX treatment of subjects with new onset T1D can safely induce remission of disease and decrease the amount of required insulin.

P45

Progression of diabetic retinopathy in pregestational diabetes mellitus

R Guerrero[1], MA Martinez Brocca[1], MA Pomares[1], S Palma[1], I Gonzalez[1], D Acosta[1], E Moreno[2] & F Villamil[1]
[1]Departament of Endocrinology and Nutrition, Hospital Universitario Virgen del Rocío, Sevilla, Spain; [2]Departament of Obstetrics and Gynecology, Hospital Universitario Virgen del Rocío, Seviila, Spain.

Background
Pregnancy may adversely affect the progression of diabetic retinopathy and can have serious implications for the pregnant women.
Aim
To asses the impact of pregnancy on the progression of diabetic retinopathy in women with type 1 and type 2 diabetes mellitus and to identify risk factors for the progression of retinopathy during pregnancy.
Methods
306 diabetic women, 229 (75.4%) with type 1 and 77 (23.9%) with type 2 diabetes, referred to the Diabetes and Pregnancy Unit of the Hospital Virgen del Rocio from January 1995 through February 2004 were studied retrospectively. Dilated fundal examination was performed at booking, second and third trimester. At early postpartum was performed fluorescein angiographies.
Results
Retinopathy at booking was seen in 54 (17.6%). Any women without retinopathy at booking developed retinopathy during pregnancy or in early postpartum. Progression to proliferative retinopathy was seen in one patient (0.32%), while progression to moderate or severe non proliferative retinopathy was found in eight (2.6%). One women developed during pregnancy macular edema (0.32%). Progression of retinopathy was significantly increased in women with duration of diabetes >10 years (6.9% vs 0%, $P<0.05$). Laser therapy was needed in four (1.3%). Although glycaemic haemoglobin A1C (HbA1c) at booking was higher (7.95 ± 1.81 vs 7.02 ± 1.27) and the fall in HbA1c between booking and 16 weeks was greater (1.66 ± 1.33 vs 1.34 ± 1.08) in those women showing progression of retinopathy, these changes were not significant.
Conclusions
Progression of retinopathy in pregnancy was uncommon, but significantly more frequent in women with duration of diabetes more than 10 years. Laser therapy

was necessary in one percent of pregnancies, which is much lower than reported in earlier studies.

P46

Comparison of insulin monotherapy and combination therapy with insulin and metformin or insulin and rosiglitazone or insulin and acorbose in type 2 diabetes

Hamiyet Yilmaz, Alptekin Gursoy, Mustafa Sahin, Neslihan Bascil Tutuncu & Nilgun Guvener Demirag
Baskent University Faculty of Medicine, Department of Endocrinology and Metabolic Diseases, Ankara, Turkey.

The aim of this study was to compare the efficacy of treatment with insulin alone, insulin plus acarbose, insulin plus metformin or insulin plus rosiglitazone in type 2 diabetic subjects who were previously on insulin monotherapy, and to evaluate the effects of these treatments on cardiovascular risk factors including lipid profile, C-reactive protein (CRP) and fibrinogen. Sixty-six poorly controlled type 2 diabetic patients on insulin monotherapy were involved. They were randomized to insulin alone, insulin plus acarbose, insulin plus metformin or insulin plus rosiglitazone groups for six months period. Mean fasting and postprandial glucose values as well as HbA1c levels significantly decreased in all groups except for insulin plus acarbose group. The greatest improvement in HbA1c was observed in insulin plus rosiglitazone (2.4%) and insulin plus metformin (2%) groups. Daily total insulin dose increased 12.7 units/day in insulin alone group, decreased 4.7 units/day in insulin plus rosiglitazone group, 4.2 units/day in insulin plus metformin group, and 2.7 units/day in insulin plus acarbose group. Least weight gain occurred in insulin plus metformin group (1.4 kg) and greatest weight gain occurred in insulin plus rosiglitazone group (4.6 kg). Except for the improvement of total cholesterol levels in insulin plus rosiglitazone group, no significant change in lipid levels was observed in any groups. CRP levels decreased significantly both in insulin plus metformin and insulin plus rosiglitazone groups. Fibrinogen levels decreased in insulin alone, insulin plus metformin, and insulin plus rosiglitazone groups. All groups were comparable in hypoglycemic episodes. No serious adverse event was noted in any group.

P47

Competition between catecholamines and glucose for binding sites on proteins of erythrocytes

Michael Lepschy, Erich Moestl & Rupert Palme
Veterinary Medicine Vienna, Vienna, Vienna, Austria.

Glucose is slowly linked to haemoglobin in a non-enzymatic reaction and the determination of a glycated protein (HbA1c) is used for long term monitoring blood glucose concentrations. Radiometabolism studies in sheep showed that a haemoglobin-adduct formation also takes place with epinephrine or norepinephrine.

The aim of our study was to elucidate if there is a competition between catecholamines and glucose for binding sites on proteins of the erythrocytes.

Heparinised canine blood was obtained and centrifuged at 1500 g and the cells were washed three times with isotonic NaCl-solution. Afterwards, 5 portions of erythrocytes (0.7 ml each) were re-suspended in 7 ml TCM 'Eagle' and incubated with epinephrine and norepinephrine (1 and 10 ng/ml) for 3 days at 38.6 °C. One portion served as control.

Afterwards the erythrocyte portions were split into sub-samples of 0.2 ml each. One half was incubated with ^{3}H-norepinephrine for 1 h (unhaemolysed), the second half with ^{14}C-glucose for 10 days after haemolysis by freezing. To determine the uptake of ^{3}H-norepinephrine, samples were centrifuged and the radioactivity of the supernatant was measured. In total 410 ± 7 Bq of the added 574 ± 16 Bq were measured in the control samples, whereas all groups preincubated with non-radioactive catecholamines showed significantly ($P < 0.05$) higher radioactivity. To determine the binding of ^{14}C-glucose, proteins were precipitated. After centrifugation, 567 ± 24 Bq of the added 1916 ± 80 Bq were measured in the supernatant of the control samples. As in the experiment using ^{3}H-norepinephrine, significant ($P < 0.05$) higher values were measured in the supernatant of the preincubated erythrocytes, indicating a lower binding of ^{14}C-glucose to the proteins.

Therefore we conclude that catecholamines are blocking binding sites on proteins of erythrocytes for additional adduct formation with ^{3}H-norepinephrine or ^{14}C-glucose.

P48

Body composition, emotional state and quality of life in patients with diabetes mellitus type 2

Lina Lasaite & Jurate Lasiene
Institute of Endocrinology, Kaunas University of Edicine, Kaunas, Lithuania.

Diabetes mellitus type 2 (DM2) is affecting physical and psychological health.
Objective
Compare anthropometric data, body composition, lipids levels, emotional state, quality of life (QoL) of DM2 patients and that of healthy persons of the same age. 39 persons (58.1 ± 9.6 years) with DM2 (18 male, 21 female) and 41 healthy persons (54.3 ± 9.9 years) (22 male, 19 female). Profile of Mood State (POMS) used for emotional state evaluation, WHO Brief Quality of Life Questionnair – for QoL.
Results
In male weight (107.6 ± 30.1 vs 86.7 ± 23.1 kg, $P = 0.008$), body mass index (35.0 ± 9.9 vs 28.1 ± 5.4 kg/m^2, $P = 0.013$), fat mass (37.7 ± 21.0 vs 25.1 ± 12.3 kg, $P = 0.041$), lean mass (69.7 ± 10.6 vs 61.6 ± 11.7 kg, $P = 0.022$), water mass (52.1 ± 9.1 vs 45.1 ± 7.6 kg, $P = 0.007$, waist-to-hip ratio (0.97 ± 0.06 vs 0.91 ± 0.05, $P = 0.018$) were significantly higher in DM2 patients than in controls. In female weight (90.5 ± 14.6 vs 74.5 ± 18.7 kg, $P = 0.002$), body mass index (34.9 ± 6.2 vs 28.0 ± 5.7 kg/m^2, $P = 0.013$), fat mass (42.0 ± 10.4 vs 30.6 ± 12.2 kg, $P = 0.003$), lean mass (48.5 ± 6.4 vs 43.5 ± 7.8 kg, $P = 0.05$), water mass (38.1 ± 4.8 vs 33.5 ± 4.9 kg, $P = 0.004$), waist-to-hip ratio (0.90 ± 0.4 vs 0.83 ± 0.1, $P = 0.002$) were significantly higher in DM2 patients than in healthy female. In male and female no significant differences between research and control groups in high and low density cholesterol were found. In male, but not female QoL (79.3 ± 8.6 vs 85.3 ± 8.7, $P = 0.032$), POMS vigor (-11.8 ± 3.8 vs -15.8 ± 4.8, $P = 0.009$) were significantly lower in DM2 than in control group. Significant correlations were found in male between vigor and waist-to-hip ratio ($r = 0.347$, $P = 0.041$), in female between vigor and water mass ($r = 0.313$, $P = 0.049$), POMS total and waist-to-hip ratio ($r = 0.362$, $P = 0.046$), depression and low density cholesterol ($r = 0.430$, $P = 0.028$), vigor and lean mass ($r = 0.385$, $P = 0.014$).
In conclusion
Weight, body mass index, fat mass, lean mass, water mass, waist-to-hip ratio were significantly higher in male and female; quality of life and vigor were significantly lower in DM2 male than in healthy persons of the same age.

P49

Deleterious effects of beta-blockers on arterial stiffness and central pulse pressure in menopausal women: baseline findings from the Cashmere trial

S Laurent[2], S Christin Maitre[1], A Gompel[3], P Boutouyrie[2], P Jaillon[1], Ch Thuilliez[4], F Zannad[5], B Pornel[7], B Pannier[8], H Struijker-Boudier[9], L Van Bortel[10], Ch Kuntz[11], A Keanry-Schwartz[5], I Pithois-Merli[6], D Simonneau[6] & T Simon[1]
[1]Saint Antoine-UMPC-ParisVI university Hospital, Paris, France; [2]HEGP-Paris V-University Hospital, Paris, France; [3]Hotel Dieu-Paris V-University Hospital, Paris, France; [4]CHU Rouen, Rouen, France; [5]CHU Nancy, Nancy, France; [6]Pfizer, Paris, France; [7]Centre de menopause, Bruxelles, Belgium; [8]Department of cardiology, fleury-merogis, France; [9]Department of cardiology, Maastricht, Netherlands; [10]Department of cardiology, Ghent, Belgium; [11]CHU strasbourg, Strasbourg, France.

Beta-blockers (BB) may be less effective than other antihypertensive drugs for stroke prevention in patients with primary hypertension (ASCOT and LIFE studies). Our study compares arterial stiffness and central PP between users (BB+) and non users of BB (BB-), among menopausal women with hypercholesterolemia and no history of CV disease.
Methods and Results
We used the baseline data of 664 menopausal women, screened for the Cashmere study, a12-month double-blind randomized trial comparing the effects of atorvastatin (80 mg/day), vs placebo, ±with hormone therapy, on the progression of CCA-IMT and arterial stiffness. Aortic stiffness was measured by carotid-femoral pulse wave velocity (PWV); central PP and augmentation index (AI, wave reflection) were determined by applanation tonometry; carotid stiffness was calculated from relative stroke change in diameter (echotracking system) and carotid PP. BB were used in 104 women for treating headache, tachycardia, arrhythmia, and hypertension. 97% BB used were devoid of vasodilating properties. Age (60 ± 6 vs 58 ± 5 years, $P < 0.0001$) and mean BP (MBP: 91 ± 12 vs 88 ± 11 mmHg, $P < 0.0001$) were slightly but significantly higher in BB+ than in BB- ($n = 560$). After

adjustment to age and MBP, BB+ had 10% higher central PP ($P<0.0001$), 6% higher AI ($P<0.001$), 4% higher PWV ($P=0.04$), and 5% higher carotid stiffness ($P<0.01$) than BB-. BB+ had 4% higher central SBP ($P<0.0001$) than BB-, despite a non significantly higher brachial SBP only (1%, $P=$NS). To rule out an influence of hypertension on arterial parameters, we compared users of anti-hypertensive drugs ($n=110$) to non users ($n=554$). No significant difference was observed concerning the above parameters, excluding or not BB- users.
Conclusions
In menopausal women with hypercholesterolemia and no CV disease, the use of non-vasodilating BB was associated with higher aortic and carotid stiffness. These data are consistent with the results of the CAFÉ trial. Whether the deleterious effects of BB on large arteries increase the risk of CV events in women remains to be determined.

P50

Prevalence of GADA and IAA in elderly patients with type 2 diabetes

Maria Kurowska[1], Jerzy S Tarach[1], Helena Jankowska[2], Mariusz Kowalczyk[1], Joanna Malicka[1] & Andrzej Nowakowski[1]
[1]Dpt Endocrinology, Skubiszewski Medical University, Lublin, Poland; [2]Dpt Nuclear Medicine, Skubiszewski Medical University, Lublin, Poland.

Little is known about the prevalence and significance of islet cell immunity in elderly patients with type 2 diabetes. The low antibody titers against islet-cell antigens in LADA elderly patients may be a sign of a less aggressive autoimmune diabetes.
The objective
To establish the changing frequency and titers of GADA and IAA in elderly diabetics.
Material
83(56F;27M)diabetic patients (60–91 y) divided in age related groups. Group 1: 55 (36F;19M) patients (60–69 y). Group 2: 14 (9F;5M) pts (70–79). Group 3: 14 (11F;3M) pts (80–91). Mean duration of diabetes 5.6 ± 6.4 y.
Method
GADA and IAA determined by RIA (ANTI-INSULIN RIA and GAD-AB kits), (CIS). IAA estimated in patients not treated with insulin. The positive GADA and IAA titers were over 1 U/ml and 5.5%B/T.
Results
Group 1: Positive GADA were found in 13(27%) assays, 5(10.2%) patients with the level 7.1–64.5 U/ml and 8(16.5%) subjects 1.02–2.1 U/ml. In 11(22.9%) patients GADA titers 0.38–0.98 U/ml were found (method sensitivity >0.3 U/ml). The positive IAA were in 20(40.8%) assays (5.6–13.2%B/T). Group 2: In 3(21.5%) patients, the GADA were >1 U/ml (1.63; 38.5;68.5 U/ml). 4(28.6%) patients had GADA 0.93–0.99 U/ml. The positive IAA were obtained in 4(28.6%) patients (9.1–19.7%B/T). Group 3: There were positive GADA in 4(30.8%)assays (1.3–12.1 U/ml). In 8(61.5%%) patients GADA ranged 0.61–1.42 U/ml. In 6(42.9%) subjects the positive IAA was obtained (1 patient 36.1%B/T and the rest 5.6–6.95%B/T).
Summary
The percentage of patients with high GADA titer didn't significantly change with the age. In the older patients the frequency of GADA low titers (close to 1 U/ml) clearly increased. The IAA frequency and titer didn't significantly change with the age.
Conclusion
Eldery diabetic patients are characterized by increasing frequency of GADA sublimited titers as they aged. The autoantibodies low level may signify a less aggressive beta-cell autoimmunity as well as instability of the immunological system related to aging or both.

P51

Comparison of plasma homocysteine concentrations (HYC) in patients with acute coronary syndrome (ACS) and newly or previously diagnosed type 2 diabetes

Maria Kurowska[1], Jerzy S Tarach[1], Wojciech Gernand[2], Janusz Kudlicki[3], Adam Tarkowski[3], Andrzej Nowakowski[1], Teresa Czekajska[3] & Janusz Solski[2]
[1]Dpt Endocrinology, Skubiszewski Medical University, Lublin, Poland; [2]Dpt Laboratory Medicine, Skubiszewski Medical University, Lublin, Poland; [3]Dpt Cardiology, Skubiszewski Medical University, Lublin, Poland.

Introduction
The patients with ACS and scheduled for an elective coronary angiography have high frequency of both newly and previously diagnosed diabetes. The diabetic patients with acute myocardial infarction have an increased risk of death. Elevated blood HYC is strongly related to an increased risk for atherosclerosis and cardiovascular disease. This association is particularly evident in patients with diabetes.
Aim of the study
An attempt to evaluate whether cardiovascular risk expressed by serum HYC in ACS patients differs between groups of patients with newly or previously diagnosed type 2 diabetes.
Group of patients
95 cases (30F and 65M) of which 71 pts (18F; 53M) without previously diagnosed disorders of carbohydrate metabolism and 24 patients (12F and 12M) with previously diagnosed type 2 diabetes. Patients aged 41–90 years.
Methods
In all patients the following parameters have been measured: 1 The blood glucose level in the course of acute coronary disorders (admission glucose); 2 Fasting blood glucose in the next day; 3 Serum HYC applying chemiluminescence method (IMMULITE, DTC reagents). Diagnosis of type 2 diabetes has been established according WHO criteria.
Results
Patients with recent diagnosed t. 2 diabetes constituted 13% of group without previously known symptoms of carbohydrate disorders. The mean admission glucose level in the group with newly diagnosed diabetes was 151.8 ± 26.9 mg/dl; in the group with previously known diabetes was 218.8 ± 127.1 mg/dl. Mean HYC in the former group was 18.4 ± 7.3 μmol/l (F-20.2 ± 9.9; M- 17.5 ± 6.6 μmol/l) and 15.3 ± 5.2 μmol/l (F-15.3 ± 4.9; M-15.4 ± 5.6 μmol/l) in the latter, respectively. In the group with normoglycemia the mean serum HYC were 15.02 ± 5.2 μmol/l (M-15.5 ± 5.5 μmol/l, F-13.6 ± 4.6 μmol/l).
Conclusions
The cardiovascular risk estimated according to serum HYC is higher in ACS patients with newly diagnosed type 2 diabetes.

P52

The association between carotid artery intima-media thickness and cardiovascular mortality and morbidity in Type 2 diabetes: a retrospective study

Hayriye Esra Ataoglu, Mustafa Yenigun, Tayyibe Saler, Zehra Yigit, Levent Temiz, Zuhal Saglam, Faik Çetin, Baki Kumbasar, Fuat Sar & Suleyman Ahbap
Haseki Training and Research Hospital 4th Clinic of Internal Medicine, Istanbul, Turkey.

Carotid artery intima-media thickness (CCA-IMT) highly correlates with cardiovascular events in type 2 diabetes (T2DM). We aimed to determine the cardiovascular mortality and morbidity incidence regarding CCA-IMT and Framingham Score compared with preceding results of T2DM individuals. Our aim was to determine whether ultrasonographic evaluation of carotid arteries may predict cardiovascular mortality, morbidity and diabetic complications in T2DM patients.
Method
Demographic and clinical data of 102 T2DM individuals were registered including blood pressure, HbA_{1c}, lipid parameters, albumin excretion rate (AER), ECG and ultrasonographic evaluation of carotid IMT and reevaluated seven years later (2004). Primary end point was defined as cardiovascular mortality and morbidity. Student-t test, regression analysis and $[chi]^2$ tests were used. $P<0.05$ was significant.
Results
The percentage of patients reaching primary end point was 45.10%. Age ($P=0.043$), diastolic blood pressure (DBP) ($P<0.0001$), systolic blood pressure (SBP) ($P=0.004$), A_{1c}% ($P=0.042$), (AER) ($P=0.017$), triglyceride levels ($P=0.038$), IMT/CCA ($P=0.001$) and percentage of coronary risk assessment by Framingham Score were significantly high($P=0.001$) in patients presenting with any of the primary end points. Reevaluation at the end of 7 years revealed that measuring DBP, SBP and IMT/CCA was statistically important at assessing the risk of presenting with any primary end points in T2DM patients (Constant:$P<0.0001$).
Conclusion
Although Framingham Score predicts 10-year risk for cardiovascular mortality and morbidity in diabetic patients, we suggest that DBP, hypertriglyceridemia and microalbuminuria should also be included in risk scoring as well as the measurement of carotid IMT.

P53

Comparison of the effects of gliclazide and glibenclamide on insulin resistance and metabolic parameters

Rabia Gokturk, Hayriye Esra Ataoglu, Mustafa Yenigun, Suleyman Ahbap, Zuhal Saglam, Levent Temiz, Faik Çetin, Fuat Sar & Baki Kumbasar
Haseki Training and Research Hospital 4th Clinic of Internal Medicine, Istanbul, Turkey.

Introduction
It has been shown with many studies that sulphonylureas may have a negative effect on parameters of insulin resistance while improving glucose regulation. However not all of the sulphonylureas have the same effect. This analysis assessed the different effects of sulphonylureas on some metabolic parameters of insulin resistance.
Method
Newly diagnosed 25 T2DM individuals who were naive of oral antidiabetic therapy were recruited and randomized to either long lasting gliclazide (30–90 mg/day;$n=13$) or glibenclamide (1–3 mg/day;$n=12$) group. Body-mass index (BMI), waist-hip ratio and blood pressure as well as biologic parameters like blood glucose, $A1_c$(%), BUN, creatinine, uric acid, lipid parameters, microalbuminuria, CRP, insulin, c-peptide, glucagon, proinsulin and IGF1 levels were recorded at baseline and at the end of the third month. The ratios of glucose/insulin, proinsulin/insulin, HOMA-IR were assessed for each patient. Comparisons between groups were performed by Students t test. $[Chi]^2$ test was used for categorical variables. All analyses were two sided with a significance level of [alpha]=0.05.
Results
By the end of three months, gliclazide caused a decrease in c-peptide and insulin levels whereas glibenclamide resulted with a significant increase. Although insulin resistance was decreased in both groups it was evident in glibenclamide group. Creatinine levels were elevated in both groups which was significant with glibenclamide group. Uric acid levels were decreased in gliclazide group contrary to glibenclamide group in which uric acid levels were elevated.
Conclusion
Sulphonylureas have different effects on metabolic parameters of insulin resistance. These data suggest that gliclazide has a lowering effect on hyperinsulinemia. Yet this study is an observation based on small number of patients, studies with bigger numbers and longer duration are required for confirmation.

P54

24-hour ambulatory blood pressure and aortic dimensions in women with Turner syndrome

Britta Hjerrild[1], Keld Sorensen[2], Erik Morre Pedersen[3], Erik Lundorf[3], Arne Horlyck[4], Jens Sandahl Christiansen[1] & Claus Hojbjerg Gravholt[1]
[1]Medical Department M (Endocrinology and Diabetes) and Medical Research Laboratories, Aarhus University Hospital, Aarhus, Denmark; [2]Department of Cardiology, Skejby Hospital, Aarhus University Hospital, Aarhus, Denmark; [3]The MR Centre, Skejby Hospital, Aarhus University Hospital, Aarhus, Denmark; [4]Department of Radiology, Skejby Hospital, Aarhus University Hospital, Aarhus, Denmark.

Study objective
To study blood pressure (BP) levels and aortic dimensions in women with Turner syndrome (TS).
Materials and methods
102 women with TS (mean age 37.7 years; 18–62 years). 24 hour ambulatory BP measurement and echocardiography was performed on participants.
Results
Mean BP systolic (sys) and diastolic (dia) values were ($\pm$SD): sysBPday 128.0$\pm$15.3; diaBPday 81.6$\pm$11.8; sysBPnight 110.4$\pm$14.0 and diaBPnight 68.1$\pm$11.5. Heart rate (HR): 77.5$\pm$9.7.
Hypertension was found in a large proportion of the women: sysBPday. 36/97 (37%); diaBPday 44/97 (45%); sysBPnight 27/96 (28%) and diaBPnight 49/96 (51%). 34/97 (35%) did not have elevated BP levels, 22/97 (23%) had elevated levels in all 4 measures. 19 women already received antihypertensive treatment, and sys BP was significantly higher in this group. Aortic diameters (cm):

	Mean	SD	% above cutoff
Aortic Annulus	1.83	0.18	0
Sinus	2.80	0.41	15
Sinotubular level	2.63	0.42	11
Brachial trunk	2.08	0.40	0

17 individuals had aortic diameters above expected levels. A positive correlation was found between systolic BP ($r=0.36$; $P=0.001$) and age, but not weight or BMI. HR correlated negatively to VO_2max ($r=0.22$; $P=0.038$). We found no correlation between BP and aortic diameters or age and aortic diameters. There was however a significant increase in aortic diameters in TS with karyotype 45,X compared to others ($P<0.02$) and in TS with bicuspid aortic valves ($P<0.02$).
Conclusion
Hypertension is common in TS, affecting more than 50% of the study group, and subjects on antihypertensive treatment were insufficiently treated. Aortic dimensions are larger in TS (17%), especially with the karyotype 45, X. In this study we found no correlation between BP and aortic dimensions.

P55

Plasma marker of lipid peroxidation and type 2 diabetes in subject with coronary artery disease in Iranian subjects

Fariborz Haghparast & Jaffar Nourooz-Zadeh
Islamic Azad University-Larestan Branch, Larestan, Iran.

Objective
Abnormal lipid profile is an important risk factor in the development of macrovascular atherosclerotic complications in patients with type 2 diabetes mellitus (T2D). The aim of this study was to investigate the relationship between lipid profile and lipid peroxidation in type 2 diabetics with and without coronary artery disease (CAD).
Materials and methods
We studied 80 patients with T2D, 40 with CAD and 40 without CAD. We also studied 50 non-diabetics, 30 with CAD, and 20 without CAD. Lipid profile was estimated by the total, HDL, LDL cholesterol and triglyceride (TG). To evaluate the oxidative status we measured circulating malondialdehyde (MDA), plasma levels of superoxide dismutase (SOD), glutathione (GSH), as well as vitamin E and C.
Results
No significant difference was found in the lipid profile in patients with T2D and CAD patients. There was significantly difference in the level of MDA between the groups. In diabetics, MDA positively correlated with the total cholesterol, LDL -C, total lipid, and the relations between LDL/HDL and TG/HDL ($P<0.001$). In non-diabetic with CAD group, MDA positively correlated with total cholesterol, ($P<0.05$). There was significant difference in the SOD, glutathione, vitamin E / total lipid and vitamin C between the groups of diabetics and were lower in the diabetes group with CAD ($P<0.05$). There were significant negative correlations between MDA and vitamin E and C in groups with T2D, but it was statistically significant in the non-diabetic with CAD ($P<0.05$).
Conclusion
Type 2 diabetes is associated with excess risk of CAD and primary therapy should be directed first at lowering lipid peroxidation. CAD and T2D alone and combined carry similar atherosclerotic burden concerning lipid profile, enzymatic and nonenzymatic antioxidative status and lipid peroxidation.

P56

Abstract unavailable

P57

Effect of testosterone replacement therapy on adipocytokines in hypogonadal men with Type 2 diabetes

D Kapoor[1], S Clarke[3], R Stanworth[1], KS Channer[2] & TH Jones[3]
[1]Centre for Diabetes and Endocrinology, Barnsley NHS Foundation Trust Hospital, Barnsley, United Kingdom; [2]Department of Cardiology, Royal Hallamshire Hospital, Sheffield, United Kingdom; [3]Academic Unit of Diabetes, Endocrinology and Metabolism, University of Sheffield, Sheffield, United Kingdom.

Serum testosterone level is known to inversely correlate with insulin sensitivity and obesity in men. Furthermore, there is evidence to suggest that testosterone replacement therapy reduces insulin resistance and visceral adiposity in Type 2 diabetic men. Adipocytokines are hormones secreted by adipose tissue and contribute to insulin resistance. We report a double-blind placebo controlled crossover study in 20 hypogonadal Type 2 diabetic men examining the effect of

testosterone replacement therapy on adipocytokines and CRP. Patients were treated with testosterone (Sustanon 200 mg) IM every 2 weeks or placebo for 3 months in random order followed by a wash-out period of 1 month before the alternate treatment phase. At baseline, leptin levels significantly correlated with BMI ($r=0.71$; $P<0.001$) and waist circumference ($r=0.78$; $P<0.001$). There was also a significant inverse correlation between IL-6 levels and total testosterone ($r=-0.68$; $P=0.002$) and bioavailable testosterone levels ($r=-0.73$; $P=0.007$). CRP levels also correlated significantly with total testosterone levels ($r=-0.59$; $P=0.01$). Testosterone treatment reduced leptin (-7141.9 ± 1461.8 pg/ml; $P=0.0001$) and adiponectin levels (-2075.8 ± 852.3 ng/ml; $P=0.02$). There was a significant reduction in waist circumference (-2.1 ± 0.81 cm; $P=0.02$). No significant effects of testosterone therapy on resistin, TNF alpha, IL-6 or CRP levels were observed.

In conclusion, testosterone replacement treatment decreases leptin and adiponectin levels in Type 2 diabetic men. Moreover, low levels of testosterone in men are associated with inflammation, though testosterone treatment over 3 months had no effect on inflammatory markers.

P58

A role of the liver in the infringements of lipid metabolism of patients with diabetes mellitus type 2 and metabolic syndrome

Vadim Korpachev[1], Alla Kovalchuk[1], Natalija Kushnareva[1] & Volodymyr Kovalchuk[2]
[1]V.P. Komissarenko Institute of Endocrinology and Metabolism, Kyiv, Ukraine; [2]O.O. Bogomolets National Medical University, Kyiv, Ukraine.

Different interdependences with symptoms of insulin resistance give us the possibility to consider steatosis as a disorder of the liver with metabolic syndrome (MS).

The aim of study is to assess the role cholesterol-HDL in rise of diabetic steatohepatosis.

40 patients with Diabetes Mellitus type 2 (DM) and signs of MS were examined to determine spreading of steatohepatosis as one of the factors of insulin resistance. Only 8 of them didn't have diabetic hepatopathy, while 32 patients had adipose infiltration of the liver (according to the results of the ultrasonic examination).

Actual difference between the two groups was revealed in the rate of HDL decrease. So, if the patients with DM type 2 and symptoms of MS with steatohepatosis have the rate of cholesterol-HDL decrease which is $34.36\pm4.2\%$ from the low norm measure, the patients with the same symptoms, but without steatohepatosis, had $6.8\pm0.2\%$ ($P<0.05$). We distinguished a group of patients who had prevalent fasting hyperglycemia. Those patients who had prelevant postprandian hyperglycemia formed the group of comparison. Analyzing the findings, it is necessary to mention that the group of patients with prevalent fasting hyperglycemia were effected by more serious disorders with lipid metabolism, they had a lower level of cholesterol-HDL than those who had rather high postprandian hyperglycemia (0.89 ± 0.03 vs 1.027 ± 0.05 mmol/l, $P<0.05$) and rather high percentage of a waste circle growing that indicates of a greater aggressiveness of MS factors.

Thus, it was determined that prevalent fasting hyperglycemia which effects patients with DM type 2 and diabetic hepatopathy in condition of adipose infiltration confirmed by echographic results is a proof of a major role of the liver in the infringement of lipid metabolism that contributes to increasing of insulin resistance due to, so called, 'lipid toxity'.

P59

Radionuclide study of hepatobiliary system function in patients with type 2 diabetes and metabolic syndrome

Valentin Slavnov, Alla Kovalchuk, Vadim Korpachev & Natalija Kushnareva
V.P. Institute of Endocrinology and Metabolism, Kyiv, Ukraine.

The aim of the research is to study the functional state of liver parenchyma in patients with type 2 diabetes and to analyze hepatobiliary system disorders depending on marker of metabolic syndrome (MS).

The study involved 22 patients with type 2 diabetes and MS and 8 healthy persons. Dynamic hepatoholecystoscintigraphy was performed using RKC 301T gamma camera after Tc-99m mesida administration and bile-expelling meal.

We established a reliable increase of maximum accumulation time of radiopharmaceutical in parenchymatous cells of the liver (22.0 ± 3.76 vs 14.6 ± 0.83, $P<0.01$) comparing to healthy. Also these patients have infringements of secretory functions that are confirmed by meaningful increase of radionuclide half-deduction time (T1/2) from the liver (60 ± 4.16 vs 45.2 ± 3.49, $P<0.03$). Also a reliable T1/2 delay occurs in patients with type 2 diabetes and metabolic syndrome comparing to healthy. But in patients with smaller body mass index was found significant lowering of time of radiopharmaceutical occurrence in intestine that testifies the hypotonia of Oddi's sphincter. In patients with decompensation stage of carbohydrate metabolism comparing to subcompensation occurs meaningful increase of liver T1/2 that points on excretory function delay. Nevertheless we have not found any significant relations between delay of liver excretory function and HOMA, level of C-peptide, triglycerides, cholesterol, HDL-cholesterol, LDL-cholesterol, WHR, arterial hypertension in patients with type 2 diabetes and metabolic syndrome. Different impacts of per oral hypoglycemic drugs displayed significant lowering of excretory function in patients taking metformin comparing to those who were taking sulfonylurea due to T1/2 elongation of liver (61.75 ± 5.54 vs 39.75 ± 6.62, $P<0.05$). The obtained findings suggest that absorbing and excretory functions of liver slow down at increase of BMI and decompensation stage in patients with type 2 diabetes and metabolic syndrome. But other markers of metabolic syndrome are not defining in early disturbances of liver excretory function in mentioned patients.

P60

Omega-3 polyunsaturated fatty acids in the treatment of cardiovascular autonomic neuropathy in Type 2 diabetes mellitus patients

Victoria Serhiyenko, Alexander Serhiyenko & Ludmila Serhiyenko
Medical University, Lviv, Ukraine.

Background and aims
The aim of this study was to assess the effects of docosahexaenoic (DHA) and eicosapentaenoic acid (EPA) on the heart rate variability (HRV), some biochemical parameters in patients (pts) with Type 2 diabetes mellitus and cardiovascular aunonomic neuropathy (CAN).
Materials and methods
39 pts with CAN (54 ± 5 yrs) were allocated in two groups: A ($n=26$) were receiving capsules of fish oil every day (2.0 g EPA, 2.0 g DHA and 0.1% α-tocopherol acetate), B ($n=13$) - placebo capsules of olive oil. We investigated the activities of protein-kinase C (PK-C), Na^+, K^+-ATPase, Ca^{2+}, Mg^{2+}- ATPase in the membranes of RBC's, levels of the ^{125}I-6-ketoprostaglandin F1alpha (6--ketoPGF1alpha), ^{125}I-thromboxane B_2 (TXB_2) in the blood plasma.
Statitics
ANOVA.
Results
The manifestation of the CAN is accompanied by decrease of the Na^+, K^+-ATPase, Ca^{2+}, Mg^{2+}-ATPase activities ($P<0.001$), 6-ketoPGF1alpha, EPA level ($P<0.001$) with increase of TXB_2, PK-C activity, stage of platelet's aggregation, QTc interval. After four months of treatment there were a decrease of TXB_2 level (141.2 ± 15.4 pg/ml, $P<0.001$), activity of PK-C (14.46 ± 4.52 pmol ^{32}P/mg protein per 1 min, $P<0.001$), degree and speed of an aggregate of thrombocytes with simultaneous increase activities of Na^+, K^+-ATPase (0.1 ± 0.004 mMol P_i/mg protein per 1 hour, $P<0.001$), Ca^{2+}, Mg^{2+}-ATPase and the level of the 6-ketoPGF1alpha in the group A marked. Also, we observed significant improvement of HRV parameters, decrease of QTc interval ($P<0.01$).
Conclusion
DHA and EPA at moderate doses may exert antithrombotic effects and may be used for prophylaxis and treatment of patients with Type 2 diabetes mellitus and cardiovascular aunonomic neuropathy.

P61

Nicotinamide and alpha-lipoic acid in the treatment of cardiovascular autonomic neuropathy in Type 2 diabetic patients

Ludmila Serhiyenko, Alexander Serhiyenko & Victoria Serhiyenko
Medical University, Lviv, Ukraine.

Background and aims
The present study has examined the effect of α-lipoic acid (ALA) and nicotinamide (NA) on the heart rate variability (HRV), superoxide dismutase (SOD), gluthation peroxidase (GPO), catalase activities, reduced gluthatione (GSH), malondialdegide (MDA) contents in the RBCs' in Type 2 diabetic patients (T2DM) with cardiovascular autonomic neuropathy (CAN).
Materials and methods
59 patients with T2DM and CAN (59.3 ± 7.9 years) were allocated to three treatment group: (1) daily per os dose of ALA 600 mg ($n=29$); (2) NA 700 mg ($n=18$); (3) ALA 600 mg and NA 700 mg ($n=12$) during 2 months.

Statistics
ANOVA.
Results
The progress of CAN is accompanied by decrease of the activities of SOD (7.38 ± 0.29, $P < 0.001$), GPO and catalase ($P < 0001$), the content of GSH and increase of the MDA ($P < 0.001$) in RBC's. After 2 months of a treatment course with ALA it the increasing spectral power in the low- and high frequency ($P < 0.01$), coefficient of variation ($P < 0.05$). Simultaneously, activity of SOD, GPO ($P < 0.001$) and GSH concentration were authentically augmented, and the contents of MDA, QTc interval parameters (0.52 ± 0.057, $P < 0.05$) was reduced ($P < 0.01$). Simultaneously introduction of NA and ALA is conducted with more significant increasing SOD - 9.14 ± 1.25 IU/ml Rbc's, $P < 0.001$; GPO - 298.14 ± 19.45 mcmol GSH/min Hb, $P < 0.001$) and GSH concentration (1.97 ± 0.04 mcM/g Hb, $P < 0.001$), TRAC ($P < 0.001$), HRV, decreasing of MDA concentration ($P < 0.001$) and QTc interval parameters.
Conclusion
Usage of ALA and NA is accompanied by improvement of HRV, QTc interval, antioxidant defence parameters and may be used for the treatment of CAN.

P62

Plasma measures of oxidative stress and antioxidant status in type 2 diabetes mellitus

Fariborz Haghparast & Jaffar Nourooz-Zadeh
Islamic Azad University-Larestan Branch, Larestan-Fars, Iran.

Objective
The aim of this study was to test the hypothesis that type 2 diabetes mellitus is associated with increased oxidative stress in Iranian subjects.
Materials and methods
The study population consisted of Fifty-nine patients with type 2 diabetes (mean age 62.5 ± 8.7 years). Type 2 diabetes was diagnosed according to the American Diabetes Association criteria. 36 patients had diabetes complications and 23 patients had no complications. For the normal control subjects, fifty-five age- and sex- matched healthy control subjects (mean age 63 ± 5.7 years) were included. Plasma α-tocopherol (α-ToH) was analyzed with HPLC. Malondialdehyde (MDA), plasma glutathione (GSH), vitaminC and superoxide dismutase (SOD) were spectrophotometrically measured. Total cholesterol, triacylglycerol, LDL-cholesterol, HDL-cholesterol, HbA1c, uric acid, blood urea nitrogen (BUN) and creatinine (Cr) were studied.
Results
Plasma α–TOH-to-lipid ratio, glutathione and vitamin C levels were significantly decreased in type 2 diabetes compared with controls (all $P < 0.05$). Plasma vitamin C and glutathione levels in diabetic patients with complications were significantly lower than in those without complications (51.86 ± 2.6 vs. 62.31 ± 2.7 μmol/L, $P < 0.001$, 64.02 ± 7.6 vs. 125.33 ± 25.6 nmol/L, $P < 0.05$, respectively). MDA concentration was significantly higher in patients compared with controls ($P < 0.005$) as well as diabetes with complication compared to without complications ($P < 0.05$). Plasma levels of α–TOH/ total lipid was similar in diabetic patients with or without complications. Plasma concentration of uric acid and SOD were significantly lower in patients with diabetes than in control subjects.
Conclusions
Our results support the oxidative stress hypothesis for type 2 diabetes mellitus. We therefore suggest that oxidative stress is an early stage in the disease pathology, which may contribute to the development of complications.

P63

Does screening of primary hyperaldosteronism only lead to diagnosis of more adrenal hyperplasia?

Helga Sigurjonsdottir[1], Mikael Gronowitz[2], Ove Anderson[3], Robert Eggertsen[4], Hans Herlitz[5] & Gudmundur Johannsson[1]
[1]Dept. of Endocrinology and Metabolism, Sahlgrenska University Hospital, Gothenburg, Sweden; [2]Nödinge Primary Care Center, Nödinge, Sweden; [3]Hypertension outpatient clinic, Sahlgrenska University Hospital, Gothenburg, Sweden; [4]Mölnlycke outpatient clinic, Mölnlycke, Sweden; Nephrology outpatient clinic, Sahlgrenska University Hospital, Gothenburg, Sweden.

Background
The prevalence of primary hyperaldosteronism (PA) has recently been reported as high as 7.5% in patients with hypertension from unselected populations (USP) and up to 22% in selected populations (SP). Whether increased screening and diagnosis of PA will lead to more findings of curable PA (aldosterone producing adenoma, APA) is unclear.
Methods
Three-hundred fifty-three consecutive patients with hypertension, age 20 to 88 years were included from two primary care centers (230 USP) and specialized university hypertension outpatient clinics (123 SP) in the same catch-up area. Plasma renin activity and serum aldosterone levels were sampled. Patients with criteria for PA did positional and salt-loading test for confirmation. Further investigation was with CT-adrenals, ^{131}I-chol-scintigraphy and adrenal vein sampling (AVS), with few exceptions. The local ethical committee approved the study.
Results
Forty-six patients, 28 SP (22.8%) and 18 USP (7.8%), had criteria for PA. Six USP and 22 SP were available for further investigation. Confirmation tests found 8 patients to be normal. Nine of eighteen (53%) CT-adrenals had positive findings. Three of 17 (18%) ^{131}I-chol-scintigraphies had positive findings. Ten of 14 AVS (71%) had positive findings. Four AVS were unsuccessful. Supplementary information from ^{131}I-chol-scintigraphy and CT-adrenals lead to clear diagnosis in 3 of them, 1 AH and 2 APA. Further investigation after screening lead to clear diagnosis in 27 of 28 (96%) patients, 8 AH, 11 APA.
Conclusion
The study confirms high prevalence of PA found in recent studies, higher in SP than USP as expected. Our results indicate that screening for PA finds more patients with curable cause of PA. All APA were from the SP group. Our findings indicate that ^{131}I-chol-scintigraphy is less accurate for diagnosing APA than CT-adrenals and that the AVS is superior to both of them, further comparison of the methods are needed.

P64

Liquorice in moderate doses decreases serum levels of vitamin B12 but does not affect the serum lipid levels

Helga Sigurjonsdottir[1], Margret Arnadottir[2] & Sven Wallerstedt[3]
[1]Dept. of Endocrinology and Metabolism, Sahlgrenska University Hospital, Gothenburg, Sweden; [2]Dept. of Nephrology, Landspitali University Hospital, Reykjavik, Iceland; [3]Dept. of Internal Medicine, Sahglrenska University Hospital, Gothenburg, Sweden.

Background
Liquorice in moderate doses increases blood pressure (BP) in healthy individuals (NT) as well as patients with hypertension (HT) due to increased cortisol effect. Glycyrrhetinic acid, the active substance in liquorice, inhibits 11beta-hydroxysteroid-dehydrogenase type 2 (11betaHSD2) which converts the active hormone cortisol to the inactive hormone cortisone. Recently it has been reported that treatment with glucocorticoids decreases serum levels of cobalamin (B12), it is also known that increased cortisol levels negatively affect different metabolic risk-factors as serum lipids. Hence, it is possible that liquorice due to its increased cortisol effect can decrease serum levels of B12 and affect the lipid levels negatively.
Methods
Thirty-six individuals, 25 NT (13 men and 12 women) and 11 HT (8 men and 3 women), 22–44 years old, consumed 100 g of liquorice (150 mg GA) daily for 4 weeks. Blood tests were taken, 24-hour-urin collected and BP measured before and after the liquorice consumption. The study was approved by the local ethical committee.
Results
Serum-B12 decreased from 299 ± 78 pmol/L to 284 ± 78 pmol/L in the whole group ($n = 36$, $P = 0.005$), from 322 ± 77 pmol/L to 303 ± 74 pmol/L ($P = 0.005$) in the NT group and from 288 ± 82 pmol/L to 270 ± 77 pmol/L ($P = 0.007$) in men. Serum levels for total-cholesterol, LDL-cholesterol, HDL-cholesterol, triglycerides, apolipoprotein A1 and lipoprotein(a) did not change after liquorice consumption. Serum levels for apolipoprotein B (ApoB) decreased from 0.83 ± 0.22 g/L to 0.81 ± 0.22 g/L ($P = 0.04$) in the whole group ($n = 36$). The ratio for urinary free cortisol/cortisone (Q, an indicator of 11betaHSD2-activity) increased significantly in all groups ($P < 0.001$ in all groups). No statistical difference was found between the genders or between the NT-and HT-groups.
Conclusion
The glucocorticoid-effect induced by liquorice consumption in moderate doses for 4 weeks is sufficient to significantly decrease the serum concentration of B12, which is a novel finding. Even if the decrease is not substantial it can be of clinical importance. This moderate dose of liquorice does not affect the serum lipid levels.

P65

Decreased insulin sensitivity in young lean hypertensive men is not associated with increased visceral fat and changes in plasma adipocytokines

Adela Penesova[1], Zofia Radikova[1], Eva Cizmarova[2], Vitazoslav Belan[3], Milan Vigas[1] & Juraj Koska[1]
[1]Institute of Experimental Endocrinology SAS, Bratislava, Slovakia; [2]Outpatients Clinic of Pediatric Cardiology, Karlova Ves, Bratislava, Slovakia; [3]Radiological Clinic of Faculty Hospital, Bratislava, Slovakia.

Objective
Increased abdominal visceral adipose tissue (VAT) deposition is associated with insulin resistance in obese and/or hypertensive patients. We investigated the association of insulin sensitivity with the amount of VAT in young, lean, non-treated males with recently established high normal blood pressure or hypertension grade 1 (HT).
Subjects and methods
Twenty-one subjects with HT (age 20.3±0.6 years, BMI 22.4±0.5 kg/m^2, systolic BP 141±2, diastolic BP 73±2 mmHg, mean ±SE) and 19 normotensive controls (NT; age 23.1±1.0, BMI 22.1±1.4 kg/m^2, systolic BP 117±3, diastolic BP 67±2) underwent a 75-g oral glucose tolerance test (OGTT) and magnetic resonance imaging for measurement of abdominal adipose tissue distribution. Fasting concentrations of leptin and adiponectin, and fasting and post load concentrations of glucose and insulin were measured in plasma. Indices of insulin sensitivity Cederholm (ISI_{CED}), Matsuda (ISI_{MAT}) and insulin resistance (IR HOMA) were also estimated. Abdominal VAT and subcutaneous adipose tissue depots (SAT) were measured from single transverse MRI scan in the space between L4 and L5. The study was approved by the Ethics Committee of the IEE.
Results
All subjects had normal fasting glucose levels and normal glucose tolerance. HT patients had higher IR HOMA (2.4±0.4 vs. 1.2±0.1, $P=0.007$) and lower ISI_{CED} and ISI_{MAT} (58±3 vs. 77±4, $P=0.0001$ and 5.1±0.6 vs. 9.0±0.8, $P=0.001$, respectively) than NT subjects. The two study groups did not differed in amount of VAT and SAT (31.80±8.63 vs. 47.35±6.78; 93.58±15.66 vs. 111.05±10.80 cm^2, NS), and in plasma levels of leptin and adiponectin (3.82±0.52 vs. 3.45±0.49 ng/ml; 1.71±0.40 vs. 1.40±0.21 μg/ml NS).
Conclusions
These results demonstrate that even lean subjects with recently established higher blood pressure and with normal fasting and post-load glucose levels display signs of insulin resistance. These changes were however not related to abdominal adipose tissue distribution or circulatory levels of leptin and adiponectin.

P66

Comparison of twice daily NPH insulin versus once daily glargine insulin in the frequency of nocturnal hypoglycemia in Type2 diabetic patients with congestive heart failure

Neslihan Kurtulmus[1], Fatma Demirdogen[2] & Tufan Tukek[2]
[1]Vakif Gureba Educational Hospital, Department of Endocrinology, Istanbul, Turkey, [2]Vakif Gureba Educational Hospital, Department of Internal Medicine, Istanbul, Turkey.

Aim
We had the aim to determine the insulin treatment strategy that could prevent or decrease the occurrence of hypoglycaemia while providing better regulation of blood glucose in Tip 2 diabetic patients with cardiac failure.
Method
The patients demonstrating similar characteristics with respect to the age, body mass index, the duration of diabetes and heart failure were randomized into two groups as insulin glargine (*n*: 19) and NPH (*n*: 11). The subjects have been prospectively followed up for 12 weeks.
Results
Basal blood glucose level was detected as 197.21±69.01 in insulin glargine group(group1), it was 175.45±52.26 in NPH insulin group(group2) ($P=0.339$). Basal postprandial blood glucose in group1 was found to be 191.42±63.42, it was 186.18±81.82 In group2 ($P=0.857$). The nocturnal(3.00 am) blood glucose was 191.42±63.42 in group1, it was 186.18±81.82 in group2 ($P=0.857$). In group1, basal HbA1c value was 8.11±1.98, which was found to be 7.88±1.49 in group2 ($P=0.728$). At week 12 of insulin therapy, HbA_{1c} value was 6.86±1.59% in group1, markedly decreased compared to initial HbA_{1c} value ($P<0.001$). In NPH group, HbA_{1c} was found to be 7.31±1.36% at week 12, which was also lower than that at the beginning of the treatment, however this result was not statistically significant ($P=0.417$). The frequency of nocturnal hypoglycaemia in group1 was detected to be 10.5%, compared to 9.1% in group2. In two groups did not show any statistical difference related to the frequency of nocturnal hypoglycaemia.
Conclusion
In our study, while the use of insulin glargine provided a better metabolic control compared to NPH insulin,but it failed to decrease the frequency of nocturnal hypoglycaemia in diabetic subgroup with cardiac failure.

P67

Intravenous constant ghrelin infusion in healthy young men: sustained cardiovascular effects of supraphysiological ghrelin levels

Esben Thyssen Vestergaard, Niels Holmark Andersen, Troels Krarup Hansen, Lars Melholt Rasmussen, Niels Moller, Keld Sorensen, Erik Sloth, Jens Sandahl Christiansen & Jens Otto Lunde Jorgensen
Aarhus University Hospital, Aarhus, Denmark.

Objective
The short-term cardiovascular effects of continuous ghrelin infusion in healthy humans remain to be studied.
Methods
Fifteen healthy, young and normal-weight men volunteered to participate in a randomized double-blind, placebo-controlled cross-over study. The local ethics committee approved the study. We used a constant infusion of human ghrelin at a rate of 5 pmol/kg body weight per minute for 180 minutes and measured peak left ventricular myocardial systolic velocity V_{max}, tissue tracking *TT* (GE Vivid Seven with a 2.5 MHz transducer) and endothelium-dependent flow-mediated vasodilatation of the radial artery (Acuson Sequoia C256, 8 MHz linear array vascular ultrasound transducer).
Results
Ghrelin infusion increased serum ghrelin levels ~6-fold (5.2 to 6.5) ($P<0.001$) , V_{max} increased ~9% ($P=0.002$), *TT* increased ~10% ($P=0.004$), while endothelium-dependent flow-mediated vasodilation did not change ($P=0.10$). Concomitantly, growth hormone peaked after 60 minutes of infusion (36.8±4.7 ng/ml, $P<0.001$), glucose levels increased 0.5±0.1 mmol/l ($P<0.001$), free fatty levels increased 1.7-fold ($P=0.002$), cortisol levels increased 1.4-fold (P 0.002), while insulin levels were constant.
Conclusion
Supraphysiological levels of ghrelin persistently improve left ventricular function in healthy young normal-weight men without changing endothelium-dependent flow-mediated vasodilation. It remains to be studied whether ghrelin exerts direct myocardial effects or indirect effects through the concomitant changes in glucose, growth hormone, free fatty acids and cortisol levels.

P68

Circulating retinol binding protein 4 and protein C inhibitor are not related to insulin resistance

Miriam Promintzer[1], Michael Krebs[1], Anton Luger[1], Martin Georg Bischof[1], Peter Nowotny[1], Christoph Binder[2], Harald Esterbauer[2] & Christian Anderwald[1]
[1]Division of Endocrinology and Metabolism, Department of Internal Medicine III, Medical University of Vienna, Vienna, Austria; [2]Clinical Institute of Medical and Chemical Laboratory Diagnostics, Medical University Vienna, Vienna, Austria.

Recent data suggest that circulating retinol binding protein-4 (RBP4) is involved in the pathogenesis of insulin resistance in rodents and humans. Moreover, protein C inhibitor (PCI) which specifically binds retinoic acid was found to be increased in myocardial infarction survivors who are also insulin-resistant.

Therefore, we investigated the association of insulin resistance with plasma retinol binding factors (RBP4 and PCI active antigen) in nondiabetic humans with high (IS; $n=20$, f/m=14/6, age: 47.2±1.9 years, BMI: 26±1 kg/m2) and low (IR; $n=20$, f/m=14/6, age:45.5±1.7 years, BMI:28±1 kg/m2) insulin-stimulated glucose-disposal (M), measured by 2-h hyperinsulinemic-(40 mU•min-1•m-2)-isoglycemic clamp-tests.

M (80–120 min) was higher in IS (10.9±0.6 mg•min-1•kg-1) than in IR (4.0±0.2; $P<10$–12). Fasting plasma RBP4 concentrations were comparable in IS (4.4±0.3 mg/dl) and IR (4.6±0.3). Fasting plasma PCI active antigen was similar in both groups (IS: 106.6±15.6%; IR: 95.3±4.0%). Plasma RBP4 and PCI were not significantly related to M.

In conclusion, our data demonstrate that healthy, nondiabetic, insulin-resistant humans do not show altered plasma retinol binding factors, such as RBP4 and PCI. Both do not significantly correlate with insulin sensitivity. Thus, our findings do not support the hypothesis of insulin sensitivity modulation by proteins involved in retinol transport.

P69

KCNJ11 and ABCC8 promotor variants in congenital hyperinsulinism

Klaus Brusgaard[1], Khalid Hussain[2], Lone Svargo[1], Bendt Brock-Jacobsen[1] & Henrik Christesen[1]
[1]Odense University Hospital, Odense, Denmark; [2]Great Ormond Street Hospital, London, United Kingdom.

Congenital hypoglycemic hyperinsulinemia (CHI) is a clinical and genetic heterogeneous entity. Clinical manifestations can vary from serious life threatening to milder difficultly identifiable cases. Children who don't react adequate to medical treatment are subject to pancreatic recession. The molecular ethiology are from recessive mutations of the *ABCC8* (SUR1) and *KCNJ11* (Kir6.2) to dominant mutations of the *GCK* or *GDH* genes. Focal dysplasia characterised by loos of maternal Chromosome 11 and hereby *ABCC8* and *KCNJ11* is a common cause of CHI. In some studies mutations in the *ABCC8* promotor have been shown to cause CHI. In approximately 50% of the incidences the disease is still genetically unexplained necessitating the search for other genetic factors.

The purpose of the present study was to identify new genetic causes of CHI in patients with a hitherto unexplained manifestation.

46 children and there parents was tested for mutations in the *ABCC8* and *KCNJ11* promotors by D-HPLC and sequencing. Samples with deviating chromatographic patterns were sequenced.

The a region covering 1063 bp including the minimal *KCNJ11* promoter and a region covering 930 pb including the *ABCC8* minimal promoter was analysed. In 13 samples a c.-507 del T mutation was found in the *KCNJ11* gene. This variant has not previously been described. Using SIGSCAN and TRANSFAC software possible transcription factor binding sites was predicted in this region site. No other variants were found in either of the two genes. If the c.-507 delT variant is a common cause of CHI in Denmark has to be further investigated.

P70

Effect of L- carnitine supplementation on glycemic profile in patients with type 2 diabetes mellitus

Rahebeh Sakerhosseini, Alireza Rahbar, Avid Saadat, Amir hamzeh Pordal, Feroug azam Taleban & Banefsheh Golestan
National Nutrition and Food Technology Research Institute, Tehran, Iran.

Background
It has been thought that L-carnitine is effective in improving insulin-mediated glucose disposal either in healthy subjects or in type 2 diabetic patients,and carnitine plays an important role in diabetes mellitus omplications (cardiovascular disease).
Objective
We designed this study to investigate the effects of oral L-carnitin administration on fasting plasma glucose (FPG) and glycosylated hemoglob (HbA1c), in patients with diabetes mellitus type II.
Materials and methods
The effect of L-carnitine on FPG and lipid parameters was investigated in 22 male and 14 female type II diabetic patients, mean age $\pm$ SD was 51.3 $\pm$ 3.7 years. The patients were randomly divided into 2 groups (i.e. test and control groups). One gram of L-carnitine or placebo was given orally three times a day to the test and control groups respectively for a period of 12 weeks.
Results
Fasting plasma glucose in the test group decreased significantly from 143 $\pm$ 35 mg/dl to 130 $\pm$ 35 mg/dl ($P = 0.03$. There were no significant changes in HbA1c, between the two groups.
Conclusion
L-carnitine significantly lowers fasting plasma glucose in type II diabetic patients

P71

Raised serum, adipocyte and adipose tissue retinol binding protein 4 (RBP4) in women with polycystic ovary syndrome

Bee Kang Tan[1], Jing Chen[1], Sheryl Pua[1], C Richard Kennedy[2] & Harpal S Randeva[1]
[1]Warwick Medical School, University of Warwick, Coventry, West Midlands, United Kingdom; [2]University Hospitals Coventry & Warwickshire NHS Trust, Coventry, West Midlands, United Kingdom.

Context
Polycystic ovary syndrome (PCOS) is associated with insulin resistance (IR) and obesity, both predisposing factors to type 2 diabetes. A very recently described adipokine, retinol-binding protein 4 (RBP-4), has been shown to modulate insulin signalling and possibly lead to IR. At present, there is no data that depict the relative expression of RBP-4 in either serum or adipose tissue of PCOS women.
Objectives
In women with PCOS compared to matched control women, we studied the mRNA expression of RBP-4 from subcutaneous (sc) and omental (om) adipose tissue and sc adipocytes. Furthermore, RBP4 protein levels were assessed in adipose tissue; serum RBP4 was also determined.
Methods
Real-time RT-PCR and western blotting were used to assess the relative mRNA and protein expression of RBP4. Biochemical measurements were also conducted. The Local Research Ethics Committee approved the study and all patients involved gave their informed consent, in accordance with the guidelines in The Declaration of Helsinki 2000.
Results
There was significant upregulation of RBP4 mRNA in both sc ($P < 0.05$) and om ($P < 0.01$) adipose tissue of PCOS women, when compared to normal controls; these findings were also reflected in isolated sc adipocytes (PCOS > controls; $P < 0.01$). In addition to elevated serum RBP4 levels in women with PCOS ($P < 0.05$), when compared to normal controls, RBP4 protein levels were significantly greater in both sc and om adipose tissue of PCOS women ($P < 0.05$ and $P < 0.05$, respectively).
Conclusions
RBP4, a new adipokine, is elevated in PCOS women. Our findings potentially introduce a novel concept into the aetiopathogenesis of insulin resistance in these women.

P72

Polymorphisms of von Willebrand factor gene promoter modulate the corticosteroid-mediated increase of VWF levels in Cushing's syndrome.

Viviana Daidone[1], Francesca Sartorello[2], Nora Albiger[1], Franco Mantero[1], Antonio Pagnan[2], Alessandra Casonato[2] & Carla Scaroni[1]
[1]Division of Endocrinology, Department of Medical and Surgical Sciences, University of Padova, Padova, Italy; [2]Division of Intenal Medicine, Department of Medical and Surgical Sciences, University of Padova, Padova, Italy.

Cushing's syndrome (CS) is associated with hypercoagulable state, mainly dependent on corticosteroid-induced increase of von Willebrand factor (VWF) levels, even though this does not affects all patients. In normals plasma VWF levels are genetically determined by ABO blood groups and polymorphisms G/C −1793, C/T −1234, A/G −1185, G/A −1050 of VWF promoter. These SNPs segregate as haplotype 1 (G/C/A/G) and haplotype 2 (C/T/G/A) with genotype 1/1 (GG/CC/AA/GG) associated with higher VWF:Ag levels than genotype 2/2 (CC/TT/GG/AA), and intermediate VWF values in heterozygote subjects (genotype 1/2). In this study we aim to investigate the relationship between SNPs of VWF promoter and VWF levels in CS patients, in order to evaluate whether glucocorticoid effects may be influenced by VWF promoter genotypes.

50 patients with Cushing's syndrome and 200 normal subjects were analyzed.

Patients were divided by ABO blood group into groups A (increased VWF) and B (normal VWF). While a significant difference in VWF levels was observed between the two groups ($P < 0.001$), cortisol values were similar ($P = 0.44$). A direct correlation between cortisol and plasma VWF levels was observed in group A ($P < 0.001$), while no correlation was found in group B ($P > 0.1$).Genotype distribution differed significantly between the two groups being 25.8% genotype 1/1, 22.6% type 2/2 and 38.7% type 1/2 in group A, as opposed to 0% type 1/1, 57.9% type 2/2 and 31.6% type 1/2 in group B ($P = 0.03$) and their genotypes also differed from the controls ($P = 0.003$ for group A, $P = 0.03$ for group B). Our findings suggest that corticosteroid-mediated increases of VWF, and its associated prothrombotic state, are dependent on peculiar haplotypes of VWF gene promoter. CS patients presenting genotype 1/1 have a higher risk of developing thrombosis than patients with genotype 2/2.

P73

Circulating pro- and anti-inflammatory cytokines in women with gestational diabetes

Mariusz Kuzmicki[1], Beata Telejko[2], Anna Zonenberg[2], Jacek Szamatowicz[3], Adam Kretowski[2], Agnieszka Nikolajuk[2] & Maria Gorska[2]
[1]Department of Pathophysiology of Pregnancy, Medical University, Bialystok, Poland; [2]Department of Endocrinology, Diabetology and Internal Medicine, Medical University, Bialystok, Poland; [3]Department of Gynecology, Medical University, Bialystok, Poland.

Gestational diabetes mellitus (GDM) identifies a population of women at high risk of subsequent type 2 diabetes mellitus, representing an early stage in the natural history of the disease. Systemic inflammation is associated with the development of type 2 diabetes but the data concerning pro- and anti-inflammatory cytokines in patients with GDM are limited. The aim of our study was to investigate serum concentrations of interleukin-8 (IL-8), IL-18 and IL-10 in pregnant women with various degree of glucose intolerance. The group studied consisted of 58 patients with GDM, 31 pregnant women with normal glucose tolerance (NGT) and 32 women with an abnormal result of a 50 g glucose challenge test (GCT) but a normal result of 75 g oral glucose tolerance test (OGTT). Serum IL-8, IL-10, IL-18 and CRP concentrations were measured by immunoenzymatic assays. Patients with GDM had markedly higher IL-8 and IL-18 levels than women with NGT (3.86 ± 5.44 vs 0.8 ± 0.57 pg/ml, $P=0.00001$ and 264.4 ± 111.98 vs 203.57 ± 108.14 pg/ml, $P=0.0005$, respectively), as well as significantly lower IL-10 concentrations (1.37 ± 2.04 vs 2.86 ± 1.53 pg/ml, $P=0.00001$). There were no significant differences in interleukin levels between patients with NGT and abnormal GCT. There were significant correlations between IL-8 concentration and prepregnancy BMI ($R=0.2093$, $P=0.031$), insulin ($R=0.42075$, $P=0.00004$), HOMA-IR ($R=0.45857$, $P=0.000001$), and glucose ($R=0.2030$, $P=0.03$), as well as between IL-18 level and insulin ($R=0.20055$, $P=0.0301$) and HOMA-IR ($R=0.20385$, $P=0.028$). IL-10 correlated inversely with insulin ($R=-0.26822$, $P=0.0036$) and HOMA-IR ($R=-0.29127$ $P=0.0016$). CRP correlated with insulin ($R=0.28875$ $P=0.0017$) and HOMA-IR ($R=0.28836$, $P=0.0019$). Our results suggest that GDM is associated with elevated concentrations of pro-inflammatory cytokines IL-8 and IL-18, as well as with low level of anti-inflammatory IL-10. This association seems to be mediated in part by the indices of insulin resistance.

P74

Abstract unavailable

P75

Metabolic improvement in diabetic patients with glucose continuous monitoritation in real time

Cristobal Morales Portillo, Isabel Serrano Olmedo, Silvia Maraver, Amaya Fernandez Arguelles, Juan Manuel Garcia-Quiros Muñoz, Guillermo Martinez De Pinillos, Carmen De La Cuesta Mayor & Angel Sendon Perez
Virgen Macarena Hospital, Seville, Spain.

Objective
To study if the use of continuous glucose monitoritation systems in real time (GUARDIAN REAL TIME®) in the diabetic patients could improve the metabolic control.
Material and method
15 type 1diabetic patients studied (age: 33.8 ± 13.40, years of evolution: 17.06 ± 11.32) in intensive treatment (5 multidoses, 10 insulin pump) which were made one first blind 4 days long monitoritation to them, PERIOD 1 (P. BLIND) with sensor CGMS Gold®. Next it was made another monitoritation with a real time system with glycemia and alarms of hyperglycemia and hypoglycemia, GUARDIAN RT®, PERIOD 2 (P. Real time). These 2 monitoritations were consecutive in each patient, establishing in the second period the levels of alarm of hypoglycemia in 50 mg/dl and hyperglycemia in 200 mg/dl. We studied in both periods: Average glycemia Glycemia Variability, Percentage of time in hiperglycemia (>180), normoglycemia and hipoglycemia (<70). A non-parametric test for matched up data was made (Wilcoxon)
Results

	PERIOD 1 (BLIND)	PERIOD 2 (R.T)	P
Average glucose	**157.17 ± 34.30** (110–224)	**137.49 ± 21.99** (115–203)	**0.053**
Variability	**73.72 ± 19.60** (38–103)	**48.23 ± 13.20** (25–75)	**<0.005**
% High	**32.56 ± 19.25** (7.0–75.0)	**19.90 ± 13.87** (1.23–56.03)	**<0.05**
% Euglucemia	**55.49 ± 17.79** (25.0–86.0)	**74.98 ± 14.22** (43.97–98.77)	**<0.005**
% Low	**11.94 ± 8.04** (0.00–29.00)	**5.10 ± 5.16** (0–17.00)	**<0.005**

Conclusions
In our patients we observed during the monitoritation in real time: – longer time in normoglycemia with decrease of the frecuency in hipoglycemia and hiperglycemia. – smaller glycemia variability.The monitoritation in real time could be a useful tool at the time of assuring a better metabolic control and to diminish the exhibition to hipoglycemias.

P76

The role of stress related aldosterone secretion in essential hypertension

Athina Markou[1], Ioannis Androulakis[1], Aggeliki Gouli[1], Kostantina Dimitriou[1], Achilleas Zacharoulis[2], Kostantinos Papadopoulos[2], Theodoros Barlogiannis[2], Evangelos Chariatis[2], Antony Halapas[2], Eleni Kourlaba[2], Despoina Ragkou[1], Georgia Kondyli[1] & George Piaditis[1]
[1]General Hospital of Athens "G. Gennimatas", Department of Endocrinology & Diabetes Center, Athens, Greece; [2]General Hospital of Athens "G. Gennimatas", Cardiology Department, Athens, Greece.

Introduction
Approximately 90% of the hypertensive population is characterized as having essential hypertension. Apart from renin and K^+, ACTH plays an important role in aldosterone secretion, being a potent stimulant under situations of stress. Up to date, the association between stress and aldosterone-related hypertension has not been studied and that is the aim of our study.
Methods
36 hypertensive patients and 14 matched for age and sex controls (BP < 140/90 mmHg), had baseline biochemical profile, TSH, cortisol, ACTH, aldosterone, active renin and 24 hr urine Na^+/K^+ measurements, followed by a Bruce protocol exercise test aiming at the 80% of maximal effort according to Froelicher normograms and repeated the hormonal profile at peak exercise. 17 hypertensines and 7 controls had a 0.03 mcg ACTH stimulation test. Hypertensive patients on treatment were switched to a calcium channel blocker for at least 3 weeks before. Exclusion criteria were any cause of secondary hypertension, renal, hepatic or heart failure, ischemic heart disease and diabetes mellitus. CT scan of the adrenals was performed in both groups.
Results
Exercise test: baseline ACTH and aldosterone to renin ratio (ARR) did not differ but at peak exercise hypertensives had statistically higher ACTH and ARR levels compared to controls [35.97 ± 5.59 (mean $\pm$ S.E.M.) vs 23.24 ± 4.25 pg/ml, $P=0.046$ and 138.83 ± 34.22 vs 55.22 ± 34.45 pmols/L/pg/ml, $P=0.015$].
0.03 mcg ACTH test: there was a trend towards higher values in ARR at peak in hypertensives that did not reach statistical significance probably due to the low number of patients.
Conclusions
Using an exercise test at sub maximal effort in order to mimic every day's life physical stress, we observed a higher response of aldosterone to stress in patients with hypertension. Therefore, stress related aldosterone hyper secretion may play a causative role in essential hypertension with major implications in its treatment.

P77

Dialysis therapy and its complications

Evgeniya Bublik, Gagik Galstyan, Galina Melnichenko, Victor Safonov, Euginy Shutov, Pavel Filipcev & Oleg Udovichenko
[1]National Research Centre of Endocrinology, Moscow, Russia; [2]Moscow Sechenov Medical Academy, Moscow, Russia.

Aim
To study prevalence of foot complications in patients on dialysis therapy and evaluate the role the haemodynamic changes during dialysis procedure in development of foot problems.

Methods
109 dialysis patients, mean age 49 years. 60 of them had diabetes mellitus (DM): 29 on haemodialysis (HD), 31 on peritoneal dialysis (PD). Non-diabetic patients (NDM): 24 HD, 25 PD.
Vascular status: doppler, photoplethysmography. Polyneuropathy: NDS. Monitoring of BP: during and after dialysis (follow-up period 14 months).
Results
Peripheral vascular disease (PVD) was associated with DM (16 DM vs. 1 NDM). Polyneuropathy: 51 DM, and in 7 NDM.
Ulcers were diagnosed only in DM patients (prevalence 25%). The number of patients with diabetic ulcers was increased after starting dialysis therapy (from 4 to 12) and it was due to increasing neuroischemic ulcers (from 0 to 8, $P=0{,}016$). Transient intra- and postdialysis hypotension (TH) was determined in 15 HD, 5 PD. This group had significant fall of pressure in toe arteries during TH. Neuroischemic ulcers were frequently diagnosed in the group with PVD and HD, than with PVD and without HD; $P=0{,}001$.
Conclusions
Diabetic patients on dialysis therapy have high risk of neuroischemic ulcers. TH can intensify PVD and provoke neuroischemic ulcers.

P78

The effect of interferon treatment on glucose metabolism in patients with chronic hepatitis

Atilla Alev[1], Ayse Kubat Uzum[2], Ali Murat Tatli[1], Fatih Oner Kaya[1], Iskender Dik[1] & Burhan Bedir[1]
[1]Istanbul Training and Research Hospital, Istanbul, Turkey; [2]Istanbul University, Istanbul Faculty of Medicine, Department of Internal Medicine, Division of Endocrinology and Metabolism, Istanbul, Turkey.

Introduction
In recent years, interferon (IFN) is used in treatment of chronic hepatitis and the studies about the side effects of IFN therapy are increasing.
Objective
We aimed to investigate the effects of IFN therapy on glucose metabolism.
Materials and methods
Study group was consisted with 30 patients who were diagnosed as chronic hepatitis. Sixteen of 30 were chronic hepatitis B and 14 were chronic hepatitis C. Diagnose was confirmed by serology and liver biopsy. Patients with chronic hepatitis B were prescribed alpha-IFN, 9–10 MU/three times/week and chronic hepatitis C were given alpha-IFN, 3 MU/three times/week, subcutaneously. All patients were evaluated by fasting plasma glucose concentrations (FPG) and oral glucose tolerance test (OGTT) at the beginning and and at the 4th week of IFN treatment. Diagnose of impaired fasting glucose (IFG), impaired glucose tolerance (IGT) and diabetes mellitus (DM) was approved by American Diabetes Association (ADA) criteria.
Results
The study group was consisted of 16 (53.3%) female and 14 (46.7%) male patients. Mean age was 42 ± 13.67 years. Twenty eight patients had normal FPG concentrations, whereas two had IFG. No patient had DM. Mean FPG concentrations of chronic hepatitis B and C was 91.13 ± 8.25 and 96.5 ± 97.07 mg/dl, respectively. At the 4th week of the therapy, we reevaluated the patients for glucose metabolism. Difference between FPG levels before and after treatment were not statistically significant (93.63 ± 10.54 and 94.33 ± 16.01 mg/dl; $P>0.05$). However OGTT results were affected by the therapy. Nineteen patients (63.3%) had normal, six had IGT anf 5 had DM. Mean glucose concentrations during initial and second OGTT were 106 ± 26.53 and 132 ± 17 mg/dl respectively ($P<0.001$).
Conclusion
IFN treatment alteres glucose metabolism. Therefore, patients who had chronic hepatitis and treated with IFN should be followed-up closely for diabetes mellitus during and after the therapy.

P79

Unacylated ghrelin (UAG) enhances the early insulin response to meal, improves glucose metabolism and decreases free fatty acids levels in healthy volunteers

Flavia Prodam[1], Fabrizio Riganti[1], Elena Gramaglia[1], Andrea Benso[1], Barbara Lucatello[1], Fabio Broglio[1], Thierry Abribat[2], Aart Jan van der Lely[3] & Ezio Ghigo[1]
[1]Dept. Internal Medicine – University of Turin, Turin, Italy; [2]Alizé Pharma, Lyon, France; [3]Dept. Internal Medicine – Erasmus University, Rotterdam, Netherlands.

Ghrelin circulates in two different forms. Acylated ghrelin (AG), a natural ligand of the GH Secretagogue receptor (GHS-R) type 1a, exerts several biologic central and peripheral actions including stimulation of GH secretion, but also modulation of insulin secretion, glucose and lipid metabolism. Unacylated ghrelin (UAG), despite unable to bind the GHS-R1a, is biologically active showing some influence *in vitro* and *in vivo* on glucose and lipid metabolism likely mediated by still unknown receptors. Based on these data, the aim of our study was to investigate the endocrine and metabolic effects of prolonged UAG administration in humans in physiological conditions. To this goal, the effects of UAG (1.0 mcg/kg/h infused iv over 16 hours from 21.00 to 13.00 h) or saline were studied in 8 normal subjects who had isocaloric balanced standardized meals at h21.20 and h09.00. Blood samples were collected every 20 min. Compared to saline, UAG infusion significantly modified the profile of all parameters, except glucagon. Compared to saline, UAG decreased glucose ($P<0.01$) and FFA AUCs ($P<0.01$). The glucose decrease during UAG was particularly relevant at fasting during nighttime ($P<0.01$) while FFA profile was reduced both post-prandially and at fasting($P<0.01$). UAG did not modify total insulin AUC; however, the early insulin response to both dinner ($P<0.01$) and breakfast ($P<0.05$) was enhanced by UAG infusion that was associated to decrease in the nighttime HOMA index ($P<0.01$). During UAG, cortisol ($P<0.01$) and GH ($P<0.05$) AUCs were lower than those during saline, but cortisol levels remained within physiological values. Thus, the intravenous infusion of UAG in normal subjects enhances the early insulin response to meals, improves glucose metabolism and insulin sensitivity, and inhibits lipolysis. Thus, UAG displays a remarkable metabolic impact suggesting a promising anti-diabetogenic action through an original mechanism of action.

P80

Weight-related concentrations of steroid hormones in patients of both sexes with preserved gonadal function suffering from coronary artery disease

Zbigniew Sablik[1], Anna Samborska-Sablik[2], Jan Henryk Goch[1] & Krzysztof Kula[3]
[1]Department of Cardiology and Cardiac Surgery, Medical University of Lodz, Lodz, Poland; [2]Department of Emergency Medicine and Disaster Medicine, Medical University of Lodz, Lodz, Poland; [3]Department of Andrology and Endocrynology of Fertility, Medical University of Lodz, Lodz, Poland.

Aim
To define concentrations (conc) of steroid hormones in patients (pts) with preserved gonadal function, suffering from coronary artery disease (CAD), and to assess their relations to patients' weight.
Material
Pts with coronarographically proved CAD: C-W group (gr)-52 women (W) in the age 43 ± 3 years, with stable menstrual rhythm, C-M gr-46 men (M) in the age 52 ± 6 years. Healthy volunteers: H-W gr-15 W (H-W) in the age 41 ± 4 years, H-M gr-13 M in the age 51 ± 6 years.
Methods
In all pts occurrences of common risk factors of CAD including values of body mass index (BMI) and waist-hip-ratio (WHR) were defined. To assess concentrations of hormones in pts of all grs blood samples from cubital vein were taken at 8.00 a.m., in W in 4-7 day of sexual cycle. Using immunological methods conc of estradiol (E2), testosterone (T), dehydroepiandrosterone sulphate (DHEAS), follicle-stimulating hormone (FSH), luteinizing hormone (LH), progesterone (P) and cortisol (Cort) were measured.
Results
Only conc of T was significantly higher in C-W than in H-W (3.5 ± 1.7 vs 2.4 ± 1.0 nmol/l, $P<0.02$). In C-W a negative correlation between BMI or WHR and conc of T and DHEAS was found.
In C-M, comparing to H-M, conc of P and conc of Cort were higher (3.5 ± 1.6 vs 1.4 ± 1.0, $P<0.001$, and 345 ± 97 vs 246 ± 96 nmol/l, $P<0.01$, respectively) and a there was a trend towards lower conc of T (10.3 ± 3.8 vs 12.2 ± 3.3 nmol/l, $P<0.1$). In C-M we found a negative correlation of BMI or WHR with conc of P and DHEAS and positive correlation with conc of E2. Because in C-M a positive correlation between conc of P and T, and conc of P and Cort was present, there was an indirect negative relationship between BMI or WHR and conc of T and Cort.
Conclusions
T is involved in pathogenesis of CAD and plays proatherogenic role in young women and probably antiatherogenic role in men. In both sexes excessive weight is a potent risk factor of CAD, because it influences conc of steroid hormones of gonadal and adrenal origin including changing conc of T in unfavourable manner.

P81

Diabetic patient's evaluation of continuous glucose monitoring sensors versus capillary glucose measurements

Guilermo Martínez De Pinillos Gordillo, Amaya Fernández-Argüelles & Juan Manuel Garcí-Quirós Jm
Hospital Virgen Macarena, Sevilla, Spain.

Objective
To evaluate the monitoritation systems acceptance: capillary glucose measurements and continuous glucose sensors (CGSM and GUARDIAN).
Research design and methods
15 diabetics patients were monitoritatied in two diferents periods of time. (Period 1: Guardian, 86 hours long. Period 2: CGSM 72 hours long). Later, they had to fill a satisfaction questionnaire concerning several aspects which were valued from 0–6.

Table 1

Results	Capillary	*P*	Guard	*P*	CGMS
System satisfaction	4.4	Ns	4.2	Ns	3.8
Information given	4.8	Ns	4.9	Ns	5.1
Recomend-able system	4.9	Ns	5.1	$P<0.05$	4.1
Wish to continue	4.4	Ns	3.8	Ns	3.1
Uncomfort-ability	2.1	Ns	3.2	Ns	4.1
Anxiety	1.0	Ns	1.8	Ns	1.9
Interference with: work	1.8	Ns	1.3	$P<0.05$	2.5
social life	1.5	Ns	1.1	$P<0.05$	2.1
physical activity	1.1	$P<0.05$	2.7	Ns	3.1
hygiene	0.5	$P<0.005$	2.3	$P<0.005$	4.2
sexual life	0.5	$P<0.05$	2.5	$P<0.05$	3.5
dream quality	0.3	$P< 0.05$	1.5	Ns	2.3
clothing	0.6	Ns	2.1	Ns	3.0

Conclusions
The information given both by capillary mesearuments and continous clucose sensors was valued positively by our patients without significative differences between them but with a bigger acceptance with the Guardian. Real time monitorization did not generate greater anxiety than the blind registry. Glucemia sensors interfere in the daily life of the patients in most of the studied aspects but less with the Guardian than the CGSM sensor.

P82

Dehydroepiandrosterone therapy in men with verified coronary heart disease: the effects on fibrinogen, plasminogen activator inhibitor-1 (PAI-1) and tissue plasminogen activator (tPA)

Michal Rabijewski, Lucyna Papierska & Wojciech Zgliczynski
Department of Endocrinology; Medical Center for Postgraduate Education, Warsaw, Poland.

Objectives
The aim of this study was to analyze the influence of DHEA therapy on fibrinogen, plasminogen activator inhibitor-1 (PAI-1) and tissue plasminogen activator (tPA) plasma concentrations in men with decreased serum DHEAS levels and angiographically verified coronary heart disease (CHD).
Material and methods
The study included thirty men aged 41–60 years (mean age 52 ± 0.90 yr) with serum DHEAS concentration <2000 μg/l, who were randomized into a double-blind, placebo-controlled, cross-over trial. Subjects completed the 80 days study of 40 days of 150 mg oral DHEA daily or placebo, and next groups were changed after 30 days of wash-out. Fasting early morning blood samples were obtained at baseline and after each treatment to determine serum hormones levels (testosterone, DHEA-S, LH, FSH and estradiol) and also fibrinogen, plasminogen activator inhibitor-1 (PAI-1) and tissue plasminogen activator (tPA) plasma concentrations.
Results
Administration of DHEA was associated with 4.5-fold increase in DHEA-S levels. Estrogen levels significantly increased after DHEA from 22.1 ± 0.7 pg/mL to 27.4 ± 1.6 pg/L (mean $\pm$ S.E.M.; $P<0.05$), while testosterone levels did not changed. Fibrynogen concentrations significantly decreased in DHEA group from 4.5 ± 0.3 g/L to 3.83 ± 0.2 g/L ($P<0.05$ vs placebo). Changes of tissue plasminogen activator (tPA) and plasminogen activator inhibitor-1 (PAI-1) were not statistical significant (respectively: 8.37 ± 0.4 ng/mL vs 8.93 ± 0.5 ng/mL and 82.3 ± 6.3 ng/mL vs 92.7 ± 9.1 ng/mL (mean $\pm$ SEM; NS vs placebo). Mean testosterone levels did change. Tolerance of the treatment was good and no adverse effects were observed.
Conclusions
DHEA therapy in dose of 150 mg daily during 40 days in men with DHEAS levels <2000 μg/l and angiographically verified coronary heart disease (CHD) was connected with significant decreasing of fibrinogen concentration and increasing of estradiol levels, and did not influence on plasminogen activator inhibitor-1 (PAI-1) and tissue plasminogen activator (tPA) plasma concentrations.

P83

Insulin resistance and insulin secretion in non-diabetic acromegalic patients

Dan Niculescu[1], Mariana Purice[2], Radu Lichiardopol[1] & Mihail Coculescu[1]
[1]Carol Davila University, Bucharest, Romania; [2]C. I. Parhon Institutute of Endocrinology, Bucharest, Romania.

Aim
To set up, in acromegaly without diabetes mellitus, a correlation between the disease activity in GH-secreting adenoma (AA) - assessed by minimum GH serum level during an oral glucose tolerance test (OGTT) - and severity of insulin resistance (IR), assessed by HOMA-IR index.
Methods
75 out of 88 consecutive patients with acromegaly hospitalized in our department were included in this study. 13 patients proved to have diabetes mellitus and were excluded. Serum glucose, GH and insulin levels were measured by immunor-adiometricassay basal and at 30, 60 and 120 minutes after a 75 g OGTT in 88 patients with active or cured acromegaly. IR was assessed using HOMA-IR index. A value over 2.5 was considered indicating IR. An Ethical Committee approval has been obtained for this study.
Results
Out of 75 patients without diabetes mellitus, 36 subjects (48%) were presenting with IR (34 with active disease, 2 cured). We found a significant positive correlation ($r=0.56$, $P<0.001$) between AA and HOMA-IR. The GH minimal level corresponding to the intersection of the exponential regression curve with the HOMA-IR level of 2.5 was 8.8 ng/mL, a cut-off point indicating IR with 82% specificity and 78% sensitivity. The odds ratio for developing IR becomes significant at a minimum GH level during OGTT of 2 ng/mL (odds ratio 7.6, 95% confidence interval 2–29).
Conclusions
The severity of IR revealed by acromegaly correlates with GH production. A GH serum level higher than 2 ng/mL during OGTT indicates an increased risk for developing IR. This cut-off level of GH can be used as one of criteria of cured disease, regarding the lack of metabolic effects.

P84

Response to metformin treatment in adolescent siblings with familial partial lipodystrophy of the dunnigan variety (FPLD) due to the R482W LMNA gene mutation

James Ryan[1], Patrick Kiely[2], Vivion Crowley[3], Michael Maher[4], Rosemary O'Connor[2] & Domhnaill O'Halloran[1]
[1]Department of Endocrinology, Cork University Hospital, Cork, Ireland; [2]Department of Biochemistry, University College Cork, Cork, Ireland; [3]Department of Clinical Chemistry, Trinity College, Dublin, Ireland; [4]Deparment of Radiology, Cork University Hospital, Cork, Ireland.

FPLD is a rare monogenic cause of insulin resistance. We document responses to treatment with metformin in 2 adolescent sisters with FPLD due to heterozygosity for R482W *LMNA* gene mutation.

The probands, aged 14 and 16 years, presented with secondary amenorrhoea, hirsutism and progressive acanthosis nigricans. Phenotypically they showed central obesity, nuchal enlargement, and thin muscular arms. These changes occurred post-pubertally. Anthrpometric and metabolic parameters of the probands, their R482W

mutation positive father and three R482W negative sisters are shown in Table 1. Proband B had impaired glucose tolerance at diagnosis. Limb MRI of the probands showed almost complete absence of subcutaneous fat; neck MRI showed lipohypertrophy. Liver ultrasound of the probands and father showed diffuse fatty infiltrate. Both probands had cystic ovaries. A therapeutic trial with metformin in both probands showed a modest improvement in insulin resistance scores(Table 1). Proband A had regression of acanthosis nigricans, Proband B regained normal glucose tolerance. Both regained menses.

Table 1

	Proband A	Proband B	Father C	Sibling D	Sibling E	Sibling F
Fasting Insulin(mIU/L)/ C-peptide(μg/L)	71.9/6.52	44.92/6.29	32.64/6.02	9.62/1.75	0.37/2.2	0.81/0.98
Baseline HOMA-IR	13.74	9.78	7.25	1.84	0.067	0.14
Baseline OGIS (ml/min/m2)	159	195	285	426	449	543
HOMA – IR @6/12	10	8.37				
@6/12 OGIS (ml/min/m2) @6/12	185	184				

This kindred demonstrate the classical phenotype associated with FPLD, including marked insulin resistance. While FPLD may be rare, it is nonetheless vital to recognise this condition, as it is associated with significant morbidity and mortality. Furthermore, while lamin mutations are associated with different diseases this particular mutation is not well studied. We document a modest decrease in insulin resistance and regression of secondary amenorrhoea in response to metformin. Further longitudinal studies are required to fully evaluate metformin as a treatment modality for FPLD.

P85

Insulin sensitivity and lipid levels in pateints with primary hyperparathyroidism

Goran Cvijovic, Dragan Micic, Aleksandra Kendereski, Svetlana Zoric, Mirjana Sumarac-Dumanovic, Danica Pejkovic & Maja Georgiev
Institute of endocrinology, diabetes and diseases of metabolism, Belgrade, United States.

Patients with primary hyperparathyroidism (PHPT) are insulin resistant. The effect of PHPT on lipid levels and low-grade inflammation leves is unknown. The aim of our study was to estimate the cardiovascular risk profile in patients with PHPT. Methods: In patients with PHPT ($N=19$; age: 58.15 ± 8.38 years; PTH 180.83 ± 104.15 ng/l, calcium 2.97 ± 0.19 mmol/l) insulin sensitivity (measured using euglycemic hyperinsulinemic clamp - M value), lipids (total cholesterol, HDL-C, LDL-C, triglycerides, ApoA1 and ApoB) and CRP levels were measured. Results: There were low-normal level of insulin sensitivity (M value: 4.29 ± 0.52), slightly elevated levels of total cholesterol (6.07 ± 1.39 mmol/l) and LDL-C (3.72 ± 1.04 mmol/l) and normal levels of HDL (1.28 ± 0.08 mmol/l), triglycerides (1.80 ± 0.19 mmol/l), ApoA1 (1.54 ± 0.09 g/l), ApoB (1.19 ± 0.09 g/l) and CRP (1.58 ± 0.52 mg/dl) levels. There were negative correlations between M index and total cholesterol ($r=-0.56$, $P<0.05$) and Apo B ($r=-0.77$, $P<0.05$) levels, while there was positive correlation between PTH and CRP levels ($r=0.55$, $P<0.05$). In conclusion, low-normal insulin sensitivity and elevated levels of total holesterol and LDL-C were observed in our group of patients with PHPT. Further evaluation of low-grade inflammation is necessery in this group of patients.

P86

The effect of surgical treatment on insulin sensitivity in pateints with primary hyperparathyroidism

Aleksandra Kendereski, Dragan Micic, Goran Cvijovic, Svetlana Zoric, Mirjana Sumarac-Dumanovic, Danica Pejkovic & Maja Georgiev
Institute of endocrinology, diabetes and diseases of metabolism, Belgrade, United States.

It was reported that patients with primary hyperparathyroidism (PHPT) are insulin resistant, and that surgical treatment may improve insulin sensitivity in this group of patients. The aim of our study was to evaluate the effect of surgical treatment on insulin sensitivity in patients with PHPT. Methods: In patients with PHPT ($N=19$; age: 58.15 ± 8.38 years) insulin sensitivity was estimated using euglycemic hyperinsulinemic clamp (M value) before and 3 months after surgical treatment. Results: There was significant reduction of PTH (180.83 ± 104.15 vs 46.11 ± 19.45, $P<0.05$) and calcium serum levels (2.96 ± 0.19 vs 2.29 ± 0.17 mmol/l, $P<0.05$) after surgical treatment. We have observed low-normal insulin sensitivity before operation with significant improvement after surgical treatment (M value: 4.29 ± 0.52 vs 8.21 ± 1.44, $P<0.01$). There was no change in BMI (25.72 ± 3.70 vs 24.93 ± 3.33 kg/m2, $P>0.05$) and waist/hip ratio (0.82 ± 0.11 vs 0.85 ± 0.13, $P>0.05$) before and after operation (when the tests were performed). There were no correlations between chages (%Δ) of M index and PTH ($r=0.32$, $P>0.05$) and calcium ($r=0.05$, $P>0.05$) levels. In conclusion, surgical treatment improves insulin sensitivity in patients with PHPT. The mechanism of insulin resistance and its improvement after surgical treatment remains unclear in patients with PHPT.

P87

Insulin-sensitivity and glycaemic control improve on rosuvastatin (RSV) treatment in hypertriglyceridaemic type-2 diabetes (T2DM)

Giorgio Silvani, Andreas Tartaglia, Silvia Acquati, Anna Laura Vacirca, Antonella Bondi, Lorenza Gagliardi & Maurizio Nizzoli
Unit of Endocrinology, GB Morgagni-L Pierantoni Hospital, Forlì, Italy.

Aims
Studies on statins and insulin-sensitivity in T2DM are highly controversial. We aimed to evaluate the effect of RSV in type-2 diabetic people and whether its action may be phenotype-dependent, i.e. triglyceride (TG)-related (study approved by the local Ethical Committee).
Methods
48 type-2 diabetic pts (22 M:26 F), in a poor glycaemic control with oral agents, insulin or a combination therapy (unchanged over the study), were given RSV 10 mg for 12 weeks and stratified in 2 groups (23:25) by fasting TG-levels (<150 and 150–400 mg/dl), matched for age (59.7 ± 9.8 vs 60.0 ± 10.2 years), BMI (29.8 ± 4.9 vs 29.9 ± 5.5 kg/mq), waist (103.9 ± 10.8 vs 105.7 ± 11.1 cm), HbA1c (8.3 ± 1.0 vs $8.5\pm1.2\%$), total cholesterol (TC) (245.8 ± 33.6 vs 264.7 ± 26.1 mg/dl), LDL-C (167.2 ± 31.2 vs 176.6 ± 35.0), HDL-C (45.7 ± 11.6 vs 44.0 ± 14.4), and Apo-B (152.4 ± 34.8 vs 170.2 ± 28.1). Baseline- and 12-wk samples were taken for TC, LDL-C, HDL-C, TG, Apo-B, HbA1c, fasting plasma glucose and insulin. Homeostasis Model Assessment for Insulin-Resistance (HOMA-IR) was calculated, baseline score being higher in the 2nd group (4.68 ± 1.0 vs 6.32 ± 1.5 $P<0.05$).
Results
In both groups RSV lowered LDL-C (-47.2 vs -45.8%) and Apo-B (-40.7 vs -39.6%) significantly and to a similar extent. HDL-C was significantly increased ($+5.3$ vs $+4.4\%$) irrespective of changes in TG levels, mostly affected by RSV in the 2nd group: 133.5 ± 47.9 (-17.2%) vs 250.5 ± 60.1 (-25.9%) $P<0.001$. HOMA-IR correlated with TG ($r=0.21$) and was significantly decreased by RSV-treatment in hyper-TG-group (3.35 ± 0.9 vs 6.32 ± 1.5 $P<0.001$), as far as HbA1c showed a slight but significant improvement (-0.7% $P<0.05$), while no change was detected in HOMA-score or in HbA1c level in normo-TG-one, BMI and waist being not modified in both.
Conclusions
Perturbations in large-VLDL- and TG-metabolism generate an atherogenic lipid profile in T2DM and are closely linked with insulin-resistance. So in our data RSV improves HOMA-IR and HbA1c in hyper-TG type-2 diabetic pts by lowering TG-levels and seems to have both phenotype-independent and –dependent (TG-related) actions.

P88

Gestational diabetes mellitus and adiponectin levels

Cavit Culha, Suheyla Gorar, Rustu Serter, Yavuz Demir, Pinar Karakaya & Yalcin Aral
Ankara Education and Research Hospital, Department of Endocrinology, Ankara, Turkey.

Aim
Hypoadiponectinemia is known to be associated with insulin resistance, diabetes and obesity. Since gestational diabetes is together with increased body mass index (BMI) and decreased insulin sensitivity, the evaluation of adiponectin levels in these patients is interesting. We investigated the relationship between adiponectin and glucose tolerance during pregnancy and after delivery.

Materials and methods
We evaluated plasma adiponectin levels, insulin resistance and glucose tolerance in women with gestational diabetes mellitus (GDM, $n=16$) and in normal pregnancies (controls, $n=18$). Measurements were performed two times as in 28–31 weeks of pregnancy and at 3 months after delivery. Insulin resistance was calculated by the homeostasis model assessment (HOMA-IR).
Results
Four of the GDM patients remained as impaired glucose tolerance after delivery. Adiponectin levels during pregnancy were significantly lower in women with GDM compared to normal pregnancies (7.68 ± 6.26 μg/ml vs 12.72 ± 3.72 μg/ml; $P<0.01$). Adiponectin levels increased significantly after delivery both in GDM and control groups. Despite the increment after delivery, adiponectin remained significantly lower in women with GDM compared to controls (11.75 ± 6.11 μg/ml vs 16.55 ± 3.05 μg/ml; $P<0.01$). In HOMA-IR, the differences between two groups before and after delivery, and also the changes with delivery within the groups, were not found statistically significant. Adiponectin was correlated negatively with HOMA-IR ($r=-0.39$, $P<0.05$), third-trimester BMI ($r=-0.37$, $P<0.05$) and one-hour plasma glucose ($r=-0.33$, $P<0.05$); and positively with HDL-cholesterol ($r=0.34$, $P<0.05$) in women with GDM. These correlations including the adiponectin-HOMA-IR one disappeared following the delivery.
Conclusion
Decreased adiponectin levels in GDM do not normalise instantaneously following the delivery. The difference is more apparent in adiponectin levels than in HOMA-IR. There is a moderate correlation between adiponectin and one-hour plasma glucose in GDM.

P89

Lipoprotein Lp(a) in patients with systemic lupus erythematosus. Relationship with disease activity and anticardiolipin antibodies

Panagiotis Athanassiou[1], Ifigenia Kostoglou-Athanassiou[2], Despina Papadopoulou[1], Chrysoula Galanaki[1], Thomais Kalogirou[3], Athanasios Kordalis[3], Christodoulos Antoniadis[1] & Philippos Kaldrymidis[2]
[1]Department of Rheumatology, Asclepeion Hospital, Athens, Greece; [2]Department of Endocrinology, Metaxa Hospital, Piraeus, Greece; [3]1st Department of Internal Medicine, Asclepeion Hospital, Athens, Greece.

Systemic lupus erythematosus (SLE) is a multisystem multifactorial autoimmune disorder. The survival of SLE patients has been improved by the administration of immunomodulatory therapy. Patients, however, are affected by late onset complications of the disease such as atherosclerosis. Lipoprotein Lp(a) is a known risk factor for the development of atherosclerosis.

The aim was to study Lp(a) levels and their relationship with disease activity in SLE patients.

Patients with SLE, $n=74$, aged 21–64 years, and normal controls, $n=74$, of the same age and sex were studied. In patients and controls the levels of hematocrit, hemoglobin, erythrocyte sedimentation rate, C-reactive protein, antinuclear antibodies, complement, anti-dsDNA antibodies, cholesterol, triglycerides, HDL, LDL and lipoprotein Lp(a) were measured.

Lp(a) levels (normal values <30 mg/dl) were found increased in 23 of 74 (31.1%) patients with SLE and in 9 of 74 (12.2%) controls. Within the group of 23 SLE patients with increased Lp(a) levels 17 (73.9%) had active disease. In 11 of 23 (47.8%) SLE patients with increased Lp(a) levels anticardiolipin antibodies were detected, while anticardiolipin antibodies were found in 12 of 51 (23.5%) patients with Lp(a) levels within the normal range. All patients with active disease and increased Lp(a) levels had renal and/or central nervous system involvement. A strong relationship was observed between Lp(a) levels and anti-dsDNA antibodies.

Lp(a) levels were higher in SLE patients. Increased Lp(a) levels were found to be related to disease activity in SLE, specifically with renal and central nervous system involvement and anticardiolipin antibodies. Increased Lp(a) levels may contribute to the development of atherosclerosis and cardiovascular disease in SLE patients.

P90

Lipoprotein Lp(a) in patients with rheumatoid arthritis and its relationship with disease activity

Panagiotis Athanassiou[1], Ifigenia Kostoglou-Athanassiou[2], Despina Papadopoulou[1], Chrysoula Galanaki[1], Thomais Kalogirou[3], Athanasios Kordalis[3], Christodoulos Antoniadis[1] & Philippos Kaldrymidis[2]
[1]Department of Rheumatology, Asclepeion Hospital, Athens, Greece; [2]Department of Endocrinology, Metaxa Hospital, Piraeus, Greece; [3]1st Department of Internal Medicine, Asclepeion Hospital, Athens, Greece.

Epidemiological studies indicate that rheumatoid arthritis (RA) patients have increased mortality. Cardiovascular disease seems to be one of the major causes of death in patients with RA. Lipoprotein disorders are observed in patients with systemic autoimmune disorders as well as in patients with RA. Lipoprotein Lp(a) is an independent risk factor for the development of cardiovascular disease.

The aim of the study was estimate lipoprotein Lp(a) levels and their relationship with disease activity in RA patients.

Patients with RA, $n=92$, aged 22–71 years and normal controls, $n=92$, of the same age and sex were studied. All the patients fulfilled the American College of Rheumatology criteria for the diagnosis of RA. In patients and controls the levels of hematocrit, hemoglobin, erythrocyte sedimentation rate, C-reactive protein, cholesterol, triglycerides, HDL, LDL and lipoprotein Lp(a) were measured. DAS28 disease activity index was calculated in all RA patients.

Lipoprotein Lp(a) levels (normal values <30 mg/dl) were found increased in 24 of 92 RA patients (26.1%) and in 11 of 92 controls (12%). Within the group of 24 RA patients with increased Lp(a) levels 18 (75%) had increased inflammation markers and increased DAS28. A strong relationship was observed between Lp(a) levels, erythrocyte sedimentation rate ($P<0.01$) and C-reactive protein ($P<0.01$).

Lipoprotein Lp(a) levels were found increased in RA patients. RA patients are at an increased risk for the development of atherosclerosis. The increase in Lp(a) levels seems to be observed specifically in patients with active RA. Inflammation may be the factor responsible for the increase in Lp(a) levels in RA patients.

P91

Impaired proinsulin secretion before and during oral glucose stimulation in HIV-infected patients, who display fat redistribution

Steen B Haugaard[2], Ove Andersen[2], Ian Halsall[4], Johan Iversen[1], Charles Nicholas Hales[4] & Sten Madsbad[3]
[1]Dept. Infectious Diseases, Hvidovre University Hospital, Copenhagen, Denmark; [2]Clinical Research Unit, Hvidovre University Hospital, Copenhagen, Denmark; [3]Dept. of Endocrinology, Hvidovre University Hospital, Copenhagen, Denmark; [4]Dept. of Clinical Biochemistry, University of Cambridge, Cambridge, United Kingdom.

Beta-cell function of HIV-infected patients on highly active antiretroviral therapy (HAART), who display lipodystrophy, may be impaired. An early defect in beta-cell function may be characterized by an increased secretion of 32–33 split proinsulin (SP) and intact proinsulin (IP).

To address this issue the secretion pattern of SP and IP of 16 HIV-infected men with lipodystrophy (LIPO) and 15 HIV-infected men without lipodystrophy (NONLIPO) were studied during an oral glucose tolerance test (OGTT). All patients received HAART. Insulin secretion rates were determined by deconvolution of plasma C-peptide concentrations.

More LIPO than NONLIPO patients displayed diabetes mellitus and impaired glucose tolerance than a normal glucose tolerance (LIPO 2/8/6 vs NONLIPO 1/2/12, $P=0.05$). LIPO had increased fasting SP, IP, ratio of SP/IP, area under the curve (AUC) of SP and IP during early phase of the OGTT (0, 10, 20 minutes), and AUC-SP and AUC-IP during the late phase of the OGTT (45, 75, 105 minutes), respectively, compared to NONLIPO (Ps <0.05). LIPO exhibited significantly increased fasting SP/IP ratio, fasting SP/insulin ratio and ratios of total proinsulin to C-peptide during the OGTT. LIPO displayed increased incremental secretion of IP during the first 10 minutes of the OGTT ($P<0.05$), despite the fact that the incremental insulin secretion during this period did not differ between LIPO and NONLIPO.

These data suggest that HIV-infected patients with lipodystrophy display major perturbations of proinsulin secretion in the fasting state and during an OGTT, which is compatible with the notion of a beta-cell dysfunction of such patients.

P92

Concentration of vasopressin and of N- terminated naturetic propeptyde type B – potent predictors of survival of patients after cardiac arrest

Anna Samborska-Sablik[1], Zbigniew Sablik[2], Wojciech Gaszynski[3], Jan Henryk Goch[2] & Krzysztof Kula[4]
[1]Department of Emergency Medicine and Disaster Medicine, Medical University of Lodz, Lodz, Poland; [2]Department of Cardiology and Cardiac Surgery, Medical University of Lodz, lodz, Poland; [3]Department of Anaesthesiology and Intensive Therapy, Medical University of Lodz, Lodz, Poland; [4]Department of Andrology and Endocrynology of Fertility, Medical University of Lodz, Lodz, Poland.

Aim
To assess concentration (conc) of arginine vasopressin (AVP) and conc of N-terminated naturetic propeptyde type B (NTpBNP) in patients (pts) after cardiac arrest (CA) and their role for clinical state of pts after CA.
Material
52 pts after CA, 36 men and 16 women, in the age 62±13 years. CA was caused by ventricular fibrillation in 31 cases, by asystolia in 15 and by pulseless electrical activity in 6. 28 pts died after CA (P-CA-D), 24 survived and were discharged from hospital (P-CA-S).
Methods
Clinical state of pts after CA was assessed by common scales used in critical care: Glasgow Coma Scale (GCS) and Acute Physiology and Chronic Health Evaluation II (APACHE II). Venous blood samples to measure conc of AVP and NTpBNP were taken from each patient just after admission to hospital and in 2 consecutive days at 8.00.
Results
Just after CA mean conc of AVP was higher in P-CA-D than in P-CA-S (87±58 vs 60±46 pmol/l, but $P<0.1$). Mean conc of NTpBNP was higher in P-CA-D in 3 consecutive days, significantly in 1 day after CA (11,4000±112000 vs 45000±58,000 pmol/l, $P<0.027$).
In logistic regression analysis as well in Kaplan-Meyer survival analysis an important relation between conc of AVP just after CA and survival after CA, and between levels of NTpBNP just after CA and in first day after CA and survival was revealed.
We proved negative correlations among blood conc of AVP, conc of NTpBNP and values of GCS and positive correlations among levels of them and values of APACHE II.
Conclusions
1-Mechanisms involving biological function of AVP and of brain naturetic peptide play an important role for covalescence in early stage after CA.
2- Conc of AVP and NTpBNP are important predictors of survival after CA.

P93

Changes in plasma adiponectin during the treatment of diabetic ketoacidosis

A Emre Yildirim[1], Yavuz Selim Demir[2], Abidin Ozturk[1], A Ozden Barazi[3], Hacer Cetiner[1], Gokcen Kilic[1], Tugrul Ozcan[1], Yasar Acar[1], Berrin Demirbas[1] & Gul Gursoy[1]
[1]Ankara Education and Training Hospital Department of Internal Medicine, Ankara, Turkey; [2]Ankara Education and Training Hospital Department of Endocrinology and Metabolism, Ankara, Turkey; [3]Ankara Numune Education and Training Hospital Department of Biochemistry, Ankara, Turkey.

Background
Low plasma adiponectin concentrations are associated with diabetes mellitus. Resuts from animal studies suggest that adiponectin plays an important role in regulating insulin action. Leptin levels found to be low in patients with diabetic ketoacidosis (DKA). The recent studies showed that insulin replacement during DKA increased leptin concentrations. In our study, we aimed to determine the effect of insulin replacement on serum leptin and adiponectin concentrations in patients with DKA.
Methods
Our study included 31 patients (F/M) with DKA. 18 of 31 patients have been treated with oral anti diabetic agents and 13 of 31 patients have been treated with insulin treatment before admission. Leptin and adiponectin concentrations are analyzed in samples which are collected on admission and at resolution of ketoacidosis in 24–48 hours.
Results
Mean age of the the patients was 46.7±17.3 years, mean period of diabetes was 7.1±7.3 years, and body mass index was 26.5±4.3 kg/m^2. There was a signifcant negative correlation between plasma adiponectin levels and blood ketone concentrations on admission ($r=-0.66$). Significant positive corelation between age and body masss index was also determined ($r=0.477$). While plasma adiponectin levels didn't change after DKA treatment ($P=0.095$), leptin levels increased significantly ($P<0.001$). In patients using oral antidiabetic agents adiponectin levels didn't change ($P=0.103$) but leptin levels increased significiantly ($P=0.002$) at the end of treatment. In the patients using insulin therapy, adiponectin levels didn't change ($P=0.56$), however leptin levels increased significantly ($P<0.001$).
Conclusions
In our study leptin levels are increased after the treatment of DKA. This result can be described with the increase of calori intake and the regulation of glucose utilization in adipocytes caused by insulin. Adiponectin levels found to be still low in DKA after the treatment. These results could be explained by early period of treatment.

P94

Power spectral analysis (PSA) of heart rate variability (HRV) in the detection of cardiac autonomic neuropathy (CAN) in subjects with diabetes mellitus

Amjed Khamis, SR Rizvi, Shu Hoashi, Marie Byrne, RG Firth & Brendan Kinsley
Diabetes and Endocrine department, Mater Misercorea University Hospital, Dublin, Ireland.

Standard Autonomic Function tests AFT may not detect subclinical CAN. Modalities exist (Frequency Domain) using PSA of HRV, which may detect subclinical CAN.

We performed standard AFTs (HR response to deep breathing, Valsalva, Tilt at 1 min and 6 min) and PSA of HRV on these tests in 46 subjects (29 DM and 17 Controls) matched for age and sex. We sought to establish if those DM subjects considered normal by AFT would exhibit abnormalities in PSA of HRV. We measured mean spectrum RR interval and LF/HF ratio (measures of sympathovagal tone).

Diabetic Subjects were divided into group A (normal AFT), group B (any abnormal AFT), and to subgroup B1–3 by number of abnormal AFTs.

We then sought to identify if subjects in Group A would demonstrate differences in results of PSA of HRV tests compared to controls, which might suggest subclinical CAN.
Results
In standard AFT, HRV in deep breathing was 22±10 beats/min in Group A and 20±6 beats/min in Control, $P=$NS. In contrast results of PSA of HRV show that LF/HF ratio in deep breathing was 7.1±3.3 (Control), 3.3±1.6 (Group A) and 1.8±1.5 (Group B), 1.2±0.6 (GroupB2), 2.3±1.6 (Group B3), $P<0.01$ Control vs Group A and Group B. $P<0.001$ Control vs Groups B1, B2, B3.
Summary
LF/HF ratio to deep breathing is a useful parameter to detect presence of subclinical CAN and to stratify severity of CAN. It may be useful as a screening test for the presence of subclinical CAN in subjects with diabetes mellitus.

P95

Glicoregulation in obese diabetics treated with glargin insulin in combination with metformin and with glargin in combination with glimepirid

Zelija Velija-Asimi
University Clinical Centre of Sarajevo, Sarajevo, Bosnia and Herzegovina.

Aims
The aim of study was to evaluate glucose control in obese diabetics during six months of treatment with glargin insulin in combination with metformin and glimepirid.
Methods
In beginning of study excluded patients who had coronary heart and kidneys disease before. In study included 43 obese diabetics with type 2 diabetes [23 male and 20 female, BMI=29.82±1.91 kg/m^2, aged 42–65 yr], who had previously been treated with different orally antidiabetics. Previous treatment was without results, because all treated patients had bed glicoregulation. Patients divided in two groups. Both groups were without significant difference in BMI, age and sex. Twenty five patients (14 male and 11 female) received glargin s.c. once a day and metformin orally at the dose of 3×850 mg/d. Eighteen diabetics (10 male and 8 female) received glargin s.c. once a day and glimepirid orally at the dose of 2–4 mg/d. Glicoregulation evaluated by measuring fast blood glucose (FBG), postprandial blood glucose (PBG) and HbA1c. Duration of study was six months. Percentile, average and correlation analysis have been utilized in statistical analysis.
Results
The results of study, after six months treatment with glargin and metformin, show statistical significantly decreasing of FBG (6,7+/−1.4 mmol/l, vs 9.9+/−2.9 mmol/l, $P<0.05$), PBG and HbA1c (7.0+/−1.3% vs 9.1+/−1.3%, $P<0.05$). BMI decreased for 10% (27.1+/−0.9 kg/m^2 vs 29.82±1.91 kg/m^2). In group treated with glargin and glimepirid FBG, PBG and HbA1c (7.7+/−1.2% vs 9.3+/−1.1%, $P<0.05$) as well decreased but no more then group treated with glargin and metformin.
Conclusion
Glargin in combination with metformin is more effective in treatment of obese diabetics then glargin in combination with glimepirid.

P96

High-sensitivity C-reactive protein in diabetes mellitus type II according to micral test findings

Zarida Hambali
University Putra, Malaysia.

Cardiac complications account for three quarter of deaths among diabetic patients. Many studies have shown that high-sensitivity C-reactive protein (hs-CRP) correlated with the inflammatory process of atherosclerosis in the coronary artery. This study is designed to determine the levels of plasma hs-CRP in Type II diabetic patients with microalbuminuria and its association with other biochemical markers used for diabetic monitoring. All biochemical parameters were analyzed using HITACHI 919 Analyzer. Microalbuminuria levels were assessed using Micral Test in 120 diabetics and 100 normal subjects (control). hs-CRP is significantly higher among diabetics ($P<0.05$) as compared to the control group. The concentrations of hs-CRP increases significantly with increasing levels of microalbuminuria which are classified into 0 mg/dL, 20 mg/dL and more than 50 mg/dL ($P<0.01$). Among diabetics, hs-CRP is significantly higher in those with miroalbuminuria compared to those without microalbuminuria ($P<0.001$). In contrast, hs-CRP is not significantly correlated with fasting blood glucose, LDL-chol, total cholesterol and triglyceride ($P>0.05$). This case-control study confirms the findings of higher concentration of hs-CRP among diabetic patients and may suggest the ongoing inflammation associated with atherosclerosis. This study suggests that by measuring the concentration of plasma hs-CRP in addition to other biochemical parameters as recommended by the Malaysian Clinical Practice Guideline, a proper planning to monitor complications of coronary artherosclerosis among diabetic patients with or without microalbuminuria can be done.

Endocrine tumors and neoplasia – presented on Sunday

P97

Localization of an ectopic adrenocorticotropin-secreting tumour using ^{18}F- Dopa PET/CT

Séverine Dubois[1], Olivier Morel[2], Patrice Rodien[1], Christian Jeanguillaume[2], Bernard Enon[3], Frédéric Illouz[1], Jean-Paul Saint-André[4], Sylvie Girault[2] & Vincent Rohmer[1]
[1]Pôle d'Endocrinologie, Diabètes et Nutrition, Centre Hospitalier et Universitaire, Angers, France; [2]Pôle de Médecine Nucléaire et Cancérologie, Centre Hospitalier et Universitaire, Angers, France; [3]Pôle de chirurgie thoracique et cardiaque, Centre Hospitalier et Universitaire, Angers, France; [4]Département d'anatomo-pathologie et de cytologie, Centre Hospitalier et Universitaire, Angers, France.

Ectopic adrenocorticotropin secretion (EAS) accounts for 10–15% of cases of Cushing's syndrome and comprises a spectrum of lesions from highly malignant tumours to a variety of less aggressive neuroendocrine tumours. Selective removal of the primary lesion is the optimal management. It is therefore mandatory to localize the source of ectopic ACTH.

As no single test is accurate enough to distinguish the ectopic from the pituitary sources of ACTH, no single imaging technique can itself identify every tumour responsible for EAS.

We report on the use of Photon Emission Tomography (PET) scanning using ^{18}F-fluoro-Dopa in the localization of an occult ACTH-secreting carcinoid tumour.

An 18-yr-old man was referred for evaluation of EAS. Evidence for EAS included: plasma ACTH and ß LPH levels above the normal reference range, no serum cortisol suppression after high-dose dexamethasone suppression test, normal pituitary MRI and lack of central to peripheral gradient on bilateral inferior petrosal sinus sampling. The patient had a history of post-infectious bronchiectasis since 6 years. The chest computed tomographic (CT) scan showed a widespread lobar disease already known and compatible with bronchiectasis. In-111 pentreotide scintigraphy was interpreted as normal. A low-intensity uptake was seen on ^{18}FDG PET scanning located in the middle right pulmonary lobe. As the patient suffered from a respiratory infection, interpretation of this image was difficult. An ^{18}F-fluoro-dopa PET scanning revealed a pathologic uptake localized in the right lung middle lobe.

The pulmonary lesion was surgically treated after adrenolytic medication. Histology revealed a bronchial carcinoid tumor. Hypercortisolism was replaced by prolonged corticotropic insufficiency. Until now, hypercortisolims did not relapse.

In conclusion, no imaging technique should be neglected in the localization of an occult EAS.

P98

Adrenocortical carcinosarcoma: first european case report

Frédéric Somda[1], Julie Leger[1], Laurent Guy[2], Olivier Norha[3], Françoise Desbiez[1], Jean-Paul Boiteux[2], Jean-Louis Kemeny[3], Philippe Thieblot[1] & Igor Tauveron[1]
[1]CHU Gabriel Montpied, Endocrinology, Clermont-Ferrand, France; [2]CHU Gabriel Montpied, Urology, Clermont-Ferrand, France; [3]CHU Gabriel Montpied, Pathology, Clermont-Ferrand, France.

Adrenocortical sarcoma is an extremly rare tumour associated with a quite pejorative evolution.We report the case of a fifty-eight years old woman presenting a chronic asthenia and acute flank pain. She had no hypertension, no clinical sign of virilization or hypercorticism. Abdominal ultrasonography revealed an 8 centimeters mass above the right kidney. CT scan aspect evoked an adrenal carcinoma embolizing vena cava. Hormonal assays did not reveale any inappropriate secretion (17 alpha hydroxyprogesterone, 11 desoxy-cortisol, cortisol, dehydroepiandrosterone-sulfate, delta-4 androstenedione, testosterone, aldosterone, renin, 24-hour urine metanephrine and normetanephrine). A radical adrenalectomy associated with a nephrectomy was performed. Tumour measured $13\times7.5\times5$ centimeters, weighed 760 grams. Histological study confirmed the diagnosis of adrenal carcinoma, but described a sarcomatous component occupying nearly twenty percent of the total mass. Immunohistochimical labelling was positive for anti-vimentin, anti-desmin and anti-actin antibodies. In addition to surgical resection, the patient received mitotane as adjuvant treatment (6 g per day, mitotanaemia: 20.6 mg/l). After a 16 month evolution, physical exmination, CT scan, PET scan and hormonal monitoring don't show any evidence of local reccurence or metastasis. In the last twenty years, only four cases of adrenocortical carcinosarcoma have been reported in literature. One was a non secreting tumor, the three others were revealed by aldosterone, androgen or catecholamine secretion. Considering pathology, one had an osteogenic and chondroid differenciation, the two others a rhabdomyosarcomatous differenciation. To our knowledge, this is the first observation of an adrenal carcinosarcoma expressing a smooth muscle phenotype. The strikingly good evolution in our patient is also particularly unusual. Indeed adrenocortical sarcoma is a cancer with a very poor prognosis since in all other cases, life expectancy after diagnosis has never exceeded 8 months.

P99

The genetic association of medullary thyroid carcinoma with Hirschsprung's disease

Sarka Dvorakova[1], Eliska Vaclavikova[1], Richard Skaba[2], Petr Vlcek[3] & Bela Bendlova[1]
[1]Dept. of Molecular Endocrinology, Institute of Endocrinology, Prague, Czech Republic; [2]Dept. of Pediatric Surgery of the 2nd Faculty of Medicine, Charles University and Hospital, Prague, Czech Republic; [3]Dept. of Nuclear Medicine and Endocrinology of the 2nd Faculty of Medicine, Charles University and Hospital, Prague, Czech Republic.

Medullary Thyroid Carcinoma (MTC) can be associated with Hirschsprung's disease (HSCR). Mutations in exon 10 of the RET proto-oncogene were found in patients with co-occurrence of HSCR and MTC. The aim of the study was to screen the MTC risk exons in patients with HSCR. The genetic analysis comprised 73 HSCR patients (53 males, 20 females) who were operated on and followed-up during 2001-2006. The cohort consisted of 48 patients with classical HSCR, 11 with long colonic aganglionosis and 14 with total colonic aganglionosis (TCA). DNAs were isolated from blood after signing informed consent approved by ethical committee. HSCR patients and 10 available family members were tested for RET mutations in exons 10,11,13,14,15 and 16. Direct sequencing revealed RET mutations in 7 (9.6%) HSCR patients. Three groups of mutations were detected. Typical MTC risk mutations were found in 2 HSCR patients with TCA: Cys609Tyr and Cys620Arg (both exon 10). Atypical mutation Tyr791Phe (exon 13) was detected in 2 classical HSCR patients. This mutation is causative for MTC only and has not been associated with HSCR till now. Novel mutations with unknown function for HSCR and MTC were found in 3 patients – del603(A) (exon 10), Gly798Ser (exon 13) and Ser649Leu (exon 11). Two of these patients had TCA and the third one had classical HSCR. MTC developed in 2 patients and 2 family members with typical mutations for HSCR-MTC. These mutation carriers underwent total thyroidectomy (TTE), the other RET positive patients are screened for calcitonin level and they are without TTE till now. Results showed the benefit of systematic RET mutation screening in HSCR families in order to identify the risk of MTC. We recommend to investigate not only exon 10 but also other MTC risk exons in all HSCR patients. This work was supported by grant GACR 301/06/P425.

P100

Inhibition of $C_{17,20}$-lyase activity by new 17β-exo-heterocyclic androsterone derivatives

Mihály Szécsi[1], István Tóth[1], János Wölfling[2], Gyula Schneider[2] & János Julesz[1]
[1]Endocrine Unit and Research Laboratory, University of Szeged, Szeged, Hungary; [2]Department of Organic Chemistry, University of Szeged, Szeged, Hungary.

17α-Hydroxylase-$C_{17,20}$-lyase ($P450_{17\alpha}$) is a key regulator enzyme of the steroid hormone biosynthesis in both the adrenals and the testes. Inhibition of this enzyme can block androgen synthesis in an early step, and may thereby be useful in the treatment of prostatic carcinoma, which is androgen-dependent in the majority of cases. Abiraterone and its analogues have been found strong inhibitors of $P450_{17\alpha}$ suggesting that steroid derivatives with heterocyclic substituent on the C-17 position may bear such potential.

We investigated inhibitory effect on $C_{17,20}$-lyase exhibited by newly synthesised androsterone derivatives with heterocyclic 17β substituents. $C_{17,20}$-lyase inhibition was tested via conversion of 17α-hydroxy-progesterone to androst-4-en-3,17-dione in the homogenate of rat testis *in vitro*. Incubation was carried out with ^{14}C labeled substrate at 37 °C for 20 min. Following an extraction procedure and isolation by thin layer chromatography, the enzyme product and the residual substrate were quantified by their radioactivities. Ketokonazole, a $P450_{17\alpha}$ inhibitor applied in medical practice was used as a reference compound. Among test compounds the non-substituted tetrahydrooxazolone and tetrahydrooxazinone derivatives were found to be the best $C_{17,20}$-lyase inhibitors; IC_{50} values were 4.2 and 6.0 μM, respectively. The N-phenyl-tetrahydrooxazinones did not show substantial inhibition ($IC_{50} > 50$ μM).

The 17β-exo-heterocyclic androsterone derivatives which proved to be potential $C_{17,20}$-lyase inhibitors in the present study, also exhibited marked inhibition against prostatic 5α-reductase activity in our previous investigations. This dual effect might be particularly beneficial in the therapy of prostate cancer.

P101

Cigarette smoking increases high calcitonin levels

Michele d'Herbomez[1], Catherine Bauters[2], Laurence Leclerc[2], Emmanuelle Leteurtre[3], Bruno Carnaille[4], Jean-Louis Schlienger[5], Philippe Caron[6] & Jean-Louis Wémeau[2]
[1]Nuclear Medicine, Lille, France; [2]Endocrinology, Lille, France; [3]Pathology, Lille, France; [4]Endocrine Surgery, Lille, France; [5]Internal Medicine, Strasbourg, France; [6]Endocrinology, Toulouse, France.

Increased basal or pentagastrin-stimulated calcitonin level is the cornerstone for the biological diagnosis of medullary thyroid carcinoma, but is also observed in patients with C-cell hyperplasia (CCH) of the thyroid. In a prospective multicenter study we re-evaluated the reference ranges of basal calcitonin (bCT) in 287 euthyroid controls without thyroid disease (142 men-45 smokers, 3 deprived, 145 women-27 smokers). The CT levels were measured using 2 different assays (Cis-Bio International, Advantage Nichols). After exclusion of the main causes of increased CT levels, 11 (8%) male controls had bCT concentrations > 10 pg/mL within the two assays. All, except one, were active or deprived smokers. Then, we evaluated preoperative bCT and pentagastrin-stimulated CT levels in patients with CCH of the thyroid (more than 50 C cells per field at 3 low-power magnification microscopic fields). In 27 smokers or deprived patients (23 men and 4 women, median age 53 years) total thyroidectomy was performed for nodular pathology. CCH was diffuse and bilateral ($n=17$), diffuse and unilateral ($n=4$), nodular ($n=1$) or diffuse and nodular ($n=5$). Preoperative bCT was normal (< 10 pg/mL), between 10 and 20 pg/mL or > 20 pg/mL in 8, 13, and 6 patients, respectively. Pentagastrin- stimulated CT level was normal (< 50 pg/mL), between 50 and 100 pg/mL, and > 100 pg/mL in 2, 3, and 15 patients respectively.

In conclusion, there are evidences that cigarette smoking induces: 1) diffuse and bilateral CCH of thyroid, 2) increased bCT level, 3) abnormal pentagastrin-simulated test, particularly in men.

P102

Cortisol excess, inflammatory markers and echocardiographic alterations in adrenal incidentalomas

Federica Ermetici[1], Alexis Malavazos[1], Calin Coman[2], Lelio Morricone[1], Massimiliano Corsi[3] & Bruno Ambrosi[1]
[1]Endocrinology Unit, Department of Medical and Surgical Sciences, IRCCS Policlinico San Donato, University of Milan, San Donato Milanese, Italy; [2]Echocardiographic Unit, IRCCS Policlinico San Donato, San Donato Milanese, Italy; [3]Institute of General Pathology, Laboratory of Clinical Pathology, University of Milan, Milan, Italy.

An increased cardiovascular risk has been described in patients (pts) with adrenal incidentaloma (AI), similarly to pts with overt Cushing's syndrome (CS). Some echocardiographic abnormalities and alterations in adipokine secretion involved in insulin resistance, inflammation and atherosclerosis have been reported in pts with CS. In this study the possible correlation between echocardiographic parameters and adipokine levels in pts with AI was evaluated.

Subjects and methods

Morphological and functional echocardiographic characteristics and plasma IL-6, TNF-α, MCP-1 and resistin levels (ELISA methods) were studied in 7 pts (60.0 ± 2.5 yrs, BMI 31.1 ± 2.1) with AI and subclinical Cushing's syndrome (SCS) and in 17 pts (58.8 ± 2.3 yrs, BMI 29.5 ± 1.2) with non functioning masses. All adrenal masses were identified as cortical adenoma. In all pts plasma ACTH, serum cortisol and urinary free cortisol (UFC) were measured.

Results

In pts with SCS the interventricular (IV) septum thickness was significantly greater than in pts with non functioning masses (13.2 ± 0.1 vs 10.7 ± 0.03 mm, $P<0.05$) and in 8 obese normotensive subjects (10.5 ± 0.5 mm, $P<0.001$). Plasma IL-6, TNF-α, MCP-1, and resistin levels were higher in pts than in 20 normal subjects (60.3 ± 2.5 vs 5.5 ± 0.6 pg/ml, 27.2 ± 1.3 vs 22.1 ± 1.4 pg/ml, 164.3 ± 17.0 vs 104.3 ± 19.4 pg/ml, 12.9 ± 2.4 vs 5.1 ± 0.2 ng/ml, respectively, $P<0.05$). The other echocardiographic parameters and adipokine values were not different in pts with SCS and with non functioning AI. In all patients, UFC excretion positively correlated with left ventricular (LV) diameter end-systole ($r=0.549$, $P=0.01$) and with LV mass ($r=0.479$, $P<0.05$). Significant correlations were found between early wave diastolic filling velocity and IL-6 and TNF-α levels ($r=-0.633$, $P=0.01$ and $r=-0.547$, $P<0.05$, respectively), and between late wave diastolic filling velocity and TNF-α levels ($r=-0.520$, $P<0.05$), in all pts.

Conclusions

In AI a long-lasting exposure to an even slight cortisol excess and inflammatory stimuli might be responsible for a gradual impairment of both diastolic function and cardiac morphology.

P103

Prognostic value of anti-thyroperoxidase antibodies in high malignancy degree breast cancer

Emilio Fiore[1], Claudio Giani[1], Elisa Giustarini[1], Ilaria Muller[1], Claudia Mammoli[1], Daniela Campani[3], Manuela Roncella[2], Loredana Fustaino[2] & Aldo Pinchera[1]
[1]Department of Endocrinology and Metabolism, Pisa, Italy; [2]Department of Surgery, Unit of Senology, Pisa, Italy; [3]Department Pathology, Pisa, Italy.

A high incidence of serum anti thyro-peroxidase antibodies (TPOAb) has been found in breast cancer (BC). Aim of this study was to evaluate the predictive value of TPOAb in BC. The study group included 47 women submitted to mastectomy for high malignancy degree BC (tumor size > 5 cm and/or n° lymph-nodes > 3), followed for 5 years. No patient had distant metastases. All were evaluated for thyroid disorders after breast surgery and before any anti-tumoral adjuvant therapy. Thirty-one out of 47 (65.9%) patients were alive 5 years after BC diagnosis (survivors group: SG), 16/47 (34.1%) were dead during follow-up (deaths group: DG), (mean age 53.1 ± 10.9 yrs and 53.3 ± 8.5 yrs, respectively) (p NS).

Estrogen Receptor (ER) was measured in 43/47 (91.5%) BC speciments. ER was detected (ER+) in 19/30 (63.0%) patients in SG and 3/13 (23.1%) in DG ($P=0.01$, x^2 5.9). Five year mortality in ER- BC was 10/21 (47.6%), and in ER+BC was 3/22 (13.6%) ($P=0.008$). The overall prevalence of TPOAb was 15/47 (31.9%); 14/31 (45.1%) patients in SG and 1/16 (6.2%) in DG were TPOAb+ ($P=0.008$). Five years mortality was 15/32 (46.9%) in TPOAb- and 1/15 (6.7%) in TPOAb + ($P=0.01$). TPOAb were detected in 8/21 (38.1%) ER- patients and in 7/22 (31.8%) ER+; no relation was found between ER expression and TPOAb positivity (x^2 0.2; p 0.7). Age at diagnosis was not significantly related to five years survival (O.R. 0.98; 95%C.I. 0.92–1.04; $P=0.6$). Absence of ER expression (O.R. 6.54; 95%C.I. 1.70–25.21; $P=0.006$) and absence of TPOAb (O.R. 9.37; 95%C.I. 1.21–72.67; $P=0.03$) were related to a higher mortality rate. RE+ and TPOAb+ are positive prognostic parameters in BC and the absence of any relationship between them seems to propose an independent role on the prognosis of BC patients.

P104

Bone density in patients with non-functioning pituitary adenomas (NFA)

Stefan Fischli, Christoph Stettler, Rahel Sahli, Silvia Schwab, Bernard Chappuis, Stefan Jenni, Peter Diem & Emanuel Christ
Department of Endocrinology, Diabetology and Clinical Nutrition, University Hospital of Bern, Inselspital, Bern, Switzerland.

Background
Surgically treated patients with NFA often present with secondary hypogonadism. Hypogonadism is a well known risk factor for osteopenia or osteoporosis. The aim of this study was to assess (a) the frequency of osteopenia/osteoporosis in a single centre Swiss cohort of patients with operated NFA and (b) whether gender or hypogonadism impacts on bone density at follow up.
Methods
Data of patients with NFAs diagnosed between 1967 and 2005 were analysed. Clinical and endocrinological parameters were recorded before, immediately after surgery and at last follow-up. Bone densitometry (DEXA) was performed during follow up. Data were analyzed using Fisher's Exact Test for calculating relative risks (RR) and p-values.
Results
121 patients with NFA were included (71% male and 29% female). Mean age at diagnosis was 55.2 ± 14.7 years. 74% of male and 25% of female patients had secondary hypogonadism at follow up, 57% (20) of female were menopausal prior to surgery. DEXA was performed in 68% ($n=82$) of all patients. Overall, DEXA showed a normal bone density (T-score $\geqq -1$) in 26%, in 30% signs of osteopenia (T-score between -1 and -2.5) and in 12% signs of osteoporosis (T-score ≤ -2.5). The relative risk (RR) for osteopenia/osteoporosis in all patients with secondary hypogonadism at follow up compared to patients with normal gonadale function at follow up was 0.84 (95% CI 0.61–1.16; $P=0.36$) [men: 1.19 (0.59–2.40; 0.74), women: 1.50 (0.67–3.34;$P=0.37$)]. The RR for osteopenia/osteoporosis in female patients with hypogonadism (incl. menopausal females) compared to men with hypogonadism at follow up was 1.57 (95% CI 1.16–2.14; $P=0.013$).
Conclusions
(1) Osteopenia and Osteoporosis is a common problem in patients with NFA. (2) A diminished bone density is not only related to impaired gonadale axis in patients with NFA. (3) The influence of gender on bone density appears to be critical.

P105

Echo-enhanced ultrasound has a higher sensitivity than high-resolution CT in the detection of hepatic metastasis of adrenocortical carcinoma

Jürgen Bauditz[1], Wolfram Wermke[1], Dirk Beyersdorff[2], Herbert Lochs[1], Christian J. Strasburger[1] & Marcus Quinkler[1]
[1]Medicine, Center for Gastroenterology, Hepatology and Endocrinology, Charite Campus Mitte, Charite Universitaetsmedizin Berlin, Berlin, Germany, [2]Institute for Radiology, Charite Campus Mitte, Charite Universitaetsmedizin Berlin, Berlin, Germany.

Background
Adrenocortical carcinoma (ACC) is a rare and heterogeneous malignancy with incompletely understood pathogenesis and poor prognosis. Computerized tomography (CT) and magnetic resonance imaging (MRI) are routinely performed for imaging of the adrenal mass and for standard staging of chest and abdomen as lung and liver are the primary organs for metastatic spread in ACC. Contrast ultrasound is a non-invasive procedure which has been shown to have a high sensitivity and specifity for differentiation of hepatic and neuroendocrine tumours.
Methods
patients (7 women, 5 men; aged 24 to 77 years) with ACC were treated in our centre from 2004 to 2006. Patients received staging with HR-CT as well as with contrast ultrasound (Sonovue/Bracco, Acuson Sequoia/Siemens, CPS) of the liver.
Results
Contrast ultrasound demonstrated liver metastases in 8 of 12 patients (67%), HR-CT showed liver metastases in 6 of 12 patients (50%). In 2 of 8 patients (25%) HR-CT missed detection of liver metastases. Even retrospectively and with knowledge of the ultrasound results, the hepatic lesions were not recognized by HR-CT, but were detectable by HR-CT at a later time point. All hepatic lesions diagnosed by HR-CT were also seen by ultrasound. The detection of liver metastases by ultrasound resulted in a change of therapy in the 2 patients.
Conclusions
Contrast ultrasound has a higher sensitivity than HR-CT in detecting highly vascularized liver metastases of ACC and should be included in the staging algorithm of ACC.

P106

Characteristics of metabolic syndrome in patients with adrenal incidentaloma

Miomira Ivovic, Svetlana Vujovic, Milos Stojanovic, Milina Tancic-Gajic, Ljiljana Marina, Marija Barac & Milka Drezgic
Institute of Endocrinology, Belgrade, Serbia.

Several studies show that characteristics of metabolic syndrome are often seen in patients with adrenal incidentaloma. The aim of our study was to evaluate metabolic factors in these patients. 208 patients (148 female and 60 male, age 55.08 ± 11.02 y's and BMI 27.91 ± 4.6 kg/m^2) were admitted and biochemical, endocrine testing were performed. Lipid status: cholesterol 5.77 ± 1.26 mmol/L, triglyceride 1.92 ± 0.98 mmol/L. 113(55%) patients were hypertensive (mean systolic pressure was 150.3 ± 30.12 mmHg, diastolic 92.93 ± 16.48 mmHg). 34 (16.35%) patients had type 2 diabetes. According to OGTT (performed in 131 patients) more than 50% were diabetic or showed glucose intolerance. Insulin sensitivity was calculated by HOMA, QUIQI formula and 56,86% of patients had insulin resistance. After endocrine evaluation we divided them in two groups: first with subclinical hypercorticism and second without hypercorticism. First group: 46 patients (38 woman and 8 man mean age 56.6 ± 9.25 y's and BMI 27.83 ± 4.37 kg/m^2). Second group: 162 patients (110 women and 52 men, age 54.66 ± 11.45 years and BMI 27.93 ± 4.67 kg/m^2). No statistically significant difference was found for cholesterol (5.68 ± 1.24 vs. 6.09 ± 1.29 mmol/L; $P > 0.05$) and triglyceride (1.88 ± 0.9 vs. 2.08 ± 1.22 mmol/L; $P > 0.05$) between these subgroups. We also find no statistically significant difference in insulin resistance between groups (QUIQI: 0.34 ± 0.05 vs. 0.33 ± 0.03; $P > 0.05$). Mean systolic and diastolic blood pressure was not significantly higher in subgroup with sublinical hypercorticism (149.6 ± 29.92 vs. 152.7 ± 31.02 mmHg; $p > 0.05$ and 92.38 ± 16.42 vs. 94.89 ± 16.74 mmHg; $P > 0.05$).

Significant number of patients with adrenal incidentaloma had characteristics of metabolic syndrome even without proved endocrine hypersecretion. These patients are at high risk for cardiovascular events. Well-defined international study protocols should include screening for metabolic syndrome.

P107

The role of radio-guided surgery (RGS) with the use of ^{99m}Tc-EDDA/-HYNIC-octreotate in detection of unknown primary and secondary sites of neuroendocrine tumours of the gastrointestinal tract (GEP-NET) and improving the final outcome of patients

Alicja Hubalewska-Dydejczyk[1], Jan Kulig[2], Piotr Szybinski[2], Renata Mikolajczak[3], Anna Sowa-Staszczak[1], Katarzyna Fröss-Baron[1] & Bohdan Huszno[1]
[1]Department of Endocrinology, Medical College at Jagiellonian University, Cracow, Poland, [2]Gastrointestinal and General Surgery Department, Medical College at Jagiellonian University, Cracow, Poland, [3]Radioisotope Centre POLATOM, Otwock-Swierk, Poland.

Despite a wide spectrum of imaging diagnostics, GEP-NETs often stay undetectable until the time of dissemination. Removing of a primary tumour together with disseminated lymph nodes even with the presence of liver metastases is the most appropriate treatment to delay progression of the disease. SRS followed by RGS gives a possibility to detect occult GEP-NET intra-opratively. ^{99m}Tc-HYNIC/EDDA-octreotate, a somatostatin analogue with high affinity to sst2 was applied in the study. **The aim of the study** was to determine whether intra-operative radio-detection with the use of ^{99m}Tc-EDDA/HYNIC-octreotate, is able to reveal unknown primary tu and metastases of GEP-NET thereby improving surgical treatment and final prognosis.
Materials and methods
There were ten patients under examination with GEP-NET (with positive SRS and negative different pre-operative imaging tests). Insulinoma was suspected in 5 pts, non-functioning pancreatic NET - 1, and carcinoid in 5 cases. At surgery, suspected lesions were measured in vivo and ex vivo (Navigator-GPS) and the exact exploration of the abdominal cavity was performed.
Results
Amongst patients with pancreatic NET, ^{99m}Tc-EDDA/HYNIC-octreotate SRS followed by RGS detected 4 insulinomas, 1 glucagonoma and in one patient false

positive result appeared to be a cyst but nesidoblastosis was finally recognised. Three carcinoids with metastases were detected; in two cases the use of hand-held gamma probe extended the surgical procedure resulting in the successful excision of the metastatic lymph nodes. In one case the liver metastases were confirmed previously revealed by SRS only. Another false positive result was caused by ileitis.
Conclusion
In our study ^{99m}Tc-EDDA/HYNIC-octreotate SRS followed by RGS localized all primary GEP-NETs undetected with other imaging diagnostics. The main advantage of RGS in comparison to SRS is high sensitivity in detection of metastatic lymph nodes. The imaging properties of the ^{99m}Tc-EDDA/HYNIC-octreotate creates abilities for more common application of this tracer followed by RGS in oncology.

P108

Ascl1 is abundantly expressed in endocrine pancreatic tumors

Téresè Johansson[1], Margareta Halin Lejonklou[1], Sara Ekeblad[1], Peter Stålberg[2] & Britt Skogseid[1]
[1]Institution of Medical Sciences, Uppsala, Sweden, [2]Institution os Surgical Sciences, Uppsala, Sweden.

Background
Apart from inactivation of the *MEN1* gene, molecular events essential for tumorigenesis of the endocrine pancreas are poorly characterized. A potentially useful approach for understanding tumor progression is to study transcription factors operating in fetal pancreatic development. The Notch signaling cascade with expression of the transcription factors *Hes1*, *Hey1*, and *Ascl1* plays a vital role in sustaining the balance between cell proliferation, differentiation and apoptosis during the pancreatic development. They may play a similar role in the development of endocrine pancreatic tumors (EPT).
Aim
To study the expression of *Notch1*, *Hes1*, *Hey1* and *Ascl1* in EPT, by quantitative PCR (qPCR) and Immunohistochemistry (IHC)
Material and methods
Notch1, *Hes1*, *Hey1*, and *Ascl1* mRNA and protein expression were investigated in 26 EPT (ten were MEN1 associated). Immunohistochemistry was also performed on 11 normal pancreatic tissues adjacent to the tumor (five MEN1 and six sporadic). The immunoreactivity was graded (negative, weak, moderate or strong), and sublocalization of expression as nuclear and/or cytoplasmatic was determined.
Results
The statistical analysis of the qPCR data revealed a correlation between the *Notch1-Hes1* expressions in EPT. All tumors displayed *Ascl1* immunoreactivity, which was graded as strong in 85%. *Hes1* expression in EPT was graded as invariably weak, or completely absent (30%). In normal islets a weak nuclear *Hes1* staining was observed. *Hey1* and *Notch1* were expressed in the cytoplasm and nucleus of tumor cells and normal endocrine tissue.
Conclusions
Ascl1 is invariably and abundantly expressed in EPT. *Hes1* is either lacking or weakly expressed and confined to the cytoplasm of EPT. The lack of *Hes1* in tumor cell nuclei could contribute to the prominent *Ascl1* expression in EPT. These results show that *Notch1*, *Hes1*, *Hey1* and *Ascl1* are variable expressed in EPT and normal pancreatic tissues; and that they may be involved in endocrine pancreatic tumorigenesis.

P109

Histopathological and molecular studies in patients with goiter and hypercalcitoninemia: reactive or neoplastic C-cell hyperplasia?

Uberta Verga[1], Stefano Ferrero[3], Leonardo Vicentini[2], Tatiana Brambilla[3], Valentina Cirello[1], Marina Muzza[1], Paolo Beck-Peccoz[1] & Laura Fugazzola[1]
[1]Endocrine Unit, Dept. of Medical Sciences, University of Milan and Fondazione Policlinico IRCCS, Milan, Italy, [2]Endocrine Surgery Unit, Fondazione Policlinico IRCCS, Milan, Italy, [3]Pathology Unit, Dept. of Medicine, Surgery and Dentistry, University of Milan, Ospedale S. Paolo, Milan, Italy.

The cut-off values able to differentiate between reactive or neoplastic C cell hyperplasia (CCH) or to predict sporadic medullary thyroid cancer (MTC) are still debated both for basal and stimulated calcitonin (bCT and sCT). Aim of the present study was to define the prevalence and the histological patterns of CCH in 15 patients with multinodular goiter (MNG), bCT $>$ 10 pg/ml and sCT levels $>$ 50 pg/ml. These data were compared with those from 16 patients with MNG and bCT levels $<$ 10 pg/ml, and with those from 4 patients with familial medullary thyroid cancer (FMTC). For each case, 6 paraffin blocks were selected, CT immunoreactive cells were counted in sixty consecutive high power fields (400x) and classified as focal, diffuse, nodular or neoplastic. *RET* genetic analyses were performed at the germline and tissue level in MTC and, for the first time, in CCH cases. In patients with MNG, sCT levels $>$ 50 pg/ml were associated with CCH or with MTC, being the total number of C cells/60 fields significantly higher than that found in MNG with normal bCT ($P = 0.0008$), and comparable to that detected in FMTCs. Interestingly, in the group with sCT $>$ 50 pg/ml, the C cells displayed a neoplastic phenotype, concerning morphology, distribution and localization. No *RET* mutations were found neither at the germline nor at the somatic level.

In conclusion, sCT levels $>$ 50 pg/ml were associated with CCH in all cases and with MTC in 4 patients, without correlation between CT levels and the number of C cells or the final diagnosis. After serial blocking and high power field magnification, an elevated number of C cells were counted, often showing a morphology and a distribution pattern consistent with neoplastic CCH, thus strengthening the hypothesis that CCH might be the precursor also of sporadic MTC in the absence of *RET* mutations. Hence, sCT levels $>$ 50 pg/ml indicate the presence of CCH with a possible preneoplastic potential, suggesting the opportunity to perform a "prophylactic" surgical treatment.

P110

Thyroid cancer and pregnancy: clinical outcome and time of diagnosis in a series of 94 women

Perrino Michela[1], Vannucchi Guia[1], Vicentini Leonardo[2], Deborah Mannavola[1], Paolo Beck-Peccoz[1] & Laura Fugazzola[1]
[1]Endocrine Unit, Dept. of Medical Sciences, University of Milan and Fondazione Policlinico IRCCS, Milan, Italy, [2]Endocrine Surgery Unit, Fondazione Policlinico IRCCS, Milan, Italy.

Thyroid cancer represents the second more frequent tumor among those diagnosed during pregnancy. Indeed, during pregnancy thyroid volume increases by 20–30% and new nodules can appear, due to the effect of choriogonadotropin which stimulates thyroid growth. Hence, it has been proposed that thyroid cancer diagnosed during pregnancy could harbour a poorer prognosis. Aim of the present study was to compare the clinical outcome in the following 3 groups of patients affected with thyroid cancer: group 1 (Gr.1): 12 women with tumor diagnosed during pregnancy and submitted to thyroidectomy during the second trimester or in the first year after delivery; group 2 (Gr.2): 33 women with diagnosis of tumor at least 1 year after the delivery; group 3 (Gr.3): 49 women with diagnosis and treatment of the tumor before pregnancy or nulliparous. The 3 groups were matched for age, treatment, histology and follow-up. In particular, all patients of group 1 were treated with total thyroidectomy and radiometabolic treatment. Remission or persistence of disease were defined on the bases of basal thyroglobulin (Tg) levels before and after rhTSH, in the absence of anti-Tg antibodies, and of Total Body Scan. No significant differences in tumor size, capsular invasion and local/distant metastases were observed between the 3 groups. As far as the outcome is concerned, patients with the tumor diagnosed during pregnancy showed more frequently persistence or relapse of the disease with respect to the patients of the other groups (Gr. 1 *vs* Gr. 2: $P = 0.0035$; Gr.1 *vs* Gr. 3: $P = 0.0057$; Gr1 *vs* Gr. 2+3, $P = 0.018$; Gr.2 *vs* Gr.3: $P =$ NS). In particular, 9/12 patients of Group1 showed persistence of disease, with lymph-node metastases in 2 cases, distant metastases in 2 cases and elevated Tg levels in 5 cases.

In conclusion, the present data show that thyroid cancer diagnosed during pregnancy is associated with a poorer prognosis with respect to tumors developed in a non gravidic period, thus suggesting the need for an aggressive treatment by thyroidectomy during the second trimester and radiometabolic therapy soon after delivery.

P111

RET genotypes comprising specific haplotypes of polymorphic variants are associated with sporadic medullary thyroid cancer

Laura Fugazzola[1], Marina Muzza[1], Caterina Mian[3], Daniela Cordella[2], Susi Barollo[3], Valentina Cirello[1], Maria Elisa Girelli[3], Giuseppe Opocher[3], Paolo Beck-Peccoz[1] & Luca Persani[2]
[1]Endocrine Unit, Dept. of Medical Sciences, University of Milan and Fondazione Policlinico IRCCS, Milan, Italy, [2]Dept. of Medical Sciences, University of Milan and Istituto Auxologico Italiano IRCCS, Milan, Italy, [3]Endocrinology Unit, Dept. of Medical and Surgical Sciences, University of Padova, Padova, Italy.

Many single nucleotide polymorphisms (SNPs) of the *RET* gene have been described both in the general population and in patients with sporadic medullary thyroid cancer (sMTC), MEN2 or Hirschsprung disease. Some association studies reported a higher prevalence of these variants in the affected patients, suggesting a possible role in modifying the risk of occurrence of the disease. However, data from different cohorts of sMTC are discrepant and the aim of the present study was to determine if a variant *per se* or a combination of variants predispose to sMTC. Thus, a possible association of RET haplotype(s) and disease was looked for in 82 patients affected with sMTC and 49 age matched controls. Six *RET* SNPs were studied by PCR and direct sequencing. The most frequent SNPs were those in intron 1 (30 and 32% in sMTC and controls, respectively), exon 2 (22 and 24%) and exon 13 (24 and 26%). No significant differences were observed in the prevalence of single SNPs between patients and controls, including G691S, which is the only non-synonymous variant. Accordingly, functional analyses did not reveal an increased autophosphorylation for G691S. Twelve unique haplotypes, labelled A-N, were obtained. The distribution of haplotypes between cases and controls were significantly different ($P<0.05$). The study of the association of these different haplotypes in cases and controls lead to the identification of 30 different genotypes. Inspection of the genotypes in the two groups showed that the genotype distribution between cases and controls was different ($P<0.05$). In particular, there were 7 genotypes unique to controls, 13 unique to sMTC and 11 shared by the 2 groups. For example, AA, AC, AD and AH, all of which containing one allele without polymorphisms, are prominently or uniquely represented in sMTC. These data suggest that genotypes comprising specific pairs of RET haplotypes are associated with predisposition to sMTC. In this series, the absence on both alleles of the 6 SNPs analyzed was recorded only for MTC cases, indicating that the presence of RET variants could be protective against cancer development.

P112

Isolation of the Side population (SP) from murine adrenal glands renders cells with adrenocortical stem cell properties

Urs Lichtenauer[1], Igor Shapiro[1], Klaus Geiger[2], Anne-Marie Pulichino[3], Jaques Drouin[3] & Felix Beuschlein[1]
[1]Institute of Molecular Medicine and Cell Research, Albert-Ludwigs-University Freiburg, Freiburg, Germany, [2]Department of Internal Medicine I, Albert-Ludwigs-University Freiburg, Freiburg, Germany, [3]Laboratoire de Genetique Moleculaire, Institut de Recherches Cliniques de Montreal, Montral, Canada.

Radioactive and transgenic tracing experiments indicate that in the adult adrenal stem cells persist in the periphery of the cortex, which migrate centripetally and populate the inner cortical zones upon differentiation. However, investigation of these cells has been hampered by the lack of known marker genes. Vital Hoechst dye exclusion has been described as a method for isolating a side population (SP) from mouse bone marrow, which was enriched with stem cells. Utilizing this technique, we demonstrate the presence of SP cells in a variety of adrenal derived cell populations including normal mouse (0.71–0.83%) and human (0.01%) adrenals. After FACS sorting, isolation of SP and non SP (NSP) cells from murine adrenal glands revealed self-renewal and long-term culture capacities only for the SP fraction, which grew in a fibroblast-like manner, whereas the NSP cells did not proliferate. In addition, adrenal SP cells expressed adrenocortical markers such as MC2 receptor, StAR, and P450scc by means of RT-PCR and IHC. Interestingly, in a mouse model of ACTH deficiency (Tpit knock out animals, Tpit$-/-$), the proportion of SP cells was significant higher in comparison to heterozygous animals (Tpit$-/-$ $0.45\pm0.16\%$ *vs.* Tpit$+/-$ $0.13\pm0.04\%$;$P<0.004$). This higher SP cell proportion was associated with an increased width of the subcapsular cell compartment (Tpit$-/-$ $100\pm12.3\%$ *vs.* Tpit$+/-$ $259\pm10.7\%$; $P<0.0001$), which was characterized by the lack of expression of steroidogenic enzymes such as 3ßHSD. Short term ACTH treatment of Tpit$-/-$ animals resulted in a decrease of SP proportion (0.09%) and a shrinkage of the subcapsular zone similar to that of untreated Tpit$+/-$ controls ($130\pm10.2\%$;$P=0.33$). In summary, the adrenal Side Population displays certain stem cell properties. Moreover, we present indirect evidence that ACTH might be required for adrenocortical stem cell differentiation thus affecting adrenal zonation *in vivo*. Current studies including *in vitro* stimulation and *in vivo* transplantation experiments aim at the further characterization of this cell population.

P113

Selective intra-arterial calcium stimulation with hepatic venous sampling in investigation of hyperinsulinemic hypoglycemia

Mohd Shazli Yusof, Tomas Ahern, Thomas P Smith & Donal O'Shea
Department of Endocrinology and Diabetes Mellitus, Elm Park, Dublin 4, Ireland.

A retrospective analysis of the results of all intra-arterial calcium stimulations performed at St. Vincent's Hospital, Dublin, in the years 2001-2006. All patients with symptoms suggestive of hypoglycemia had 72 hour fasting test with evidence of inappropriately elevated insulin and c-peptide at the time of hypoglycemia. These patients were investigated further with pancreatic imaging and selective intra-arterial calcium stimulation with hepatic venous sampling (ASVS). Analysis of the results was performed using the Wilcoxon signed rank test. Results were available in 9 patients. The overall catherisation success rate was: minimum four arteries in 7/9, three arteries 1/9 and two arteries in 1/9. CT was positive in 2/7 patients, MRI 0/2, octreotide scan 0/2 and endoscopic ultrasound 0/2. Mean insulin increment was 11.91 fold (95% CI 6.51–17.30) in tumour area versus 1.61 fold (95% CI 1.21–2.01) $P=0.002$. ASVS was positive in 8 patients. 7 patients were found to have insulinoma and 2 patients were diagnosed with adult nesidioblastosis by means of histological diagnosis. One of nesidioblastosis patient had negative calcium stimulation test but had diffuse hyperinsulinemic picture on ASVS. Our results suggest that selective intra-arterial calcium stimulation with hepatic venous sampling remains a powerful tool for diagnosis of insulinoma. CT pancreas alone combined with ASVS should be the standard of investigation in biochemically proven insulinoma. Three fold insulin levels increment should be used as the cut-off point for positive test after calcium stimulation. We reported a case of failure ASVS. ASVS use should be restricted to units with expertise in this area.

P114

Somatostatin and dopamine receptor regulation and effects of a new somatostatin/dopamine chimeric compound on cell proliferation of the human androgen-dependent prostate cancer cell line LNCaP

Marica Arvigo[1], Massimiliano Ruscica[2], Elena Dozio[2], Michael D Culler[3], Francesco Minuto[1], Marcella Motta[2], Paolo Magni[2] & Diego Ferone[1]
[1]Department of Endocrinological & Metabolic Sciences and Center of Excellence for Biomedical Research, Genoa, Italy, [2]Center for Endocrinological Oncology, Istituto di Endocrinologia,, Milan, Italy, [3]Biomeasure Incorporate/IPSEN, Milford, United States.

The increasing use of somatostatin (SRIF) analogues prompted extensive investigations on SRIF receptor (SSR) in human tumours. Human prostate cancer (PCa) may differentially express SSRs from the normal tissue. Moreover, SSR and dopamine (D) receptors (DR) may interact to form homo- and heterodimers with enhanced functional activity. In the present study, using the human androgen-dependent PCa cell line LNCaP, we investigated: 1) SSR and DR subtype expression in different culture conditions (10% and 2% FBS); 2) the effects of SRIF and of a new SRIF/D chimeric molecules, BIM-23A760, able to bind with high affinity both sst_{2A} and D_2R on cell proliferation. LNCaP expressed sst_1, sst_{2A}, sst_3, sst_5, and D_1R and D_2R subtypes at gene (RT-PCR) and protein (Western blot) level. SSRs and D_2R expression was differentially regulated by the culture conditions: sst_{2A}, sst_5 and D_2R expression was not modified by serum concentration, whereas sst_1 and sst_3 were inversely correlated to serum concentration. Dose-response studies were performed at 24 and 48 h with a dose range from 10^{-11} to 10^{-7} M. SRIF inhibited cell proliferation ([^{3}H]thymidine incorporation) after 24 and 48 h at all doses. The clinically available analogue lanreotide inhibited cell proliferation after 24 and 48 h with a maximum effect at 10^{-11} M. However, the chimeric BIM-23A760 resulted more potent than lanreotide and significantly inhibited cell proliferation after 24 h at 10^{-9} M and after 48 h in a dose range from 10^{-7} to 10^{-11} M. These data indicate a heterogeneous expression of SSRs and DRs in PCa, depending on the culture conditions and show an enhanced potency of the chimeric BIM-23A760 in inhibiting cell proliferation, suggesting an important role of the dopaminergic pathway in PCa. Hence, LNCaP provides a model to study the interaction between membrane receptors and to further investigate chimeric SRIF/D compounds in human cancer.

P115

Somatostatin receptor regulation and effects of somatostatin and somatostatin analogues on cell proliferation of the human androgen-dependent prostate cancer cell line LNCaP

Massimiliano Ruscica[1], Marica Arvigo[2], Elena Dozio[1], Michael D Culler[3], Francesco Minuto[2], Marcella Motta[1], Diego Ferone[2] & Paolo Magni[1]
[1]Center for Endocrinological Oncology, Istituto di Endocrinologia, Università degli Studi di Milano, Milan, Italy, [2]Department of Endocrinological & Metabolic Sciences and Center of Excellence for Biomedical Research, University of Genoa, Genoa, Italy, [3]Biomeasure Incorporate/IPSEN, Milford, Massachusetts, United States.

Somatostatin (SRIF) has been demonstrated to inhibit *in vitro* proliferation of normal and transformed cells via SRIF receptors (SSRs). Moreover, like other neuroendocrine molecules, SRIF and SSRs may play a significant role in the progression and neuroendocrine differentiation of human prostate cancer (PCa). However, conflicting results have been reported in the literature on SSR heterogeneity and specific cell localization in PCa. In the present study, using the human androgen-dependent PCa cell line LNCaP, we investigated: 1) SSR subtypes expression in different culture conditions (10% and 2% FBS); 2) the effects of SRIF and of new agonists on cell proliferation. LNCaP expressed sst_1, sst_{2A}, sst_3, sst_5, at gene (RT-PCR) and protein (Western blot) level. SSR level of expression was differentially regulated by the culture conditions: sst_{2A} and sst_5 expression was not modified by serum concentration, whereas sst_1 and sst_3 expression was inversely correlated to serum concentration. Dose-response studies were performed at 24 and 48 h with a dose range from 10^{-11} to 10^{-7} M. SRIF inhibited cell proliferation ([^{3}H]thymidine incorporation) after 24 and 48 h at all doses. The sst_1 (BIM-23926), sst_2 (BIM-23120) and sst_5 (BIM-23206) preferential compounds did not affected cell proliferation. Conversely, lanreotide, inhibited cell proliferation after both 24 and 48 h with a maximum effect at 10^{-11} M, whereas, the bispecific sst_2/sst_5-preferential ligand BIM-23244 inhibited cell proliferation after 24 h at the dose of 10^{-9} M. The bi-specific sst_1/sst_2-preferential ligand BIM-23704 inhibited LNCaP proliferation after 48 h treatment, (dose range 10^{-10} M to 10^{-11} M). SSR subtype expression in PCa can be actively regulated by culture conditions, suggesting that receptor profile in PCa may depend from the tumor microenvironment. Finally, LNCaP represents a useful model for studying SSR regulation in PCa, intracellular subtype-linked signalling, and validate new analogues with different receptor affinities in PCa treatment.

P116

Conjugated and unconjugated serum steroid hormone concentrations in relation to tumour receptor status in postmenopausal breast cancer patients

Borbála Vincze, Ferenc Pommersheim, Nora Udvarhelyi, Zsolt Horváth, Bence Kapuvári, Mariann Boldizsár, István Köves, Zsolt Orosz & István Láng
National Institute of Oncology, Budapest, Hungary.

Human breast cancer tissue is able to concentrate estrogens. 17-beta-estradiol (E2) and estrone (E1) are produced locally through several mechanisms, e.g. from conjugated and unconjugated steroid hormones uptaken from the circulation. This study was aimed to investigate the correlation between endogenous serum sex steroid concentrations and tumour receptor status in postmenopausal breast cancer patients undergoing surgical intervention. The study involved 740 postmenopausal patients with primary breast cancer of Stage I-II prior to surgical intervention. None of them took hormone preparations and received chemo or radiotherapy. Serum levels of sexual hormones and precursors, sex hormone-binding globulin (SHBG), insulin-like growth factor-1 (IGF-1) and IGF binding protein-3 (IGFBP-3) were measured by fully automated equipment using RIA and IRMA methods. Estrogen (ER) and progesterone receptors (PR), HER2/neu expression in tumour tissues were determined using immunohistochemical methods (NCL-ER-6F, 11/2; NCL-L-PgR-312; CB11-RTU, Novocastra; Hercep Test, DAKO). In the ICH 2+/3+ cases HER2/neu gene amplification was confirmed by fluorescence in-situ hybridization. MedCalc Software was used for statistical analysis. Our investigation revealed significant correlations among steroid receptor status of tumour tissue and the serum E1 and androstenedione (AD) levels. Close relationship was observed among serum value of E1-sulfate, IGF-1, testosterone (TE), dehydroepiandrosterone sulphate (DHEA-S) and HER2/ER status of tumour tissue. Results demonstrate that the positivity of tumour tissue receptor status can be predicted on the basis of increased serum unconjugated (E1, DHEA, AD, TE) and conjugated (E1-S, DHEA-S) sexual hormone concentrations. It is suggested that circulating E1-S and DHEA-S might play a major role in the intratumoral estrogen synthesis. Our study supports the hypothesis that the serum E1, AD, E1-S, DHEA-S, TE and IGF-1 levels might also be useful for predicting the magnitude of response to postoperative chemotherapy.

This research was supported by the Hungarian Research Fund (OTKA No.T 049814).

P117

A novel activating germline mutation in the RET gene (Y606C) in a patient with medullary thyroid carcinoma

Michaela Luconi, Tonino Ercolino, Adriana Lombardi, Lucia Becherini, Elisabetta Piscitelli, Maria Sole Gaglianò, Mario Serio & Massimo Mannelli
University of Florence, Dept. Clinical Physiopathology, Florence, Italy.

Germline mutations in the RET gene cause MEN2, an inherited cancer syndrome associated with medullary thyroid carcinoma (MTC). We performed genetic analysis on DNA from whole blood of a 58 yr old female affected by a multifocal MTC. Exons 10, 11, 13, 15 and 16 of RET gene were amplified by PCR using specific primers and characterised by direct automatic sequencing. Here, we report a new RET point mutation: a heterozigous missense mutation Y606C, a G to A nucleotide substitution leading to a Tyrosine (Y) to Cysteine (C) amino acid change in exon 10. We approached the functional effects of such a mutation in an *in vitro* system by cloning the wild-type RET, the Y606C mutation as well as the C620Y mutation, previously described as less strong RET oncogene associated with MTC, in an expression vector and transiently transfecting NIH3T3 fibroblasts. All mutations were obtained by site-directed mutagenesis. We first demonstrated by western blot analysis using a specific antibody an increased tyrosine phosphorylation in the Y905 residue in the RET/Y606C, corresponding to receptor activation. Since RET activation results in an intracellular signalling cascade leading to extracellular signal regulated kinases (ERKs), we investigated ERK activity in our transfected cells. Results demonstrate a significant increase in ERK2 phosphorylation/activation in the RET/Y606C *versus* the wild type and RET/C620Y. We finally showed by gel electrophoresis of transfected cell lysates in non reducing conditions that the introduction of a C due to the Y606C mutation results in an increased dimerization of the receptor. All these findings suggest that the Y606C mutation confers constitutive activation of RET signalling.

P118

Novel germline VHL mutations associated to uncommon clinical presentations

Tonino Ercolino[1], Lucia Becherini[1], Lisa Simi[1], Maria Sole Gaglianò[1], Gabriella Nesi[2], Andrea Valeri[3] & Massimo Mannelli[1]
[1]University of Florence, Dept. Clinical Physiopathology, Florence, Italy, [2]University of Florence, Dept. Human Pathology, Florence, Italy, [3]AOUC, General and Vascular Surgical Unit, Florence, Italy.

The von Hippel-Lindau (VHL) syndrome is an inherited multi-tumor disorder characterized by clinical heterogeneity and high penetrance. Pheochromocytoma (Pheo) is present in 10-15% of cases. It can be isolated or associated with other lesions such as hemangioblastomas, kidney cysts or cancer, and pancreatic lesions. Pheos secrete norepinephrine and are generally located in the adrenals. While performing genetic testing in patients affected by apparently sporadic pheos or PGLs, we found two novel different VHL germline mutations in two patients presenting two uncommon clinical pictures (an adrenal incidentaloma and a neck tumor, respectively).

Coding regions and exon-intron boundaries of RET (exons 10, 11, 13, 14, 15), VHL, SDHD, SDHB and SDHC genes were amplified and sequenced. We identified two novel point mutations: a L198V missense mutation in a 32 yr old female affected by a right adrenal compound and mixed tumor constituted by an epinephrine secreting Pheo, a ganglioneuroma and an adrenocortical adenoma and a T152I missense mutation in a 24 yr old female affected by a left carotid body tumor. An extensive clinical, laboratory and radiological examination of the patients and the mutated relatives did not show any other lesion.

We also analyzed the three-dimensional structure of the wild-type and the mutated VHL protein showing that the mutations are located in functionally relevant sites.

These cases enlarge the list of VHL mutations and add new insights in the clinical variability of VHL disease, thus confirming the importance of genetic testing in patients affected by apparently sporadic Pheos or PGLs.

P119

The expression of alternatively spliced forms of type 1 deiodinase is changed in clear cell renal cell carcinoma

Agnieszka Piekielko-Witkowska[1], Adam Master[1], Tanski Zbigniew[2] & Nauman Alicja[1]
[1]Medical Center of Postgraduate Education, Warsaw, Poland, [2]Specialistic Hospital, Ostroleka, Poland.

Type 1 deiodinase (D1) catalyses deiodination of tyroxine (T4), which leads either to synthesis of triiodothyronine or reverse triiodothyronine (rT3). T3 can influence the process of neoplasia through its receptors which act as transcription factors and regulate the expression of many tumor suppressor genes and oncogenes. The aim of the study was to analyze the changes in expression of alternatively spliced variants of D1 mRNA in clear cell Renal Cell Carcinoma (ccRCC), which is the most common type of renal cancers (75% of primary renal

malignancies). Tissue samples were obtained with the permission of the local Ethical Committee of Human Studies. Using quantitive real-time PCR we have analyzed: 33 samples of ccRCC with their controls (the contralateral pole of the same kidney not infiltrated by cancer, assigned C) as well as control samples from patients suffering from other, nonneoplastic kidney abnormalities (6 samples, assigned N). The expression of the whole pool of D1 transcript variants was dramatically lowered in ccRCC tissues. The separately performed expression analysis of alternatively spliced D1 transcript variants, which differ in the presence or absence of subexon 1b, also exhibited about 90% decrease of mRNA in both transcript variants of cancer tissues. Simultaneously, the comparison of these alternatively spliced mRNA groups revealed that ratio: (whole pool of D1 transcripts)/(transcripts containing the1b exon) as well as relation: (whole pool of D1 transcripts)/(transcripts devoid of the1b exon) were increased several times in the ccRCC in comparison with controls. This observation suggests the existence of at least one another alternatively spliced variant, which extends the whole poll of D1 transcripts and possibly is overexpressed in ccRCC. Our results indicated that the alternative splicing process of deiodinase type I can be disturbed in ccRCC.

P120

Ret expression reduces estrogen-induced lactotrope hyperplasia

Carmen Cañibano, Noela Rodríguez, Sulay Tovar, María Jesús Vázquez, Montserrat Lavandeira, Carlos Díeguez & Clara Álvarez
Department of Physiology, School of Medicine, University of Santiago de Compostela, santiago de Compostela, Spain.

RET is a tyrosine kinase receptor activated by GDNF, NTN, ART and PSPN through GFRa1,2, 3 and 4 respectively. Activation of the receptor elicits intracellular pathways such us Ras/MAPK and PI3K/AKT leading to differentiation and proliferation. Our group has previously shown that RET is expressed specifically in the somatotroph cell population within the pituitary gland, both in rats and in humans. We have also shown that, in absence of its ligand GDNF, RET induces activation of caspase 3 PKCd/JNK/c/EBPa and CREB, causing apoptosis in cell cultures. Cell death is dependent on Pit-1 and p53 induction. This findings confirm previous hypothesis and strongly indicate that RET acts as a dependence receptor. Now we provide evidence that the same biological and biochemical mechanisms work *in vivo*.

For doing so, we have used a model of lactotroph hyperplasia induced by estrogen administration in rat. Hyperplasic pituitary glands were infected with purified high-titer retroviruses encoding RET or the corresponding empty virus as control. Viral delivery was achieved by estereotaxia, injecting the retrovirus directly into the pituitary of living anesthetized rats. Following treatment and infection rats were sacrificed and pituitary weights recorded. As expected, estrogen treatment induced a marked increase in pituitary size. Interestingly, viral-mediated RET expression caused a significant reduction compared to mock-infected pituitaries (26.6+/− 1.8 mg *vs* 18.0+/− 1.0 mg), restoring pituitary weight to values similar to pituitaries not treated with estrogens. We were able to detect RET expression in lactotrophs, suggesting that ectopic expression of the dependence receptor caused lactotroph cell death and hyperplasia reversal. Moreover, we show activation of the caspase 3-PKCd-JNK-c/EBPa-CREB apoptotic pathway, indicating that the same molecular events are elicited by RET in cell culture models and *in vivo*.

P121

Analysis of BRAF point mutation in papillary thyroid carcinoma

Vlasta Sykorova[1], Sarka Dvorakova[1], Jan Laco[2], Ales Ryska[2], Daniela Kodetova[3], Jaromir Astl[4], David Vesely[4] & Bela Bendlova[1]
[1]Institute of Endocrinology, Prague, Czech Republic, [2]Fingerland's Institute of Pathology, Hradec Kralove, Czech Republic, [3]Institute of Pathology and Institute for Pathological Anatomy and Molecular Medicine FN Motol, Prague, Czech Republic, [4]Department of ENT and Head and Neck Surgery FN Motol, Prague, Czech Republic.

BRAF point mutations are found in 29-69% of papillary thyroid carcinoma (PTC). BRAF is a serine-threonine kinase involved in the phoshorylation of MAPK signaling pathway. The mutation is located in the exon 15 of BRAF, resulting in the substitution of valine to glutamate at codon 600 (V600E). Mutation generates unregulated B-Raf activity that leads to increased cellular proliferation. The aim of this study was to determine the frequency of BRAF mutation in the Czech population and its changes in 1960-2006. We examined 145 of PTC: 92 paraffin-embedded formalin-fixed tissue samples, 44 fresh frozen tissues and 9 wash-out material from fine-needle aspiration biopsies (FNAB) after signing informed consent approved by ethical committee. For assessment of influence of Chernobyl nuclear accident we devided samples into 5 periods - one period before and four periods after the accident. DNAs from paraffin-embedded samples were extracted using the QIAamp DNA Blood Mini Kit and frozen samples using Trizol reagent. BRAF gene was screened using the single strand conformation polymorphism method (SSCP) and verified by direct sequencing. The V600E mutation was detected in 56 samples (38,6%). All BRAF mutations except one were heterozygous. Surprisingly, in the period before Chernobyl nuclear accident no BRAF mutation was found, in other periods 56 mutations were detected (41,2%). The female to male ratio was 3,7:1, mutation was found in 48,4% of male and in 36% of female patients. In our series difference between age at diagnosis in patients with and without mutation was not significant. Our study confirms a high rate of BRAF V600E mutation in Czech PTC patients. Results indicate that the mutation is the most frequent genetic alteration found in PTC and it could be used as a reliable genetic marker of PTC and applied for FNAB before surgery.

This study was supported by IGA MZ CR NR/7806-3.

P122

Effect of surgery on carotid vascular remodeling in patients with pheochromocytoma

Giampaolo Bernini, Fabio Galetta, Michele Bardini, Ferdinando Franzoni, Chiara Taurino, Angelica Moretti, Matteo Bernini & Antonio Salvetti
Department of Internal Medicine, Universiyt of Pisa, Pisa, Italy.

In vitro and *in vivo* studies suggest that catecholamines, in addition to their hemodynamic effect, exert a direct influence on the vascular wall, leading to eutrophic and hypertrophic remodeling. This finding is in agreement with that recently reported by our group on patients with pheochromocytoma (PHEO) who show carotid intima media thickness (IMT) and vascular fibrosis higher than essential hypertensives matched for classic cardiovascular risk factors, including blood pressure. To further confirm the direct vascular influence of catecholamines in humans, we compared carotid IMT, by ultrasound imaging, and vascular fibrosis, by imaging backscatter signal (IBS) analysis, in a group of patients with PHEO and high-normal blood pressure (n = 10; mean ± SD age 51 ± 13.2 yr, range 28–70 yr) before and after surgical cure (mean ± SD 20.5 ± 5.98 months, range 12–29 months). After removal of the tumor, no significant variation in systolic (126.5 ± 6.5 *vs* 138.3 ± 5.6 mmHg, mean ± SE) and diastolic (83.6 ± 3.1 *vs* 87.0 ± 4.1 mmHg) blood pressure and in total cholesterol (207.0 ± 9.6 *vs* 198.8 ± 12.6 mg/dl), HDL-cholesterol (62.8 ± 4.5 *vs* 61.3 ± 4 mg/dl), and LDL-cholesterol (118.3 ± 8.5 *vs* 117.9 ± 13.1 mg/dl) was observed, while a reduction in urinary metanephrines (normetanephrine: 480.0 ± 51.2 *vs* 2264.8 ± 681.4 μg/24 h, $P < 0.003$; metanephrine: 178.7 ± 23.5 *vs* 879.2 ± 290.8 μg/24 h, $P < 0.03$) and in catecholamines (plasma noradrenaline: 442.9 ± 54.7 *vs* 623.9 ± 115.0 pg/ml, N.S.; plasma adrenaline: 36.1 ± 7.2 *vs* 183.8 ± 99.3 pg/ml, $P < 0.02$; urinary noradrenaline: 49.4 ± 8 *vs* 86 ± 27.4 μg/24 h, N.S.; urinary adrenaline:8.6 ± 0.7 *vs* 18.0 ± 7.7 μg/24 h, NS) was shown. After surgery, IBS values significantly decreased (−22.82 ± 0.40 *vs* –21.17 ± 0.61 dB, $P < 0.005$) and a similar pattern was observed for carotid IMT (0.86 ± 0.06 *vs* 0.88 ± 0.06 mm, $P < 0.06$), though at not significant extent. A direct and significant correlation was found between the absolute reduction in IBS values and the absolute decrement in urinary normetanephrines levels ($r = 0.54$, $P < 0.03$). In conclusion, our results confirm that high catecholamine levels directly affect the vascular wall structure, indipendently of the hemodynamic discharge.

P123

A case report of ectopic Cushing's disease presented with trombocytopenia

Demet Ozgil Yetkin[1], Sayid S Zuhur[1], Ekrem Kara[1], Ayla Gurel Sayin[2], Buge Oz[3] & Ertugrul Tasan[1]
[1]Istanbul University Cerrahpasa Medical faculty Endocrinology and Metabolism Department, Istanbul, Turkey, [2]Istanbul University Cerrahpasa Medical faculty Cardiovascular Surgery Department, Istanbul, Turkey, [3]Istanbul University Cerrahpasa Medical faculty Pathology Department, Istanbul, Turkey.

PURPOSE
To report a case of Cushing's syndome caused by ectopic ACTH secretion related to a thymic carcinoid presented with trombositopenia.

CASE
years old male presented with fatigue, skin rash. At presentation, physical findings showed Cushingoid appearance, with moon face, hyperpigmentation, easy bruising and buffalo hump. His laboratory findings showed platelet: 90.000 (150.000–450.000), ACTH: 609 pg/ml (0–46 pg/ml), baseline cortisol level 60.8 μgr/dl (6.2–19 μgr/dl), potassium: 2.4 mEq/l (3.5–5 mEq/l), midnight cortisol level: 57.17 μgr/dl, urine cortisol level: >1000 μgr/24 hour. Serum cortisol levels failed to supress after low and high dose dexamethasone (DST) (1 mg: >60 μgr/dl, 8 mg: 42 μgr/dl), therefore confirming the diagnosis of ectopic ACTH production. Laboratory evaluation for trombositopenia showed, normal eritrocyte series, deficient trombocyte. PT, aPTT and FDP were normal, fibrinojen: 606 mg/dl (<350). Megacaryocyte level was elevated and platelet count was normal in bone marrow aspiration. His sella MRI was normal, thorax CT showed 2×1.5 cm lesion at anterior mediastinum, and surrenal hyperplasia on his abdomen CT. His octreoscan was normal. There was a hypermetabolic focus in anterior mediastinum and bilaterally adrenal gland on his 5FDG PET/CT. Under the diagnosis of ectopic ACTH production in anterior mediasten, he underwent mediastinotomy and thymectomy. Pathological examination showed ACTH, chromogranin and synaptophsin positive thymic benign carcinoid. After the operation his cortisol levels returned to normal (corisol:11 μgr/dl, ACTH: 54 pg/ml) and low dose DST was 1.6 μgr/dl. Three weeks after the operation his platelet count was 411.000, with exclusions of other causes of tromposytopenia and reversal of platelet counts to normal after the operation we concluded that his trombosytopenia was due to a paraneoplastic immune trombocytopenic purpura (ITP).
CONCLUSION
Thymic ACTH secreting carcinoid tumors are rare phenomenon of ectopic Cushing's syndome. To our knowledge this is the first case of ectopic Cushing's disease with paraneoplastic ITP.

P124

Influence of Lanreotide Autogel on insulin sensitivity among patients with acromegaly

Melek Eda Ertorer[1], Okan Bakiner[1], Inan Anaforoglu[1], Emre Bozkirli[1], Neslihan Bascil Tutuncu[2] & Nilgun Guvener Demirag[2]
[1]Baskent University Faculty of Medicine, Endocrinology and Metabolism, Adana, Turkey, [2]Baskent University Faculty of Medicine, Endocrinology and Metabolism, Ankara, Turkey.

There are limited data inquiring the relationship between somatostatin analogues and insulin sensitivity among acromegalic cases. This study was conducted to evaluate short-term effects of lanreotide autogel (LA), administered every 28 days by deep subcutaneous injection, on insulin sensitivity among acromegalic patients with pituitary tumors. Before and following six months of LA treatment, insulin resistance and beta-cell function were calculated by using homeostasis model assessment of insulin resistance (HOMA-IR) and beta-cell function (HOMA-beta) formula, and euglycemic hyperinsulinemic clamp test was performed for evaluating the whole insulin sensitivity. Naïve acromegalic patients (Case 1, Case 3) and cases who experienced any prior unsuccessful treatment modality and approved to consume LA (Case 2, Case 4, Case 5) were included. The study was approved by the local ethics committee. Euglycemic hyperinsulinemic clamp defined by De Fronzo was used and insulin sensitivity was derived from glucose disposal rate expressed as mg/kg/min and indicated as 'M' index. The characteristics of the cases regarding serum growth hormone (GH) levels and insulin sensitivity markers during follow-up are shown in Table. Although there were statistically insignificant difference between baseline and final GH, HOMA-IR, HOMA-beta% and M values ($P=0.150$, $P=0.447$, $P=0.158$, $P=0.151$, respectively), remarkable M value improvement was observed in Case 1, Case 2 and Case 3. This finding might be explained by the prominent decrease in their GH levels following LA treatment.

	Case 1	Case 2	Case 3	Case 4	Case 5
GH (ng/ml)*	34.20/15.30	4.25/0.74	5.0/0.66	1.2/1.0	5.8/3.2
HOMAI-IR*	2.32 / 2.25	2.31 / 0.41	4.29/ 5.59	3.23 / 2.59	4.36/3.45
HOMAI-beta (%)*	95/ 58.48	289.15 / 83.63	152.25/ 98.06	76.87 /67.14	228.91/218.05
M value*	1.03 / 8.22	2.98 / 4.70	5.09 / 13.09	5.72 /5.53	3.90/3.35

*Baseline/ following 6 months of treatment.

P125

A newly detected mutation of the RET proto-oncogene in exon 8 as a cause of multiple endocrine neoplasia Type 2A

S. Bethanis[1], Th. Palouka[1], Ch. Avgoustis[1], G. Koutsodontis[2], T. Bei[2], D. Yannoukakos[2] & S. Tsagarakis[1]
[1]Department of Endocrinology, Athens' Polyclinic, Athens, Greece, [2]BioGenomica, Centre for Genetic Research and Analysis, Athens, Greece.

Multiple endocrine neoplasia type2A (MEN 2A) is a syndrome of familial cancers characterized by medullary thyroid carcinoma (MTC), pheochromocytoma and hyperplasia of the parathyroid glands. RET protooncogene is the responsible gene for MEN 2A; in more than 96% of MEN 2A families mutations in RET exon 10 or exon 11 are identified. Herein we report a MEN 2A case affected by a mutation ($Gly^{533}Cys$) in exon 8. A 66-yr-old male patient was referred to our Department due to bilateral adrenal nodules, revealed incidentally on a computed tomography of the abdomen. Patient's family history was remarkable for pheochromocytoma in his mother. On physical examination there were no features of von Hippel-Lindau disease (VHL) or neurofibromatosis type 1(NF1). Biochemical evaluation (elevated normetanephrines and metanephrines excretion) and findings of the adrenals' magnetic resonance imaging (hyperintense adrenal nodules on T_2-weighted image) were compatible with the diagnosis of bilateral pheochromocytomas. The patient underwent laparoscopic bilateral adrenalectomy and histological examination confirmed the preoperative diagnosis of pheochromocytoma. Absence of phenotypic characteristics of VHL or NF1 and elevated basal and stimulated by pentagastrin serum calcitonin levels raised the possibility of MEN 2A syndrome. Total thyroidectomy was performed and histological examination showed the presence of MTC. Genetic testing for the presence of a RET mutation was also recommended. Direct sequencing of exon 8 from patient's genomic DNA revealed the mutation c.1597G–>T (Gly533Cys). So far, the above missense point mutation has been associated with familial MTC (FMTC) but, to the best of our knowledge, mutations in exon 8 have never been identified in a MEN 2A case. In conclusion, in patients with clinical suspicion of MEN 2A syndrome the analysis of RET exon 8 should be considered when routine evaluation of mutations in exons 10, 11 and 13 is negative.

P126

Clinical and biochemical effects of adjuvant mitotane treatment in patients with adrenocortical cancer (ACC)

Fulvia Daffara[1], Silvia De Francia[2], Giuseppe Reimondo[1], Barbara Zaggia[1], Paola Sperone[3], Francesco Di Carlo[2], Alberto Angeli[1], Alfredo Berruti[3] & Massimo Terzolo[1]
[1]Dipartimento di Scienze Cliniche e Biologiche - Medicina Interna I, ASO San Luigi, Orbassano, Italy, [2]Dipartimento di Scienze Cliniche e Biologiche - Laboratorio di Farmacologia, ASO San Luigi, Orbassano, Italy, [3]Dipartimento di Scienze Cliniche e Biologiche - Oncologia Medica, ASO San Luigi, Orbassano, Italy.

Objective
Seventeen patients (9 women, 8 men aged 36 years, 22–58) radically resected for ACC were treated with adjuvant mitotane and prospectically followed from 2000 to 2006.
Methods
Stage of ACC: was: 1 stage I,; 12 stage II, 4 stage III; Weiss score 6, 3–9; Ki67% 20, 4–67. Eleven patients had functional tumors. Median duration of treatment was 15 months (range:4–84) and 14 patients are currently on mitotane, 2 died, 1 discontinued treatment after 5 years. All patients were treated with a low-dose regimen (till to 3–4 g/die) and underwent monitoring of plasma mitotane level every 3 months. None of the patients discontinued mitotane definitively for side effects and 16/17 patients reached the therapeutic levels after a median time of 3 months. At the last follow up, 6/17 (35%) patients have relapsed, 15 patients are still alive.
Results
Hyperprolactinemia was observed in 50% of men and 40% of women, 62% of men become partially hypogonadic: reduction of free testosterone was greater than total testosterone. Central hypothyroidism developed in 9 patients who were treated, while 4 patients already on thyroxine required dose increment. Fifteen patients developed overt hypoadrenalism, while 1 patient showed normal cortisol and elevated ACTH, 11 patients developed hypoaldosteronism. Total cholesterol level were slightly enhanced with increase of HDL and reduction of LDL, triglicerides were normal. Reduction of folate level and consequent increase of homocysteine was also observed. Mitotane levels were inversely correlated with cortisol ($P=0.007$), aldosterone ($P=0.01$) and FT4 levels ($P=0.03$), while they were positively correlated with PRA ($P=0.004$) and HDL levels ($P=0.005$).
Conclusions
In conclusion, a low-dose regimen of adjuvant mitotane is well tolerated and able to reach the therapeutic interval. Adequate supplementation of adrenal and sex steroid and thyroid hormones is necessary. Some effects of mitotane may be ascribed to either adrenolytic or estrogen-like actions of the drug.

P127

Effectiveness of retinoic acid treatment for redifferentiation of thyroid cancer in relation to recovery of radioiodine uptake

Cristián A. Fernández Fernández[1], Manel Puig-Domingo[1], Francisco Lomeña[1], Montserrat Estorch[2], Angel L Bittini[3], Mónica Marazuela[5], Javier Santamaría[4], Tomás Martín[7], Purificación Martínez de Icaya[6], Immaculada Moraga[5], María Cuesta[7], Ana Mejías[8], Manel Porta[9], Dídac Mauricio[2] & Irene Halperin[1]
[1]Hospital Clinic i Provincial de Barcelona, Barcelona, Spain; [2]Hospital de Sant Pau, Barcelona, Spain; [3]Hospital Gregorio Marañon, Madrid, Spain; [4]Hospital de Cruces, Barakaldo, Spain; [5]Hospital de la Princesa, Madrid, Spain; [6]Hospital de Leganés, Madrid, Spain; [7]Hospital Virgen Macarena, Sevilla, Spain; [8]Hospital Juan XXIII, Tarragona, Spain; [9]Hospital de Vic, Vic, Spain.

Retinoic acid (RA) treatment has been used in the last decade for redifferentiation of metastatic thyroid cancer that have lost radioiodine uptake (RIup) with heterogeneous results.
Aim
To evaluate the improvement of RIup after a course of RA treatment.
Method
Retrospective analysis of 29 patients with radioiodine negative metastatic disease (17 men /12 women; 22 papillary, 4 follicular and 3 oncocytic tumours). RA was given at a dose of 0.66–1.5 mg/kg for 5–12 weeks, followed by a therapeutic ^{131}I dose (3700–7400 MBq). Thyroglobulin levels and CT imaging control after 3 months of RA were performed.
Results
In 44.8% of the patients (14 out of 29 cases, 11 papillary/3 follicular) a positive radioiodine scan was observed; in 7 additional cases (5 papillary, 2 oncocytic) a weak RIup was also apparent (total responders 21/29, 72.4%), and in the remaining 8 the RIup persisted negative (6 papillary, 1 follicular and one oncocytic). No correlation was observed between changes in thyroglobulin levels and recovery of RIup. In 11 RA positive treatments a stabilization of mestastasic growth was observed in 5, while in 6 tumoural mass increased at short term. No major side effects were detected.
Conclusion
A relatively high rate of reinduction of RIup after RA treatment may be possible in advanced stage papillary and follicular thyroid cancer patients, with uncertainty in relation to a potential modification of the natural course of the disease. Further studies, aiming to identify potential responders to RA treatment by a better characterization of the biological nature of these tumours, will be required for an improved indication of RA coadjuvant treatment of thyroid cancer in the future.

P128

Expression of the neuropeptide cortistatin in haematological malignancies

Virgil Dalm, Frank Staal, Leo Hofland, Aart-Jan van der Lely & Martin van Hagen
Erasmus MC, Rotterdam, Netherlands.

Introduction
Cortistatin (CST) is a 17 amino-acid neuropeptide involved in sleep regulation. Due to its structural resemblance to somatostatin (SS), CST binds with high affinity to the 5 known SS receptors. CST also binds to the putative MrgX2 receptor. Previously we demonstrated that various types of human immune cells and tissues as well as lymphoid cell lines express CST mRNA. We suggested that CST plays a regulatory role in immune cell function both in physiological and pathophysiological conditions.

In the present study we investigated CST expression in human haematological malignancies, in order to gain more insight in the potential significance of CST in these diseases.
Patients and methods
Bone marrow and peripheral blood samples of 38 patients with T-ALL and B-ALL were studied using micro-array technique (Affymetrix) and 5 lymph node biopsies from patients with non-Hodgkin's lymphoma (NHL) using Q-PCR. Expression of both SS and CST mRNA was investigated in all samples.
Results
In 11 out of 22 patients with B-ALL CST expression was found, whereas in only 1 patient SS expression could be detected. Moreover, in 14 out of 16 patients with T-ALL CST expression was detected, while SS expression was present in only 1 patient. In all 5 NHL biopsies low expression of CST mRNA was detected, while no SS mRNA was found.
Conclusion
In the present study we demonstrated that CST mRNA is widely expressed in samples of patients with leukemic disease and in malignant NHL. On the other hand, expression of SS is absent in most cases. These findings suggest that, in line with our findings in normal human immune cells, CST might play a regulatory role, potentially with respect to control of proliferation or cytokine secretion, in these diseases, rather than SS. Further studies will be necessary to evaluate the role of CST and the potential therapeutical implications of CST or CST-like peptides.

P129

A loss-of-function polymorphic mutation in the P2X7 receptor gene in patients with papillary thyroid cancer

Angela Dardano[1], Simonetta Falzoni[2], Antonio Polini[1], Alessia Bemi[1], Anna Solini[1], Nadia Caraccio[1], Francesco Di Virgilio[2] & Fabio Monzani[1]
[1]University of Pisa, Pisa, Italy; [2]University of Ferrara, Ferrara, Italy.

Extracellular nucleotides, via specific plasma membrane receptors (P2Rs) of the X and Y subtype, modulate several cell functions, including cell-to-cell cross talk. We have previously demonstrated the expression of several functional P2XRs subtypes, including $P2X_7$ in primary human thyrocytes. P2X7 is the main player in inflammation and immunomodulation; a strong expression of this receptor has been shown in several human solid tumors. Polymorphisms of the gene encoding for $P2X_7$ have been described; among these, 1513A>C induces loss-of-function while 489 C>T gain-of-function of the receptor.

We evaluated the presence of 1513A>C and 489C>T polymorphisms in patients with papillary thyroid carcinoma (PTC).

$P2X_7R$ genotypic analysis was performed in 83 patients with PTC (70 women; mean age 43±13 yrs; 29 with diameter <1 cm; 33 with follicular and 50 with classical variant) and 100 healthy subjects (Bone Marrow Bank donors, Ferrara). The single nucleotide polymorphisms were analyzed in genomic DNA samples by the TaqMan MGB probe technique. Results are summarized in the table.

Table 1

Polymorphism 1513A>C	Minor Allele Frequency	Genotype (%) A/A	A/C	C/C	p
Controls	0.2	62	36	2	
Patients	0.3	48	43	9	0.0004

Increased homozygous substitution 1513A>C was detected only in patients with the follicular variant (22%). A significant correlation with PTC dimension was also observed ($P=0.02$). No differences were detected in the allelic frequencies for 489C>T.

Overall, our data demonstrate an increased prevalence of 1513A>C polymorphism in patients with PTC. This loss-of-function polymorphism characterized the follicular variant and correlated with cancer dimension. Further studies are needed to evaluate the role of 1513A>C polymorphism as a novel clinical marker of differentiated thyroid carcinoma.

P130

Enhanced expression of functional $P2X_7$ receptor in human papillary thyroid cancer

Angela Dardano[1], Davide Ferrari[2], Sabina Cuccato[1], Eleonora Santini[1], Nadia Caraccio[1], Sara Gulinelli[2], Giulia Callegari[2], Pinuccia Faviana[1], Anna Solini[1], Francesco Di Virgilio[2] & Fabio Monzani[1]
[1]University of Pisa, Pisa, Italy; [2]University of Ferrara, Ferrara, Italy.

Extracellular ATP modulates several biological processes via activation of plasma membrane receptors (P2Rs) in normal human thyrocytes (NT). We characterized P2Rs expression and function in two thyroid cancer cell lines: FB1 (anaplastic cancer) and FB2 [papillary cancer (PTC)]. P2Rs expression was evaluated by RT-PCR and WB, intracellular Ca^{2+}changes by fluorimetric technique (Fura2-AM), IL-6 release by ELISA, intracellular [*i*(ATP)] and extracellular ATP [*e*(ATP)] concentration by luminometry.

FB1 and FB2 showed significantly higher *e*(ATP) and *i*(ATP) concentration than NT ($P<0.001$ for both). [Ca^{2+}] fluxes induced by *e*(ATP) (1 mM, in the presence of external Ca^{2+}) were higher in both FB1 and FB2 than NT cells, ($P<0.01$). Moreover, the addition of ATP (0.25 and 1 mM) induced a significantly higher IL-6 release respect to NT ($P<0.001$at both ATP concentrations) in both cell lines. The $P2X_7$ agonist BzATP, almost ineffective in NT, induced a huge IL-6 release in FB2 (from 6315 ± 328 to 11764 ± 1652 and to 25661 ± 2815 pg/ml/1.5x10^5 cells with BzATP 0.25 and 1 mM, respectively) and FB1, although at a lesser extent (from 7388 ± 170 to 8721 ± 1332 and to 10620 ± 2216, respectively). Moreover, IL-6 release was prevented either by oxidized-ATP or KN-62, selective blockers of human $P2X_7$. Accordingly, FB2 cells showed a strong expression of $P2X_7$, less evident in FB1 cells. These findings demonstrated an enhanced expression of functional $P2X_7$ receptors in thyroid cancer cell lines. Therefore, we checked $P2X_7$ expression in 33 human PTC histological samples, confirming an increased $P2X_7$ expression in cancer than in normal thyroid tissue both by RT-PCR ($P<0.0001$) and immunostaining (avidin-biotin method) ($72\pm15\%$ *Vs* $8\pm3\%$ of cells, respectively).

In conclusion, human thyroid cancer is characterized by an enhanced P2XRs function; specifically, PTC shows a strong $P2X_7$ expression in comparison to normal thyroid tissue. The increased $P2X_7$R function may play a role in the modulation of the inflammatory response to neoplasia.

P131

Results 90Y-DOTATATE therapy in patients with neuroendocrine tumours (NETs) - own experience

Jolanta Kunikowska[1], Alicja Hubalewska-Dydejczyk[2], Anna Sowa-Staszczak[2], Leszek Krolicki[1], Pawel Ochman[1], Renata Mikolajczak[3], Dariusz Pawlak[3], Malgorzata Kobylecka[1], Joanna Maczewska[1] & Bohdan Huszno[2]
[1]Nuclear Medicine Department, Medical Academy, Warsaw, Poland; [2]Department of Endocrinology, Medical College at Jagiellonian University, Cracow, Poland; [3]Radioisotope Centre POLATOM, Otwock-Swierk, Poland.

In the 1980 s the discovery of expression of somatostatin receptors on NET cells made the use of somatostatin analogues in diagnosis and therapy possible.
The aim
Of the study was to assess response of targeted radio-nuclide therapy with radio-labelled somatostatin analogue ^{90}Y-[DOTA^0,D-Phe^1,Tyr^3]-octreotate (DOTATATE) in treatment of disseminated NETs.
Material and methods
12 patients (aged 56.7+/−11.2): carcinoid-5 pts, insulinoma-1pt, gastrinoma-2 pts, pancreatic NET-2 pts, ca neauroendocrinale without primary tumor-1, stomach NET-1 pt) were enrolled in the study. Before the therapy, blood tests for hematology, kidney and liver function and CgA were performed. All patients underwent CT scans and ^{99m}Tc-HYNIC/EDDA-octreotate SRS. Treatment with ^{90}Y-DOTATATE) was repeated every 4–6 weeks up to the total of 200 mCi/m^2. Amino acids infusion was used for kidney protection.
Results
One year observation: regression of disease (PR -decrease of size and number of metastases, ↓CgA level, good clinical response) was observed in 6 pts, stable disease (SD-stable size and number of metastases, ↓CgA) in 3 pts. 3 patients died. No nephrotoxicity was observed. WBC and PLT levels were stable during therapy in 3 pts (without chemotherapy). In 1 pt with previous chemotherapy (last course a month before radiotherapy), PLT level decreased (220×10^3/mm^3 ® 47×10^3/mm^{3}after the first course); the patient died 2 months after the beginning of the therapy. In 8 pts leucopoenia was observed ($<4\times10^3$/mm^3) but serious neutropenia ($<2\times10^3$/mm^3) was found in 3 pts with previous chemotherapy. Thrombocytopenia (PLT $<100\times10^3$/mm^3) was observed in 2 patients with previous chemotherapy.
Two-year observation: prolonged PR – 4 pts; SD – 3 pts, progression of disease in 2 pts: with gastrinoma and stomach NET without hormonal activity (4 and 9 months after radiotherapy). Blood tests stable.
Conclusion
PR and SD were observed in 9/12 patients with disseminated NET. Severe haematologic toxicity was mainly observed in patients after prior chemotherapy –the question of optimising the time between chemotherapy and radiotherapy is still open.

P132

Results of treatment of patients with pituitary somatotroph adenomas

Andrey Grigoriev, Natalye Molitvoslovova, Galina Kolesnikova & Anatolij Kuzmin
Endocrinology Scientific Centre, Moscow, Russia.

In ESC for the period since October 2004 till October 2006 were operated 69 patients with acromegaly. Men were 22 (32%), women – 47 (68%). Age of patients changed from 24 till 68 (middle – 47).

All patients were separated into 2 groups: surgery (group 1) and combination treatment (group 2), which consist of surgery and somatostatin analogues therapy before and after surgery.

In most cases were macroadenomas, only 5 patients (7%) had microadenomas. Suprasellar invasion had 21 patients (30%), infrasellar – 28 (41%) and 32% patients had invasion to one or both cavernous sinuses.

50 patients operated by transnasal approach and 19 with endoscopic techniques. In 47 cases (69%) tumor was total removal, in 17 – subtotal (not less 90% tumor mass was removal), and in 5 cases (7%) – partial removal.
Results
Significant clinical improvement is seen in most patients – 66 (97%). Reduce diabetes mellitus we observed at 43% patients (6 from 14), visual improvement had 78% patients (14 from 18).
Nobody had CSF leak after operation. Diabetes insipidus had 6 patients (9%). Pulmonary embolus had 3 patients (1 patient died).
After 6–12 months were examination 14 patients from group 1 and group 2. GR was normalized in 79% of patients of each group. IGF-1 was normalized in 75% of each group. And postglucose GH level was normalized in 46% into group 1 and 58% into group 2.
Conclusion
Transsphenoidal surgery for acromegaly is safe and effective treatment with minimal mortality and morbidity.
Obvious distinctions in postoperative dynamics IGF-1 and postglucose GH in both groups it is not revealed. There is a tendency in greater efficiency of the combined treatment.

P133

Adrenal incidentaloma, an oncological or endocrinological enigma? Clinical analysis of 1300 cases observed at a single endocrinological centre

Anna Kasperlik-Zaluska[1], Elzbieta Roslonowska[1], Jadwiga Slowinska-Srzednicka[1], Wojciech Zgliczynski[1], Wojciech Jeske[1], Lucyna Papierska[1], Maciej Otto[2], Rafal Slapa[2], Andrzej Cichocki[3] & Jaroslaw Cwikla[4]
[1]Centre for Postgraduate Medical Education, Warsaw, Poland; [2]Warsaw University of Medicine, Warsaw, Poland; [3]Centre and Institute of Oncology, Warsaw, Poland; [4]Hospital of MSWiA, Warsaw, Poland.

Objective
Incidentally found adrenal tumour (adrenal incidentaloma = AI) is the most frequent adrenal disorder. Every patient with AI has to be evaluated carefully to choose the best method of management. We present our experience with a group of 1300 patients with AI, registered at our department.
Material and methods
Material consisted of 1300 patients (female/male ratio 2.6, age 10–87 years) with AI ranging in size from 0.8 to 23.0 cm. Methods: clinical examination, biochemical assays, hormonal determinations (cortisol, androgens, ACTH, aldosterone, metanephrines), imaging studies (ultrasound scans, CT, MRI), histological/immunocytochemical investigations in 420 patients treated by surgery.
Results
Basing on these examinations we diagnosed in our material 116 patients with adrenal cancer, 14 – with other primary malignant adrenal tumours, 48 – with metastatic tumours and 1122 with probably benign tumours. The most important criteria for surgery were imaging phenotype (mainly high density, over 20 HU in the I phase of CT), size (≧5 cm) rapid growth of the tumour and suspicion of a clinically silent chromaffin tumour (for fear of an unexpected metanephrines crisis). In some cases of adrenal cancer elevated levels of androgens have been noted. The most frequent form of subclinical hyperactivity has been pre-Cushing's syndrome (6.5%).
Conclusions
1/Malignant adrenal tumours were found in 178 patients (14%), in this number adrenal cancer in 9%. 2/ The oncological criteria for surgery were of primary importance in our material, with the elevated density in CT (I phase) as the main single indication.

P134

Frequency and type of adrenal tumors in our patients

Milica Medic-Stojanoska[1], Branka Kovacev-Zavisic[1], Tijana Radovanov[1], Ivana Bajkin[1], Jovanka Novakovic[1], Milena Mitrovic[1], Dusan Z Tomic[1], Nilkola Curic[2] & Ljiljana Todorovic-Djilas[1]
[1]Clinic for Endocrinology, Institute for Internal Medicine, Clinical Center of Vojvodina, Novi Sad, Vojvodina, Serbia; [2]Department for pathological physiology, Clinical Center of Vojvodina, Novi Sad, Vojvodina, Serbia.

In recent years, adrenal tumors (AT) are no rare disease. They may arise from all zones of adrenal cortex and medulla, benign or malignant, sometimes as metastases of distant malignances. Patient's present hormone excess or mass effect, but part of them is clinically silent. The aim of this study was to investigate the frequency, hormonal secretion and pathohistology of AT in our patients lasting years. All patients with AT which are hospitalized in the period from January 1st, 2000. to October 15th, 2006. in our Clinic are included in study. Data of clinical feature, hormonal secretion, imaging and pathohistology of AT are collected in our hospital register of admitted patients and medical records. Patients with AT are divided according to hormonal secretion and pathohistology per years. Linear trend are calculated.
Results
During this 7 years in our Clinic are admitted 102 patients with AT, 65 (63,72%) females and 35 (36,28%) males. It has been 2,38% of all hospitalized patients. Hormonally inactive are presented 64,71%. Patients with hormonally active AT be demonstrated as Cushing's syndrome (18,63%), Syndrome Conn (8,82%) and pheochromocytoma (3,92%). According to data of histology and immunohistology after surgery, 89,22% be presented as benign and 10,78% as malignant. Only 5,88% of malignant tumors has been metastases of distant tumors. Linear trend is pointed the increase of incidence patients with AT during period of observation.
Conclusion
The incidence of patients with AT have tendency to increase lasting years in our region. Benign and non-functionally AT are the most common.

P135

Papillary thyroid cancer – the possible role of death ligands in tumor immunology

Emese Mezosi[1], Ferenc Gyory[2], Agnes Borbely[2], Bernadett Ujhelyi[2], Gyorgyike Soos[2], Endre V. Nagy[2], Zsuzsa Varga[2] & Laszlo Bajnok[1]
[1]University of Pecs, School of Medicine, Pecs, Hungary; [2]University of Debrecen, Medical and Health Science Center, Debrecen, Hungary.

Papillary thyroid cancer (PTC) cells and immune cells can kill each other by death ligands. Death ligands induce apoptosis only in sensitive cells. The sensitivity to apoptosis is regulated in a complex and poorly understood manner. The aim of this study was to investigate the Fas ligand (FasL) and Tumor Necrosis Factor-Related Apoptosis Inducing Ligand (TRAIL) expression in PTC cells and tumor infiltrating immune cells. Twenty-six PTCs without and fifteen PTCs with cervical lymph node metastasis were examined by immunohistochemistry. Lymphocytic and macrophage infiltration, HLA-DR, FasL and TRAIL expressions were investigated. The intensity of positive staining was evaluated by a semiquantitative score system. Macrophages and lymphocytes infiltrated the majority of tumor samples. FasL expression of cancer cells was universal and did not show any correlation with the intensity of lymphocytic infiltration and lymph node metastasis. A small subgroup of lymphocytes in close proximity to tumor cells was strongly positive for FasL. Lymphocytes did not express TRAIL. TRAIL expression of tumor cells was increased in PTCs with lymph node metastasis ($P=0.01$). Macrophages were negative for death ligands. In summary, increased TRAIL expression of tumor cells may inhibit the anti-tumor immunity and promote the formation of lymph node metastasis. A subgroup of lymphocytes can use FasL for tumor cell killing.

This work was supported by a grant from the Hungarian Medical Research Council (ETT 186/2003).

P136

Leptin and adiponectin interact in regulating prostate cancer cell growth

Tina Mistry[1], Janet Digby[1], Ken Desai[2] & Harpal Randeva[1]
[1]University of Warwick Medical School, Coventry, United Kingdom; [2]University Hospital Coventry and Warwickshire, Coventry, United Kingdom.

Introduction
Leptin and adiponectin have opposing properties and are implicated as molecular mediators between obesity and (aggressive) prostate cancer. Adiponectin, circulates inversely proportional to visceral fat accumulation, and has demonstrated anti-proliferative effects in prostate cancer cells; circulating leptin levels increase with visceral fat accumulation and has shown mitogenic effects. We propose that adiponectin and leptin interact in prostate cancer cell growth regulation.
Materials and Methods
We studied the effect of full-length (fAd) and globular (gAd) adiponectin (0.01 nM–100 nM) $\pm$ 100 nM leptin on LNCaP and PC3 prostate cancer cell proliferation. *p53* tumour suppressor and *bcl-2* oncogene expression was measured using quantitative RT-PCR.
Results
LNCaP: co-incubation of fAd with leptin resulted in decreased cell proliferation; fAd alone had little effect. gAd alone slightly increased proliferation and had little effect when co-incubated with leptin. fAd alone increased *p53* mRNA expression and rescued leptin-induced inhibition of *p53* expression; both fAd and gAd alone increased *bcl-2* expression, but reduced expression to below basal when co-incubated with leptin. PC3: fAd decreased proliferation at 100nM, but reduced proliferation to half of basal when co-incubated with leptin; gAd alone increased proliferation but reduced proliferation to basal when co-incubated with leptin. Both fAd and gAd demonstrated significant dose-dependent increases in *p53* mRNA expression when co-incubated with leptin; both fAd and gAd reduced *bcl-2* expression to negligible levels despite the addition of leptin.
Conclusion
We show an interaction between adiponectin and leptin in the regulation of prostate cancer cell proliferation through modulation of *p53* and *bcl-2* expression; this is most marked in the advanced PC3 cell line. Concurrent hyperleptinaemia and hypoadiponectinaemia in obese patients may modulate prostate cancer progression, and serum leptin:adiponectin ratio could represent a new prognostic marker; increasing circulating fAd in these patients may be a novel treatment for this disease.

P137

A novel role for Visfatin/Pre-B cell colony-enhancing factor 1 (PBEF) /Nicotinamide phosphoribosyltransferase (NMPRTase) in prostate carcinogenesis

Tina Mistry[1], Janet Digby[1], Joe Cross[1], Ken Desai[2] & Harpal Randeva[1]
[1]University of Warwick Medical School, Coventry, United Kingdom; [2]University Hospital Coventry and Warwickshire, Coventry, United Kingdom.

Introduction
Visfatin/PBEF is a novel adipokine circulating inversely proportional to visceral fat mass and exerts insulin-mimetic effects; it is expressed in normal, inflamed and tumour tissues. Visfatin/PBEF has also been identified as NMPRTase, a key intracellular enzyme involved in NAD^+ metabolism, replenishing NAD^+ during cellular respiration. Inhibition of NMPRTase by the anti-cancer agent FK866 has been shown to induce apoptosis in tumours. Prostate cancer progression is associated with obesity and its metabolic sequelae, and we propose a role for visfatin/PBEF/NMPRTase in prostate carcinogenesis.
Materials and Methods
Visfatin expression was studied in normal and malignant prostate cancer tissue and LNCaP and PC3 human prostate cancer cell lines using RT-PCR, immunocytochemistry and confocal analysis. Regulation of visfatin expression by testosterone, 5-alpha dihydrotestosterone (DHT) (10^{-6}M) interleukin-6 (30 ng/ml) and insulin-like growth factor-1 (IGF-1) (10 ng/ml) was studied using quantitative RT-PCR and Western blotting. We also investigated the effect of visfatin $\pm$ IGF-1 on LNCaP and PC3 cell proliferation.
Results
Visfatin mRNA and protein were detected in LNCaP and PC3 cells and normal and malignant prostate cancer tissue; visfatin protein demonstrated cytoplasmic and nuclear distribution. Testosterone, DHT and IGF-1 increased visfatin mRNA and/or protein expression in both the androgen-sensitive LNCaP and androgen-insensitive PC3 cell line. Treatment of PC3 cells with visfatin resulted in a dose-dependent increase in PC3 cell proliferation which was enhanced in the presence of IGF-1; co-incubation of visfatin and IGF-1 showed a synergistic dose-dependent increase cell proliferation in LNCaP cells.
Conclusions
Our novel findings demonstrate a multifunctional (intra- and extra-cellular) role for visfatin in prostate carcinogenesis, and provide greater insight into the molecular association between obesity and prostate cancer. High visfatin expression in prostate cancer cells may indicate poor prognosis, and inhibition of visfatin may represent a novel therapeutic target for treatment of this disease.

P138

Initial presentation of patients with acromegaly - analysis of the German acromegaly register

Stephan Petersenn[1], Martin Reincke[2], Michael Buchfelder[3], Holger Franz[4] & Hans-Jürgen Quabbe[5]
[1]Division of Endocrinology, University of Duisburg-Essen, Essen, Germany; [2]Klinikum Innenstadt, University of Munich, Munich, Germany; [3]Department of Neurosurgery, University of Erlangen-Nuremberg, Erlangen, Germany; [4]Lohmann & Birkner Health Care Consulting GmbH, Berlin, Germany; [5]Prof. emer., Free University Berlin, Berlin, Germany.

Due to its rarity, initial endocrine abnormalities in acromegaly are difficult to investigate in a large cohort, especially with respect to cofounding variables. We searched the German Acromegaly Register for data on the first presentation of patients with acromegaly.

Up to November 2005, 1485 patients with acromegaly had been entered into the database. Male patients demonstrated significantly higher random GH (21.0 (0.2–620.0) ng/ml, median (range)) and IGF-1 (773.0 (118–2000) ng/ml) levels than females with 14.0 (0.06–556.0) ng/ml ($P<0.005$) and 679.0 (136–2103) ng/ml ($P<0.0001$). Furthermore, comparison of biochemical parameters for various age decades demonstrated a significant association between increasing age and decreasing random GH and IGF-1 levels. Gonadal insufficiency occurred in 18.8%, secondary adrenal insufficiency in 11.8%, TSH deficiency in 7.5%, and diabetes insipidus in 1.3% of subjects. Pituitary insufficiencies occurred with higher frequency in male patients (39.1% vs. 22.0%, $P<0.0001$), and in a significantly higher percentage of patients with macro- (31.6%) compared to microadenomas (18.1%, $P<0.005$). During initial biochemical analysis, 6.4%, 1.5%, and 3.7% of subjects revealed non-pathological results for random GH (<2.5 ng/ml), minimal GH during oGTT (<1 ng/ml), and IGF-1, respectively. None had normal, and 91,4% had pathological results for all three parameters. Whereas the combination of GH during oGTT and IGF-1 raised suspicion of acromegaly in all subjects, 0.5% and 1.1% of subjects demonstrated normal values with combinations of random GH and IGF-1, or random and glucose suppressed GH, respectively.

In conclusion, biochemical activity of acromegaly may depend on age and sex. Therefore, therapy may need to consider and being adapted according to these parameters. Patients with acromegaly may need to be evaluated for pituitary insufficiencies, even with microadenomas. The combination of glucose-suppressed GH and IGF-1 may be the best screening parameters for acromegaly.

Endocrine tumors and neoplasia – presented on Tuesday

P139

Survivin – a promising target for immunotherapy in patients with adrenocortical carcinoma

Martin Fassnacht[1], Sebastian Wortmann[1], Silviu Sbiera[1], Dorothee Kuehner[1], Marion Wobser[2], Patrick Adam[3], Juergen C. Becker[2] & Bruno Allolio[1]
[1]University of Wuerzburg, Dept. of Medicine I, Wuerzburg, Germany; [2]University of Wuerzburg, Dept. of Dermatology, Wuerzburg, Germany; [3]University of Wuerzburg, Dept. of Pathology, Wuerzburg, Germany.

Objectives
Adrenocortical carcinoma (ACC) is a rare tumor with poor prognosis and limited therapeutic options. Survivin is an anti-apoptotic molecule expressed by neoplastic and tumor-specific endothelial cells of various carcinomas, but rarely or only weakly in normal differentiated tissue. In melanoma and pancreatic cancer, preliminary results of a survivin vaccination trial (www.clinicaltrials.gov) indicated that an immunological response in patients is often paralleled by tumor control. Hence, we investigated, whether survivin may also be a reasonable target for an immunotherapy in ACC.
Methods
We performed survivin real-time-PCR in 14 ACCs and 13 normal adrenals. In addition, survivin protein was analysed by immunohistochemistry in 78 ACC samples and 5 normal adrenals using a tissue array (scoring of expression: 0–3). Finally, the presence of spontaneous survivin-recognizing T-cells in the peripheral blood of 7 ACC patients were investigated by indirect interferon-gamma-ELISPOT using HLA-A1, -A2 or -B35 restricted survivin peptides.
Results
Survivin RNA was detectable in 11/12 ACCs and 8/13 normal adrenals. However, the mean expression in ACC was an order of magnitude higher than in normal adrenals ($9071\pm5561\%$ vs. $100\pm25\%$, $P<0.001$). Immunohistochemistry confirmed survivin protein expression in 89% of ACCs. Moreover, in 38/78 of the ACCs but in none of the normal adrenals the expression was judged as moderate-to-high (score 2 or 3). Notably, in 1/7 ACC patients spontaneous HLA-A2-restricted survivin-specific T cells response was detected suggesting that the used epitope might be of immunotherapeutic value.
Conclusion
This is the first study addressing survivin expression in a large series of ACC patients. Since antiapoptotic survivin is overexpressed in many ACCs and exhibits immunogenic properties, it is an intriguing target for immunotherapy also in this rare disease. Especially in patients with refractory ACC having progressed after several cytotoxic therapies an experimental vaccination approach seems to be justified and promising.

P140

Thyroid cancer: with an unexpected location – in the pancreas and in an unexpected combination with Boeck's sarcoidosis

Zsuzsanna Valkusz[1], Éva Csajbók[1], János Gardi[1], Lilla Kardos[2], László Tiszlavicz[3] & László Pávics[4]
[1]Endocrin Unit, University of Szeged, Szeged, Hungary; [2]International Medical Center, Szeged, Hungary; [3]Dept. of Pathology, University of Szeged, Szeged, Hungary; [4]Dept. of Nuclear Medicine, University of Szeged, Szeged, Hungary.

The incidence of differentiated thyroid cancer gradually increased in the last few decades. Primery thyroid cancer is usually located in the thyroid gland and can be classified into well differentiated and poorly differentiated forms. Sooner or later, these cancers metastatize into local lymphnodes or distant organs.

We present the histories of two patients with anusual forms of thyroid cancer.

A woman of 64 was admitted in our department in 2004, due to an inoperable tumor in the pancreas. Histological sampling revealed a well differentiated ectopic follicular thyroid cancer. After total thyroidectomy (no malignancy in the thyroid), 131-I scintigraphy showed isotope accumulation int he pancreas.

Repeated high-dose 131-I therapy shrank the size of the pancreatic tumor and markedly decreased the thyroglobulin level in the serum. One year after these interventions, the patient feels well, has no further distant metastases and is treated for insulin-dependent diabetes mellitus; TSH is sctrictly suppressed by thyroxine medication.

A man 28 was admitted in our department for severe dyspnea in 2004. The computed tomography of the chest detected disseminated patches in the lung with enlarged lymphnodes both int he mediastinum and ont he neck. Total thyroid surgery plus modified cervical and mediastinal lymphnode dissection showed a papillary type thyroid cancer metastatizing into the lung and combined with Boeck's sarcoidosis. Postoperative thyroglobulin level was foud extremely high and 131-I scintigraphy showed pulmonary accumulation. Repeated radioiodine treatment resulted in decreasing thyreogobulin level and strongly improved picture of the chest by computed tomography. The patient is under TSH suppressing therapy.

P141

Thyroglobulin-antibodies in the "normal" range may decrease the diagnostic accuracy of thyroglobulin in the care of patients with differentiated thyroid cancer

Locsei Zoltán[1], Toldy Erzsébet[1], Szabolcs István[2] & Kovács L. Gábor[3]
[1]Markusovszky Hospital, szombathely, Hungary; [2]National Medical Centre, Budapest, Hungary; [3]University of Pécs, Pécs, Hungary.

Issue
The use of thyroglobulin (Tg) as tumor marker in differentiated thyroid cancer (DTC) is limited in the presence of thyroglobulin-antibodies (TgAb) but it is generally believed that this is true only for TgAb concentrations over the normal 'cut off' point.
The aim
Of this study was to investigate if TgAb-s in the normal range, considered to be physiological, may also influence the accuracy and clinical relevance of Tg measurement.
Methods
Recombinant human TgAb (Roche) was added stepwise to serum-samples ($n=45$) with TgAb concentrations near to the analytical sensitivity of the method (10 IU/ml), aiming to have TgAb concentrations of 50–100–150 and 200 IU/ml (ECLIA Elecsys 2010 Roche, normal 'cut off' <115 IU/ml). After this, Tg levels were measured at all TgAb concentrations by electrochemiluminescence immunoassay (ECLMA, Elecsys 2010, Roche). Additionally, 134 samples from 27 patients with DTC were measured for Tg, Tg-recovery (Tg%) and TgAb.
Results
In the *in vitro* experiment, TgAb and Tg concentrations showed strong correlation ($r=0.93$, $P<0.01$) both at normal and elevated TgAb levels, which could be described mathematically as: Loss of $Tg=-0.43\,Ln(TgAb\ IU/ml)+1{,}06$. Patients with non-detectable Tg had higher antibody levels than those with detectable Tg. There was a rather weak negative correlation ($r=-0{,}32$ $P<0.001$) of Tg% to TgAb and in 19% of the samples the results were clinically discordant. In 2/27 patients, on-T4 Tg levels of <2.0 ng/ml were corrected to be >2,0 ng/ml by using the above function. Subsequent off-T4 Tg levels appeared to be significantly elevated in both.
Conclusion
Physiological (normal) TgAb concentrations may also decrease serum Tg but their effect can be calculated from the actual Tg and TgAb concentrations by the

mathematical model described. The findings stress the importance of parallel Tg and TgAb measurements in patients with DTC expected to have undetectable or low Tg.

P142

Dopamine receptor expression and dopamine agonist effectiveness in post-surgical persistent medullary thyroid cancer

Rosario Pivonello[1], Piero Ferolla[2], Antongiulio Faggiano[1], Diego Ferone[3], Enrica Cerasola[3], Gabriella Angeletti[2], Fausto Santeusanio[2], Francesco Minuto[3], Gaetano Lombardi[1], Steven WJ Lamberts[4], Leo J Hofland[4] & Annamaria Colao[1]
[1]Department of Molecular and Clinical Endocrinology and Oncology, "Federico II" University, Naples, Italy; [2]Department of Internal Medicine and Endocrinological Sciences, University of Perugia, Perugia, Italy; [3]Department of Endocrinological and Metabolic Sciences, University of Genova, Genova, Italy; [4]Department of Internal Medicine, Erasmus Medical Center, Rotterdam, Netherlands.

Dopamine receptors was suggested to be expressed in medullary thyroid cancer (MTC). The aim of the current study was to evaluate the expression of D_2 dopamine receptor in MTC and the effectiveness of the dopamine agonist cabergoline in patients with MTC. Five paraffin-embedded cases of MTC obtained after thyroidectomy were used to evaluate D_2 receptor expression by immunohistochemistry. Fifteen patients (7 males, 8 females, 36–78 years) with post-surgical persistent and not operable MTC were treated with cabergoline for 4 months, in order to evaluate its effect on clinical syndrome, serum calcitonin (CT) and CEA levels, and metastasis number and size. Cabergoline was administered at the dose of 1 mg/week for the first month and 3.5 mg/week for the following 3 months. D_2 receptor was variably expressed in all 5 cases of MTC. Before treatment, all patients had progressively increasing serum CT and/or CEA levels. Lymph node metastasis were visible in 4, whereas liver and lung metastasis were identified in 1 and 2 patients, respectively. At the 4-month follow-up, a significant decrease of serum CT ($P=0.027$) but not CEA ($P=0.244$) levels was found. A $>50\%$ decrease in serum CT levels was found in 3 (20%), a 25–50% decrease was found in 10 (66.7%) and an increase in serum CT levels was found in 2 (13.3%) patients. A significant improvement in flushing ($P=0.039$) and fatigue ($P=0.023$) and a slight improvement in diarrhoea ($P=0.066$) score was also found. No significant change was found in body weight. No significant change was observed in metastasis number and size, although one patient experienced a disease progression. In conclusion, the results of this study demonstrated that D_2 receptor is expressed in MTC and that cabergoline treatment improve clinical syndrome and decrease serum CT levels in patients with post-surgical persistent MTC. Further studies on a larger number of patients and longer period of treatment are mandatory to draw definitive conclusions on the usefulness of cabergoline treatment in patients with MTC.

P143

Somatostatin analogues and the PI3K-AKT-MTOR-P70S6K pathway: how do they control the proliferation of neuroendocrine tumours?

Giulia Franchi, Simona Grozinsky-Glasberg, Antonio Ribeiro de Oliveira, Nabila Salahuddin, Márta Korbonits & Ashley B. Grossman
Department of Endocrinology, William Harvey Research Institute, Barts and the London, Queen Mary School of Medicine, University of London, EC1M 6BQ, UK, London, United Kingdom.

Background
Somatostatin analogues are very useful in the treatment of symptomatic neuroendocrine tumours, but effects on proliferation remain unclear. Over-expression of the proto-oncogene protein kinase Akt has been demonstrated in certain endocrine tumours, and activates downstream proteins including mTOR and p70S6K, which play a significant role in cell growth and proliferation. We have therefore explored the site of action of somatostatin in causing inhibition of proliferation in a neuroendocrine cell line.
Aims
To confirm the anti-proliferative effects of SS analogue treatment in a rat insulinoma cell line (INS-1), and to investigate whether the SS analogues act on the PI3K-Akt-p70S6K pathway.
Methods
RT-PCR was used to demonstrate SS receptors (SSTR) in the INS-1 cell lines. MTS and thymidine incorporation were used to determine the effects of the SS analogues octreotide (SSTR2 agonist) and pasireotide (SOM230, Novartis; activation of SSTR-1, 2, 3 and 5) on cell proliferation. Western blotting was used to characterise phosphorylated-Akt and p70S6K expression in the SS-treated cells.
Results
The INS-1 cells expressed SSTR 1, 2, 3 and 5. Treatment with octreotide and pasireotide caused significant dose-responsive inhibition of proliferation. No difference in phospho-Akt (either Ser473 or Ser308) expression was detected in the octreotide-treated INS-1 cell lysates. However, phospho-p70S6K (Thr389) expression was significantly reduced at 10 minutes-6 hours treatment with octreotide 10^{-9}M ($P=0.01$), while no effect on phospho-p70S6K (Thr229) expression was observed at 30 and 60 minutes. It is known that Thr229 site of phosphorylation is affected by PDK1 upstream of Akt. Treatment with IGF-1 (10nM) increased both phospho-p70S6K (Thr389) and phospho-Akt expression.
Conclusions
Octreotide and pasireotide treatment inhibited proliferation of INS-1 cells and, at a concentration achieved in clinical human use, octreotide attenuated p70S6K (Thr389) phosphorylation, but not Akt phosphorylation. We conclude that SS analogues acts downstream of Akt to inhibit the mTOR-p70S6K pathway.

P144

Angiotensin 4–8 and angiotensin 5–8 inhibit cell proliferation in GH3 rat pituitary lactosomatotroph tumor cell culture

Dorota Ptasinska-Wnuk, Jolanta Kunert-Radek, Hanna Lawnicka & Marek Pawlikowski
Medical University of Lodz, Lodz, Poland.

Introduction
In many tissues angiotensin peptides acts as the auto/paracrine growth factors. Their effects are dependent on activation of various intracellular signaling pathways, including mitogen-activated protein kinases (MAPK).

Angiotensin II (ang II) is the best known angiotensin peptide. The ang II derivatives, angiotensin III (ang III) and angiotensin IV (ang IV) posses biological activity as well. Both ang II and ang IV are known to promote the proliferation of rat prolactinoma cells *in vitro* and rat anterior pituitary cells *in vivo*. The role of ang IV degradation products, angiotensin 4–8 (ang 4–8) and angiotensin 5–8 (ang 5–8) in the regulation of cellular growth has not already been investigated.
Aim
In our study we examined the influence of ang 4–8 and ang 5–8 on the GH3 cells (rat pituitary lactosomatotroph tumor cells line) proliferation and the possible role of two MAPK pathways (p44/42 and p38) in ang 5–8 regulatory action.
Material and Methods
GH3 cells were cultured in F-10 medium and then plated at 96-multiwell plates (10×10^3 cells/well). After 12 hours of preincubation cells underwent to 72-hours treatment either with ang 4–8 or ang 5–8 alone or with the combination of ang 5–8 and p44/42 MAPK-kinase or p38 MAPK inhibitor (PD98059 or SB203580 respectively). Cell proliferation was evaluated using two colorimetric assays: based on the measurement of cell activation and on the BrdU incorporation during DNA synthesis.
Results
Ang 4–8 and ang 5–8 decreased both the cell activation and BrdU incorporation in GH3 cells culture. SB203580 prevented only the ang 5–8-induced inhibition of cells activation. Non of ang 5–8 effects was abolished by PD98059.
Conclution
Ang 4–8 and ang 5–8 inhibit GH3 cell proliferation. This mechanism is independent of both MAPK p44/42 and MAPK p38. They probably exert additional proapoptotic effect, mediated by MAPK p38.

P145

Epidermal growth factor receptor (EGFR) as a potential new target in the treatment of patients with adrenocortical carcinoma – results of pre-clinical studies

Martin Fassnacht[1], Stefanie Hahner[1], Barbara Heinrich[1], Patrick Adam[2], Sarah Johanssen[1], Marcus Quinkler[3], Ann-Cathrin Koschker[1], Philipp Stroebel[4], Alexander Marx[4] & Bruno Allolio[1]
[1]University of Wuerzburg, Dept. of Internal Medicine I, Wuerzburg, Germany; [2]University of Wuerzburg, Dept. of Pathology, Wuerzburg, Germany; [3]Charite University, Dept. of Medicine, Campus Mitte, Berlin, Germany; [4]University of Heidelberg, University Hospital Mannheim, Dept. of Pathology, Mannheim, Germany.

Objectives
Adrenocortical carcinoma (ACC) is a rare malignancy with incompletely understood pathogenesis and poor prognosis. Overexpression of epidermal growth factor receptor (EGFR) has been demonstrated in several tumors and is partly associated with a more aggressive phenotype and a worse prognosis. In addition, targeting the EGFR tyrosine kinase represents a successful new therapeutic strategy, e.g. in non-small cell lung cancer. Therefore, we investigated the role of EGFR in ACC as a potential therapeutic target.
Methods
EGFR expression was analyzed by immunohistochemistry in 95 ACCs and 5 normal adrenals using paraffin sections and tissue arrays (scoring of expression: 0–3). Utilizing the clinical data from the German ACC registry, Kaplan Meier survival analyses were performed. In 30 patients the tumor DNA was sequenced for mutations of the 'hot spot' exons 19–21 of the EGFR gene. In addition, cells of the ACC cell line NCI-h295 were incubated with the EGFR antibody cetuximab (1–100 μg/ml) and cell proliferation was measured by MTT tests.
Results
Immunohistochemistry revealed EGFR expression in 78% of ACCs. In 55/95 (58%) of the ACCs and 0/5 of the normal adrenals the expression level was judged as moderate-to-high (score 2 or 3). However, the expression level did not correlate with the clinical outcome in these patients. In addition, none of the sequenced tumor DNA samples showed a mutation in exon 19–21. Cetuximab exhibited a dose dependent antiproliferative effect in NCI-H295 cells (cell viability: 1 μg/ml: 95$\pm$2%; 10 μg/ml 90$\pm$3%*; 100 μg/ml 85$\pm$4%* vs untreated control cells: 100$\pm$3%; *=$P<0.01$).
Conclusion
EGFR is overexpressed in the majority of ACC. Moreover, *in vitro* experiments demonstrated that inhibition of EGFR signalling lead to moderate growth inhibition in ACC cells. Therefore, in patients with ACC refractory to established cytotoxic therapies the experimental use of EGFR inhibitors (combined with cytotoxic therapy) seems to be justified.

P146

Time necessary to achieve the maximum effect of goserelin, LH-RH agonist, in therapy of hormone dependent breast cancer

Jure Murgic, Martina Matovinovic, Lucija Fabijanic, Darko Katalinic & Milan Vrkljan
University hospital Sestre milosrdnice, Zagreb, Croatia.

Nearly one third of women diagnosed with invasive breast cancer are younger than 50 years with regular menstrual cycles. 60% of these tumors express estrogene and progesterone receptors. Common treatment procedure is surgery followed by chemotherapy, radiotherapy and hormone therapy. Often in younger patients chemotherapy causes permanent amenorrhoea. In case that menses afterwards occurs ovarial suppression is needed, mostly by goserelin, LH-RH agonist. The principle of therapy is to cause inhibition of LH and FSH pituitary secretion (medicamental ovariectomy). Sometimes in premenopausal women ovarian suppression is added to standard chemohormonal therapy. In this review two high-risk node positive premenopause breast cancer patients are presented, diagnosed at the age of 38 and 28. Both had hormone receptors positive tumor and underwent breast surgery followed by FEC regimen chemotherapy and radiotherapy. Chemotherapy caused them temporary amenorrhoea, but soon after radiotherapy and tamoxifen introduction regular menstrual cycle began. Due to high-risk node positive cancer combined therapy with tamoxifen 20 mg daily and goserelin 3,6 mg s.c. monthly was introduced. The first patient needed a three months goserelin application to obtain amenorrhoea but the other patient needed only one. After six months of goserelin plus tamoxifen therapy gynaecological and endocrinological evaluation was preformed. In both patients LH value was lower than 1.0 IJ/L but in the first one FSH, estradiole and progesterone values were within menopausal ranges with ultrasound proof of ovarial and endometrial inactivity. In the other patient FSH, estradiole and progesterone values were within fertile range, with present ovary follicles, although ammenorrhoic. This review refered that numerous individual factors influence the effect of adiuvant LH-RH agonist therapy in high-risk breast cancer patient and that different time period is needed to obtain its maximum effect.

P147

Acromegaly due to a lung carcinoid: a case report

Rezzoug malika & Chentli Farida
Bab El Oued Hospital, Algiers, Algeria.

Acromegaly secondary to a lung carcinoïd is a very rare entity. Secretion of GHRH (Growth hormone releasing hormone) or GHRH like by neuroendocrine tumor induces pituitary hyperplasia and a production of Growth hormone (GH) with or without others anterior pituitary hormones. Total resection of lung tumor induces normalisation of pituitary function as in our observation.

AD, 37 years, male, came to our unit for diabetes mellitus and acromegaly. His chest X ray showed a 7 cm right lung tumor. On hormonal exploration there was a very high GH =92 to 132 ng/ml ($N<5$), high prolactine (PRL)=120 ng/ml ($N<20$), elevated ACTH=70 pg/ml ($N=0$–46)) and cortisol =262 ng/ml ($N=50$–210) without clinical signs of Cushing's syndrome. Thyreotrop function was preserved but there was a gonadotrop deficit: testosterone =0,91 ng/ml ($N=3$–5). On MRI there was a huge pituitary process impading the third ventricle and a destroyed sella turcica.

GHRH and 5 HIA (5 hydroxyindolacetic acid) were not evaluated. Surgical exploration and pathology study showed typical picture of carcinoïd in the right lung. On post operative period there was a dramatic fall of GH (=1,2 ng/ml). PRL, ACTH and cortisol normalized and diabetes mellitus disappeared. Three month after surgery MRI showed a significant reduction of pituitary process with partial empty sella.
Conclusion
In this observation even if evaluation GHRH assay and immunohistochimy of the tumor was not available, clinical, biological and radiological evaluation confirmed that all endocrine abnormalities observed in our patient were due to lung carcinoïd.

P148

Intra- and supra-sellar immature teratoma mimicking pediatric craniopharyngioma

Chentli Farida
Bab El Oued Hospital, Algiers, Algeria.

Immature teratoma arising from the brain is very rare. The intra and supra sellar localization is very exceptional. Its clinical symptoms and radiological aspects on TDM are similar to those of craniopharyngiomas but on MRI the fat signal characterize teratomas, but only the histological exam gives the confirmation of this last lesion and makes differential diagnosis with mature tumor whose prognosis is better than the immature one. Our observation illustrates all these problems.
Observation
LM, 6 years, female, is referred to our unit for craniopharyngioma. She complaints of headaches, vomiting and a decrease in visual acuity. On clinical exam we noted a blindness, diabetes insipidus and a statural deficit with hypothyroidism wich are confirmed by hormonal results. On TDM there is huge (60$\times$32 mm) solid intra and supra sellar tumor with cysts and calcifications wich arrives to the third ventricule but on MRI there is a fat signal evocating a teratoma. histological exam of this very hemorrhage tumor argue for an immature teratoma.
Conclusion
This observation prouves that clinical and TDM aspects of craniopharyngiomas are similar with those of teratomas. Only the fat signal on the MRI argue for the teratoma. Histological exam is the only one wich makes the proof and the differential diagnosis between craniopharyngioma and mature or immature teratoma. The last one has the worst prognosis.

P149

Pituitary microprocess

Chentli Farida
–Bab El Oued Hospital, Algiers, Algeria.

Nowadays neuro-radiological explorations are so frequent that radiologists discover more and more pituitary lesions. In this work we would like to study pituitary microlesions (PML: inferior or equal to 10 mm) in order to analyze age and sex repartition, clinical symptoms leading to the diagnosis, position in the pituitary area and the apparent etiologies.

All our patients are examined and hormonal exploration is as complete as possible.

Results

Among 85 subjects with PML proved by TDM and or MRI, there are 79 women and 6 men (sex ratio = 13/1). Age at diagnosis = 30.8 years (14–73), most of them are between 21 and 30 years old. The complaints are: Gonadal dysfunction = 72%, galactorrhea = 10.5%, headaches = 5.8%, metabolic abnormalities = 6.7% and visual troubles = 4.3%. The diagnosis is really fortuitous in 2 subjects = 2.3%. For the apparent etiology there are 58 prolactinomas, 12 ACTH (19.2%), 10 non functioning (11.8%) and 5 somatotrop adenomas = 5.7%. The average size = 6.45 mm (3–10). 58% are in right pituitary area, 23%, in the left and 13% in the middle.

Conclusion

In our population the diagnosis of pituitary microlesions is rarely fortuitous. Gonadal abnormalities are the most complaints. This may be explained by the high frequency of female cases and secreting tumors. The diagnosis is relatively late (mean size = 6.5 mm). PML are frequently located in the right area. ACTH PML are the smallest and the GH one are the biggest

P150

Adrenal incidentalomas and insulin sensitivity – are there any differences between adenomas and hyperplasia?

Daniela Dudasova, Ivica Lazurova, Hedviga Wagnerova & Ingrid Dravecka
–Faculty of Medicine, P. J. Safarik University, Kosice, Slovakia.

It is well known that adrenal masses, particularly adenomas are frequently related to metabolic syndrome and insulin resistance. However, there are no reported data about the differences between adenomas and hyperplasia.

Authors examined the prevalence of symptoms of the metabolic syndrome and insulin resistance in 25 patients with adrenal incidentalomas (10 men, 15 women) of the mean age 57.9 + 15 years. 15 patients had adrenal adenoma determined by CT or MR scan and 10 had unilateral or bilateral hyperplasia. The prevalence of obesity was 72%, arterial hypertension 60%, diabetes mellitus or impaired glucose tolerance 28%, hyperlipidemia 56% and hyperuricemia 20%, respectively, which is more frequent occurrence than that in normal human population. Patients with adrenal adenomas had mildly but significantly higher body mass index (BMI, $P < 0.05$) and insulin resistance calculated as HOMA IR ($P < 0.05$) and FIRI ($P < 0.05$) and significantly higher values of serum ferritin ($P < 0.01$). Plasma cortisol values were slightly but not significantly higher in the group with adrenal adenomas.

Authors conclude that adrenal adenomas are probably more related to the metabolic syndrome than adrenal hyperplasia.

P151

Frequency of occurence of MEN1 syndrome in patients admitted with primary hyperthyroidism

Agata Baldys-Waligorska, Grzegorz Sokolowski, Malgorzata Trofimiuk, Filip Golkowski & Bohdan Huszno
Department of Endocrinology, Collegium Medicum of the Jagiellonian University, Krakow, Poland.

Background

Primary hyperparathyroidism (HPT) is the most common endocrinopathy in MEN1 and usually its first clinical manifestation. Yet MEN1 is a rare disease, representing only 2–4% of all cases of HTP. We studied the frequency of MEN1 syndrome in HPT patients admitted to our Department.

Methods

In a retrospective analysis of 84 suspected HPT patients hospitalized in 1999–2006, case reports of 11 patients with suspected MEN1 were analysed. MEN1 was stated if two of the three main MEN1-related endocrine tumours occurred.

Results

HPT diagnosis was confirmed in 69 patients: of mean age 55.4 ± 14.1 yrs. Median values of PTH and total calcium concentration were 57.4 pg/ml (min – 60.6, max – 1580) and 2.95 mmol/l (min – 2,2, max – 4,0), respectively. In parathyroid scintigraphy equivocal tracer accumulation was found in 72% of cases. MEN1 was diagnosed in 9 patients of mean age 51.3 ± 12,0 yrs, in 8 of whom (89.0%) HPT was confirmed. Pituitary adenoma was found in 7 patients: 3 prolactinomas, 1 acromegaly, 1 Cushing disease and 2 non-functioning tumours. In 2 patients pancreatic tumours were diagnosed: somatostatinoma and gastrinoma were confirmed by laboratory tests and immunohistochemistry. Four carcinoids: 3 gastric and one bronchial were found. Mean 5-HIAA (5-hydroxyindoloacetic acid) urine excretion in the carcinoid patients was 144.0 μmol/24hrs (norm: up to 40), mean serum concentration of CgA (chromogranin-A) 728.7 U/L (norm: up to 18.0). Moreover, in the patient with HPT and somatostatinoma concurrent von Recklinghausen's disease was diagnosed and in the HPT and prolactinoma patient, meningioma was found. Adrenal tumours were observed in two cases: one pheochromocytoma and one non-functioning tumour.

Conclusions

The frequency of MEN1 occurrence in our patients (13%) is much higher than that quoted in the literature (2–4%), clearly, due to referral of complicated cases to our Department. Patients with symptoms atypical for HPT should be screened towards MEN1.

P152

Evaluation of the efficacy of sandostatin LAR in the treatment of acromegaly

Agata Baldys-Waligorska, Anna Krzentowska, Filip Golkowski & Bohdan Huszno
Department of Endocrinology, Collegium Medicum of the Jagiellonian University, Krakow, Poland.

Background

Somatostatin analogues are used to treat acromegaly patients who, following surgery, have not fulfilled cure criteria (hGH < 2,5 ng/ml, IGF-1 below normal range for age and post-OGTT hGH < 1,0 ng/ml). We evaluated the efficacy of Sandostatin LAR in managing such patients.

Material and method

In our Clinic, 81 acromegaly patients (mean age 51.6 ± 14.4 yrs) were registered over the years 1983-2005. Based on CT i MRI, macroadenoma and microadenoma were stated in 63% and 37% of these patients, respectively. 70 patients (86.5%) underwent surgery, 6 (7.4%) refused surgery and 5 (6.1%) underwent radiotherapy., Independently of time after surgery, 60 patients underwent diagnostic tests to qualify them for Sandostatin LAR treatment. Treatment efficacy was based on measuring concentration of hGH i IGF-1 3, 6, 9 and 12 months, and performing control MRI 6 and 12 months after the beginning of Sandostatin LAR treatment (20 mg/month, increased to 30 mg/month if unsatisfactory).

Results

Criteria of post-surgery cure were not fulfilled by 40 patients (66.6% of the 60 evaluated). Due to poor tolerance, one patient was treated with Pegvisomant. 19 patients (31.6%) required no further treatment. After 6 months of treatment, hGH < 2.5 ng/ml was stated in 63%, and IGF-1 below normal ranges for age in 58.8% of patients, and after 12 months – in 68.4% and 36.8% of patients, respectively. In control MRI, recurrence, correlated with enhanced concentration of IGF-1, was stated in 7 patients (17,5%).

Conclusions

In terms of hGH and IGF-1 levels, satisfactory acromegaly control was obtained in about 40% of patients treated with Sandostatin LAR. This result may be biased by the high number of macroadenoma, and possible non-radical surgery in our patients. Due to evident disparity between 12-month normalization of hGH and of IGF-1 levels, measurements of IGF-1 concentration are of considerable diagnostic value in assessing the activity of acromegaly.

P153

The beta-HLH transcription factor neurogenin-2 is preferentially expressed by secreting pituitary adenomas

Amato Fratticci[1], Fabio Grieco[1], Cristina Spilioti[1], Felice Giangaspero[2], Vincenzo Esposito[2], Antonio Santoro[3], Luca Ventura[4], Edoardo Alesse[1] & Marie-Lise Jaffrain-Rea[1]
[1]Experimental Medicine, University of L'Aquila, L'Aquila, Italy; [2]Neuromed, IRCCS, Pozzili, Italy; [3]Neurosurgery, University "La Sapienza", Rome, Italy; [4]Pathology, S. Salvatore Hospital, L'Aquila, Italy.

Beta-HLH transcription factors are involved in the ontogenesis of neural/neuroendocrine cells, and may play a role in the pathogenesis of neuroendocrine tumours. Neurogenin 2 (Ngn2) is expressed by the developing mouse pituitary. After preliminary data indicating its expression in the normal human pituitary, we have studied its phenotypic expression in normal and adenomatous pituitary tissues.
Methods
Fifty-two pituitary adenomas (PA) – 23 clinically non-secreting (CNS) and 29 clinically secreting (CS) (13 GH-, 8 PRL-, 6 ACTH- and 2 TSH-secreting PA, respectively) - and 4 normal pituitaries (NP) were studied. Ngn2 transcripts were determined by realtime qRT-PCR and compared to beta-actin transcripts, using Taqman on-demand assays (Applied Biosystems). Immunohistochemistry was performed on 21 PA and 2 NP, using a rabbit polyclonal antibody (Chemicon). Mouse monoclonal antibodies for pituitary hormones (Dako) were used for co-localization experiments.
Results
Ngn2 transcripts were observed in all NP and 39/52 (75%) of PA, with a higher frequency in CS versus CNS PA (89.6% vs 56.5%, $\chi 2 = 7.51$, $P = 0.006$). Accordingly, Ngn2 levels were higher in CS than in CNS PA ($P = 0.006$, Mann-Whitney). Only a subset of PA ($11/52 = 21.1\%$) were found to moderately overexpress Ngn2 as compared to NP: 8 were CS and 3 were CNS, including 2 silent-secreting PA. Nuclear immunopositivity for Ngn2 was detected in scattered cells of the NP, co-localizing with most pituitary hormones, and in 17/21 PA (14/15 CNS and 3/6 CNS, respectively). No significant correlation was found between Ngn2 expression and tumour volume, invasiveness or Ki-67 labelling index.
Conclusions
Ngn2 is expressed by the NP and a significant subset of PA. Its preferential expression by CS PA, the lack of significant overexpression or correlation with tumour aggressiveness, suggest that Ngn2 may contribute to maintain a differentiated secreting phenotype in PA but plays no role in pituitary tumorigenesis itself.

P154

TGFß1 signalling in human insulinomas compared with human islets.

Anastasiya Nabokikh[1], Martin Bilban[2], Wolfgang Gartner[1], Greisa Vila[1], Bruno Niederle[3], Jens Nielsen[4], Christoph Zielinski[5], Anton Luger[1] & Ludwig Wagner[1]
[1]Medical University of Vienna, Department of Medicine III, Vienna, Austria; [2]Medical University of Vienna, Department of Medical and Chemical Laboratory diagnostics, Vienna, Austria; [3]Medical University of Vienna, Department of Endocrine Surgery, Vienna, Austria; [4]University of Copenhagen, Department of Medical Biochemistry and Genetics, Copenhagen, Denmark; [5]Medical University of Vienna, Department of Medicine I, Vienna, Austria.

Insulinomas are thought to be the result of reduced ß-cell death and hyperproliferation of this specific and highly differentiated cell type. Specific growth- factors are responsible for inducing ß- cell replication and might therefore be involved in insulinoma formation. Pluripotent islet progenitor cells are thought to be located at pancreatic ducts, which can give rise to novel islets as well as exocrine pancreas formation. TGFß1 signalling disruption has been shown to result in premalignant ductal lesions in mouse models as well as in humans.

The **specific objective** of this study was to evaluate the gene expression profile of human insulinoma tumors compared with human islets. The gene expression profile of three human insulinomas originating from different individuals was compared to one islet donor. The comparative Affimetrix gene chip analysis of 8000 spotted genes revealed 1102 upregulated ($>$ 1.5x) and 210 downregulated (>1.5x) genes. The results revealed significant differences in the expression of members of the TGFß signalling pathway. Insulinomas contained reduced TGFß1 and TGFß-induced proteins, but overexpressed TGFß receptors. These data were confirmed by quantitative real-time PCR expanding the numbers of insulinomas to 7 and islets-donors to 3. Our results suggest a novel important function of TGFß in development of human insulinomas and cell growth regulation at the islet of Langerhans. Furthermore they are in accordance with earlier data on the exocrine counterpart, where impairment of TGFß signalling is documented in ductal progenitor cells and premalignant ductal lesions leading to pancreatic adenocarcinomas. Apparently, in the presence of aberrant TGFß signalling, these unique pluripotent progenitor cells might be able to give rise to both endocrine and exocrine neoplasias.

P155

The effect of SOM230 on cell proliferation and cortisol secretion in the human adrenal carcinoma cell line H295R

Barbara Mariniello, Sara Cervato, Giuseppe Opocher, Nora Albiger, Gianluca Occhi, Carla Scaroni & Franco Mantero
Division of Endocrinology, Department of Medical and Surgical Sciences, University of Padova, Padova, Italy.

Adrenocortical carcinoma (AC) is a rare neoplasm with poor prognosis. Medical treatment of AC is actually based on the use of op'DDD (mitotane) with or without traditional chemotherapeutic agents. Only very few information are available about the effectiveness of somatostatin analogs in AC. In human adrenal gland the expression of all five somatostatin receptor (SSTR) subtypes was previously demonstrated. A differential expression was shown in adrenal adenomas and carcinomas.

SOM230 is a new somatostatin analog able to interact with SSTR type 5. The effect of SOM230 on cell proliferation and hormone secretion was demonstrated in corticotroph pituitary adenomas primary cultures, but no data are available on adrenal gland.

The aim of the present study was to evaluate the effect of SOM230 on H295R, a human cell line derived from adrenal carcinoma. Cell proliferation was assessed by MTT-assay, whereas cortisol secretion was determined, with and without forskolin stimulation, using a competitive chemiluminescence immunoassay. Moreover, SSTR expression profile study was performed by RT-PCR.

SSTR 3, 4 and 5 were expressed in H295R cells, whereas no expression of SSTR1 and 2 was shown instead. The effect of SOM230 on H295R was determined in a 5 days treatment. A slight decrease of cell proliferation (11.4%) was observed after 72 h of treatment with a high dose of SOM230 (10^{-5}M). At the same high dose (10^{-5}M) SOM230 significantly ($P < 0.05$) inhibits cortisol secretion already after 24 h. A lower concentration of the drug (10^{-8}M) is effective only after 72 h of treatment.

These preliminary data show that SOM230 seems to have an effect on adrenal cell proliferation only at high dose, while a significant dose dependent effect on suppression on cortisol release was observed at 72 h also at low doses. Further studies are required to determine if SOM230 might be used for treatment of patients with AC.

P156

MEN2B – Two simultaneous cases of a rare syndrome

Ágnes Sallai, Éva Hosszú[1], Péter Gergics[2], Zsolt Tulassay[2], Károly Rácz[2] & György Fekete[1]
[1]Semmelweis University 2nd Dep. of Paediatrics, Budapest, Hungary; [2]Semmelweis University 2nd Dep. of Medicine, Budapest, Hungary.

A 17-year-old boy was referred to our Department. In his medical history Crohn's disease had been supposed because of abdominal pain and distention. He had previously undergone minor surgery as having large tongue with neuromas and hypertrophic gums. Due to his marfanoid appearance, arachnodactyly, massive eyebrows and lips together with his medical history, multiple endocrine neoplasia type 2B (MEN2B) was suspected, which is a very uncommon hereditary disease. It consists of typical dysmorphia, mucosal neuromas, ganglioneuromatosis, medullary thyroid carcinoma (MTC) and phaeocromocytoma, and the prognosis depends on the presence of MTC.

Two weeks later a 10-year-old girl presented with a hard mass at her neck. She had massive lips, neuromas on the tongue and solitary thyroid nodule. Thyroid scan showed a cold nodule in the right lobe, and fine needle aspiration cytology suggested MTC.

Genetic analysis was carried out in both patients and revealed a point mutation at codon 918 (M918T) of the proto-oncogene RET. Adrenomedullary function tests showed normal levels of serum and urinary fractionated catecholamines, however, high levels of plasma calcitonin related to MTC. Imaging studies did not identify metastases. Both patients underwent total thyroidectomy and lymph node dissection. Histological examination verified MTC in the thyroids and in the lymph nodes, too. After the operation the plasma calcitonin level of the girl decreased, but it remained high in the boy, so PET-CT was performed to look for metastases. These were found at his cervical region, therefore a reoperation was made with a more extensive node dissection. Since the operations (2006) both patients have been doing well.

Our conclusion is that whenever the M918T mutation of proto-oncogene RET is found total thyroidectomy should be done right after the diagnosis, or if possible within the first 6 months of life.

P157

Prevalence of autonomous cortisol and aldosterone secretion in patients with a single benign cortical adrenal adenoma after modification of the diagnostic tests

George Piaditis[1], Gregory Kaltsas[1], Theodora Kounadi[1], Athina Markou[1], Nikos Mazarakis[1], Aggeliki Gouli[1], Polyzois Makras[1], Dimitrios Papadogias[1], Kostantina Dimitriou[1], Alexandra Stathopoulou[1], Kostantinos Kossyvakis[1], Ragkou Despoina[1], Vamvakidis Kyriakos[2], George Zografos[2], George Kontogiorgos[3] & George Chrousos[4]
[1]Department of Endocrinology & Diabetes Center, General Hospital of Athens G. Gennimatas, Athens, Greece; [2]3rd Department of General Surgery, General Hospital of Athens G. Gennimatas, Athens, Greece; [3]Department of Pathology, General Hospital of Athens G. Gennimatas, Athens, Greece; [4]First Department of Pediatrics, University of Athens Medical School, Agia Sophia Children's Hospital, Athens, Greece.

Background
The normal cut-offs of screening and diagnostic tests for autonomous aldosterone (AAS) and cortisol (ACS) secretion are poorly defined, mainly due to the presence of adrenal adenomas among those who have served as controls and the stimulating effect of ACTH on aldosterone secretion.
Methods
We investigated cortisol and aldosterone secretion in 151 patients with benign cortical adrenal adenomas (BCAA) and in 119 healthy controls with a normal CT of adrenals. Tests for AAS were performed before and after dexamethasone suppression to eliminate the ACTH effect on aldosterone secretion. Performed tests: 1. ACTH-test (250 μg ACTH 1-24, IV) for cortisol, plasma active renin (PRC), aldosterone (PAC) and PAC/PRC ratios measurements at 0, 30 and 60 min. 2. Classical saline infusion test (SIT, 2 liters NaCl 0.9%/4 h, IV) for PRC, PAC and PAC/PRC ratios measurements, 3. LDDST (0.5 mg DEX/6hX24 h) for ACTH and cortisol measurements. 4. A further saline infusion test (POST-DEX-SIT) 2 h after the LDDST.
Results
Using ROC analysis the POST-LDDST cortisol levels (26.90 nmol/L), as well as the POST-DEX-SIT PAC (53.45 pmols/L) and POST-DEX-PAC/PRC (6.18 pmols/L/mU/L) achieved a 100% sensitivity and specificity. Using these new cut-offs the estimated prevalence of ACS and AAS among the BCAA-patients was 61.58% and 33.74% respectively, whereas simultaneous AAS and ACS was observed in 15.68% of the patients. Both systolic and diastolic blood pressure were significantly correlated with POST-DEX-SIT PAC/PRC ratio ($^{P}<0.003$ and $^{P}<0.002$ respectively) and PAC/PRC ratio at 60 min of ACTH-test ($^{P}<0.0003$ and $^{P}<0.001$ respectively) but not with the basal measurements.
Conclusions
With the newly defined normal cut-offs even mild forms of ACS and AAS were identified. As a consequence the estimated prevalence of ACS and AAS in BCAAs was found much higher than the reported previously, whereas a high prevalence of simultaneous cortisol and aldosterone secretion was identified for first time.

P158

Immunohistochemical detection of angiotensin receptors AT1 and AT2 in adrenal tumors

Marek Pawlikowski, Beata led & Katarzyna Winczyk
Department of Neuroendocrinology, Chair of Endocrinology, Medical University of Lodz, Lodz, Poland.

Immunohistochemical detection of angiotensin receptors AT1 and AT2 in adrenal tumors

Angiotensin II is well known to affect the adrenal cell growth and function. Angiotensin receptors AT1 and AT2 were found to be present in the normal adrenal gland. However, the data on the expression of angiotensin receptors in the adrenal tumors is very scarce.

To overcome this gap, the paraffin sections of the adrenal cortical tumors and of pheochromocytomas from the archival material were immunostained with antibodies raised against AT1 (sc-1173) and AT2 (sc-9040) receptor proteins. In hyperplasia of the adrenal cortex and in benign adrenocortical adenomas, both functioning and non-functioning, the AT1 immunostaining was present mainly in the cell membranes. A positive immunoreaction was also found in a subpopulation of cell nuclei and within the cytoplasm. In the adrenal cancer, as well as in pheochromocytomas neither cell membranes nor cell nuclei were immunostained with anti-AT1 antibody. However, a weak AT1 immunostaining was present within cytoplasm of the tumoral cells. With anti-AT2 antibody, in all tumors investigated, the tumoral cells were immunonegative but moderate to strong AT2 immunostaining was observed in the walls of intratumoral blood vessels and in the interstitial tissue. Our data indicates that the expression of AT1 receptors is altered in adrenal cancer and in pheochromocytomas. The expression of AT2 receptors, in turn, may be connected with the process of tumoral neo-angiogenesis.

P159

Bilateral adrenal incidentalomas: exploration of aberrant responses and comparison with unilateral lesions

Eirini Vicha, Dimitra-Argyro Vassiliadi, Christos Avgoustis, Theodossia Palouka & Stylianos Tsagarakis
Department of Endocrinology, Athens' Polyclinic, Athens, Greece.

Aberrant hormone receptors have been demonstrated in macronodular adrenal hyperplasia or, rarely, unilateral adenomas causing Cushing's syndrome but their prevalence in adrenal incidentalomas (AI) remains uncertain. Therefore we evaluated patients with bilateral AI for evidence of abnormal response to physiological stimuli. We also compared their biochemical characteristics with those of patients with unilateral AI.

Assessment of adrenal function was performed in 93 patients (27 men, 66 women, mean age 59.2+/−12 years) with AI; 27 patients (29%) with bilateral (Group A) and 66 patients with unilateral adenomas (Group B). Non-diabetic patients ($n=68$) underwent a 75g-OGTT. Eighteen patients of Group A were submitted to a meal test and 15 to a posture test. The posture test was positive in 3/15 (20%) patients and the meal test in 1/18 (5.5%). The size of the largest adenoma in Group A was significantly greater compared to Group B (3.1+/−1.1 vs. 2.3+/−1.1, $P=0.01$). No significant difference regarding the mean levels of UFC, ACTH, DHEAS and midnight cortisol existed between the groups. A significantly greater proportion of Group B patients had fully suppressed cortisol levels (<1μg/dl) post-LDDST (37.9% vs. 14.8% for Group A, $P=0.023$). The prevalence of diabetes and hypertension and mean glucose levels during OGTT were similar among groups, but in Group B the HOMA-R was significantly higher (2.74+/−1.3 vs. 1.89+/−0.78 $P=0.037$) and the QUICKI and ISI-composite indices significantly lower (0.33+/−0.03 vs. 0.35+/−0.03, $P=0.046$ and 3.3+/−1.5 vs. 4.7+/−2, $P=0.016$).

In conclusion, evidence for aberrant responses to physiological stimuli, particularly to upright posture, is occasionally found in patients with bilateral AI. Although there are no major biochemical differences between subjects presenting with bilateral or unilateral lesions, bilateral lesions tend to be larger and are more often associated with lack of dexamenthasone suppression whereas unilateral adenomas are more related to increased insulin resistance.

P160

Inhibitory effect of rosiglitazone – PPARgamma receptor ligand on growth of human adrenocortical tumor cells in vitro

Katarzyna Winczyk, Hanna Lawnicka, Julita Fuss-Chmielewska, Krzysztof Kolomecki, Jacek Kuroszczyk & Marek Pawlikowski
Department of Neuroendocrinology, Chair of Endocrinology, Medical University of Lodz, Lodz, Poland.

Introduction
The peroxisome proliferator-activated receptors gamma (PPARγ) are nuclear receptors which are detected in normal and pathological tissues. Our earlier study showed the overexpression of PPARγ in human adrenal tumors and pituitary adenomas in comparison to normal glands. The *in vitro* experiments indicated that ligands of PPARγ inhibit growth of many tumors including pituitary adenomas, thyroid cancers and adrenal carcinomas. However, the data concerning the effects of PPARγ ligands on adrenal tumors is very scarce.
Objective
In the present study, we investigated the action of PPARγ ligands rosiglitazone on growth of human adrenocortical tumors in vitro.
Materials and methods
Ten surgically removed adenomas (five non-functioning adenomas, four aldosterone-secreting tumor and one cortisol-secreting adenoma) were examined. The adrenal tumors cells were exposed in the primary culture to rosiglitazone at the concentration of 10^{-3}, 10^{-4} and 10^{-5} M for 24 hours. To measure cell growth the modified colorimetric Mossman method detecting the viable cells was applied. Moreover, the immunohistochemical evaluation of PPARγ expression in paraffin sections of adrenal tumors was performed. The study protocol was approved by local Ethical Committee of Medical University of Lodz.

Results
We have shown that rosiglitazone significantly inhibited the cell growth in 9 out 10 examined adrenal tumor in a dose-dependent manner. Rosiglitazone was the most effective at concentration of 10^{-3} M. PPARγ receptors were found in all tissue, but the number of cells with positive immunoreaction was the lowest in aldosterone-secreting adenoma, which was insensitive to rosiglitazone.
Conclusions
Our results suggest that rosiglitazone may be useful in the treatment of human adrenocortical adenoma. However, the efficacy of PPARγ ligands requires a confirmation in study performed on the larger group of adrenal tumors.

P161

The modern pre- and intraoperative diagnostic algorithm of pancreatic NET with the use of 99mTc-EDDA/HYNIC-octreate scintigraphy – the impact of SRS on patients' management

Alicja Hubalewska-Dydejczyk[1], Katarzyna Frӧss-Baron[1], Renata Mikolajczak[2], Bogdan Huszno[1], Piotr Szybinski[3], Jan Kulig[3], Aleksandra Januszewska[1], Anna Sowa-Staszczak[1] & Dorota Pach[1]
[1]Chair and Department of Endocrinology, Medical College, Jagiellonian University, Cracow, Poland; [2]Radioisotope Centre POLATOM, Otwock-Swierk, Poland; [3]Gastrointestinal and General Surgery Department, Medical College, Cracow, Poland.

Pancreatic NETs often cause difficulties in imaging diagnostics and optimal diagnostic algorithm is searched for. According to the latest reports MDCT sensitivity amounts 60-90%, MR: 80-90%, SRS: 62-100%, EUS: 70-90%.
Aim
Assessment of the usefulness of $^{99\,m}$Tc-EDDA/HYNIC-octreotae scintigraphy in detection of primary and metastatic tumours of pancreatic NET in comparison to CT, EUS and IOUS and evaluation of the impact of scintigraphic results on clinical management of these patients.
Materials and methods
27 patients (aged 52.0 ± 17.3 y) with suspected or histopathologically confirmed pancreatic NET were qualified for the study. Imaging diagnostics was performed in order to detect the primary lesions, local recurrences an metastases. $^{99\,m}$Tc-EDDA/HYNIC-octreotate SRS, CT, EUS and IOUS were performed. The patients with positive SRS were qualified for RGS.
Results
On the basis of the imaging methods results and histopathologic verification: insulinoma- 8, glucagonoma-6, gastrinoma-5, somatostatinoma-2, NET with ACTH ectopy-2, non-functioning NET- in 4 pts were finally diagnosed. Primary lesions (16) and local recurrences (4) were revealed in 20pts, and metastases in 8pts. Sensitivity of SRS and CT was 85% vs 65% respectively. SRS visualized metastatic lesion in 100%, while CT in 87.5% of pts. IOUS revealed the primary tumours in all cases of insulinoma and gastrinoma (9/9). SRS and EUS detected 5/7 insulinoma and 2/2 gastrinoma (CT: 3 insulinomas, 1gastrinoma). SRS changed the diagnostic approach in 13 pts: 8 were qualified for ^{90}Y-DOTA-TATE therapy and 2pts with negative SRS were referred for chemiotherapy. 2 insulinomas and glucagonoma liver metastases were visualised only in SRS and detected with hand-held gamma-probe intra-operatively.
Conclusions
$^{99\,m}$Tc-EDDA/HYNIC-octreotate SRS is a sensitive method of pancreatic NET detection. It is particularly useful in visualisation of the small tumours of the pancreatic tail and small liver metastases. It has essential impact on patients treatment as it enables tumours'resection with RGS and selects patients for PRRT with ^{90}Y-DOTA-TATE.

P162

Segregation of P25L and S80I mutations of the *vhl* gene in an extended Hungarian family with von Hippel-Lindau syndrome

Attila Patócs[1], Katalin Balogh[1], Miklós Tóth[1], Ferenc Fazakas[2], István Likó[3] & Károly Rácz[1]
[1]2nd Department of Medicine, Semmelweis University, Budapest, Hungary; [2]Department of Clinical Biochemistry and Molecular Pathology, University of Debrecen, Debrecen, Hungary; [3]Gedeon Richter LTD, Budapest, Hungary.

Background
von Hippel-Lindau syndrome (VHL) is a rare autosomal dominant disease caused by alterations of the *vhl* tumor-suppressor gene. Patients with VHL are at risk for development of retinal, central nervous system and spine hemangioblastomas, clear-cell renal cell carcinomas, pheochromocytomas, endolymphatic sac tumors and cysts; and pancreatic islet cell tumors. Based on the presence or absence of pheochromocytoma as a phenotypic marker, VHL can be divided into different subtypes. According to Knudson's two-hit hypothesis, tumor formation in VHL requires inactivation of both copies of the tumor suppressor *vhl* gene. Some specific genotype-phenotype correlations have been recognized, but the majority of families have their own specific genetic alteration.
Objective
To identify the disease-causing *vhl* gene mutation in a large Hungarian VHL kindred and to study the genotype-phenotype correlations.
Patients and methods
32 family members spanning 5 generations were evaluated. Initial screening included medical history, physical examination, abdominal ultrasonography, abdominal and cranial CT or MRI, as well as ophthalmologic examination and laboratory tests. Mutation analysis of the vhl gene was performed in DNA samples obtained from peripheral blood. Written informed consent was obtained from all family members who participated in the study.
Results and conclusions
Two genetic alterations of the *vhl* gene (P25L and S80I), both resulting in an amino acid change were identified. The detailed medical examination confirmed that VHL-specific tumors were associated with the presence of S80I mutation. In three family members this mutation was associated with the presence of pheochromocytoma. To our knowledge, the S80I mutation has not been previously described in VHL patients who had pheochromocytoma. Therefore, this finding represents a novel genotype-phenotype association. The P25L variant was identified in clinically healthy family members, suggesting that this variant represents a sequence polymorphism rather than a real disease-causing mutation.

P163

High prevalence of novel mutations of the MEN1 gene in Hungarian patients with multiple endocrine neoplasia type 1

Katalin Balogh[1], László Hunyady[2], Attila Patócs[1], Peter Gergics[1], Zsuzsa Valkusz[3], Miklós Tóth[1], Ibolya Varga[1], Edit Gláz[1] & Károly Rácz[1]
[1]2nd Department of Medicine, Semmelweis University, Budapest, Hungary; [2]Department of Physiology, Semmelweis University, Budapest, Hungary; [3]Division of Endocrinology, University of Szeged, Szeged, Hungary.

Introduction
Multiple endocrine neoplasia type 1 (MEN 1) may present as a familial or a sporadic disorder with multiple endocrine tumours including parathyroid adenomas or hyperplasias, tumours of endocrine pancreatic and pituitary gland. Familial and sporadic MEN 1-related states which do not fulfill current diagnostic criteria but may be related to MEN 1 syndrome have been also described.
Aims
The aim of this study was to examine the prevalence and spectrum of *MEN1* gene mutations in Hungarian patients with familial and sporadic MEN 1 and in those with an MEN 1-related state.
Methods
We performed mutation analysis using temporal temperature gradient gel electrophoresis (TGGE) and direct sequencing of the entire coding and exon-intron boundaries of the *MEN1* gene. Genomic DNA was obtained from 32 patients (19 index patients with familial or sporadic MEN 1 and 13 index patients with familial or sporadic MEN 1-related state). Family screening was performed in families of patients with identified *MEN1* mutation.
Results
Ten different *MEN 1* gene mutations were identified in 10 index patients, including 5 novel mutations (A91V, G28A and E26X in exon 2, L301R in exon 6, and C354X in exon 8). All but one mutations occurred in index patients with familial or sporadic MEN 1; the prevalence of mutation was considerably higher in index patients with familial MEN 1 (6/6 patients, 100%) than in those with sporadic MEN 1 (3/13 patients, 23%). Of the 13 index patients with MEN 1-related state, only one patient with recurrent isolated primary hyperparathyroidism had *MEN1* gene mutation. Family screening indicated mutations in 6 symptomatic and in one asymptomatic first-degree relative.
Conclusions
These results confirm previous reports on the high prevalence of novel *MEN1* gene mutations among patient with MEN 1, and support the questionable efficacy of mutation screening in patients with sporadic MEN 1-related states.

P164

Analysis of germline mutations in patients with pheochromocytomas and paragangliomas

Aleksandra Krawczyk[1], Kornelia Hasse-Lazar[1], Jacek Ziaja[2], Agnieszka Pawlaczek[1], Jolanta Krajewska[1], Mariola Peczkowska[3], Aleksander Preibisz[3], Agata Kubaszek[3], Andrzej Januszewicz[3] & Barbara Jarzab[1]
[1]Department of Nuclear Medicine and Endocrine Oncology, MSC Memorial Cancer Center and Institute of Oncology, Gliwice, Poland; [2]Department of General, Vascular and Transplant Surgery, Medical Silesian University, Katowice, Poland; [3]Department of Hypertension, National Institute of Cardiology, Warsaw, Poland.

There are two types of neoplasms derived from chromaffine tissue: pheochromocytomas (tumors of adrenal core) and paragangliomas (tumors located extraadrenally). Majority of these tumors are sporadic, although according to literature, when DNA analysis is carried out, hereditary disease can be diagnosed in about 25% of patients: Multiple Endocrine Neoplasia type 2 (MEN2A and MEN2B), von Hippel-Lindau Syndrome (VHL), Pheochromocytoma/Paraganglioma Syndrome (PPS) and neurofibromatosis type 1 (NF-1), caused by DNA germline mutations in *RET* protooncogene and *VHL*, *SDHB*, *SDHD*, *NF-1* genes respectively. The aim of our study is evaluation of the frequency of hereditary chromaffine tissue neoplasms in group of apparently sporadic patients, diagnosed and treated by our cooperation. DNA was isolated from peripheral blood leukocytes. Analysis of *RET*, *SDHB* and *SDHD* was carried out in order to seek for DNA changes. DNA fragments were amplified with the use of the polymerase chain reaction (PCR). Multiplex Single Strand Conformation Polymorphism (MSSCP) analysis was used as the screening method. When a conformation change was observed, it was confirmed by sequence analysis. The whole analysis was completed in 63 patients. Germline mutations were found in 16 patients (25.5%); in the group with pheochromocytomas as the sole manifestation in 14 patients (26.4%). Most frequent germiline mutations in pheochromocytoma patients were mutations of *RET*: codon 634 (9 patients) and codon 791 (5 patients) and in paraganglioma patients – mutation in *SDHD* codon 33.

Conclusions

Our analysis confirms the significant contribution of inherited disease to the occurrence of apparently sporadic pheochromocytomas and paragangliomas.

P165

RET exon 13 germline polymorphism in patients with pheochromocytomas and paragangliomas

Kornelia Hasse-Lazar[1], Agnieszka Pawlaczek[1], Aleksandra Krawczyk[1], Malgorzata Wiench[1], Malgorzata Oczko-Wojciechowska[1], Mariola Peczkowska[2], Aleksander Preibisz[2], Agata Kubaszek[2], Andrzej Januszewicz[2], Jacek Ziaja[3] & Barbara Jarzab[1]
[1]MSC Memorial Cancer Center and Institute of Oncology, Department of Nuclear Medicine and Endocrine Oncology, Gliwice, Poland; [2]Department of Hypertension, National Institute of Cardiology, Warsaw, Poland; [3]Department of General, Vascular and Transplant Surgery, Medical Silesian University, Katowice, Poland.

Germinal mutations in protooncogene RET are associated with the inherited medullary thyroid carcinoma (MTC) which occurs as the sole manifestation of disease (FMTC) or, more frequently, as the part of multiple endocrine neoplasia (MEN2). The contribution of RET polymorphism to the occurrence of apparent sporadic MTCs is controversial. In our previous study we have found out that the frequency of RET 769 CTT>CTG polymorphism in patients with MTCs is not significantly higher when compared to control group.

In the present study we analyzed RET 769 polymorphism in 61 patients with apparent sporadic pheochromocytomas or paragangliomas, in whom known germline RET mutations and SDHB/D mutations were excluded.

DNA was isolated from peripheral blood leukocytes. Polymorphism 769 CTT>CTG was found in 39 patients (59%). Its frequency was 56% in patients with pheochromocytoma and 72.7% in the group of non functional paraganglioma. Simultaneously, its frequency was 23% in patients with true sporadic MTC and 27% in the control group of healthy patients ($P<0.05$).

Conclusions

The protooncogene RET exon 13 polymorphism is associated with the occurrence of apparent sporadic pheochromocytomas and paragangliomas

P166

Cabergoline suppression test in distinguishing the variability of response to dopamine agonists in prolactinomas

Corin BADIU[2], Daniela VOICU[4], Andra Caragheorgheopol[1], Dan Hortopan[1] & Luigi Silvestro[3]
[1]Institute of Endocrinology, Bucharest, Romania; [2]C. Davila University of Medicine and Pharmacy, Bucharest, Romania; [3]Pharmaserv, Bucharest, Romania; [4]Medicover, Bucharest, Romania.

Primary therapy in prolactinomas, the most frequent pituitary adenomas, consists in ergot derivatives dopamine agonists (bromocriptine or cabergoline) which lowers prolactin levels and shrink the tumour. Bromocriptine was the first drug used, but the therapeutic levels are attained after several days/weeks, therefore an acute suppression test is not possible. However, the biological response is variable and 10% of prolactinomas are resistant to medical therapy. In order to evaluate the degree of response to dopamine agonists, we tempted a short (48 h) cabergoline (CAB) suppression test. Twenty-nine patients with hyperprolactinemia, 21 prolactinomas (14 women and 7 men), 2 GH-PRL secreting adenomas (2 women) and 6 idiopathic hyperprolactinemia (5 women, 1 man), received a single cabergoline dose (0.5 mg) and were sampled for PRL at baseline, 12 h, 24 h and 48 h after CAB administration. Simultaneously, CAB levels were determined by mass spectrometry. Subsequently, patients were treated with Cab in doses up to 2 mg/twice a week. The final response to treatment was evaluated after completion of 6 months of therapy. According to this the 21 prolactinomas were divided into 13 sensitive and 8 resistant to dopamine agonists.

Mean PRL levels decreased from 384.37 ng/mL to 101.9 ng/ml at 12 h, 94.7 ng/mL at 24 h and 73.31 ng/ml at 48 h, in the senstitive group, and from 1508.37 ng/mL to 1060.34 ng/ml at 12 h, 755.33 ng/mL at 24 h and 600.84 ng/ml at 48 h, in the resistant group. Average cabergoline levels were similar in both groups. PRL decrease at 48 h as compared to baseline, was at 40% from basal level in resistant and at 20% in responsive cases, $P<0.005$. In acromegalic patients, co-secretion of PRL was suppressed at 65% basal level at 48 h, while in functional hyperprolactinemia, normal values were attained at 48 h. Suppression level was not influenced by the tumour size. In conclusion, cabergoline suppression test could be used as early predictor of PRL suppression and biological response in prolactinomas.

P167

Predictive value of pituitary histology on clinical outcome in acromegaly: a retrospective cohort study

Birgit Steffin[1], Wolfgang Saeger[2], Hans-Jürgen Quabbe[3], Stephan Petersenn[4], Dieter K Ludecke[5], Jürgen Honegger[6], Michael Buchfelder[7] & Martin Reincke[1]
[1]Medizinische Klinik Innenstadt, Klinikum der Universität, München, Germany; [2]Marienkrankenhaus, Hamburg, Germany; [3]Deutsches Akromegalie-Register, Berlin, Germany; [4]Universitätsklinikum, Essen, Germany; [5]Universitätsklinikum Eppendorf, Hamburg, Germany; [6]Universitätsklinikum, Tübingen, Germany; [7]Universität Erlangen-Nürnberg, Erlangen, Germany.

Immunohistochemistry is commonly performed on tumour specimen obtained during transsphenoidal surgery, but its predictive value for clinical outcome is largely unknown. The aim of this study was to compare clinical and biochemical outcome characteristics after surgery with histological tumour properties. This was achieved by matching data from the German Acromegaly Register with those of the Pituitary Tumour Registry of the German pituitary working group. From 285 out of 1543 acromegalic patients of the German Acromegaly Register (145 f,140 m), data on morphological properties analyzed by a single pathologist (W.S) in the department of pathology, Marienkrankenhaus, Hamburg were available. Using immunohisto-chemistry, the density of cytoplasmatic granules, pattern of hormone expression and mitotic activity (Ki67) were analyzed. Tumours were stratified according to growth hormone (GH) and prolactin expression and Ki67 index. Clinical and biochemical parameters predicting disease outcome such as post-surgical GH and IGF-1 were analyzed. Control of acromegaly was defined as random GH <2.5 µg/l and normal IGF-1. Results are presented as range, mean and SEM. Before surgery, GH and IGF-1 concentration did not differ between patients with sparsely ($n=93$) and densely granulated ($n=145$) adenomas. However, after transsphenoidal surgery, patients with densely granulated adenomas had significantly higher GH (0–100, 5.4 ± 1.14 vs 0–57, 2.98 ± 0.917 µg/l, $P=0.03$) and IGF-1 (94–1963.522 $\pm$ 14.81, vs.12–1002.456 $\pm$ 36.38 ng/ml, $P=0.006$) concentrations compared to sparsely granulated adenomas. These patients had a lower rate of biochemical control (31% vs 54%, $P=0.01$). Co-expression of prolactin was found in 14% of adenomas. This was associated with higher postsurgical GH and IGF-1 (GH

10.5 ± 8.31 vs 3.3 ± 1.23μg/l, IGF-1 437 ± 149.01 vs 348 ± 27.3 ng/ml) compared to tumours not expressing prolactin. Ki67 staining (Ki67 index $<1\%$ vs $>1\%$) did not have impact on clinical and biochemical variables (P = n.s.). The granulation density of GH producing adenomas is a useful parameter predicting patient's biochemical outcome in acromegaly.

P168

Somatostatin receptor immunohistochemistry in neuroendocrine tumors: a proposal of scoring system for clinical characterization and therapy selection

Antongiulio Faggiano[2], Marco Volante[1], Maria Pia Brizzi[1], Stefano La Rosa[3], Ida Rapa[1], Anna Ferrero[1], Anna De Chiara[4], Luisella Righi[1], Carlo Capella[3], Gaetano De Rosa[4], Luigi Dogliotti[1], Annamaria Colao[2] & Mauro Papotti[1]
[1]Dept. of Clinical and Biological Sciences, San Luigi Hospital, University of Turin, Orbassano, Turin, Italy; [2]Dept. of Molecular and Clinical Endocrinology and Oncology, "Federico II" University, Naples, Naples, Italy; [3]Section of Anatomic Pathology, Dept. of Human Morphology, University of Insubria and Ospedale di Circolo, Varese, Varese, Italy; [4]Dept. of General Pathology, Medicine, Human Pathology, and Clinical Pathology, "Federico II" University of Naples, Naples, Italy.

Background
Typing somatostatin receptor (SSTR) expression in neuroendocrine tumors (NETs) is of relevance to target an octreotide-based diagnostic approach and treatment. The expanding use of immunohistochemistry to detect SSTR is to date not paralleled by an accurate methodological setting and standardized interpretation of the results.
Objective
A multicentric study was designed to compare SSTR immunohistochemical expression with *in vivo* scintigraphic data and verify its usefulness in the clinical management of NETs.
Design
After methodological setting by testing different SSTR antibodies, 107 cases of NETs with available OctreoScan data and pathological material (both surgical and preoperative) were retrospectively analyzed for SSTR type 2A immunohistochemical expression, and the results combined in a four grade scoring system (0 to 3) and compared with scintigraphic images and, whenever available, with the clinical response to somatostatin analogue treatment.
Results
Restricting "positive cases" to the presence of a membrane pattern of staining (proposed scores 2 and 3), an overall SSTR type 2A immunohistochemistry/OctreoScan agreement of 77% (Chi-square test $P<0.0001$) was reached. Lower concordance ratios were detected in preoperative and metastatic tumor samples, possibly as a consequence of SSTR expression heterogeneity. Pure cytoplasmic staining showed poor correlation with OctreoScan images (54% concordance rate). In a pilot series, SSTR type 2A immunohistochemistry correlated with clinical response in 82% of 22 patients undergone to therapy with somatostatin analogs on the basis of a positive OctreoScan uptake.
Conclusions
A standardized scoring system for SSTR type 2A immunohistochemistry is proposed as a useful and reliable adjunct to OctreoScan in the clinical management of NET patients. A membranous SSTR type 2A staining well predicts clinical response to somatostatin analogue therapy and provides additional information on receptor distribution into a given tumor tissue and among primary and metastatic lesions.

P169

Prevalence of primary aldosteronism among hypertensive patients (preliminary results)

Joanna Matrozova, Sabina Zacharieva, Georgi Kirilov, Mihail Boyanov & Vladimir Hristov
University Hospital of Endocrinology 'Akad.Ivan Penchev', Sofia, Bulgaria.

Until recently primary aldosteronism /PA/ was thought to be rare, accounting for no more than 0.05–2% of the hypertensive patients. Studies published in the last decade demonstrate that primary hyperaldosteronism is a much more common cause of secondary hypertension than was previously thought, accounting for as many as 5% to 25% of hypertensives in some series. For the present, there are no data concerning the prevalence of PA in Bulgaria which determined the realization of the present study. A total of 200 patients/126 females, 74 males/were studied until now, including 160 patients, referred to the Clinical Center of Endocrinology and Gerontology, 20 patients referred to the Endocrinology Clinic, Internal Medicine Department, and 20 out-patients. The screening was effectuated using the aldosterone to renin ratio. Blood samples for aldosterone (pmol/l) and PRA (ng/ml/h) were taken under standardized sampling conditions and after correction of antihypertensive medications. We used 750 pmol/l/ng/ml/h as a cut-off for the ratio aldosterone/renin. The captopril test and the measurement of aldosterone in urine were used for confirmatory testing. The diagnosis of PA was confirmed in 13 cases, which suggests a prevalence of 6.5% among hypertensive patients. Adrenal tomography was performed in all biochemically confirmed cases of PA. The presence of different types of PA was as follows: 7 cases/54%/ of adrenal adenomas and 6 cases /46%/ of idiopathic PA. Among the confirmed cases of PA 1 normokalaemic and 12 hypokalaemic patients were found. Our study confirms the results obtained by other recent investigations for an increased prevalence of PA. In contrast to other studies in our research work the cases of Conn's adenoma are predominant, as well as the hypokalaemic forms of PA.

P170

Leptin modulates the growth of murine Colon 38 cancer and interferes with the cytotoxic effect of fluorouracil *in vitro*

Gabriela Melen-Mucha & Hanna Lawnicka
Department of Immunoendocrinology, Chair of Endocrinology, Medical University of Lodz, Lodz, Poland.

Epidemiological studies underline that obesity represents a significant risk factor for development of several cancer among them colon cancer. Moreover, multiple recent data indicate that some of adipose tissue-derived hormones may influence the growth of malignant cells. Leptin, the product of the *ob* gene, is one of them. However, research is still contradictory regarding the role of leptin in colon cancer.

The aim of our study was to examine the direct effect of leptin at various concentrations (from 10^{-5} to 10^{-12} M) applied alone or jointly with fluorouracil (the classical cytotoxic drug for colon cancer) at two concentrations (0.25 μg/ml and 2.5 μg/ml) on the growth of murine Colon 38 cancer cells *in vitro*.

Colon 38 cancer cells were preincubated in RPMI 1640 medium supplemented with fetal calf serum for 24 hours. Then the cells were cultured for 72 hours in the presence of various concentrations of the examined substances applied either alone or jointly. The growth of Colon 38 cell line was assessed by the colorimetric Mosmann method.

We have found that leptin increased the growth of murine Colon 38 cancer at the concentrations of 10^{-6}, 10^{-7} M and 10^{-10}, 10^{-11}, 10^{-12} M. Its stimulatory effect was rather slight with enhancement of cancer growth by 8% to 15% as compared to controls. Fluorouracil, at both concentrations (0.25 μg/ml and 2.5 μg/ml) inhibited the growth of Colon 38 cancer up to 28% and 40% of controls, respectively. Leptin did not modulate the cytotoxic effect of fluorouracil applied at higher concentration (2.5 μg/ml) but unexpectedly it enhanced at the concentrations of 10^{-9} and 10^{-10} M the cytotoxic effect of fluorouracil given at lower concentration (0.25 μg/ml).

These data indicate that leptin is involved in the regulation of colon cancer growth and it may even enhance the cytotoxic effect of fluorouracil.

Grant No. 502-11-295 from the Medical University of Lodz.

P171

Muscle mitochondrial function is impaired in patients with prior acromegaly

Julia Szendrödi[1], Elisabeth Zwettler[2], Albrecht Ingo Schmid[3], Marek Chmelik[3], Giovanni Pacini[4], Gertrud Kacerovsky[5], Christoph Schnack[6], Guntram Schernthaner[6], Oswald Wagner[7], Klaus Klaushofer[2] & Michael Roden[5]
[1]Karl-Landsteiner Institute for Endocrinology and Metabolism, Vienna, Austria; [2]Dept. Intern. Med. 4 and Ludwig Boltzmann Institute for Osteology, Hanusch Hospital, Vienna, Austria; [3]MR Centre of Excellence, Med. Univ. Vienna, Vienna, Austria; [4]National Research Council, Padova, Italy; [5]Dept. Intern. Med. 1 and Karl-Landsteiner Institute for Endocrinology and Metabolism, Hanusch Hospital, Vienna, Austria; [6]Dept. Intern. Med. I, Rudolfstiftung Hospital, Vienna, Austria; [7]Clinical Institute of Medical and Chemical Laboratory Diagnostics, Medical University Vienna, Vienna, Austria.

Acromegaly is amongst other symptoms associated with myopathy, alterations of energy expenditure and insulin action which are mediated by growth hormone (GH) and insulin-like growth factors (IGFs). It is unclear to which extent these abnormalities remain after treatment. Thus, we examined glucose metabolism, intracellular fat deposition and mitochondrial function in patients with prior acromegaly (AM).

Six AM (4f/2 m, age: 49 ± 10 years, body mass index, BMI: 27 ± 3 kg/m^2) with an at least 7-years history of successful treatment and age-/BMI-matched healthy volunteers (CON: 3f/3 m, 43 ± 12 years, 26 ± 4 kg/m^2) were studied. Insulin sensitivity (OGIS) and first-phase insulin secretion were assessed from the frequently sampled OGTT (insulinogenic index, ISEC). Mitochondrial function was assessed from ATP synthetic flux (fATP) during fasting using ^{31}P magnetic resonance spectroscopy (MRS) of calf muscle. Intracellular lipid contents of tibialis anterior (IMCLt) and soleus muscles (IMCLs) as well as liver (HCL) were measured with ^{1}H MRS. The protocol was approved by the local institutional ethics board.

IGF-1 did not differ between groups (AM: 177 ± 88 ng/ml; CON: 145 ± 51 ng/l). Fasting plasma glucose was ~16% higher in AM (99 ± 8, CON: 85 ± 6 mg/dl, $P <$ 0.05), OGIS was comparable (395 ± 74, CON: 415 ± 14), but ISEC was ~87% lower in AM (0.9 ± 0.9, CON: 6.7 ± 4.3, $P < 0.05$). fATP was ~22% lower in AM (10.1 ± 1.5 vs. 12.9 ± 2.4 mmol.l^{-1}.min^{-1}, $P < 0.05$) and related positively to ISEC ($r =$ 0.687, $P < 0.01$). IMCLt and IMCLs and HCL were not different different between groups. IMCLs related negatively to insulin sensitivity ($r = -0.745$, $P = 0.005$).

Successfully treated acromegaly patients exhibit reduced insulin secretion and muscle ATP synthesis despite normal insulin sensitivity. The impairment of mitochondrial function could be explained by previous long-term GH/IGF exposure and/or chronically increased plasma glucose concentrations resulting from impaired ß cell function.

P172

Diagnosis and treatment of the ACTH-secreting neuroendocrine pancreatic tumors

Gilis-Januszewska Aleksandra, Hubalewska-Dydejczyk Alicja, Trofimiuk Malgorzata, Pach Dorota, Szurkowska Magdalena, Buziak-Bereza Monika, Mazurek Elwira & Huszno Bohdan
Chair and Department of Endocrinology, Collegium Medicum, Jagiellonian University, Krakow, Poland.

Introduction
Neuroendocrine tumors secreting ACTH are a rare cause of Cushing's syndrome. Diagnostic and therapeutical difficiulties might be caused due to different clinical picture of of neuroendocrine tumors.
Patients, diagnostic and therapeutic approach
During 2004–2005 2 female patients 32-years old AL and 67-years old ZS were hospitalized in Endocrinology Department due to severe hypercorticism signs and symptoms. In both patients biochemical and functional tests revealed ACTH-dependent Cushing syndrome due to ectopic secretion of ACTH. In both patients ultrasonography and computed tomography revealed not well defined pancreas region lesions and multiple hepatic metastases. ^{99m}Tc-EDTA/HYNIC-Octreotate scinitigraphy showed the uptake of the tracer in similar locations. Neuroendocrine cells were found in bioptic examination. Due to the dissemination of the disease process and bad clinical condition in both patients no surgical treatment could be performed. Important clinical and biochemical improvement was noted after introduction of aminoglutethymide (AL, ZS) and long acting somatostatin analogue (Sandostatin LAR) (AL).

The palliative chemotherapy with 5-FU was implemented in AL. Both patients were approved for therapy with somatostatin analogue labeled with ^{90}Y (^{90}-Y DOTA-Tate). Patient ZS after three series of ^{90}Y (^{90}-Y DOTA-Tate) was approved to continous somatostatin analoque treatment; patient in relatively good condition remains under Endocrinology Outpatient Department control (actually 12 month after diagnosis). Unfortunately Patient AL before admission to the hospital, suddenly died for massive pulmonary embolism.
Conclusions
^{99m}Tc-EDTA/HYNIC-Octreotate scinitigraphy become an important localising technique in neuroendocrine tumors diagnosis.

Somatostatin analoques and ^{90}Y (^{90}-Y DOTA-Tate) therapy seem to be promising treatment methods in non-operative neuroendocrine tumor cases.

P173

Novel mutations in genes encoding succinate dehydrogenase complex subunits B (SDHB) and von Hippel-Lindau protein (VHL) in patients with nonsyndromic pheochromocytoma

Jovana Vignjevic, Jelena Nikolic, Tatjana Isailovic, Katarina Mirkovic, Milan Petakov, Jadranka Antic, Djuro Macut, Bojana Beleslin-Cokic, Bojana Popovic, Ivana Bozic, Sanja Ognjanovic & Svetozar Damjanovic
Institute of Endocrinology, Diabetes&Metabolic Diseases, Clinical Center of Serbia, Belgrade, Serbia.

Background
Several susceptibility genes have been found to be associated with development of pheochromocytoma (PHEO): RET, VHL, SDHB and SDHD. We investigate the frequency of germ-line mutations in SDHB and VHL genes in patients with apparently sporadic PHEO.
Material and methods
Fifty patients (38 women, mean age 42) with apparently sporadic adrenal and extra-adrenal PHEO were screened. DNA was extracted from whole blood and from paraffin embedded tumors using standard phenol-chloroform method. For detection of SDHB and VHL mutations PCR method followed by direct sequencing gene was used.
Results
In 5/50 (10%) patients, five novel germ-line variants were identified: four heterozygous germ-line mutations (nonsense: W218X; frameshift: c.661delG, p.Asp221ThrfsX27; splicing:c.424-12delTCTT and missense: R116M) of the SDHB gene and one heterozygous germ-line mutation (V84M) of the VHL gene. In the patient with adrenal PHEO and heterozygous germ-line W218X mutation, the same heterozygosity state in the tumor tissue was found. The patient with c.661delG mutation was found to have extra-adrenal retroperitoneal malignant PHEO. Family members were also tested and they are negative for the mutation.The patient with c.424-12delTCTT is 12 years old boy with adrenal PHEO. He inherited the mutation from his father who is clinically asymptomatic for PHEO. The patient with V84M mutation was found to have adrenal PHEO. His family history is negative and he doesn't have any other tumors associated with VHL syndrome.
Conclusion
Patients with SDHB mutations are in an increased risk for the development of extra-adrenal and malignant PHEO. Our patient with extra-adrenal disease needs careful follow-up, since he is in higher risk for the development of metastases or novel adrenal/extra-adrenal PHEO. The patient with VHL mutation (V84M) is apparently classified as 2C. Until now genotipe/phenotipe correlation is not proven. This patient may develop some other tumors than PHEO.

P174

Evaluation of plasma and urinary metanephrines as well as serum chromogranin A for the diagnosis of pheochromocytoma

Nicole Unger[1], Christian Pitt[2], Martin K Walz[2], Klaus Mann[1] & Stephan Petersenn[1]
[1]Division of Endocrinology, Medical Centre, Essen, Germany; [2]Department of Surgery and Centre of Minimally Invasive Surgery, Kliniken Essen-Mitte, Essen, Germany.

Adrenal pheochromocytomas are neoplasms characterized by catecholamine excess. We recently reported on the diagnostic value of plasma metanephrines measured by RIA for the diagnosis of pheochromocytoma. However, RIA may not be used in many laboratories.

This study evaluated plasma and urinary metanephrines determined by a newly available ELISA as well as serum chromogranin A (CgA) for the diagnosis of pheochromocytoma. Spontaneous blood samples and 24h-urine samples were collected in 154 subjects, including 24 histologically proven pheochromocytomas, 17 aldosterone-secreting and 21 cortisol-secreting adrenal adenomas, 30 nonfunctioning adrenal masses, 16 patients with essential hypertension and 42 healthy normotensive volunteers. Plasma and urinary metanephrine (MN) and normetanephrine (NMN) as well as CgA were determined and putative thresholds calculated by ROC analysis.

Plasma NMN showed highest sensitivity (89.5%) and specificity (98.3%) using a threshold of 167 pg/ml, with lower sensitivity (85.7%) and specificity (91.8%) for urinary NMN by a threshold of 318 µg/24 h. Plasma and urinary MN demonstrated a much lower sensitivity (68.4% resp. 71.4%) and specificity (90.0% resp. 77.6%) using a threshold of 26 pg/ml and 90 µg/24 h respectively. Analysis of the combination of plasma metanephrines revealed a sensitivity of 89.5% and a specificity of 90.0%. Considering both urinary parameters demonstrated a slightly higher sensitivity (92.9%) with lower specificity (77.6%). ROC analysis revealed a threshold of 215pg/l for CgA with rather low sensitivity (73.9%) and specificity (74.2%). A weak positive correlation was found between the tumor size of pheochromocytomas and plasma MN ($r = 0.53$, $P <= 0.05$) as well as CgA ($r = 0.60$, $P <= 0.01$).

In conclusion, plasma metanephrines measured by ELISA are convenient and reliable parameters for the diagnosis of pheochromocytoma. In contrast, CgA demonstrated poor sensitivity and specificity.

P175

^{11}C-5-hydroxytryptophan PET scan in diagnosis of ectopic Cushing's syndrome from typical lung carcinoid: a case report

Djuro Macut[1], Svetozar Damjanovic[1], Jelica Bjekic[2], Bojana Popovic[1], Ivana Bozic[1], Milan Petakov[1], Sanja Ognjanovic[1], Tanja Isailovic[1], Miloje Joksomovic[3], Radoslav Jakovic[4], Jelena Mirkovic[1] & Jelena Stojsic[4]
[1]Institute of Endocrinology, Clinical Center of Serbia, Belgrade, Serbia; [2]Department of Endocrinology, CHC B. Kosa, Belgrade, Serbia; [3]Institute of Neurosurgery, Clinical Center of Serbia, Belgrade, Serbia; [4]Department of Thoracic Surgery, Clinical Center of Serbia, Belgrade, Serbia.

A 34-year-old woman was initially presented with clinical signs of Cushing's syndrome (CS). On endocrinological examination, a diagnosis of ACTH dependent CS was established (serum cortisol: 08.00 h: 1245 nmol/l; 24.00 h: 275; plasma ACTH concentration 104 ng/l; inadequate cortisol suppresion on LDDST (787) and suppresion to 318 following HDDST). A magnetic resonance imaging (MRI) confirmed a microadenoma in the left part of the pituitary. Ultrasound examination confirmed hyperplastic adrenals. Hypercortisolism persisted after the transsphenoidal operation of the pituitary adenoma; immunohistochemical staining was positive only on FSH and LH. Subsequently, she developed ankle edema, hypokalemia and hormonal profile suggestive on ectopic CS (plasma ACTH 171.9 and failure to suppress serum cortisol following HDDST) confirmed by CRF and DDAVP test. Neuroendocrine origin of the ectopic ACTH production was further suspected with elevated chromogranin A (489.2 ng/ml). Normal levels of 5-HIAA and PTH were obtained. A genetical analyses excluded mutation in menin. A subsequently repeated CT/MRI scans of neck, thorax, abdomen and pelvis were negative. Scintigraphy with ^{111}In-pentreotide did not show any accumulation of the tracer in the body. Whole-body chateterization and sampling did not reveal an ectopic ACTH source. Positron emission tomography (PET) using ^{11}C-5-hydroxytryptophan (5-HTP) (Uppsala, Sweden) showed increased tracer uptake in the retrocardial region of the left lung. In meantime, octreotide in a dose of 900 μg/day s.c. was applied producing complete normalizatioon of arterial blood pressure, restoration of menstrual cyclicity, and complete normalization of cortisol and ACTH. She was successfully operated 14 months after the onset of first signs of CS with pathological confirmation of 11 mm typical lung carcinoid. We presented an unusual case of ectopic CS produced from the typical lung carcinoid that was detected only by means of 5-HTP PET, and associated with coincidentally diagnosed gonadotroph pituitary adenoma.

P176

Mutational analysis in patients with nonsyndromic MEN1

Katarina Mirkovic, Bojana Beleslin-Cokic, Jovana Vignjevic, Jelena Nikolic, Jadranka Antic, Bojana Popovic, Tatjana Isailovic, Ivana Bozic, Milan Petakov, Djuro Macut, Sanja Ognjanovic & Svetozar Damjanovic
Institute of endocrinology, Clinical Center of Serbia, Belgrade, Serbia.

Background
Mutational screening of the *MEN1* gene has been recommended for patients who fulfill clinical criteria for familial or sporadic MEN1 and those suspicious or atypical of MEN1.
Patients and methods
Eighteen apparently unrelated individuals (6 males; 12 females, age range 16–71) with clinical manifestations of MEN1 were analised. In addition, we evaluated 7 relatives. Genomic DNA from peripheral blood leucocytes was extracted using standard procedures. PCR amplification followed by bidirectional sequencing of the entire coding region and exon-intron boundaries of the *MEN1* gene was used to detect mutations.
Results
In 9/18 (50%) of the index cases we identified 9 independent germline *MEN1* mutations: 3 nonsense (R527X, Y77X, Y341X), 3 frameshift (c.1089delT, c.865del4, c.960delG), 2 missense (H317Y, G225V) and one splice-site mutation (IVS4-1G>A). Three mutations were not previously reported. In addition, we detected 3 benign polymorphisms: S145S, R171Q and D418D. The patient with c.865del4 mutation was presented with insulinoma and primary hyperparathyroidism. This mutation is in exon 4 of the MEN1 gene and is predicted to cause truncation of the protein after 28 amino-acids (p.Asp252AspfsX28). Frameshift - deletion c.960delG is located in exon 6 and creates stop codon after three amino-acids (p. A263GfsX3). Patient in whom we detected this mutation had pituitary tumor and primary hyperparathyroidism. Third novel mutation, G225V, is located in exon 4 of the MEN1 gene. This patient had hyperparathyroidism, carcinoid and adrenal gland tumor. Four out of seven relatives were found to be a mutation carriers. Patient with Y341X mutation is sixteen years old boy with mixed pituitary tumor and he is at high risk for developing other MEN1 manifestations.
Conclusion
Identification of an *MEN1* mutation allows genetic testing for family members who are at risk for developing disease. Only mutation-carriers among family members need careful follow-up for the clinical manifestations of MEN1 syndrome.

P177

Screening for mutations in exon 10, 11, 13 and 14 of the RET protooncogene associated with inherited medullary thiroid carcinoma (MTC) in Serbian population

Jelena Nikolic, Jovana Vignjevic, Katarina Mirkovic, Bojana Beleslin-Cokic, Jadranka Antic, Tatjana Isailovic, Milan Petakov & Svetozar Damjanovic
Institute of endocrinology, Clinical Center of Serbia, Belgrade, Serbia.

Background
Ret protooncogene germ-line mutations are associated with the inherited multiple endocrine neoplasia type 2 syndromes (MEN2a and MEN2b) and also with familial medullary thyroid carcinoma (FMTC). In this study, we report a large scale of mutations in exon 10, 11, 13 and 14 RET protoocogene in patients from Serbia. Our study included patients with MTC.
Methods
Our study included 180 patients. Patients were tested for RET protooncogene mutations in exons 10, 11, 13 and 14 by polymerase chain reaction (PCR) followed by restriction fragment length polymorphism (RFLP) and sequencing analyses. Sequencing analysis was performed on ALFexpress II using Thermo Sequence CY5 Terminator Cycle Sequencing Kit and Applied Biosystem Genetic Analyzer 3130 using Big Dye Sequencing Kit.
Results
In 41/180 (23%) patients 7 different heterozygous germ-line mutations were identified: (C634Y, C634R, C634F, C634W in exon 11; C618Y in exon 10; Y791F in exon 13; and V804M in exon 14). Prophylactic thyroidectomy was performed in 6 C634R germline mutation carriers. Interestingly in one family with Y791F mutation MEN 2a was found while in other three components of brachi-oto-remal syndrome were found without MTC. Two patients with V804M had MTC.
Conclusions
Base on these data in Serbian population we found similar frequencies of inherited medullary thyroid carcinoma as in other European countries.

P178

The use of ^{18}F-FDG PET/CT with or without rhTSH stimulation during follow-up of patients with differentiated thyroid carcinoma

Gelsy Arianna Lupoli[1], Annalisa Panico[1], Sara Colarusso[1], Francesco Fonderico[1], Antonio Gonnella[1], Maria Rita Poggiano[1], Luigi Pucci[1], Addolorata Martinelli[1], Emanuele Nicolai[2], Marco Salvatore[2] & Giovanni Lupoli[1]
[1]Department of Molecular and Clinical Endocrinology and Oncology – University 'Federico', Naples, Italy; [2]Department of Functional and Biomorphological Sciences – University 'Federico II', Naples, Italy.

Background
Fluorine-18 fluorodeoxyglucose positron emission tomography (FDG-PET) is a new method employed in the management of differentiated thyroid cancer (DTC). The integrated FDG-PET plus computed tomography (PET/CT) fusion imaging system seems able to provide some additional advantages over PET alone, mainly related to a better anatomical localisation of the hypermetabolic metastatic lesions. The influence of serum TSH levels on ^{18}F-FDG uptake by recurrences or metastases of DTC has not been clarified yet.
Aim
To evaluate the clinical use of PET/CT during the follow-up of patients with DTC; moreover, to ascertain whether the administration of recombinant human thyrotropin (rhTSH) can increase the sensibility and specificity of PET/CT.
Patients and methods
We selected 12 pts with positive or equivocal thyroglobulin (Tg) levels and negative or equivocal ^{131}I scintigraphy and/or conventional morphological imaging techniques (ultrasound, MRI, etc); they underwent ^{18}F-FDG PET/CT during TSH suppression (<0.05 IU/L) and after rhTSH administration (> 30 IU/L).

Results
For 4 pts both basal and rhTSH-stimulated PET/CT scans were positive: in 3 cases tumour foci were detected (confirmed also by histology in 2 cases) whereas 1 of them was false positive results (due to lymph nodes inflammation). PET/CT was completely negative in 8 pts: 6 results were true negative while 2 were false negative, since scanning following rhTSH identified metastatic lesions.
Therefore, PET/CT was able to identify the metastatic foci very efficiently and to localise previously unknown tumour relapse; moreover, in 2 out of 12 patients, rhTSH administration resulted in detection of new lesions.
Conclusions
Our data confirm that PET/CT is a valuable tool in detecting residual disease in DTC patients and suggest a potential role for rhTSH in enhancing the diagnostic accuracy of this method

P179

Abstract unavailable

Growth and development – presented on Sunday

P180

Lower catch-up growth under rGH therapy at pre-pubertal pituitary dwarves diagnosed at an older age

Dumitru Branisteanu, Corina Galesanu, Cristina Cristea, Eusebie Zbranca, Carmen Vulpoi, Cristina Preda & Voichita Mogos
University of Medicine and Pharmacy 'Gr.T.Popa', Iasi, Romania.

Growth hormone deficiency leads to profoundly decreased growth velocity and, when untreated, to pituitary dwarfism. We evaluated growth evolution for one to four years under rGH therapy (0.07 IU/kg/day, subcutaneously) at seventeen idiopathic pituitary dwarves with isolated GH deficiency, 13 boys and 4 girls, with a wide span of age at therapy onset (between 4 and 24 years old). Diagnosis was set subsequent to at least two negative GH stimulation tests. All patients were pre-pubertal, with a bone age below 13 years (Grunlich and Pyle Atlas) but had normal thyroid and adrenal function. Patients were divided into two subgroups: early-diagnosed patients (12 patients younger than 14 at therapy onset) and late-diagnosed patients (5 patients, diagnosed at a chronological age of over 16 years). Growth velocity was significantly increased in the entire group, from $0.33 +/- 0.07$ cm/month before therapy onset to $0.8 +/- 0.05$ cm/month for the whole follow-up period ($P < 0.0005$). Catch-up growth was maximal during the first year of therapy, with a velocity of $1.04 +/- 0.16$ cm/month, which decreased subsequently. Both mean growth velocities for the whole follow-up period ($0.99 +/- 0.08$ vs $0.5 +/- 0.06$ cm/month) and for the first year of therapy ($1.33 +/- 0.13$ vs $0.61 +/- 0.09$ cm/month) were significantly higher at the early-diagnosed patients ($P < 0.01$), despite present radiographic growth potential. Early therapy onset in isolated GH deficiency is therefore important not only because patients have a smaller height handicap to recuperate in order to enter the normal growth channel, but also – as our data suggest - because growth cartilage seems to loose with age its reaction potential to GH administration in pre-pubertal patients. Our data show, nevertheless, that high-dose rGH therapy is still beneficial in older pre-pubertal GH deficient patients by significantly accelerating growth speed. GH dosage should be diminished to adult substitutive levels and puberty should be triggered therapeutically once growth ceases.

P181

The growth hormone – insulin-like growth factor-I axis in adult thalassemic patients

Leila Danesi[1], Agnese Cattaneo[1], Elena Valassi[1], Massimo Scacchi[1], Emanuela D'Angelo[2], Nadia Mirra[2], Laura Zanaboni[3], Maria Domenica Cappellini[3] & Francesco Cavagnini[3]
[1]Chair of Endocrinology, University of Milan, Ospedale San Luca IRCCS, Istituto Auxologico Italiano, Milan, Italy; [2]2nd Pediatric Clinic, University of Milan, Fondazione Policlinico Mangiagalli Regina Elena, Milan, Italy; [3]Department of Internal Medicine, University of Milan, Milan, Italy.

GH deficiency (GHD) can be recognized in a not negligible proportion of thalassemic children, while data on the prevalence of this disorder in adult patients are lacking. Therefore, we elected to study the GH – IGF-I axis in a large group of adult thalassemic subjects.

Study design
Ninety-four patients (69 with thalassemia major and 25 with thalassemia intermedia on stable transfusional regimen, 39 men and 55 women, aged 31.5 ± 6.8 years, receiving sex steroid replacement when necessary) underwent a GHRH (1 μg/kg as an i.v. bolus) + arginine (0.5 g/kg as a 30 min i.v. infusion) test. Severe GHD was defined by GH peaks lower than 9 μg/l, whereas partial GHD was defined by GH peaks ranging from 9 to 16.5 μg/l. Blood samples for IGF-I, ferritin and pseudocholinesterase measurement were also performed.
Results
Severe GHD was demonstrated in 21/94 patients (22.3%), while 18 additional patients (19.1%) displayed partial GHD. No correlations were found between ferritin levels on one side and GH peaks and IGF-I SDS on the other side. GH peaks were positively correlated with IGF-I SDS ($P < 0.05$), although 1 of the 21 patients with severe GHD showed normal IGF-I SDS values, and 45 of the 55 patients with normal GH reserve displayed low IGF-I SDS. A strong positive correlation ($P < 0.0001$) between IGF-I SDS and pseudocholinesterase was shown.
Conclusions
a) This study has demonstrated a high prevalence of GHD, either partial or severe, in adult thalassemic patients. b) The lack of correlation between ferritin and both GH peaks and IGF-I SDS suggests that mechanisms other than iron overload play a major role in the pathophysiology of somatotropin-somatomedin deficiency in this clinical condition. c) The finding of a positive correlation between IGF-I SDS on one side and GH peaks and pseudocholinesterase values on the other side indicates that liver protidosynthetic activity, in addition to somatotropin secretory status, is a major determinant of IGF-I production in thalassemia.

P182

The role of BMP-3B in the establishment of zona glomerulosa in the adrenal gland

Artem Bakmanidis & Peter King
William Harvey Research Institute, London, United Kingdom.

The adrenal gland is composed of the medulla and the cortex, which 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-β (TGF-β) superfamily. In a microarray analysis of transcripts from the rat adrenal zG and zF, we have discovered that some BMPs are potentially ZG specific and BMP-3B showed exclusive expression in zG by Real-Time PCR and immunohistochemistry. Adrenal H295R cells (human adrenocorticocarcinoma cell line) were used as an in-vitro model to examine the role of BMP-3B further. The cells were differentiated into a zG (by Angiotensin II) and zF (by Forskolin) phenotype in the presence and absence of exogenous BMP-3B protein. BMP-3B was able to drive the differentiation of H295R cells into a more zG phenotype while inhibiting the differentiation into a zF phenotype as judged by the inhibition of CYP11B1 expression and the promotion of CYP1B2 expression respectively. The effect of BMP-3B on differentiation was confirmed by over-expressing BMP-3B in stable cell lines and blocking endogenous BMP-3B by siRNA. These experiments imply a role for BMP-3B in steroidogenesis and by implication in adrenal zonation.

P183

Selenium supply modulates growth spurt of selenoprotein P knockout mice

Kostja Renko, Marten Michaelis, Ulrich Schweizer, Josef Köhrle & Lutz Schomburg
Institut für Experimentelle Endokrinologie, Charité Universitätsmedizin Berlin, Berlin, Germany.

Background
Selenoproteins are playing an important role in body homeostasis and development. They control thyroid hormone metabolism and are of prime importance for intracellular redox regulation and cellular defence. The SePP knockout mouse (SePP-KO) is a model of impaired Se metabolism characterized by a disrupted distribution system for organified Se. One of the major phenotypes of the homozygote SePP-KO mice is a reduced increase in size and weight during the growth spurt that can dose-dependently be rescued by Se supplementation.

Hypothesis
Se has an effect on the growth hormone axis and affects bone metabolism by modifying either growth signal synthesis or the response of target tissues.
Materials and methods
Male and female wild-type, heterozygous and homozygous SePP-KO mice were raised on regular rodent chow. At the age of 35 days, we studied the expression of growth-relevant genes in target tissues by realtime-PCR and Nothern blot analysis. Serum markers like IGF-1 and Leptin were determined by multiplex ELISA technique.
Results
On commercial diets with Se-contents not specified, we identified disarrangements in the IGF- and IGFBP-mRNA expression levels, which appeared inconclusive. On diets with defined Se content, male SePP-KO mice had a body weight of 11.3 g ($\pm$ 0.4 g) at P35 compared to 14.8 g ($\pm$0.6 g) in heterozygous or wild-type mice ($P<$ 0.001). The diets revealed a narrow window between rescue (above 0.24 ppm Se) and lethal progression of the phenotype (below 0.15 ppm). These findings now result in a well-defined model to study the impact of Se on growth and body mass.
Conclusion
Se metabolism, Se status and Se transport have an important impact on growth and body mass. Different SePP expression levels modify growth and development in transgenic SePP-KO mice. Together with specific diets this mouse model offers an ideal way to study the interaction of Se supply and growth hormone axis.

P184

Factors affecting height velocity (HV) during GnRH analog therapy in girls with central precocious puberty (CPP)

Dimitrios Thomas[1], Elpis Vlachopapadopoulou[1], Feneli Karachaliou[1], Eirini Vourliotaki[1], Philippos Kaldrimides[2] & Stefanos Michalakos[1]
[1]Dept. of Growth and Development, Children's Hosp. "P. & A. Kyriakou", Athens, Greece; [2]Dept. of Endocrinology and Metabolism, "Metaxa" Memorial Anticancer Research Hospital, Piraeus, Greece.

Aim
To assess factors affecting HV during triptorelin suppression treatment for CPP.
Materials and methods
Forty-six girls with CPP, with mean age at diagnosis 8.4 yrs who presented with signs and symptoms of puberty before the age of 8 years and were treated with triptorelin for at least 2 years, were studied. All girls were categorized into three groups according to the difference between bone age (BA) and chronological age (ΔBA-CA): group I with ΔBA-CA $<$ 11.99 months, group II with ΔBA-CA between 12 and 23.99 months and group III with ΔBA-CA $>$ 24 months. Furthermore, girls were categorized in two groups: girls with BA before treatment initiation $\leq$10 years and girls with BA $>$10 years. Four groups were formed according to Tanner breast staging: group A,B,C,D with breasts TII, TII-III, TIII and TIII-IV respectively.
Results
A statistically significant difference in mean HV during the 2nd year of treatment was observed between group I (5.99$\pm$2.21), group II (3.87$\pm$1.46) and group III (3.09$\pm$ 1.47) (P=0.012, AN.O.VA). Mean HV during the 2nd year of treatment was statistically higher in girls with BA before treatment $\leq$10 years (5.78$\pm$1.75) compared to girls with BA before treatment $>$10 years (3.17$\pm$1.27) (P=0.0001, t-test). A statistically significant difference in mean HV during the 1st year of treatment was observed between group A (6.32$\pm$0.96), group B (5.56$\pm$0.97), group C (4.96$\pm$1.07), and group D (4.26$\pm$1.66) (P=0.05, Kruskal-Wallis AN.O.VA). HV during the second year of treatment could be statistically predicted using bone age (P=0.002) and weight (P=0.036) before treatment initiation as independent factors in multivariate linear regression model, according to the following equation: $HV_{2nd\ year}$=15,026$-$0,702X(BA)-0,0892X(W).
Conclusions
Bone age, Tanner breast stage and weight seem to be important factors affecting HV during triptorelin therapy for CPP.

P185

Auxological and IGF system parameters in African in comparison with western countries normal children

Mara Boschetti[1], Sabrina Piredda[1], Marilena Sidoti[1], Eliana Di Battista[2], Daniela Larizza[3], Diego Ferone[1], Antonina Barreca[1] & Francesco Minuto[1]
[1]Department of Endocrinology and Metabolism and Center of Excellence for Biomedical Research, University of Genova, Italy; [2]Department of Pediatrics, Ospedale Gaslini, University of Genova, Italy; [3]Department of Pediatric Sciences and IRCCS Policlinico San Matteo, University of Pavia, Italy.

Growth is an integrated process, resulting from the response of cells to nutrient availability and to hormonal status. Nutrients, in turn, are important regulators of IGF-IGFBP system which are critical regulators of growth. Genetic factors seem to be very important determinant of final stature in countries with high quality of life at variance with underprivileged countries where food intake deficiency is critical. The aim of our study was to evaluate the influence of environmental conditions on IGF-I secretion and the role of GH-IGF-I system on the generational trend of stature in a selected population of children living in conditions of low dietary intake. We analyzed the auxological parameters and the circulating levels of the different components of the GH-IGF system in 38 normal African children from Ivorian Coast (NA) and 50 normal age and sex-matched Italian children (NE). The results of this study showed that in Africans the levels of all components of the circulating 150 kDa ternary complex (IGF-I, IGFBP-3, ALS) were significantly lower as compared with Italians ($P<$0.001). However, molar ALS/IGF-I, ALS/IGFBP-3, and IGF-I/IGFBP-3 ratios in African children were comparable with those found in Italians.

Clinical and auxological data of children (Mean$\pm$Standard error)

	Age	Height sds	BMI sds	IGF-I nM/L	IGFBP-3 nM/L	ALS nM/L
NE 28M/ 22F	5.0$\pm$0.3	0.3$\pm$0.1	0.2$\pm$0.2	22.4$\pm$1.6	120.4$\pm$5.5	300.7$\pm$16.4
NA 15M/ 23F	4.0$\pm$0.2	0.2$\pm$0.2	0.0$\pm$0.2	6.7$\pm$0.8	37.7$\pm$3.9	123.3$\pm$12.7
p	0.01	n.s.	n.s.	$<$0.001	$<$0.001	$<$0.001

In conclusion the levels of IGF ternary complex parameters are maintained higher in Italian than in African children by the higher dietary intake but the molar ratios and the stature were similar in both groups. It seems therefore that an optimal concentration of total IGF-I contributes to the improvement of final stature in generational trend.

P186

X-linked neuronal T_3 transport defect: Allan Herndon Dudley syndrome

Tabinda Dugal[1], Assunta Albanese[2] & Muriel Mc Entagart[3]
[1]St Georges Hospital, London, United Kingdom; [2]St Georges Hospital, London, United Kingdom; [3]St Georges Hospital, London, United Kingdom.

Thyroid hormone is absolutely necessary for early brain development.Incidence of thyroid disorders in infancy is 1:4,000.Thyroid hormones can be deficient through hormone synthesis and action or very rarely through defective transport. Some new and exciting transporters for tri-iodothyronine (T_3) have recently come to light.MCT 8 gene encodes the protein that transports T_3 into neurons. Its mutation result in inability of T_3 to enter a developing brain neuron. This leads to peripheral elevation of T_3 and TSH and low levels of T_4. Clinically this causes a spectrum of neurological features known as Allan-Herndon-Dudley syndrome (AHDS).This X-linked mental retardation syndrome was described first in 1944.

We report a case of a male child born in 2002 with intrauterine growth retardation (IUGR).He was diagnosed with cerebral palsy with supportive MRI scan. His hypotonia, poor feeding and delayed milestones were attributed to this,although the phenotypic features of AHDS ie elongated facies, bifrontal narrowing,flat ears were also present.He had severe cognitive impairment and was not walking at 42 months. He continued to be hypotonic with athetoid movements. He was under a paediatric neurologist till his raised T_3 and TSH levels were noted.He was then transferred to endocrinologist. The diagnosis of AHDS was on genetic studies.Thyroxin treatment has normalised his T_4 and TSH.T_3 remains elevated.

Thyroid hormone replacement does not correct any neurological deficit-s.Therefore ante-natal diagnosis is important.This case is unique as the mother was a mosaic carrier with no family history.Several families have been described in literature with affected male relatives. Largest series of 6 (Schwartz *et al.* 2005).It is important to recognise the defect early to plan counselling.Sex selection can also be offered for next pregnancy. Females have 1:2 chance of being a carrier while males have a 1:2 chance of inheriting the defective gene.

P187

Cephalometric analysis and dental maturation in patients with Turner's syndrome (TS)

Rafaella Shimada Gomes, Darwin Luiz Martins Oliveira, João Carlos Ramos-Dias, Alcinda Aranha Nigri, Reinaldo José Gianini, Sérgio Yassuo Nonoyama & Maria Helena Senger
Faculdade de Medicina de Sorocaba - PUC/SP, Sorocaba, São Paulo, Brazil.

Craniofacial proportions of girls with TS, compared to normal children, show reduced size of the craniofacial complex, retrognathic profile and tendency towards advanced dental age. Growth hormone (GH) treatment in TS positively affects stature, but its effects on craniofacial growth and dental development are largely unknown. The aim of this study was to analyze and to correlate the craniofacial morphology, chronological, dental and bone ages of TS patients receiving GH or not. After the study was approved by the local Ethics Committee, we evaluated 21 cephalometric measurements (lateral cephalograms), dental age (DA) (panoramic radiograph), bone age (BA) (left hand-wrist radiograph) and stature Z-escore in 22 TS patients (9 monosomy X; 10 mosaicism; 3 structural abnormalities of the X chromosome). The GH treatment lasted from 0 to 6.8 yr. The median chronological age (CA) was 16 ± 3.4 yr ($\pm$S.D.). The variations for BA and DA were 6.8 yr to 17 yr and 6 yr to 17 yr, respectively. Stature Z-escore was -2.33 ± 1.8 (mean $\pm$S.D.). Statistics were performed using the principal component analysis, simple linear regression and Pearson correlation coefficient. P values <0.05 were considered significant. Face height and mandibular length were the most affected measures and showed correlations with BA, CA and GH treatment duration ($P<0.05$). Cytogenetic status did not influence face alterations. CA was greater than BA ($P<0.05$) and did not differ from DA, while BA was lower than DA ($P<0.05$). We observed a positive correlation between CA and BA ($r=0.7$), CA and DA ($r=0.8$) and BA and DA ($r=0.7$). In conclusion, we showed that our TS patients present a short and retropositioned face, mainly in the lower third part, conferring them a convex profile. A prospective study will provide greater knowledge of GH effects on craniofacial structures, looking for better orthodontic treatment for these patients.

P188

Craniofacial development and dental maturation in growth hormone(GH)-deficient patients

Darwin Luiz Martins Oliveira, Rafaella Shimada Gomes, Maria Helena Senger, Alcinda Aranha Nigri, Reinaldo José Gianini, Sérgio Yassuo Nonoyama & João Carlos Ramos-Dias
Faculdade de Medicina de Sorocaba - PUC/SP, Sorocaba, São Paulo, Brazil.

Growth is a complex process, influenced to a large extent by GH. Children with GH deficiency (GHD) have typical somatic features, including short stature and a reduction of the craniofacial structures. Dental age (DA) is normally delayed in relation to chronological age (CA). The effect of GH replacement on craniofacial growth is still poorly understood. We studied the craniofacial development and dental maturation in 17 patients (4F, 13M) with GHD of different etiologies. The length of rhGH treatment lasted from 0–15.2 yr. The median CA was 16.2 ± 3.9 yr ($\pm$S.D.). BA varied from 5–18 yr and DA, from 7.7–17 yr. Mean stature Z-escore was -1.8 ± 1.8 (mean $\pm$S.D.). Craniofacial morphology was analysed by standardized lateral cephalometric radiographs with 21 measurements. DA was calculated by panoramic radiographs and BA was estimated by left hand-wrist radiographs. This study was approved by the local Ethics Committee. Statistics were performed using the principal component analysis, simple linear regression and Pearson correlation coefficient. P values <0.05 were considered significant. The most affected measures were the posterior cranial base, position of the temporomandibular articulation, facial height and mandibular length, that had correlation with BA and lenght of GH treatment ($P<0.05$). BA was delayed in comparison with CA and DA. There were no significant differences between CA and DA. We observed a positive correlation between BA and DA ($r=0.8$), CA and BA ($r=0.8$), and CA and DA ($r=0.7$). In conclusion, we showed that our group of GHD patients presents with a short face (mainly in the lower third) and a retropositioned mandible, conferring a more convex face profile to them. A longitudinal study will provide a greater knowledge of the effect of rhGH treatment on the craniofacial structures, looking for earlier orthodontic follow-up and better results in these children.

P189

Developing brain as an endocrine gland secreting GnRH and dopamine to general circulation

Michael Ugrumov
Institute of Developmental Biology RAS, Moscow, Russia.

This study was aimed to test our hypothesis that the brain-derived gonadotropin-releasing hormone (GnRH) and dopamine (DA) are delivered to the general circulation in fetal and neonatal rats, i.e. before the establishment of the blood-brain barrier, that is in contrast to adult rats. The GnRH and DA concentrations were measured in plasma and in the brain on the 18th embryonic day (E18), E21, 3rd postnatal day (P3), i.e. before the establishment of the blood-brain barrier, and on P30–36 after the establishment of the barrier. Moreover, the concentrations of GnRH and DA were measured in fetal plasma after microsurgical lesion of the brain regions containing most GnRH or DA neurons or after the inhibition of DA synthesis in the brain with sterotaxically injected α-methyl-p-tyrosine. According to our data, the concentrations of GnRH and DA in plasma on E18, E21 and P3 enormously exceeded those on P30–36 being as great as those in the hypophysial portal circulation in adult rats. Reverse was true for the ontogenetic dynamics of the GnRH and DA concentrations in the brain. The lesion of the local brain regions resulted in a drop of the GnRH and DA concentrations in fetal plasma. The DA concentration in plasma also decreased significantly after the inhibition of DA synthesis in the brain. The rest of circulating GnRH and DA was shown to be insufficient to provide the regulation of the respective adenohypophysial functions.

Thus, brain-derived GnRH and DA are delivered to the general circulation in fetal and neonatal rats in amounts sufficient to influence peripheral targets and the brain itself.

Obesity and metabolism – presented on Sunday

P190

Closure by iptakalim, a cardiovascular K(ATP) channel opener, of rat islet beta-cell K(ATP) channels and its molecular basis

Sechiko Suga[1], Makoto Wakui[2], Takanori Kudo[3] & Yoshiji Ogawa[3]
[1]Center for Education and Research of Lifelong Learning, Hirosaki University, Hirosaki, Aomori, Japan; [2]Division of Clinical Research, Hirosaki National Hospital, Hirosaki, Aomori, Japan; [3]Department of Internal Medicine, Hirosaki University School of Medicine, Hirosaki, Aomori, Japan.

Diabetes mellitus is a group of diseases characterized by high levels of blood glucose resulting from defects in insulin production, insulin action, or both. Diabetes patients usually have accompanying cardiovascular disorders. Sulfonylureas have been the leading oral antihyperglycemic agents for type 2 diabetes treatment, which currently still constitute the most popular anti-diabetic drugs. Nevertheless, concern has arisen over the side effects of sulfonylureas on the cardiovascular system. Here we report that iptakalim, a novel cardiovascular ATP-sensitive potassium (K(ATP)) channel opener, closed rat islet beta-cell K(ATP) channels and increased insulin release. Using whole-cell patch-clamp recordings, iptakalim depolarized beta-cells, induced action potential firing and reduced pancreatic K_{ATP} channel currents. Using single-channel recordings, iptakalim reduced K(ATP) channel open-probability independently of intracellular ATP concentrations. We demonstrated that iptakalim elevated intracellular calcium concentrations and increased insulin release as revealed by fluorescence imaging (fura-2) and biochemical measurements, respectively. In addition, iptakalim significantly inhibited the open-probability of recombinant Kir6.2/SUR1 and Kir6.2FL4A (a trafficking mutant of the Kir6.2) channels expressed in transfected human embryonic kidney (HEK) 293 cells. Collectively, iptakalim, a cardiovascular K_{ATP} channel opener, closes rat islet beta-cell K(ATP) channels, which may result from direct inhibition of the Kir6.2 subunit. Therefore, iptakalim bi-directionally regulates K(ATP) channels in cardiovascular and islet tissues, and this unique pharmacological property suggests iptakalim could be used as a new therapeutic strategy for the treatment of type 2 diabetes with the potential benefit in alleviating cardiac and/or vascular disorders frequently associated with diabetes.

P191

Plasma adiponectin and leptin levels in obese and insulin resistant postmenopausal females with type 2 diabetes

Nona Adamia, Tea Tsotsonava & Ketevan Janberidze
Tbilisi State Medical University, Tbilisi, Georgia.

Objective

Adipocytokines appear to be important in regulating insulin sensitivity. The objective of this study was to compare the levels of adiponectin and leptin in lean, obese and obese diabetic (OD) postmenopausal female (PMF) subjects during 6 months follow-up of Metformin therapy (MT).

Methods
We examined plasma levels of adiponectin and leptin in 26 OD PMF with a mean body mass index (BMI) of 36.6±1.8, 10 obese (BMI=35.9±2.2) and 10 lean (BMI=22.3±1.9) individuals. The investigation was approved by the local ethics committees. All participants gave informed, written consent before starting the trial. Insulin resistance (IR) was assessed using the homeostasis model assessment.
Results
Baseline characteristics of all groups shown that adiponectin was significantly decreased and leptin is significantly elevated in OD PMF and obese subjects in comparison with leans ($P<0.001$ and $P=0.003$, respectively). There was a tendency for adiponectin levels to be lower in OD PMF as compared with obese individuals ($P=0.053$). OD PMF were more insulin resistant than obese and lean subjects ($P<0.001$). Results of MT shown that circulating adiponectin levels were significantly increased (16.1±3.9 vs. 19.1±6.0 ng/ml, $P=0.008$) with significant reduction of BMI and IR ($P=0.005$ and $P<0.001$, respectively). Leptin levels did not change significantly.
Conclusions
Circulating adiponectin levels is significantly reduced in OD PMF in comparison with obese and lean subjects. Hypoadiponectinemia in PMF may be explained by only IR because the amelioration of whole-body insulin action by MT causes the increase of serum adiponectin levels. Leptin levels in OD PMF are not significantly different from leptin levels of obese subjects, although they significantly differ from leptin levels of lean individuals.

P192

Pioglitazone modifies the effects of growth hormone on lipolysis and insulin sensitivity

Morten B Krag[1], Søren Nielsen[1], ZengKui Guo[2], Steen B Pedersen[3], Ole Schmitz[1], Jens S Christiansen[1] & Jens OL Jørgensen[1]
[1]Medical Department M, Aarhus University Hospital, DK-8000 Aarhus C, Denmark; [2]Endocrine Research Unit, Mayo Clinic, Rochester, MN 55905, United States; [3]Medical Department C, Aarhus University Hospital, DK-8000 Aarhus C, Denmark.

Context
Thiazolidiones (TZD) improves insulin sensitivity in type 2 diabetes via effects on fat metabolism, whereas growth hormone (GH) stimulates lipolysis and induces insulin resistance.
Objective
To evaluate the effects of TZD on fat metabolism and insulin sensitivity in GH-treated GH deficient (GHD) patients.
Design
Randomized, placebo-controlled, double-blind parallel-group study including 20 GHD patients on continued GH replacement therapy. The patients were studied before and after 12 weeks.
Intervention
Patients received either tablet pioglitazone 30 mg ($N=10$) or placebo ($N=10$) once daily for 12 weeks.
Results
12 weeks of pioglitazone treatment in GH-replaced GHD patients was associated with improved insulin sensitivity ($P=0.03$) and increased basal glucose oxidation ($P=0.004$). Change in insulin-stimulated adiponectin level after pioglitazone treatment was positive correlated to the change in insulin-stimulated total glucose disposal ($R=0.69$, $P=0.04$). Pioglitazone significantly decreased basal free fatty acid levels ($P=0.02$) and lipid oxidation ($P=0.02$). Adiponectin levels almost doubled during pioglitazone treatment ($P=0.0001$).
Conclusion
The impact of GH on lipolysis and insulin sensitivity is modified by administration of PPARγ agonists.

P193

The metabolic syndrome and associated sexual dysfunction: psycho-biological correlates

Giovanni Corona[1], Edoardo Mannucci[2], Luisa Petrone[1], Riccardo Mansani[1], Giancarlo Balercia[3], Valerio Chairini[4], Gianni Forti[1] & Mario Maggi[1]
[1]Andrology Unit, University of Florence, Florence, Italy; [2]Geriatric Unit, Diabetes Section, University of Florence, Florence, Italy; [3]Endocrinology Unit, Polytechnic University of Marche, Ancona, Italy; [4]Endocrinology Unit, Maggiore-Bellaria Hospital, Bologna, Italy.

Objectives
The aim of present study is to determine psychobiological characteristics of sexual dysfunction (SD) associated with metabolic syndrome (MS; as defined by National Cholesterol Education Program's Adult Treatment Panel III, NCEP-ATP-III criteria) in a consecutive series of 803 male out-patients.
Methods
Several hormonal, biochemical and instrumental (penile doppler ultrasound, PDU) parameters were studied, along with psychopathology scores (Middlesex Hospital Questionnaire modified MHQ). The Structured Interview on Erectile DYsfunction (SIEDY), was also applied.
Results
Among subjects studied, 236 patients (29.4%) were diagnosed as having a MS. Among them 96.5% reported ED, 39.6% hypoactive sexual desire, (HSD) 22.7% premature and 4.8% delayed ejaculation. Patients with MS were characterized by greater subjective (as assessed by SIEDY) and objective (as assessed by PDU) ED and by greater somatizated anxiety than the rest of the sample. The prevalence of overt hypogonadism (total testosterone <8 nM) was significantly higher in patients with MS. Circulating TT decreased as the number of MS components increased (B=−1.35±0.182 nmol/l; $P<0.0001$, after adjustment for age). Accordingly, the relative risk for hypogonadism was significantly higher in patients reporting 3 or more risk factors for MS. Among MS components, waist circumference and hyperglycemia were the best predictors of hypogonadism. Among patients with MS, hypogonadism was present in 11.9% and 3.8% in the rest of the sample ($P<0.0001$) and it was associated with typical hypogonadism-related symptoms, such as hypoactive sexual desire, low frequency of sexual intercourses and depressive symptoms.
Conclusion
Our data suggest that MS is associated with a more severe ED and induces somatization. Furthermore, MS is associated with a higher prevalence of hypogonadism in patients with SD. The presence of hypogonadism can further exacerbate the MS-associated sexual dysfunction, adding the typical hypogonadism-related symptoms. (including HSD, 66.7%).

P194

A comparison of NCEP-ATP-III and IDF metabolic syndrome definitions with relation to metabolic syndrome associated sexual dysfunction

Giovanni Corona[1], Edoardo Mannucci[2], Luisa Petrone[1], Francesco Lotti[1], Alessandra D Fisher[1], Giancarlo Balercia[3], Valerio Chairini[4], Gianni Forti[1] & Mario Maggi[1]
[1]Andrology Unit, University of Florence, Florence, Italy; [2]Geriatric Unit, Diabetes Section, University of Florence, Florence, Italy; [3]Endocrinology Unit, Polytechnic University of Marche, Ancona, Italy; [4]Endocrinology Unit, Maggiore-Bellaria Hospital, Bologna, Italy.

Objectives
The aim of present study was to verify possible differences in the prevalence of vasculogenic ED and hypogonadism comparing two distinct new definitions of MetS, as National Cholesterol Education Program-Third Adult Treatment Panel (NCEP-ATPIII) and International diabetes Federation (IDF) in patients with sexual dysfunction.
Methods
Several hormonal, biochemical and instrumental (penile doppler ultrasound) parameters were studied. ANDROTEST Structured Interview was also applied. This a 12-item, recently validated, inventories, which assesses the degree of androgenization in male.
Results
We studied a consecutive series of 1086 patients. The prevalence of metabolic syndrome was 32.0% and 44.7% according to NCEP-ATPIII and IDF criteria, respectively. Patients with MetS according to both criteria reported lower PGE-1 stimulated penile flow (Vpmax). At multivariate analysis, only NCEP-ATPIII was significantly associated with Vpmax (B=−7.7±3.8; $P<0.05$). Patients with MetS defined according to both criteria reported lower total (13.6±6.0 vs. 17.4±7.2 and 14.7±7.4 vs. 18.2±6.0 nmol/l,) and free testosterone levels (34.8±14.0 vs. 40.8±13.7 and 36.2±14.1 vs. 42.5±13.5 pmol/l), higher prevalence of hypogonadism (34.3 vs. 11.9 and 25.3 vs. 8.7%), and higher ANDROTEST score (9.6±3.0 vs. 7.2±3.6 and 9.2±3.2 vs. 6.0±3.2) respectively for NCEP-ATPIII and IDF; all $P<0.0001$. However, when IDF, but not NCEP-ATPIII, criteria were fulfilled, the prevalence of hypogonadism was significantly lower than that observed in patients fulfilling both criteria (15.6 vs.34.8% respectively; $P<0.0001$). Conversely, those fulfilling NCEP-ATP-III, but not IDF, criteria did not show a significant different prevalence of hypogonadism than those positive for both sets of criteria (30.8 vs.34.8%; $P=$NS).
Conclusions
In patients with ED, NCEP-ATPII criteria seem to be a better predictor of hypogonadism and impaired penile blood flow than IDF.

P195

Effect of supervised structured exercise program for 16 weeks on metabolic, pulmonary and cardiovascular parameters in obese adolescents

Juan Guízar-Mendoza, Benigno Linares, Norma Amador, Gloria Barbosa & Juan Malacara
[1]Instituto Mexicano del Seguro Social, León/Guanajuato, Mexico, [2]Universidad de Guanajuato/Instituto de Investigaciones Médicas, León/Guanajuato, Mexico, [3]Universidad de Guanajuato/Facultad de Medicina, León/Guanajuato, Mexico.

Objective
To investigate whether a supervised structured exercise program by 16 weeks improves metabolic, pulmonary and cardiovascular parameters in obese adolescents.
Material and methods
We included 38 obese adolescents between 12–15 years old. They participated in a supervised exercise program by 90 minutes, 5 days a week during 16 weeks. At baseline and at the end of the exercise program, we evaluated cardiorespiratory fitness, anthropometric measurements, lipid profile, glucose, insulin, leptin, adiponectin, and blood pressure levels. Pulmonary function was evaluated by spirometry and heart sympathetic activity by spectral analysis of the R-R interval during 60 minutes to obtain indices of heart autonomic function.
Results
The exercise program increased exercise ability ($P<0.001$), maximal oxygen uptake ($P=0.01$), forced vital capacity ($P=0.004$), and adiponectin levels ($P<0.001$); while BMI ($P=0.001$), body fat (<0.001), glucose, triglycerides ($P<0.001$ in both), leptin ($P<0.001$), blood pressure levels ($P<0.001$), and heart sympathetic activity expressed as LF/HF index ($P=0.005$) significantly decreased. The change in LF/HF index was correlated with the decrease in leptin ($r=43$; $P=0.007$), diastolic ($r=0.33$; $P=0.04$) and systolic (0.35; $P=0.03$) blood pressure levels respectively.
Conclusions
A short-term supervised structured exercise decreased adiposity and improves metabolic, pulmonary, and cardiovascular parameters in obese adolescents.

P196

Continuous administration of dihydrotestosterone or letrozole to immature female rats results in polycystic ovary syndrome characteristics at adult age

Louise Mannerås[1], Theodore Lystig[2], Agneta Holmäng[1], Malin Ottosson-Lönn[2] & Elisabet Stener-Victorin[1]
[1]Institute of Neuroscience and Physiology, Göteborg, Sweden; [2]Insitute of Medicine, Göteborg, Sweden.

Introduction
Polycystic ovary syndrome (PCOS) is a complex endocrine and metabolic disorder associated with ovulatory dysfunction, hyperandrogenism, abdominal obesity and insulin resistance. Since hyperandrogenism is a PCOS key feature, the aim was to evaluate the effects of androgen receptor activation in terms of continuously administration, beginning pre-pubertal, of either the non-aromatizable androgen dihydrotestosterone (DHT) or the aromatase inhibitor letrozole (L), on ovarian morphology, as well as on the endocrine and metabolic status were investigated.
Methods
At 21 days of age, the rats were implanted subcutaneously with a pellet releasing DHT or L continuously during 90 days. Estrus cyclicity (vaginal smear), ovarian morphology, sex steroid and leptin concentrations, body composition (DEXA, MRI, and tissue dissection), mesenteric adipocyte size (computerized image analysis), and insulin sensitivity (euglycemic hyperinulinemic clamp) were examined.
Results
DHT induced polycystic ovaries (PCO) and anovulation in 75% of the rats. DHT rats also displayed increased body weight, fat mass and weight of individual abdominal fat depots, as well as enlarged mesenteric adipocyte size with a right shifted size distribution curve. Moreover, elevated leptin levels and insulin resistance were observed in DHT treated rats. Almost all L rats developed PCO morphology with similarity to human PCO, including hyperplastic theca cell layer, and anovulation. Hyperandrogenism and increased body weight without any body composition changes were other characteristics of the L group.
Conclusions
Typical PCO morphology was induced both by DHT and L treatment. In particular DHT treatment also resulted in metabolic disorders of the syndrome, while the endocrine features of the syndrome were mainly induced by L. Both models can therefore be concluded as suitable for investigation of different aspects of the human PCOS.

P197

Neonatal sex steroid exposure of female rats results in insulin resistance and enlarged mesenteric adipocytes

Camilla Alexanderson[1], Theodore Lystig[2], Elisabeth Stener-Victorin[1], Malin Lönn[2] & Agneta Holmäng[1]
[1]Instititute of Neuroscience and Physiology, Göteborg, Sweden; [2]Institute of Medicine, Göteborg, Sweden.

Introduction
Neonatal events may contribute to the development of disorders such as type 2 diabetes and obesity at adult age. We have previously shown that neonatal testosterone (T) programming of female rats is followed by insulin resistance and changes in adipose tissue distribution with centralization of body fat. Therefore, the aim of this study was to examine the effects of neonatal injection of T, estradiol (E) or dihydrotestosterone (DHT) on insulin sensitivity and size distribution of adipocytes in intra-abdominal and subcutaneous adipose tissue in female rats.
Methods
Pups received one injection of T, E, DHT or vehicle within 3 hours after birth. At 14 wks of age the rats were exposed to a euglycemic hyperinsulinemic clamp. Intra-abdominal (mesenteric) and subcutaneous (inguinal) adipose tissues were dissected and weighed. Adipocyte size was analysed using a computerized image analysis system.
Results
All groups receiving steroids were insulin resistant in comparison with controls. The mesenteric adipocyte size distribution was shifted to the right in T- and E-rats compared with controls while adipocyte size in the inguinal depot was not affected. T-rats also displayed increased mesenteric adipose tissue weight.
Analysis of all groups together showed a negative correlation between mesenteric adipocyte size and glucose infusion rate.
Conclusions
Sex hormone exposure in early life may predispose to disturbances in insulin sensitivity and adipose tissue at adult age. Directly after birth, in particular the mesenteric adipose tissue depot seems to be vulnerable to T- and E exposure which is seen as a shift to the right of the adipocyte size distribution in adulthood.

P198

Evaluation of visceral protein malnutrition in morbid obese patients operated on laparoscopic gastric bypass

Rosa Casañ, Carlos Morillas, Eva Sola, Katherinne García, Jesus Yanini, Ana Jover, Marcelino Gomez & Antonio Hernández
Endocrinology Department, Doctor Peset University Hospital, Valencia, Spain.

Morbid obesity is associated with a decreased life expectancy and a myriad of serious medical problems. The Roux-en-Y gastric bypass (RYGBP) is the most effective procedure for the treatment of these patients, but it can be responsible of early and late complications. The aim of this prospective study was to evaluate the rate of visceral protein malnutrition (VPM) in morbid obese (MO) patients two years after laparoscopic RYGBP. Albumin (Alb), prealbumin (Prealb), transferrin (Transf), retinol binding globulin (RBG), C3-complement factor (C3) plasma levels, and lymphocyte count (Lymph) were measured before and 2 years after RYGBP. Data were evaluated using paired Student t-test. Data were available for 46 patients (9 men and 37 women). Mean age: 38.5 ± 11 years; mean follow-up time: 24 ± 9 months.
Results
No differences were observed in Prealb, RBG, Transf or Lymph count. Before surgery, 1 patient (2.2%) had C3 values under normal levels, and after surgery 4 patients (8.7%) had C3 values under normal levels.

	Baseline	24 months	*P*
Weight (kg)	124.8 ± 17.7	84.8 ± 15.9	<0.001
BMI (kg/m^2)	47.8 ± 6.7	32.5 ± 6.3	<0.001
Alb (mg/dL)	3.93 ± 0.4	3.89 ± 0.3	0.08
C3 (mg/dL)	132.5 ± 20.9	103.1 ± 16.9	<0.001

Conclusions
1- There were no changes in main visceral protein plasma levels: Alb, Prealb, Transf, and RBG in MO patients after 2 years of RYGBP. 2- A significant decrease of C3 values was observed in these patients, without changes in lymphocyte count. In spite of this decrease, C3 levels remained in most patients between the normal range. 3- RYGBP seems to be an effective procedure to treat morbid obesity which does not cause VPM, but immunity should be assessed.

P199

Overconsumption of salty and sweet foods increases blood pressure in children

Vassilios Petrou[1], Athanasia Tertipi[1], Asteroula Papathanasiou[1], Ourania Zerva[2], Vassiliki Skarpa[1], Petros Papachilleos[1], Marina Anastasacou[1], Panagiotis Nicolopoulos[1] & Charalambos Hadjiathanasiou[1]
[1]Children's Hospital "P. & A. Kyriacou" Department of Endocrinology, Athens, Greece; [2]Children's Hospital "P. & A. Kyriacou" Department of Nutrition, Athens, Greece.

Aim
To estimate the impact of overconsumption of salty and sweet foods on Body Mass Index (BMI) and Blood Pressure (BP) in children.
Patients-Methods
We studied 208 children (105 girls), aged 9.2±3.0 yr, 57 (27.4%) of normal weight, 37 (17.8%) overweight and 114 (54.8%) obese. Overconsumption of NaCl was considered >5 g/day and of free sugar >0.5 g/Kg ideal Body Weight/day. BP was measured as appropriate and BMI was estimated in all children.
Results
Children overconsuming salty and sweet foods had significantly higher BMI SDS than children consuming small amount of salty and sweet foods (2.1±1.5 vs 1.2±1.5, $P<0.001$ for salty foods and 2.1±1.5 vs 1.2±1.6, $P=0.002$ for sweet foods). Thirty-three (57.9%) of children of normal weight overconsumed salty foods versus 23 (62.2%) of overweight and 98 (86.0%) of obese ($\chi^2=18.8$, $P<0.001$). Thirty-five (61.4%) of children of normal weight overconsumed sweet foods versus 32 (86.5%) of overweight and 99 (86.8%) of obese ($\chi^2=16.5$, $P<0.001$). One hundred twenty nine children (83.8%) overconsuming salty foods had Systolic BP (SBP)>50th percentile versus 35 (64.8%) of children consuming small amounts of salty foods ($\chi^2=8.6$ $P=0.006$). One hundred thirty six children (81.9%) that overconsumed sweet foods had SBP>50th percentile versus 28 (66.7%) that consumed small amounts of sweet foods ($\chi^2=4.6$, $P=0.036$). There was no difference regarding diastolic BP (DBP) among children consuming large or small amounts of salty and sweet foods respectively. BMI SDS emerged as the most important determinant of SBP>50th percentile and DBP>50th percentile in multivariate analysis.
Conclusion
Overconsumption of salty and sweet foods is related to a relatively increased BP in children through the incremental effect on BMI SDS.

P200

Expression of PKA regulatory subunits inversely correlates with BMI and insulin resistance parameters in human adipocytes from lean and obese subjects

Sara Bondioni[1], Giovanna Mantovani[1], Luisella Alberti[2], Sabrina Corbetta[3], Luisa Gilardini[2], Cecilia Invitti[2], Marco Antonio Zappa[4], Stefano Ferrero[5], Erika Peverelli[1], Andrea G Lania[1], Paolo Beck-Peccoz[1] & Anna Spada[1]
[1]Endocrine Unit, Dpt. of Medical Sciences, Fondazione Ospedale Maggiore Policlinico Mangiagalli e Regina Elena IRCCS, University of Milan, Milan, Italy; [2]Lab. of Diabetologic Research, Istituto Auxologico Italiano IRCCS, Milan, Italy; [3]Endocrinology and Diabetology Unit, Dpt. of Medical-Surgical Sciences, University of Milan, Policlinico San Donato IRCCS, San Donato Milanese, Milan, Italy; [4]General Surgery Unit, Dpt. of Surgical Sciences, University of Milan, Fondazione Ospedale Maggiore Policlinico Mangiagalli e Regina Elena IRCCS, Milan, Italy; [5]Pathology Unit, Department of Medicine, Surgery and Dentistry, A.O. San Paolo and Fondazione Ospedale Maggiore Policlinico Mangiagalli e Regina Elena IRCCS, Milan, Italy.

In human adipocytes the cAMP-dependent pathway mediates signals originating from the activation of beta-adrenergic receptors, thus playing a key role in the regulation of important metabolic processes such as lipolysis and thermogenesis. CyclicAMP effects are mainly mediated by cAMP-dependent protein kinase (PKA), a tetrameric enzyme composed by two catalytic subunits associated with two regulatory (R) subunits. There are four different R subunit genes and proteins (R1A, R1B, R2A, R2B) expressed with a tissue-specific pattern and exerting distinct roles in cell differentiation and growth control. Recent studies indicate the R2B isoform as the most expressed in mouse adipose tissue while its presence is limited elsewhere. Moreover, R2B knock-out mice are genetically lean and protected against developing diet-induced obesity and fatty-livers. The aim of this study was to investigate the expression of the different PKA regulatory subunits in 65 human subcutaneous and visceral adipose tissue samples from 10 lean subjects (BMI<25) and 55 obese patients (BMI>30). Real-time PCR showed that, as in mice, R2B is the most abundant transcript, both in obese and normal subjects, with no differences between visceral and subcutaneous adipose tissue. Moreover, a significant negative correlation was observed between R2B expression levels and BMI, insulin levels, HOMA-IR ($r=-0.280$, $r=-0.269$, $r=-0.255$, respectively; $P<0.05$), with a positive correlation with adiponectin and adiponectin receptors 1&2 mRNA levels ($r=+0.636$, $r=+0.582$, $r=+0.631$ respectively; $P<0.001$). Moreover, among obese patients, patients with metabolic syndrome showed the lowest R2B levels. Immunohystochemistry and western-blot analysis performed in 15 of the 55 samples from obese patients and in the 10 samples from lean subjects confirmed the same expression pattern. This is the first study evaluating the relative expression of the different PKA isoforms in human adipose tissue. Our results indicating important BMI-related differences in R2B expression suggest that similar differences in PKA activity may modulate the lipolytic response to beta-adrenergic activation.

P201

Insulin resistance and fasting leptin's relationship in subjects with metabolic syndrome

T Mokhort & E Shishko
Belarusian State Medical University, Minsk, Belarus.

Background and aims
The aim of our study is to investigate the possible associations between leptin and fasting insulin and index HOMA-IR in patients with Metabolic Syndrome as Leptin is involved in regulation of body weight.
Materials and methods
The study included 100 patients (32 m, 68 f) 25–65 years. They were divided into 2 groups matched by sex, age, weight, body mass index (BMI), waist-to-hip ratio (WHR). Research group included 56 patients (24 m, 32 f) with Metabolic Syndrome: abdominal obesity, insulin resistance, hyperinsulinemia, glucose intolerance, hypertension and dyslipidemia. Control group included 44 patients (16 m, 28 f) without clinical and biochemical findings of Metabolic Syndrome. The average fasting plasma glucose, 2-hour plasma glucose concentrations following a 75-g oral glucose tolerance test, total cholesterol, triglycerides, systolic and diastolic blood pressure were also evaluated. Fasting serum leptin (FL) and fasting insulin levels (FI) were detected by sensitive and specific ELISA. Index HOMA-IR was calculated by standard formula. HOMA-IR=or> 2.7 were considered as insulin resistance.
Results
In patients of the research and control groups serum leptin levels were higher in females (median 45.1 and 27.8 ng/ml respectively) than in males (15.9 and 7.7 ng/ml respectively). But only in patients of the research group correlations were between BMI and WHR (r=0.91 $P<0.001$ vs $r=0.93$ $P<0.01$ respectively). Correlation analysis showed that FL were significantly correlated with the FI ($r=0.56$ $P<0.01$) and HOMA-IR ($r=0.52$ $P<0.01$) in research group. In subjects of the control group leptin concentration correlated with the HOMA-IR only in men ($r=0.91$ $P<0.01$) and not correlated in female. The strongest correlations were between FL and total cholesterol ($r=+0.49$ $?<0.05$ in men) and triglycerides ($r=+0.8$ $?<0.05$ in women) in research group.
Conclusion
Determine positive correlation of basal leptin and index insulin resistance confirms hyperleptinemia and leptinresistance concern in formation of metabolic syndrome.

P202

Epicardial adipose tissue, hepatic steatosis and obesity

Ayse Sertkaya Cikim[1], Ergun Topal[2], Murat Harputluoglu[3], Lezzan Keskin[1], Zeynep Zengin[1], Kerim Cikim[1], Ramazan Ozdemir[2], Murat Aladag[3] & Saim Yologlu[4]
[1]Inonu University Faculty of Medicine Department of Internal Medicine Division of Endocrinology and Metabolism, Malatya, Turkey; [2]Inonu University Faculty of Medicine Department of Cardiology, Malatya, Turkey; [3]Inonu University Faculty of Medicine Department of Internal Medicine Division of Gastroenterohepathology, Malatya, Turkey; [4]Inonu University Faculty of Medicine Department of Biostatistics, Malatya, Turkey.

Objective
The measurement of epicardial adipose tissue (EAT) sonographically is reported to be related with both obesity and insulin resistance. Hepatic steatosis is one of the best known other coincidence with obesity. We aimed to evaluate the relationships between EAT thickness, hepatic steatosis and insulin resistance in obese patients.
Methods
Obese 63 subjects were enrolled into the study. Local ethical committee approval was obtained. Patients were divided into three groups according to body mass index (BMI) as follows: 20 patients with $30 \leq BMI < 35$ kg/m^2 (Group 1, mean age 39.3 ± 12.9 yrs), 25 patients with $35 \leq BMI < 40$ kg/m^2 (Group 2, mean age 41.7 ± 9.3 yrs), and 18 patients with BMI ≥ 40 kg/m^2 (Group 3, mean age 36.8 ± 13.9 yrs). EAT thickness and grade of hepatic steatosis were assessed sonographically. Anthropometrical measurements were assessed with the foot-to-foot bioelectrical impedance analysis. Insulin resistance was assessed according to basal insulin, QUICKI and HOMA equations.
Results
hsCRP was the only metabolic parameter; which was higher in Group 3 than Group 1 significantly ($P = 0.02$). EAT thickness was similarly higher in both groups 2 and 3; but groups were found to be similar for grade of hepatic steatosis. Both EAT thickness and grades of hepatic steatosis were positively and significantly correlated with whole body fat mass and abdominal adiposity. Waist circumference was the only factor affecting EAT thickness in linear regression analysis.
Conclusion
Grade of hepatic steatosis is a lesser sensitive marker for closer obesity levels than EAT, but with its significant correlations; hepatic steatosis can also be assessed as a valuable predictor for reflecting increments of whole body fat mass and abdominal adiposity as EAT thickness.

P203

Decreased 11beta-hydroxysteroid dehydrogenase type 1 activity in obese boys

Susanna Wiegand[1], Anna Richardt[1], Thomas Remer[3], Stefan A Wudy[4], Jeremy W Tomlinson[5], Annette Grüters[1], Paul M Stewart[5], Christian J Strasburger[2] & Marcus Quinkler[2]
[1]Paediatric Endocrinology, Charite Campus Virchow, Universitaetsmedizin Berlin, Berlin, Germany; [2]Clinical Endocrinology, Charite Campus Mitte, Universitaetsmedizin Berlin, Berlin, Germany; [3]Department of Nutrition and Health, Research Institute of Child Nutrition, Dortmund, Germany; [4]Steroid Research Unit, Center for Child and Adolescent Medicine, Justus Liebig University, Giessen, Germany; [5]Division of Medical Sciences, Institute of Biomedical Research, University of Birmingham, Birmingham, United Kingdom.

Objective
The incidence of childhood obesity and type 2 diabetes has reached epidemic proportions. Glucocorticoid excess causes central obesity and diabetes mellitus as seen in Cushing's syndrome. The 11beta-hydroxysteroid dehydrogenase type 1 enzyme (11beta-HSD1), which is predominantly expressed in liver and adipose tissue, regenerates active cortisol from inactive cortisone. Increased 11beta-HSD1 may cause tissue-specific Cushing syndrome with central obesity and impaired glucose homeostasis.
Design, patients and methods
Clinical and laboratory characteristics, and anthropometric measurements were determined in 15 male (aged 12–18) and 6 female (aged 12–18) obese pubertal children. In addition, analysis of 24 h excretion rates of glucocorticoids were performed in obese and age- and sex-matched non-obese children using gas chromatographic-mass spectrometric (GC-MS) analysis.
Results
11beta-HSD1 activity (urinary THF + 5alphaTHF/THE ratio) was lower in obese compared to non-obese boys. In addition, obese children had a higher total cortisol metabolite excretion than non-obese children. 11beta-HSD1 activity was significantly related to age, but not to waist-to-hip ratio, fat mass (% of body mass), or insulin resistance index (HOMA). Standard deviation score (SDS)-BMI did not correlate with 11beta-HSD1 or −2 (urinary free F/free E ratio) activity, or with total cortisol metabolite excretion. We did not find a gender difference regarding 11beta-HSD1 or −2 activity. 11beta-HSD2 activity significantly correlated to abdominal circumference in obese children.
Conclusions
In conclusion, our findings strongly suggest that 11beta-HSD1 activity increases with age and is reduced in obese boys. In addition, obese children have a higher total cortisol metabolites excretion suggesting a stimulated HPA axis.

P204

Clinical presentation of nonclassic congenital adrenal hyperplasia (NC-CAH): from suspicion to diagnosis

Anna Casteràs Román, M[a] Concepción Páramo Fernández, Javier De la Fuente Aguado, Elena Hervás Abad, Laura Fajar Rodríguez, Beatriz Mantiñán Gil, Reyes Luna Cano, M[a] Antonia Rego Iraeta, Digna Rodríguez, Domingo González Lestón & Ricardo Víctor García-Mayor
Hospital Xeral de Vigo, Vigo, Pontevedra, Spain; Hospital POVISA, Vigo, Pontevedra, Spain.

Background
Nonclassic congenital adrenal hyperplasia (NC-CAH) caused by mutations in CYP21B gene is an inherited disorder with various clinical forms in relation to the 21-hydroxylase (21OH) activity. Classic forms are recognized early during neonatal period as salt-wasting crisis or genital ambiguity, while non-classic form presents later with wide hyperandrogenic spectrum. Genetic testing has proved to be the definitive diagnostic method.
Aim
To observe the clinical presentation in relation to the genotype among subjects with clinical suspect of NC-HAC.
Subjects and methods
Ninety-seven patients (90 female, 7 male) consulting with suggestive clinical data of NC-HAC were genotyped and classified into groups (1: no mutation $n = 54$; 2: homozygotes $n = 22$; 3: compound heterozygotes $n = 11$; 4: simple heterozygotes $n = 10$). Clinical presentation was correlated with the genetic findings.
Results
Mutations in CYP21B were present in 44,3% of patients and V281L in homozygous state was the most frequent genotype in the studied population (48,8%). In general, hirsutism and premature pubarche were the most common symptoms (32,9 and 28,8% respectively).
Conclusions
Less than 50% of hyperandrogenic patients had genetic confirmation of 21OH deficiency. We did not find clinical features associated with the genotype, but precocious pubarche, which is more common in simple and compound heterozygotes than in homozigotes or without mutation ($P < 0.05$).

P205

Daily and nightly urinary free cortisol ratio as a marker of the hypothalamic-pituitary-adrenal (HPA) axis activity in abdominal obesity

Valentina Vicennati[1], Uberto Pagotto[1], Bruno Ambrosi[2], Mauro Maccario[2], Ettore Degli Uberti[2], Marco Boscaro[2], Francesco Cavagnini[2], Franco Mantero[2], Decio Armanini[2], Roberto Vettor[2], Mario Marugo[2] & Renato Pasquali[1]
[1]Endocrinology Unit, Bologna, Italy; [2]"HPA axis and obesity" Group-SIE, Bologna, Italy.

Abdominal obese (AO) women might have a hyperactivation of the HPA axis. The limitations of previous studies have been often represented by the limited and

heterogeneous number of patients enrolled. Our aim was to asses urinary free cortisol (UFC) output during daily and nightly hours in a large cohort of AO women vs. normal weight controls (CT). 107 AO women and 37 CT were enrolled in the study. In basal condition, each subject underwent OGTT, biochemical determinations. Each subject collected daily (from 0800 AM to 0800 PM, dUFC) and nightly (from 0800 PM to 0600 AM of the day after, nUFC) urine.
Cholesterol and triglycerides levels were significantly higher ($P<0.001$) in the AO, whilst HDL were significantly ($P<0.01$) lower than in CT. AO had significantly higher HOMA index than CT. There were no differences neither in dUFC nor in the nUFC between the groups. On the contrary, AO had significantly lower dUFC/nUFC than CT.
There was a negative and significant correlation between dUFC/nUFC and waist and BMI in all subjects. When AO were analyzed separately, the correlation between dUFC/nUFC and anthropometric variables was still present. Moreover, the ratio was also positively correlated to HOMA index ($P<0.05$).
In order to assess the linkage between HPA axis activity and metabolic syndrome, a multiple regression was performed in AO. dUFC/nUFC was still negatively and significantly correlated to BMI, while the correlation with waist circumference was lost. Interestingly, dUFC/nUFC was still positively and significantly correlated to HOMA index and systolic blood pressure. On the contrary, a negative and significant correlation was found between dUFC/nUFC and both HDL and diastolic blood pressure.
In conclusion, obesity by itself is characterized by high nightly UFC excretion. The HPA axis dysregulation is strictly associated to the abnormalities of the metabolic syndrome, particularly to glucose-insulin homeostasis, dyslipidemia and hypertension.

P206

Interaction of hypothalamic receptors involved in weight regulation

Anne Rediger[1], Patrick Tarnow[1], Annette Grüters[1], Michael Schäfer[2] & Heike Biebermann[1]
[1]Charité Universitätsmedizin Berlin, Inst. Exp. Ped. Endocrinology, Berlin, Germany; [2]Charité Universitätsmedizin Berlin, Inst. Pharmacology, Berlin, Germany.

Objectives
Food intake is centrally regulated in hypothalamic nuclei where many G-protein-coupled receptors (GPCRs) are expressed which are known to be involved in weight regulation. Peripheral hormonal signals activate their corresponding receptors in the arcuate nucleus. Orexigenic signals activate POMC expression in one subset of neurons and inhibit AgRP and NPY expression in a second subset. Cleavage products of POMC, α- and β-MSH then stimulate melanocortin-4-receptors (MC4R) in the paraventricular nucleus of the hypothalamus to inhibit food intake or stimulate the melanocortin 3 receptor (MC3R) in the arcuate nucleus to activate a feedback loop. Other neuropeptides or neurotransmitters are involved in hypothalamic regulation of body weight, which also act through G-protein-coupled-receptors co-expressed with melanocortin receptors (MCR) in hypothalamic nuclei. The concept of homo and hetero-oligomerization of GPCRs today is well accepted. Recently we could show homo-oligomerization of MC4R. In a systematic approach we investigated the interaction of GPCRs that are expressed on the same neurons.
Methods
We used two different methods to investigate GPCR oligomerization: a sandwich-ELISA approach with differentially N- and C-terminal tagged receptors in COS-7 cells and the FRET-acceptor-photobleaching-technique which allows monitoring of GPCR interaction in living HEK-293 cells. Furthermore we investigated receptor co-localization on the cell surface by laser scanning microscopy.
Results
Here we report data on interaction of the MC3R and ghrelin receptor (GHSR) that are coexpressed on arcuate NPY/AgRP neurons. The usage of both methods results in a strong signal of MC3R/GHSR oligomerization.
Conclusion
We could demonstrate for the first time that GPCR from different subfamilies, that are expressed on the same neuron and are involved in weight regulation form receptor oligomeres. These findings may provide a mechanistic basis of a functional interaction between melanocortin and ghrelin receptors and thereby widen our understanding of hypothalamic signalling pathways involved in weight regulation.

P207

Association of estrogen receptor-alpha gene polymorphisms with cerebrovascular disease in patients with metabolic syndrome

Nektaria Xita[1], Sophia Markoula[2], Leandros Lazaros[3], Athanasios Kyritsis[2], Ioannis Georgiou[3] & Agathocles Tsatsoulis[1]
[1]Department of Endocrinology, University of Ioannina, Ioannina, Greece; [2]Department of Neurology, University of Ioannina, Ioannina, Greece; [3]Laboratory of Reproductive Genetics, University of Ioannina, Ioannina, Greece.

Introduction
The vascular protective effects of estrogens are known to be mediated by their binding to specific estrogen receptors (ER). However, the significance of genetic variations of the ER in vascular diseases has not been reported. We have examined the association between stroke and PvuII and XbaI polymorphisms of the estrogen receptor-alpha gene in patients with metabolic syndrome.
Methods and subjects
The study population consisted by 84 male and 46 female patients with metabolic syndrome compared with 100 healthy men and 140 healthy women respectively. The body mass index was recorded and biochemical parameters were measured. PCR-RFLP and genotyping of ER PvuII and XbaI polymorphisms were performed in peripheral blood leucocytes. Multiple logistic regression analysis was used to explore the risk factors for stroke. Local Ethical Committee approval was obtained.
Results
Both polymorphisms were in Hardy Weinberg equilibrium in the study population. Genotype distributions and allele frequencies of PvuII or XbaI polymorphisms were not significantly different between control subjects and patients. No association was found between the polymorphisms and the severity of stroke. Total cholesterol, triglyceride, or HDL-cholesterol levels were not significantly different among ER genotypes. However, men homozygous for A allele of XbaI polymorphism had a stroke at a younger age compared to other genotypes ($53,3 \pm 8,1$ years vs $56,9 \pm 9,4$ years, $P<0,05$).
Conclusion
These findings suggest that PvuII and XbaI polymorphisms of ER are not associated with the prevalence and severity of cerebrovascular disease. However, the XbaI polymorphism seems to affect the age of developing cerebrovascular disease in men with metabolic syndrome.

P208

Bone mineral density and body composition in pubescent obese children endangered by metabolic syndrome

Borbala Tobisch, Andrea Kozma, Zsuzsanna Nagy-Szakall, Ferenc Peter & Laszlo Blatniczky
Buda Children's Hospital, Budapest, Hungary.

Aims
The prevalence of type 2 diabetes due to insulin resistance is increasing in puberty. Authors have investigated whether body weight excess arises only from increased fat content of the body. Different anthropometrical data were also analysed if they are able to predict degree of insulin resistance.
Materials and methods
108 obese children (50 female, 58 male, Tanner st-s1-5, mean age 12.06 years, mean BMI: 30.97 kg/m^2) with positive familiar anamnesis of metabolic syndrome were in the study. Bone density by PQCT as well as body composition by bioelectrical impedance analyser(inBody 3.0) were measured. Waist/hip-ratio and body fat% based on skin-fold thicknesses measurement were calculated. HOMA-index and ΣC-peptide/Σ*IRI* ratio (ΣC/Σ*IRI*) during oral glucose tolerance test as markers of insulin resistance were calculated
Results
Total Z-score of bone mineral density in obese children exceeded by 0.2 SD and trabecular density by 0.65 SD those of normal population of the same age. Obese children's muscle mass exceeded by 6.8 kg in average compared with same values of "sample" population of the same age. There were slack correlations ($r=0.578$ vs. 0.682) between measured and calculated body fat% as well as measured fat% and BMI. There was no significant correlation between the anthropometrical values and HOMA-index, nor the ΣC/Σ*IRI*. Waist/hip-ratio showed a mild correlation with HOMA-index ($r=0.268$) and a moderate one with ΣC/Σ*IRI* ($r=0.462$).
Conclusions
Increased BMI-values in obese children are partially caused by both increased bone mineral content and higher muscle mass. BMI-values are less helpful to estimate inappropriate body composition. Differences between measured and calculated body fat% can indirectly indicate the degree of visceral fat. Increased waist/hip ratio predicts insulin resistance better. Anthropometrical data themselves do not

predict insulin resistance in youngsters, it has to be determined individually. The $\Sigma C/\Sigma IRI$ is a more exact indicator of insulin resistance than the HOMA-index.

P209

Influence of gaining weight on metabolic syndrome in the menopause

Aleksandra Simoncig-Netjasov, Svetlana Vujovic, Milos Stojanovic, Miomira Ivovic, Milina Tancic & Milka Drezgic
Institut of Endocrinology, Clinical Center of Serbia, Belgrade, Serbia.

Metabolic syndrome (MS) represents a prominent risk factor for cardiovascular disease. Parameters of MS were compared between obese women and controls. I: 50 women (31.92 ± 5.83 kg/m^2; 54.4 ± 3.64 y's); Controls: 37 women (23.50 ± 2.13 kg/m^2; 53.92 ± 3.95 y's). Weight, height, waist and hips circumference, sagital abdominal diameter (SAD) and blood pressure (BP) were measured. Blood was taken at 8 am for: fasting glucose, triglycerides, cholesterol, HDL, LD, Lp(a), FSH, LH, PRL, E2 and OGTT was performed. Hormone analyses: RIA. Statistics: T test, Mann – Whitney U test, ANOVA. MS: 66% in I and 22% in controls. Significant differences between groups were found for: glycose (6.22 ± 2.26 vs 5.49 ± 2.43 mmol/l, $P<0.05$), weight (86.20 ± 17.82 vs 62.81 ± 7.90 kg, $P<0.01$), waist (99.96 ± 14.65 vs 79.9 ± 8.78 cm, $P<0.01$), hips circumference (114.31 ± 11 vs 96.93 ± 11.04 cm, $P<0.01$), SAD (31.9 ± 6.83 vs 24.9 ± 9.86 cm, $P<0.01$), BMI (31.92 ± 5.83 vs 23.5 ± 2.13 kg/m^2, $P<0.01$), diastolic BP (93.08 ± 13.41 vs 85.75 ± 10.54 mmHg, $P<0.01$), Lp(a) (0.50 ± 0.36 vs 0.11 ± 0.03 g/l, $P<0.01$), FSH (54.35 ± 27.16 vs 72.32 ± 30.17 IU/L, $P<0.01$), LH (20.33 ± 11.08 vs 28.77 ± 14.16 IU/L, $P<0.01$), PRL (251.52 ± 142.60 vs 370.27 ± 237.74 nmol/l, $P<0.05$). There are positive correlations between menopausal duration and waist, BMI and BP. Negative correlation was found for BMI, menopausal duration and HDL.

Conclusion

Hypoestrogenic status in the menopausal women shows a shift to a central android fat distribution and MS that can be counteracted by HRT.

P210

Effects of physiological bell-shaped elevations of free fatty acids on glucose metabolism and insulin sensitivity in humans

Lars Gormsen, Charlotte Nielsen, Niels Jessen, Esben Vestergaard, Jens Christiansen, Jens Jørgensen & Niels Møller
Aarhus University Hospital, Aarhus, Denmark.

Background

Physiological elevations of free fatty acids (FFAs) occur in a dynamic bell-shaped fashion lasting some hours, e.g. nocturnally and during exercise. In order to define the metabolic role of physiological elevations in relation to diurnal fluctuations in insulin sensitivity, the present study was designed to identify the metabolic effects of a dynamic 4 hour elevation of FFAs during a glucose clamp.

Materials and methods

8 lean, healthy men were examined twice in a cross-over design: 1) Control (saline), and 2) 4 h graded infusion of intralipid (20%)/heparin. Insulin sensitivity and EGP were assessed by the isotope dilution (3H-glucose) technique during an 8 h hyperinsulinemic-euglycaemic clamp (0.5 mU/kg/min). Before the study, the protocol was approved by the Aarhus County Ethical Scientific Committee; the purpose and potential risks of the study were explained to all subjects; and informed, written consent was obtained from all participants.

Results

Infusion of intralipid caused a significant increase of average FFA levels (Area under the curve (AUC)) compared with saline reaching peak levels $\sim$1.9 mmol/L and markedly impairing insulin sensitivity [iAUCglucose Rate of disappearance (Rd) (mg/kg): 709 ± 25 vs. 380 ± 112, $P=0.04$). There was a lag phase of 300 minutes from initiation of intralipid infusion until glucose Rd was significantly reduced. Glucose Rd returned to control levels after a further 150 minutes. Average insulin sensitivity was negatively correlated with average FFA level ($r^2=0.52$, $P=0.002$). EGP was equally suppressed by hyperinsulinaemia regardless of treatment.

Conclusions

Our data suggest that physiological FFA elevations induce insulin resistance in the periphery after a lag of 4–5 h and that normal insulin sensitivity is restored 1–2 h after FFA values have returned to normal. It is therefore likely that FFA plays an important role in circadian variations of insulin sensitivity (e.g. the Dawn phenomenon and during exercise).

P211

The metabolic changes induced by glucocorticoids: involvement of AMP-activated protein kinase

Mirjam Christ-Crain[1], Blerina Kola[1], Francesca Lolli[1], Csaba Fekete[2], Dalma Seboek[3], Gabor Wittman[2], Sharon Ajodha[1], Judith Harvey-White[4], George Kunos[4], Beat Mueller[3], Giorgio Amaldi[5], Gilberta Giacchetti[5], Marco Boscaro[5], Ashley Grossman[1] & Marta Korbonits[1]
[1]Dept of Endocrinology, William Harvey Research Institute, Barts and The London, Queen Mary School of Medicine, University of London, EC1M 6BQ London, United Kingdom; [2]Dept of Neurobiology, Institute of Experimental Medicine, Hungarian Academy of Sciences, Budapest, Hungary; [3]Dept of Endocrinology, University Hospital Basel, Basel, Switzerland; [4]Laboratory of Physiological Studies, National Institute of Alcohol Abuse and Alcoholism, National Institutes of Health, Bethesda, United States; [5]Dept of Internal Medicine, University of Ancona, Ancona, Italy.

Background

Excess glucocorticoids result in Cushing's syndrome (CS) which is characterised by increased food intake, central obesity, dyslipidaemia and insulin resistance, leading to the metabolic syndrome. AMPK is a regulator of energy homeostasis and plays an important role in the regulation of appetite, glucose uptake, lipogenesis and gluconeogenesis. We hypothesised that the effects of corticosteroids on metabolism would be mediated by changes in AMPK activity in a tissue-specific manner.

Methods

Rats were implanted with corticosterone-containing pellets and consumed chow and 30% sucrose for 2 weeks. Control animals were implanted with cholesterol pellets consuming sucrose or saline only. AMPK activity (kinase assay), metabolic enzyme expression (qRT-PCR) and hypothalamic endocannabinoid content were measured. Human visceral fat tissue of patients with CS was analysed for AMPK activity and compared to controls. In vitro experiments using human *ex vivo* differentiated adipocytes and a human hepatoma cell line.

Results

Corticosterone-treated rats demonstrated higher insulin, leptin, cholesterol and triglyceride levels and an increase in visceral fat weight (to $129 \pm 5\%$ of controls; mean $\pm$ SEM). The AMPK activity in the visceral fat of corticosterone-treated rats and CS patients was significantly lower compared to controls. The gene expression of gluconeogenic and adipogenic enzymes was increased in adipose tissue. The data on AMPK were confirmed in human adipocytes treated with dexamethasone for 24 h. In the liver, fat content was increased concomitant with an increased AMPK activity. In the heart a decrease in AMPK was observed, consonant with the cardiomyopathy observed in humans. In the hypothalamus, AMPK and the endocannabinoid content were increased concordant with the increased appetite typical of CS.

Conclusion

We demonstrate that corticosteroids change AMPK activity in various tissues in a manner that may explain the increase in food intake, lipid deposition in visceral adipose and hepatic tissue and the peripheral cardiac effects of Cushing's syndrome.

P212

Net endogenous acid production and circulating leptin are associated with potentially bioactive free glucocorticoids in healthy lean women

Christiane Maser-Gluth[1], Triantifillia Dimitriou[2] & Thomas Remer[2]
[1]Dept of Pharmacology, University of Heidelberg, Heidelberg, Germany; [2]Research Institute of Child Nutrition, Dortmund, Germany.

Recent evidence suggests that endogenous glucocorticoids (GC) may be suppressed by adipocyte-derived leptin and elevated by dietary acidity. Therefore we examined whether these factors might be predictors of potentially bioactive free glucocorticoids independently of adrenocortical activity.

Body composition, plasma cortisol, plasma leptin, 24-h urinary excretion rates of net acid (NAE) reflecting daily diet-dependent acid load, total nitrogen, urinary free cortisol (UFF), free cortisone (UFE), the main GC metabolites tetrahydrocortisone (THE), tetrahydrocortisol (THF) and 5alpha-tetrahydrocortisol (alloTHF) were examined cross-sectionally in 30 healthy adults (15 females; 22–44 yr old; BMI: 20–25 kg/m). Adrenocortical activity (AA) was assessed by the sum of the 3 major glucocorticoid metabolites (THE+THF+alloTHF), reflecting overall daily cortisol secretion. As a measure of potentially bioactive free GCs (bioactiveGCs) the sum of free cortisol and cortisone in urine (UFF+UFE) was taken, reflecting the free fraction of circulating cortisol and cortisone. Plasma leptin (mean $\pm$ SD, 2.8 ± 1.6 vs. 7.6 ± 4.9 ng/mL) and percent body fat (%BF, 16.8 ± 4.2 vs. $26.9 \pm 4.9\%$) were lower ($P<0.01$) and body surface (BS)-corrected AA higher ($P<0.01$) in males, whereas plasma cortisol and

BS-corrected bioactiveGCs were statistically undistinguishable between the sexes. Both bioactiveGCs and AA correlated positively with %BF and leptin in males ($P<0.05$), but not in females. After adjusting for AA, NAE was a positive ($P=0.011$) and leptin a negative ($P=0.046$) predictor of bioactiveGCs in females (total explained variability $R^2=0.71$). In males only AA explained variation of bioactive-GCs ($R^2=0.49$, $P=0.004$).
Our findings indicate that – at least in females – variability of potentially bioactive glucocorticoids is not only explained by individual adreno-cortical activity, but may also be affected by circulating leptin and diet-dependent daily acid load.

P213

Serum gamma-glutamiltransferase increases in type 2 diabetes mellitus but it is not related with the body mass index

Juan Carlos Ferrer-García, Xelo García-Fabra, Raquel Albalat-Galera, Patricia Sánchez-Llópez, Agustín Herrera-Ballester & Carlos Sánchez Juan
Unit of Diabetes and Endocrinology, Department of Internal Medicine, University General Hospital, Valencia, Spain.

Objectives
We have examined the relationship between the hepatic enzymes and type 2 diabetes. We have analyzed if the levels of hepatic enzymes are associated with body weight, lipid profile and the treatment with metformin, thiazolidinediones or statins.
Methods
318 patients with type 2 diabetes were included and they were compared with 100 healthy subjects. Alanine aminotransferase (ALT), aspartate aminotransferase (AST) and gammaglutamiltranspeptidase (GGT) were measured. We studied the lipid profile, the weight and the treatment with metformin, thiazolidinediones and statins, analyzing possible differences in hepatic function.
Results
Type 2 diabetic patients showed significantly increased levels of GGT that the population control (48.3 ± 5.2 vs 25.6 ± 2.1 U/l respectively; $P<0.01$). The difference was confirmed after adjustment for age, sex and body mass index (BMI). There were no differences in the levels of ALT, AST. Levels of GGT upper the normality were presented in 33.0% of type 2 diabetic patients and 13% of healthy subjects. The diabetic subjects showed higher triglycerides and more reduced LDL-c and HDL-c levels. Lipid profile and body weight were not correlated by the hepatic function. The diabetic patients treated with metformin presented lower levels of ALT (31.3 ± 2.7 vs 22.1 ± 2.2 U/l; $P<0.05$). There were no differences in patients treated with statins or thiazolidinediones.
Conclusions
Increased levels of GGT are closely associated with type 2 diabetes, and this association is independent of the BMI. Metformin has been associated with reduced levels of ALT.

P214

Effects of pharmacological stimulation or blockade of cannabinoid receptor type 1 (CB1) on gene expression in mouse cultured adipocyte cells

Luigi Bellocchio, Cristina Cervino, Flaminia Fanelli, Valentina Vicennati, Renato Pasquali & Uberto Pagotto
Endocrine Unit and C.R.B.A. Hospital S.Orsola-Malpighi, Bologna, Italy.

The endocannabinoid system has recently emerged as an important modulator of several functions of adipose tissue by altering cell proliferation and gene expression. In this work, we investigated the effects of CB1 activation/blockade in mouse 3T3-L1 adypocite cells by using WIN55,212, a CB1/CB2 agonist and rimonabant, a specific CB1 antagonist, in different experimental settings such as acute treatment on pre-adipocytes and on mature adipocytes, and chronic treatment during differentiation process. The gene expression was first analyzed by semi-quantitative RT-PCR and then confirmed by Real-TIME PCR for selected genes. We found that CB1 and FAAH mRNAs were both up-regulated by WIN55,212 and down regulated by SR141716, this effect was stronger in pre-adipocytes than in mature adipocytes. Furthermore, in pre-adipocytes, rimonabant was able to down-regulate PPAR-γ expression, whereas WIN55,212 gave an opposite effect. Moreover, rimonabant was also able to stimulate UCP1 and UCP2 mRNA expression.
Among adipokynes, adiponectin mRNA has been shown to be down-regulated by WIN55,212 and up-regulated by rimonabant, whereas visfatin, apelin and IL-6 mRNAs resulted up-regulated by WIN55,212 and down regulated by rimonabant. In the same cells, rimonabant reduced lipogenic gene expression, in particular of FAS, ACC, LPL, SCD-1, DGAT-2 mRNAs, whereas WIN55,212 up-regulated these genes suggesting a stimulatory role of endocannabinoids on fatty acids and triglycerides biosynthesis.
All together, these results indicate that endocannabinoid system is able to stimulate differentiation of pre-adipocytes towards adipocytes and to directly influence several metabolic processes of these cells including their secretory profile.

P215

Absence of TSH-induced increase in leptin levels in patients with history of differentiated thyroid carcinoma undergoing rhTSH testing

Francesca Cecoli[1], Gabriella Andraghetti[1], Carla Ghiara[2], Lucia Briatore[1], Domenico Cavallero[1], Michele Mussap[2], Francesco Minuto[1] & Massimo Giusti[1]
[1]Dipartimento di Scienze Endocrinologiche e Metaboliche, Azienda Ospedaliera Universitaria San Martino, Genoa, Italy; [2]U.O. Laboratorio Analisi, Azienda Ospedaliera Universitaria San Martino, Genoa, Italy.

Some extra-thyroid effects of TSH have been described *in vitro* and *in vivo*. TSH has recently been suggested to induce IL-6 secretion from adipocytes. Leptin is the main secretory protein from adipose tissue. Our aim was to evaluate the acute effect of rhTSH-induced TSH surge on serum leptin levels in differentiated thyroid carcinoma (DTC) patients. Ten patients (2 m, 8 f; age range 31–66 years) with stage 1-3 DTC were evaluated during scheduled standard rhTSH testing. Leptin, thyroglobulin (Tg) and TSH were measured, before and after rhTSH administration (0.9 mg i.m. for 2 consecutive days). L-T4 therapy ranged from 575 to 1050 µg/week and f-T4 levels ranged from 8 to 23 pg/ml. According to BMI data, only 2 patients were obese. One patient presented a high HOMA-IR (>4). LDL-cholesterol levels were over 130 mg/dl in 50% of patients. Baseline leptin levels were 8.4 ± 1.3 ng/ml. Only BMI correlated significantly ($P=0.05$) with baseline leptin levels. After rhTSH administration, TSH levels increased significantly ($P<0.01$), while thyroid hormones remained unchanged. According to Tg-stimulated levels and neck sonography, all but 2 patients were considered disease-free. Two patients were considered partially ablated after post-surgical radioiodine therapy. On average, leptin levels did not significantly change during rhTSH administration. Twenty hours after the last rhTSH administration, leptin levels were 8.6 ± 1.4 ng/ml, maximal leptin levels being recorded after 1 week (8.9 ± 1.5 ng/ml). No correlation between maximal TSH and leptin levels after rhTSH was noted. In conclusion our *in vivo* experimental model suggests that acute TSH increase after rhTSH testing is uneffective on circulating leptin. These results are in contrast with some literature data reporting an *in vivo* correlation between leptin and TSH in hypothyroid, hyperthyroid and obese subjects.

P216

Serum visfatin in relation to insulin resistance and markers of hyperandrogenism in lean and obese women with polycystic ovary syndrome

Irina Kowalska, Marek Straczkowski, Agnieszka Nikolajuk, Agnieszka Adamska, Monika Karczewska-Kupczewska, Elzbieta Otziomek, Slawomir Wolczynski & Maria Gorska
Department of Endocrinology, Diabetology and Internal Medicine, Medical University of Bialystok, Bialystok, Poland; Department of Reproduction and Gynecological Endocrinology, Medical University of Bialystok, Bialystok, Poland.

Visfatin is a newly discovered protein secreted by adipose tissue, which is suggested to play a role in pathogenesis of insulin resistance. Polycystic ovary syndrome (PCOS) is heterogeneous disorder, where insulin resistance might be involved in the development of endocrine and metabolic abnormalities. The aim of the present study was to asses the relation between serum visfatin and insulin sensitivity and markers of hyperandrogenism in a lean and obese PCOS patients. The study group consisted of 70 women with PCOS (23 lean and 47 overweight or obese) and 45 healthy, normally menstruating women (25 lean and 20 overweight or obese). Euglycemic hyperinsulinemic clamp and the measurements of serum visfatin and sex hormones were performed. PCOS group had lower insulin sensitivity ($P=0.00004$) and higher serum visfatin concentrations ($P=0.026$) in comparison to controls. The decrease in insulin sensitivity was present both in

lean ($P=0.0053$) and in obese ($P=0.017$) PCOS subjects, whereas increase in serum visfatin was observed only in lean PCOS ($P=0.013$). In the whole studied group, serum visfatin was negatively related to insulin sensitivity ($r=-0.27$, $P=0.004$). This relationship was also observed in the subgroup of lean ($r=-0.30$, $P=0.038$), but not obese women. Additionally, in lean women visfatin was associated with serum testosterone ($r=0.47$, $P=0.002$) and free androgen index ($r=0.48$, $P=0.002$), independently of other potential confounding factors. Obtained results pointed out that visfatin could play a role in pathogenesis of PCOS in lean women.

P217

Relationship between serum adiponectin and oxidative and non-oxidative glucose metabolism in apparently healthy humans

Agnieszka Adamska, Marek Straczkowski, Irina Kowalska, Agnieszka Nikolajuk, Monika Karczewska-Kupczewska, Malgorzata Karolczuk-Zarachowicz, Elzbieta Otziomek & Maria Gorska
Department of Endocrinology, Diabetology and Internal Medicine, Medical University of Bialystok, Bialystok, Poland.

The pathogenesis of insulin resistance is not completely understood, however, there are data that it might be associated with altered tissue carbohydrate and lipid oxidation. Adiponectin may be a key regulator of insulin sensitivity and energy metabolism. The aim of the present study was to determine the association of adiponectin, glucose metabolism (oxidation and storage) and lipid oxidation by applying the euglycemic clamp technique and indirect calorimetry.

The study was carried out on 68 young (age 26.38 ± 6.82 yr (mean$\pm$S.D.), BMI: 29.15 ± 7.24 kg/m^2 (mean$\pm$S.D.)) people. Anthropometric and biochemical parameters were measured and oral glucose tolerance test was performed. Plasma adiponectin was measured with radioimmunoassay (RIA) kit. Insulin sensitivity was evaluated with the euglycemic hyperinsulinemic clamp technique. Whole-body fat and carbohydrate oxidation was measured by indirect calorimetry at baseline (in the fasting state) and during last 30 minutes of the clamp. Nonoxidative glucose disposal rate was calculated by subtracting glucose oxidation rate from GDR.

Plasma adiponectin was positively related to insulin sensitivity ($r=0.477$, $P=0.000038$), glucose oxidation at the steady state ($r=0.326$, $P=0.006$) and non-oxidative glucose metabolism ($r=0.424$, $P=0.0003$) and was negatively associated with FFA at the end of the clamp and fat oxidation during hyperinsulinemia ($r=-0.0309$, $P=0.0137$ and $r=-0.260$, $P=0.031$). Insulin sensitivity was positively related to fat oxidation during fasting ($r=0.241$, $P=0.04$) and carbohydrate oxidation during last 30 minutes of the clamp ($r=0.308$, $P=0.001$).

We conclude that adiponectin modulates insulin sensitivity probably through influencing both oxidative and non-oxidative glucose metabolism.

P218

Relationships between serum adiponectin, interleukin 10 and interleukin 18 concentrations and muscle lipid fractions in healthy humans

Marek Straczkowski[1], Irina Kowalska[1], Agnieszka Nikolajuk[1], Marcin Baranowski[2], Agnieszka Adamska[1], Malgorzata Karolczuk-Zarachowicz[1], Monika Karczewska-Kupczewska[1], Piotr Zabielski[2], Agnieszka Blachnio[2], Jan Gorski[2] & Maria Gorska[1]
[1]Department of Endocrinology, Diabetology and Internal Medicine, Medical University of Bialystok, Bialystok, Poland; [2]Department of Physiology, Medical University of Bialystok, Bialystok, Poland.

Intramuscular lipids, including ceramide, might be responsible for the development of insulin resistance. Insulin action is also inversely associated with circulating proinflammatory cytokines, like interleukin (IL)-18 and positively related to antiinflammatory factors, like adiponectin and IL-10. The aim of the present study was to estimate the relationships between serum adiponectin, IL-10 and IL-18 concentrations and muscle lipid fractions in healthy humans.

The study group consisted of 37 male subjects with normal glucose tolerance, without morbid obesity or other serious medical problems. Euglycemic hyperinsulinemic clamp and a biopsy of vastus lateralis muscle were performed. Muscle ceramide, sphingosine and sphinganine content and the activities of the enzymes: neutral and acid sphingomyelinase, neutral and alkaline ceramidase and serine palmitoyltransferase were measured. Muscle free fatty acid (FFA), diacylglycerol (DAG) and triacylglycerol (TG) content was also assessed.

Insulin sensitivity was related to circulating cytokines (adiponectin, $r=0.38$, $P=0.021$; IL-10, $r=0.47$, $P=0.0034$; IL-18, $r=-0.37$, $P=0.023$) and to muscle lipids (ceramide, $r=-0.45$, $P=0.024$; DAG, $r=-0.43$, $P=0.031$; TG, $r=-0.52$; $P=0.01$). It was also associated with the activities of the enzymes regulating ceramide metabolism (serine palmitoyltransferase, $r=-0.58$, $P=0.002$; alkaline ceramidase, $r=-0.37$, $P=0.025$). Adiponectin was negatively related to muscle ceramide content ($r=-0.44$, $P=0.027$) and to serine palmitoyltransferase activity ($r=-0.35$, $P=0.032$). IL-10 and IL-18 were associated, in an opposite manner, with muscle DAG (IL-10, $r=-0.46$, $P=0.022$; IL-18, $r=0.40$, $P=0.049$) and muscle TG (IL-10, $r=-0.50$, $P=0.014$; IL-18, $r=0.46$, $P=0.026$). IL-10 was also related to muscle FFA pool ($r=-0.51$, $P=0.026$).

We conclude that there are multiple associations between circulating cytokines and muscle lipid pool, which possibly might influence insulin sensitivity. Adiponectin is related to muscle ceramide content, mostly through an association with de novo synthesis pathway. IL-10 and IL-18 are associated with muscle FFA-DAG-TG pathway.

P219

Prevalence of metabolic syndrome in old men and its relation to ghrelin

Sergio Rueda Alfaro[1], Mateu Serra-Prat[2], Cristián Fernández Fernández[1], Elisabet Palomera[2], Roser Casamitjana[1] & Manel Puig Domingo[1]
[1]Hospital Clinic i Provincial de Barcelona, Barcelona, Spain; [2]Hospital de Mataró, Mataró, Spain.

Aim

To study the prevalence of metabolic syndrome (MS) and its relation with ghrelin in old men.

Material and methods

Prospective-population based study (2002–2005) in which 153 independently living men older than 70 y were included. Comorbidities, physical exam, BMI, blood pressure were recorded and blood sample taken for biochemical and hormonal determinations. Metabolic syndrome was defined using IDF criteria.

Results

MS was found in 54.9%; BMI in non-MS individuals was 25.8 ± 3.3 and in MS was 28.3 ± 3.7 ($P<0.001$). No association was found between ghrelin and MS at basal evaluation (non-MS 1185 ± 445 vs MS 1106 ± 368; p:ns), even after weight adjustment.

At 3 years follow-up ghrelin level in MS were lower than in non-MS individuals (non-MS 1165.8 ± 356.0 vs MS 988.4 ± 245.8; P:0.004). Differences between ghrelin levels at the two time-points was only statistical significant in MS group (P:0,006). Ghrelin correlated with BMI ($r=-0.22$; $P=.023$) in subjects between 70–80 years and with creatinina <1.5 mg/dl. Also a correlation was found with HDL ($r=0.21$; $P=.012$). Multiple lineal regression analysis showed than age (beta$=-12.1$; $P=.049$), BMI (beta$=-22.0$; $P=.021$) and creatinine (beta$=407.7$; $P=.002$) had an independent effect on circulating ghrelin.

Conclusions

MS in old men is associated to a decrease in circulating ghrelin over time.

P220

Antipsychotic drugs and associated metabolic disorders

Kolcsár Melinda, Nagy Réka, Nagy Imre, László Annamária & Kun Imre Zoltán
U.M.Ph – Department of Pharmacology and Endocrinology, Tg.Mures, Romania.

Obesity is a major contributor to a range of metabolic disorders responsible for much of the medical morbidity and mortality. Increasing numbers of reports concerning not only obesity, but diabetes, hyperglycaemia and lipid dysregulation in patients treated with antipsychotics have raised concerns about a possible association between these metabolic effects and treatment with these medications. The objective of our study was to investigate the prevalence of obesity and other metabolic disorders in young patients treated with different antipsychotics and in the age matched general population.

Antropometric and metabolic data of the patients treated with psychotropics, hospitalized in the Endocrinology Clinic, Tg. Mures, between years 2001–2005 were compared with the data of persons selected among patients hospitalized in the same clinic and period, without psychotropic drug use. The frequency of patients treated with antipsychotics was 10.92% (4.33% typical antipsychotics and 6.59% atypical antipsychotics) with 43.1±13.6 years of mean age. In this patients the prevalence of obesity, elevated total cholesterol and triglyceride level was significantly higher than in the control group. The blood sugar didn't present difference between the two groups, but measuring HOMA-IR in 25 patients treated with atypical psychotropics and 20 other persons without treatment with psychotropic drug we found a significant difference between them. We concluded that a complete metabolic syndrome (MS) was present in 34.2% of the patients treated with antipsychotic drugs, while the frequency of MS was only 18.7% in the age matched patients group without any psychotropic drug use. Atypicial antipsychotics causes the most severe metabolic disorders in association with a significantly elevated prolactin level, when compared with the control group. The choice of a second generation antipsychotic for a given patient depends on many factors. The likehood of developing severe metabolic disease should also be an important consideration.

P221

Associations between thyroid function parameters and adipokines in euthyroid individuals

Laszlo Bajnok[1], Ildiko Seres[2], Zsuzsa Varga[2], Sara Jeges[1], Attila Peti[1], Zsolt Karanyi[2], Attila Juhasz[2], Eva Csongradi[2], Emese Mezosi[1], Endre V Nagy[2] & Gyorgy Paragh[2]
[1]School of Medicine, University of Pécs, Pécs, Hungary; [2]University of Debrecen, Medical and Health Science Center, Debrecen, Hungary.

Adipose tissue secreted hormonal mediators, adipokines play pivotal roles in the regulation of, among others, central nervous and immune systems influencing body weight, insulin action and inflammatory responses. The aim of our present study was to investigate possible associations of thyroid function with plasma levels of three of known adipokines, i.e. leptin, adiponectin and resistin in 74 Caucasian subjects without any endocrine diseases or related therapy. In order to create broad adipokine ranges, 3 age-, and sex-matched groups were formed: Group 1 and 2 consisted of non-diabetic obese patients ($n=25$ with BMI: 28–39.9 kg/m^2, $n=25$ with BMI $\geq$40 kg/m^2, respectively), while Group 3 of 24 healthy, normal weight control subjects. Level of TSH was correlated negatively with leptin ($r=-0.26$, $P<0.05$), while positively with adiponectin ($r=0.28$, $P<0.05$). Both were independent predictors of TSH level in a multiple regression model including BMI, age, gender, FT_3 or FT_4. But when both leptin and adiponectin were included into the model, only the latter remained significant. In opposite to TSH, level of FT_3 was negatively associated with adiponectin ($r=-0.27$, $P<0.05$) and showed a positive trend with leptin ($r=0.26$, $P=0.06$) of which the latter was independent predictor in multivariate analyses, beside age, BMI and FT_4. FT_4 was not correlated with any of adipokines. In univariate analysis, neither BMI, nor resistin was significantly correlated with thyroid function parameters.
In conclusion, in individuals without thyroid illness, leptin and adiponectin plasma levels are associated with TSH and FT_3 concentrations in opposite ways, and partly independently of anthropometric parameters. Adipokines may participate in the regulation of thyroid hormone axis.

P222

Human adipose tissue derived DPP-IV regulates lipolysis through NPY in cultured abdominal subcutaneous adipocytes

Katarina Kos, Adam Baker, Alison Harte, Philip McTernan, Paul O'Hare & Sudhesh Kumar
University of Warwick, Coventry, United Kingdom.

We have previously shown that the orexigenic hormone NPY is secreted by human adipocytes. The orexigenic hormone NPY(1–36) is truncated by the dipeptidyl-inhibitor IV (DPP-IV) to NPY(3–36) as consequence its affinity changes from receptor Y1 to Y4 and 5. The aim was to investigate whether DPP-IV is expressed in adipose tissue (AT) where it could modulate adipose tissue growth through modulation of NPY activity. This is relevant in light of DPP-IV inhibitors utilised as therapeutic agents and their use for treatment in Type 2 diabetes. For this purpose *ex vivo* human abdominal AT was taken from women undergoing elective surgery (BMI: 27.5(mean±S.D.)±5 kg/m^2, Age: 43.7±10 yrs, $n=18$). Isolated AbdSc adipocytes were treated with 1–100 nM rhNPY with and without DPP-IV inhibitors; a glycerol release assay was used as an index of lipolysis and DPP-IV mRNA expression assessed in AbdScAT. Treatment with NPY reduced glycerol release which was further blunted by co-incubation with DPP-IV inhibitors (baseline 234(mean±S.E.)±23 μmol/l, NPY100: 187±30μmol/l*; NPY100 with DPP-IV: 121±14 μmol/l**, *$P<0.01$, **$P<0.01$, $n=8$). Relative DPP-IV mRNA expression was reduced in AbScAT taken from obese subjects versus lean subjects (obese: 77±6 SU versus lean: 186±29 SU*, $n=10$).
In conclusion, paracrine effects of NPY may be modulated by AT-derived DPP-IV. Thus DPP-IV inhibitors may have little effect on tissue mass regulation in the obese where endogenous DPP-IV from AT is reduced, but may enhance fat accumulation in the lean through enhanced antilipolytic effects of NPY, which requires further study.

P223

The role of nitric oxide in pathogenesis of development of arterial hypertension during obesity

Diana Giorgadze[1], Ellen Giorgadze[1], Tamar Doliashvili[1], Tamar Sanikidze[2], Ketevan Asatiani[1], Ketevan Bochorishvili[1] & Arkadi Surmava[1]
[1]N4 clinical hospital, Tbilisi, Georgia; [2]State Medical University, Tbilisi, Georgia.

Background
The cardiovascular complication is the main cause of morbidity and mortality in obese patients. Endothelial dysfunction and atherosclerosis have the goal role in development of these diseases. The aim of our study was to reveal the role of nitric oxide during obesity associated arterial hypertension.
Subject and method
200 obese patients (age 35–55) were investigated. Control group comprised 25 healthy subjects. We calculated BMI, determined lipid profile, concentration of nitric oxide, activity of antioxidant enzymes – superoxiddismutase and katalaze, evaluated arterial pressure.
Results
Systolic arterial pressure insignificantly increased in overweight group ($n=50$) compared to control group (124.3±5.6 mm/hg), but significantly ($P<0,05$) – in patients with obesity of I ($n=50$) (134.4±11.7 mm/hg), II ($n=50$) (142.6±12.6 mm/hg) and III ($n=50$) (145.7±10.3 mm/hg) degree. Diastolic arterial pressure significantly ($P<0.05$) increased in patients with obesity of II (91.8±9.4 mm/hg) and III (95.6±7.2 mm/hg) degree compared to control group (81.4±6.2 mm/hg). According to weight gain the whole lipid profile (Chol, Trig, HDL, LDL) was damaged. Concentration of nitric oxide significantly reduced in obese subjects compared to control group. Significant decrease of nitric oxide in different BMI groups was revealed (overweight-11.875±0.427, I degree-11.2154±0.3113, II degree-10.2364±0.381, III degree-9.5±0.2823 $P<0,001$). Changes in concentration of NO correlated with decrease of antioxidant enzymes activity (enzymes activity decrease compared to control group and increase according to weight gain).
Conclusion
Hyper generation of oxygen causes inactivation of antioxidant enzymes and disorders in redox-status. NO oxidative degradation, stimulated by dyslipidemia, has the main role in the pathogenesis of arterial hypertension development during obesity.

P224

Effects of PGC-1α on endothelial function and apoptosis

Joong-Yeol Park[1], Jong Chul Won[1], Ki-Up Lee[1], Woo Je Lee[2], Kyung Soo Ko[2], Kee-Ho Song[3] & In-Kyu Lee[4]
[1]University of Ulsan College of Medicine, Seoul, South Korea; [2]Inje University College of Medicine, Seoul, South Korea; [3]Konkuk University School of Medicine, Seoul, South Korea; [4]Kyungpook National University, Daegu, South Korea.

Aims
Central obesity is associated with increased cardiovascular morbidity and mortality. It has been proposed that increased lipid accumulation in vascular tissue and the consequent increase in oxidative stress may be a missing link between obesity and atherosclerosis. The peroxisome proliferators-activated receptor (PPAR)-γ coactivator 1-α (PGC-1α) is a transcriptional coactivator playing an important role in energy metabolism. PGC-1α is present in vascular

cells, but its role in vascular endothelial cells has not been established. In this study, we examined the effect of adenoviral overexpression of PGC-1α (Ad-PGC-1α) in human aortic endothelial cells (HAECs) on apoptosis induced by linoleic acid (LA).
Methods
Effect of PGC-1 on HAECs apoptosis was evaluated by ELISA, WST-1 assay, and caspase activity. Using Ad-PGC-1 and ANT-1 siRNA, effect of PGC-1 and ANT-1 on reactive oxygen species (ROS) production, fatty acid oxidation (FAO) and mitochondrial membrane potential ($\Delta\psi m$) were analyzed.
Results
PGC-1α prevented LA-induced endothelial apoptosis. PGC-1α also reduced LA-induced increases of antioxidant enzyme expression and ROS accumulation at basal state. LA decreased the activity of adenosine nucleotide translocase (ANT), and increased $\Delta\psi m$. In the Ad-PGC-1α-infected HAECs, activity and the mRNA expression of ANT-1 were increased and LA did not increase $\Delta\psi m$. siRNA against ANT-1 reversed the changes induced by PGC-1α.
Conclusion
These data suggest that PGC-1α functions as a physiologic regulator of ROS generation in endothelial cells and that part of this effect is mediated by ANT-dependent increase in FAO.

P225

Changes in serum glucose metabolism and growth hormone, cortisol, prolactin, ghrelin, leptin concentrations in normal weight patients with schizophrenia before treatment with atypical antipsychotics

Mirjana Doknic[1], Nadja Maric[2], Sandra Pekic[1], Aleksandar Damjanovic[2], Dragana Miljic[1], Marko Stojanovic[1], Marina Djurovic[1], Miroslava Jasovic-Gasic[2], Carlos Dieguez[3], Felipe Casanueva[4] & Vera Popovic[1]
[1]Institute for Endocrinology, Diabetes and metabolic Diseases, Belgrade, United States; [2]Institute for Psychiatry, Belgrade, United States; [3]Departement of Physiology, School of Medicine, and Comlplejo Hospitalario Universitario de Santiago, Santiago de Compostela, Spain; [4]Departement of Medicine, School of Medicine, and Complejo Hospitalario Universitario de Santiago, Santiago de Compostela, Spain.

Schizophrenia is a devastating mental illness associated with obesity and diabetes mellitus rates that far exceed those of the general population.
The aim was to evaluate changes in positive energy balance (serum insulin, leptin and ghrelin) and hormones involved in neuroendocrine regulations (serum cortisol, growth hormone and prolactin) before treatment with atypical antypsyhotics (SGA) in normal weight patients with schizophrenia.
Thirty patients with schizophrenia (13 males, mean age 28.9 ± 1.3 years and BMI, 23.3 ± 0.6 kg/m^2) treated with antypsyhotics first generation were investigated in this study. They had neither other diseases. The control group included 27 healthy subjects (9 males, mean age 30.7 ± 1.9 years, BMI od 22.8 ± 0.6 kg/m^2). Positive family history for diabetes mellitus was similar between groups.
A oral glucose tolerance test (OGTT) with measuring glycemia, insulin, growth hormone and ghrelin was performed in all patients. Fasting samples for leptin, cortisol and prolactin were taken. Patients had normal fasting glucose levels but significantly higher peak glucose levels during OGTT as well as glucose area under the curve (AUC) than control subjects (746 ± 25 vs 650 ± 26 mmol/L/120 min; $P < 0.01$). Fasting insulin levels, as well as insulin AUC did not differ from control subjects at baseline ($P > 0.05$) but peak insulin values were significantly higher in patients with schizophrenia (95.1 ± 14.8 vs 52.2 ± 6.5 mU/L, $P < 0.05$). Growth hormone (GH) and ghrelin levels during OGTT, and leptin concentrations did not differ between patients and control subjects ($P > 0.05$). Cortisol levels (513.3 ± 29.1 vs 441.9 ± 24.3 nmol/L; $P < 0.05$) were higher in patients. Prolactin levels were higher in patients with schizophrenia than in control subjects (821 ± 135 vs 353 ± 45 mU/L; $P < 0.01$).
Normal weight patients with schizophrenia have already some abnormalities in glucose metabolism therapy and neuroendocrine responses (cortisol, prolactin) before SGA. Thus, shizophrenia could be *per se* risk factor for diabetes mellitus.

P226

Frequency of hypogonadism in males with type 2 diabetes and its relation with erectile dysfunction and obesity

Katherinne García-Malpartida, Rosa Casañ-Fernández, Jesús Yanini-García, Ana Jover-Fernández, Marcelino Gómez-Balaguer, Eva Solá-Izquierdo, Carlos Morillas-Ariño & Antonio Hernández-Mijares
Hospital Universitario Dr. Peset, Valencia, Spain.

Introduction
The aim of our study was to determine the frequency of hypogonadism (H) in males with type 2 diabetes (T2D) and its relation with erectile dysfunction (ED) and obesity.
Methods
We studied 107 diabetic males who came successively to an Endocrine consultation. The presence of H was determined by total testosterone (T) with an immunofluerescence method and free testosterone (fT) calculated with Vermeulen's equation, defining H if $T < 2$ ng/ml or $fT < 250$ pmol/l, with LH, FSH and prolactine in the normal range. We studied ED by means of the International Index of Erectile Dysfunction (IIEF) (questions 1 to 5 and 15 that determine ED). We excluded patients taking drugs that cause ED and those diagnosed of severe autonomic neuropathy. The anthropometic parameters analyzed were weight, height, waist perimeter and the calculated body mass index (BMI).
Results
We included 107 patients, aged 55.1 ± 7.8 years (range 39–70) with an average of duration of T2D of 8.2 ± 8.1 years (range 1–32). The frequency of H was 22.4%. The average of LH was 3.7 ± 1.7 mU/ml (range 1.1–9.5), FSH 5.1 ± 2.3 mU/ml (range 1.2–13.3) and prolactine 8.5 ± 2.9 ng/ml (range 2.9–16.5). ED was present in 66.7% of hypogonadal males and 66.7% of patients not presenting H. Patients with H had more weight (93.2 ± 11.9 vs 84.8 ± 13.8 kg, $P = 0.016$), more BMI (31.8 ± 3.8 vs 29.6 ± 3.8 kg/m^2, $P = 0.025$) and more waist perimeter (111.1 ± 9.2 vs 104.7 ± 10.7 cm, $P = 0.028$), compared to patients without H. The table below show the means of T and fT according to BMI:

BMI (kg/m^2)	<25	25–30	30–35	35–40	*P*-value
T (ng/ml)	5.9	5.1	4.5	3.8	<0.05
fT (pmol/l)	440.9	336.3	309.8	296.6	<0.05

Conclusions
The frequency of H is 22.4%. ED appears in the same proportion in patients with and without H. Hypogonadal patients are more obese and there is an inverse relation between BMI and T and fT.

P227

Acute phase reactants and soluble cell adhesion molecules are associated to plasma leptin levels in obese nondiabetic children

Barbara Garanty-Bogacka, Malgorzata Syrenicz, Anhelli Syrenicz, Aneta Gebala, Grazyna Dawid & Mieczyslaw Walczak
Pomeranian Medical University, Szczecin, Poland.

There are increasing evidences that leptin, a protein secreted by adipose tissue, may be an important factor contributing to the development of atherosclerosis. In this study, the relationship between plasma leptin levels and markers of inflammation and endothelial activation was investigated in 214 obese nondiabetic children and adolescents. Fasting levels of leptin, C-reactive protein (CRP), fibrinogen (FB), interleukin-6 (IL-6), interleukin-1β (IL-1β), intercellular cell adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), von Willebrand factor (vWF), glucose and insulin were determined. Insulin resistance was assessed by the homeostasis model. At multiple regression analysis leptin predicted IL-6, FB, ICAM-1, VCAM-1 and vWF independently of obesity measures and HOMA IR. There was a trend for association between leptin and CRP concentrations. Therefore, our findings showed that leptin levels is associated with inflammation and endothelial activation markers and in such way may promote the development of attherosclerosis relatively early in life

P228

Relationship between homocysteine level and low-grade systemic inflammation in obese children with metabolic syndrome

Justyna Syrenicz & Barbara Garanty-Bogacka
Pomeranian Medical University, Szczecin, Poland.

Obesity is an independent risk factor for the development of cardiovascular disease, frequently associated with various metabolic disorders defined as metabolic syndrome. High plasma total homocysteine (Hcy) concentration is

now well established as a powerful risk indicator for a wide range of vascular diseases.
The aim of this study was to investigate total Hcy levels in obese children and their possible association with both metabolic syndrome and various inflammatory biomarkers.
The study group consisted of 61 obese children, (aged 6–18 y.) with metabolic syndrome, defined according to NCEP-ATP III criteria and 122 obese counterparts without metabolic syndrome. Both group were comparable regarding to age, sex, and pubertal development.
The obese subject with metabolic syndrome presented significantly higher values for fasting insulin ($P<.001$), HOMA IR ($P<.001$), C-reactive proteine ($P<.01$), interleukin-6 ($P<.001$), interleukin-1ß ($P<.01$), and WBC ($P<.001$). In the group with metabolic syndrome plasma Hcy concentration was positively correlated with insulin ($P<.001$), HOMA IR ($P<.01$), C-reactive protein ($P<.001$), interleukin-6 (P,.01) and WBC (P,.05), but not in the group without metabolic syndrome.
Elevated plasma Hcy level in obese children with metabolic syndrome, may be causally involved in the pathogenesis of cardiovascular disease.

Obesity and metabolism – presented on Tuesday

P229

Oxidative stress and antioxidant defense is associated with adiposity in men among the urban population of south Iran

Fariborz Haghparast & Jaffar Nourooz-Zadeh
Islamic Azad University-Larestan Branch, Larestan, Fars, Iran.

Introduction
Changes in lifestyle have resulted in an increased number of boese subjects, and obesity is currently an important causative factor of health-related problems in Iran.
Aims
To investigate the direct relationship of oxidative stress and antioxidant status with obesity in men.
Materials and methods
We measured the plasma levels of malondialdehyde (MDA) as a marker of oxidative stress and vitamin E, glutathione and superoxide dismutase as antioxidants in 44 obese and 47 no obese men and evaluated their relationship with body mass index (BMI); body fat weight; waist-to-hip ratio (WHR).
Results
Compared with controls, obese men had a significantly higher body mass index (28.97 ± 2.42 vs. 16.03 ± 1.88 kg/m^2; $P=0.0002$) and waist-to-hip ratio (WHR) (0.89 ± 0.03 vs. 0.80 ± 0.01; $P=0.0004$); vitamin E, glutathione, superoxide dismutase, vitamin C levels were significantly decreased (all $P<0.05$), whereas MDA was significantly increased (114.9 ± 21.4 vs. 64.3 ± 14.2 nmol/L; $P=0.001$). MDA significantly correlated with BMI ($r=-0.34$ ($P=0.004$)) and WHR ($r=-0.63$ ($P=0.0001$)). We calculated the amount of vitamin E per LDL-cholesterol, total cholesterol and total lipids, we found all of them, significantly lower levels in obese men as compared to controls. There was also a significant correlation between the plasma levels of MDA and vitamin E, vitamin C, glutathione and superoxide dismutase in obese men and all men (all $P<0.01$).
Conclusion
In brief, these findings showed that the circulating levels of oxidative stress are related to adiposity in men. Although correlation does not prove causation, the results of this study suggest that obesity is an important factor for enhanced oxidative stress and important role of oxidative stress deleterious impact.

P230

Ghrelin basal levels in metabolic syndrome

Tatiana Mokhort
Belarusian State Medical University, Minsk, Belarus; State Research Center for Radiation Medicine and Human Ecology, Gomel, Belarus.

Ghrelin is known to play an important role in overweight formation and glucose metabolism regulation. The aim was to assess ghrelin basal secretion features in persons with metabolic syndrome (MS).
We examined 39 patients (age 35–55years) with MS (IDF criteria) and 28 healthy persons of comparable age. Ghrelin, insulin and C-peptide serum concentrations were measured by immunoenzyme method, lipid spectrum parameters - by spectrophotometry. For IR assessment we used HOMA-IR and Reciprocal of HOMA-IR indexes.
Basal insulinemia and C-peptide levels in S significantly exceeded the ones in healthy persons: 21.3 ± 3.86 vs 9.96 ± 1.18 mU/l and 2.86 ± 0.56 vs 1.28 ± 0.76 ng/ml. HOMA-IR in MS significantly exceeded the value of control group (5.03 ± 1.03 vs 2.06 ± 0.23). Reciprocal of HOMA-IR showed the opposite results. Ghrelin level was significantly lower in MS 61.06 ± 11.9 vs 88.76 ± 16.9 ng/ml in control group. Progressive decrease of ghrelin from 71.59 ± 7.09 to 50.34 ± 6.58 ng/ml was marked at BMI increase that is confirmed at correlation analysis: ghrelin levels negatively correlated with BMI ($r=0.41$; $P<0.05$), waist-to-hip ratio ($r=0.37$; $P<0.05$) and waist circumference ($r=0.39$; $P<0.05$). Ghrelin levels also showed negative correlation with systolic ($r=0.40$; $P<0.01$) and diastolic blood pressure ($r=0.39$; $P<0.01$).
We observed significant negative correlation of ghrelin and insulin ($r=0.18$), C-peptide ($r=0.15$), HOMA-IR ($r=0.23$) and positive with Reciprocal of HOMA-IR ($r=0.22$). We revealed significant negative correlation of ghrelin and atherogenecity index ($r=0.32$), while there was no significant connection with other parameters of lipid spectrum.
Conclusion
Progressive decrease of basal ghrelin levels with increase of BMI, waist-to-hip ratio and waist circumference was revealed that can testify to ghrelin influence on formation of visceral obesity. Obtained results are proved by negative correlation of ghrelin level with basal insulinemia, HOMA-IR and positive one with Reciprocal of HOMA-IR that confirms ghrelin role in formation of insulin resistance in MS and dictates essential necessity for further studies.

P231

Pioglitazone treatment significantly decreases 5-alpha reductase activity and improves metabolic risk factors in PCOS

Dorte Glintborg[1], Anne Pernille Hermann[1], Claus Hagen[1], Johannes Veldhuis[2], Anne Schmedes[3], Jan Frystyk[4], Allan Flyvbjerg[4] & Marianne Andersen[1]
[1]Department of Endocrinology and Metabolism, Odense University Hospital, DK-5000 Odense C, Denmark; [2]Division of Endocrinology and Metabolism, Department of Internal Medicine, Mayo Clinical Research Center, Rochester, United States; [3]Department of Clinical Chemistry, Vejle Hospital, DK-7100 Vejle, Denmark; [4]Medical Research Laboratories, Clinical Institute and Medical Department M (Diabetes and Endocrinology), Aarhus University Hospita, DK-8000 Aarhus, Denmark.

Objective
To investigate the effect of pioglitazone on cortisol metabolism in PCOS.
Design
Thirty insulin resistant PCOS patients were randomized to either 16 weeks of pioglitazone (30 mg/day) or placebo treatment. Before and after intervention, patients underwent 24 h 20 min.-integrated blood sampling for measurement of cortisol and 24 h excretion of cortisol, cortisone and steroid metabolites (cortisol, corticosteron, androgen, and 17-hydroxyprogesteron) were measured in urine. Fasting insulin, adiponectin, testosterone, dihydrotestosterone (DHT), and dehydroepiandrosteronsulfate (DHAS) was measured. 5-alfa reductase activity was evaluated by alloTHF/THF and androsteron/ethiocholanolon ratios. Delta values (Δ) denoted changes during the treatment period.
Results
Pioglitazone treatment was followed by significantly decreased 5-alfa reductase activity as evaluated by alloTHF/THF levels. Δ-androsteron/ethiocholanolon showed a significant negative correlation with Δ-IGF-I and Δ-peak GH level during PD-GHRH test. Furthermore, a significant negative correlation was found between Δ- alloTHF/THF and Δ- adiponectin levels.
No significant changes were measured in 24 h mean cortisol levels or urine excretion of cortisol, cortisone or steroid metabolites.
Insulin sensitivity, GH, adiponectin, and IGF-I was significantly increased during pioglitazone treatment.
Conclusion
Pioglitazone treatment was followed by significantly decreased 5-alfa reductase activity which was inversely correlated with IGF-I, GH, and adiponectin levels. These results suggest important relations between 5-alfa reductase activity and the GH/IGF-I system as well as metabolic risk factors.

P232

Plasma adiponectin and leptin levels in menopausal metabolic syndrome

David Virsaladze[1], Nona Adamia[1], Nino Charkviani[1], Ketevan Khitarishvili[1], Tea Giorgadze[1] & David Tananashvili[2]
[1]Tbilisi State medical University, Tbilisi, Georgia; [2]E. Andronikashvili Institute of Physics, Tbilisi, Georgia.

Objective
The aim of our investigation was the study of blood adiponectin and leptin levels in patients with menopausal metabolic syndrome (MMS) and their correlation with the parameters of MMS features.
Methods
40 females with menopausa have been investigated. In 38 cases diabetes mellitus type 2 has been registered, and in 2 – impaired glucose tolerance. Mean duration of postmenopausal period was 11.1 ± 7.4. Control group consisted of 10 females of postmenopusal age. The blood content of adiponectin and leptin was measured by ELISA. For MMS diagnostics WHO classification (2002) was applied.
Results
In basic group MMS was revealed in 37 patients, in control group – in 3 cases ($\chi^2 = 19.53$, $P < 0{,}001$). It was not observed significant difference in blood adiponectin levels of basic and control groups (16.4 ± 7.6 vs. 16.3 ± 6.1, $P =$ NS), but blood leptin level was significantly higher in investigated group in comparison with control (166.7 ± 105.3 vs. 60.3 ± 51.0, $P < 0.001$). It was revealed significant correlations of blood adiponectin and leptin levels with the parameters of MMS features.
Conclusions
Obtained results show that blood adiponectin level in MMS does not differed from control value. Blood leptin level is significantly higher than control one. They significantly correlated with the parameters of MMS features.

P233

Serum interleukin 6 and soluble form of interleukin 6 receptor concentrations in obese subjects with impaired glucose tolerance

Agnieszka Nikolajuk, Marek Straczkowski, Irina Kowalska, Agnieszka Adamska, Ida Kinalska & Maria Gorska
Department of Endocrinology, Diabetology and Internal Medicine, Medical University of Bialystok, Bialystok, Poland.

Background
Obesity is associated with an increased risk of impaired glucose tolerance and type 2 diabetes. Insulin resistance is the link between obesity and disturbances of glucose metabolism. It is suggested that some substances secreted by adipose tissue might play a role in the pathogenesis of insulin resistance. One of these substances is interleukin-6 (IL-6), cytokine, which regulates synthesis of the acute-phase proteins in the liver. The aim of the present study was to estimate serum IL-6, soluble form of IL-6 receptor (sIL6-R) and C-reactive protein concentrations (hs-CRP) in obese subjects with normal and impaired glucose tolerance.
Methods
The study group consisted of 107 subjects, 28 obese with impaired glucose tolerance (IGT), 44 obese with normal glucose tolerance (obese-NGT) and 35 lean healthy controls. Insulin sensitivity was measured with euglycemic hyperinsulinemic clamp technique. The protocol was approved by Ethics Committee of Medical University, and informed consent was obtained from each subject.
Results
IGT subjects had lower insulin sensitivity index in comparison to obese-NGT and controls (both $P < 0.000001$), and obese-NGT subjects had lower insulin sensitivity in comparison to controls ($P = 0.00043$). We found higher IL-6 and hs-CRP concentrations in IGT group in comparison to obese-NGT ($P = 0.042$ and $P = 0.041$ respectively) and to controls ($P = 0.00056$ and $P < 0.000001$ respectively). Differences in sIL-6R concentration between IGT subjects and the remaining groups were approaching the level of significance (obese-NGT, $P = 0.087$, control, $P = 0.066$). We found significant correlations between insulin sensitivity index and IL-6 ($r = -0.21$, $P = 0.029$), sIL-6R ($r = -0.19$, $P = 0.049$) and hs-CRP ($r = -0.34$, $P = 0.001$). IL-6, sIL-6R and hs-CRP were also associated with fasting insulin and with post load glucose and insulin concentrations. IL-6 and hs-CRP were also related to triglycerides and HbA1c and IL-6 was related to HDL-cholesterol.
Conclusions
Our data indicate that IL-6/sIL-6R system might play a role in the development of insulin resistance in obese subjects with IGT.

P234

Association of sex hormone-binding globulin (shbg) levels with measures of adiposity and metabolic profile in apparently healthy individuals

Katerina Saltiki[2], Paraskevi Voidonikola[3], Kimon Stamatelopoulos[3], Emily Mantzou[1], Eleftheria Chryssohoou[3], Christos Papamichael[3] & Maria Alevizaki[2]
[1]Endocrine Unit, Evgenidion Hospital, Athens University School of Medicine, Athens, Greece; [2]Dept Medical Therapeutics, Alexandra Hospital, Athens University School of Medicine, Athens, Greece; [3]Vascular Laboratory, Dept of Medical Therapeutics, Alexandra Hospital, Athens University School of Medicine, Athens, Greece.

Objective
The association of SHBG levels with obesity, hyperinsulinemia and metabolic abnormalities is well recognized in both men and women. Recent data suggest that SHBG levels are an important predictor of cardiovascular disease (CVD) risk. Several methods have been used for the measurement of adiposity including ultrasonography (U/S) which is a reliable and low-cost method. We used U/S to assess regional adiposity and investigated possible associations with SHBG levels.
Methods
309 apparently healthy individuals (124 men and 185 women, mean age 43.9 ± 9) without a history of diabetes or hypertension were examined for indices of the metabolic syndrome. None of the subjects was taking hormone therapy. The thickness of abdominal subcutaneous and peritoneal fat layer was estimated by U/S. Clinical parameters of obesity such as waist and hip circumference and BMI were recorded and SHBG, insulin, glucose and lipid levels were measured.
Results
SHBG levels were inversely correlated with peritoneal fat ($P = 0.003$) whereas there was no significant association with subcutaneous fat. Lower SHBG levels were associated with increased waist circumference, decreased hip circumference, increased BMI, higher HOMA - Insulin Resistance Index and insulin levels ($P < 0.02$). Step multivariate analysis showed that peritoneal fat, hip circumference and insulin levels were independently associated with SHBG levels. Significant associations were also found with age ($P = 0.047$).
Conclusions
Peritoneal but not subcutaneous adiposity, as assessed by U/S, is inversely associated with SHBG levels. U/S seems to be a simple, low-cost method for the assessment of central adiposity in apparently healthy individuals. SHBG levels, which have been recognized as a risk factor for CVD, are highly correlated with indices of either protective type (hip) or high-risk type (peritoneal and waist) regional adiposity, indirectly supporting the importance of regional adiposity to the risk for metabolic syndrome and cardiovascular disease.

P235

Comparative analysis of adiponectin, leptin and C-peptide levels in obese non-diabetic, type 1 diabetic and lean non-diabetic children

Olga Sysoyeva, Nataly Karlovich & Tatiana Mokhort
State Rehabilitation Centre, Department of Endocrinology, Minsk, Belarus.

A range of hormones which regulate energy metabolism are secreted by adipose tissue, among which adiponectin and leptin are the main adipokines regulating insulin sensitivity.

The aim of our study was to estimate and compare levels of adiponectin, leptin, C-peptide and adiponectin-to-leptin ratio (A/L) in obese non-diabetic, Type 1 diabetic (T1D) children and lean non-diabetic controls.

BMI and SDS BMI were calculated in 88 children (46f, 42m, age 14.8 ± 3.6 yrs): 32 pts with obesity, 34 pts with T1D, 22 lean non-diabetic persons. Serum levels of C-peptide, adiponectin and leptin were measured by ELISA.

Median adiponectin levels were higher in control group (22.1 mcg/ml; $P = 0.0001$) and T1D pts (21.1 mcg/ml; $P = 0.0002$) as compared with obese pts (12.6 mcg/ml). Leptin levels were higher in pts with obesity (41.2 ng/ml) as compared with control (1.8 ng/ml; $P < 0.000001$) and T1D pts (2.8 mcg/ml; $P < 0.000001$). Leptin levels in T1D pts were higher than in control group ($P = 0.027$), while adiponectin levels were practically the same.

Highest A/L ratio was in lean controls (11.6), lowest – in obese non-diabetic children (0.5), whereas in T1D pts A/L ratio was 6.6. Differences between groups were significant ($P < 0.05$).

We did not find significant correlation of adiponectin and leptin levels, adiponectin-to-leptin ratio with age at observation, BMI, C-peptide. At the same time adiponectin level and adiponectin-to-leptin ratio negatively correlate with BMI in T1D ($r = -0.37$, $P = 0.032$; $r = -0.35$, $P = 0.049$) and obese non-diabetic children ($r = -0.55$, $P = 0.001$; $r = 0.45$, $P = 0.011$).

Surprisingly, in obese non-diabetic pts we find significant correlation of adiponectin and age at observation ($r=-0.59$, $P=0.0004$).

We concluded that the older obese pts are, the lower adiponectin level is. Adiponectin-to-leptin ratio is a more useful marker of impaired adipokines secretion than adiponectin or leptin levels alone, though further study is necessary to prove reliability of this test for assessment of insulin sensitivity.

P236

Effects of pioglitazone and metformin on body weight and the insulin resistance parameters in young patients with obesity

Olya OLeksyk, Alexander Serhiyenko & Victoria Serhiyenko
Medical University, Lviv, Ukraine.

Background and Aims
The aim of this study was to evaluate the effects of pioglitazone (PGZ) and metformin (MET) plus Hypocaloric Diet (HD) in young patients with obesity and impaired oral glucose-tolerance test (75 g glucose).
Materials and methods
49 patients (17.1 ± 1.2 yrs) were allocated in groups: A ($n=14$) received PGZ 30 mg tid plus HD, B ($n=12$) - MET 1000 mg tid plus HD, C - PGZ 30 mg plus MET 1000 mg plus HD ($n=11$), D ($n=12$) were only on HD. The duration of the study was 3 months. We investigated Body Mass Index (BMI), triglyceridaemia (TG), Systolic (SBP), Diastolic Blood Pressure (DBP), insulin resistance (IR) parameters: Homeostasis Model Assesment (HOMA) - IR index; HOMA - β-cell function (HOMA-β-CF). Statitics: ANOVA.
Results
The increase of BMI, W/H, pre- and postprandial TG, HOMA-IR index ($P<0.05$), SBP and DPB ($P<0.05$) parameters, the decrease of HOMA-β-CF ($P<0.05$) were observed. PZG lead to the decrease of postprandial TG, HOMA-IR ($P<0.05$), some increase of BMI, improvement of HOMA-βCF and did not significantly influence SBP, DBP. MET was accompanied by the decrease of BMI ($P<0.05$), postprandial TG ($P<0.05$), SDP, DBP ($P<0.05$), but in a smaller degree, than PZG. The combined administration of PZG and MET lead to more expressed positive dynamics of investigated parameters. In particular, BMI made 26.4 ± 3.6 ($P<0.05$), HOMA IR index, HOMA-βCF 0.28 ± 0.004 ($P<0.01$), postprandial TG 1.77 ± 0.03 ($P<0.01$), SBP ($P<0.05$), DBP 85.4 ± 2.5 ($P<0.05$). The use of HD only lead only to some decrease of BMI ($P<0.05$).
Conclusion
The administration of PZG and MET in young patients with obesity and impaired OGTT is accompanied with more expressed positive dynamics of IR parameters, that allows to recommend their use in such patients.

P237

Plasma visfatin levels during oral glucose tolerance test in obese women

Dragan Micic[1], Mirjana Sumarac-Dumanovic[1], Aleksandra Kendereski[1], Goran Cvijovic[1], Danica Stamenkovic-Pejkovic[1], Maja Georgiev[1], Svetlana Zoric[1], Jagoda Jorga[1], Carlos Dieguez[2] & Felipe Casanueva[2]
[1]Institute of Endocrinology, Diabetes and Diseases of Metabolism, Clinical Center of Serbia, Belgrade, Serbia; [2]Department of Endocrinology, University of Santiago De Compostela, Santiago de Compostela, Spain.

Visfatin is expressed in visceral adipose tissue and is up regulated in some animal models of obesity. Insulin-mimetic actions of visfatin may be part of the feedback regulation of glucose homeostasis, so plasma glucose or insulin may have effect on visfatin levels in humans. The aim of study was to investigate plasma glucose, insulin and visfatin during oral glucose tolerance test (OGTT, 75 gr) in obese women. 22 obese women (age: 36.73 ± 1.88 yrs; BMI 34.72 ± 0.67 kg/m^2) were studied. Plasma visfatin (EIA Phoenix, ng/ml), adiponectin and leptin (Linco RIA, ng/ml), insulin (RIA Inep, mU/l) and glucose (mmol/l) were measured in basal state, while additional visfatin and insulin were measured at the peak glucose during OGTT. Insulin sensitivity (M index:mg/kgBW/min) was measured using euglycemic 2 hr clamp. Basal glucose was 4.78 ± 0.10 and peak glucose during OGTT 8.20 ± 0.42 ($P<0.005$). There were no significant differences in visfatin between basal sample and at the peak glucose levels (72.26 ± 3.34 vs. 79.46 ± 7.15, $P>0.05$). Basal insulin was 16.57 ± 1.28 and at the peak glucose 97.88 ± 13.01 ($P<0.05$). After analysis of the individual data we found that 7 obese women (Group A) had significant decrease (44.77 ± 3.87 vs.36.19 ± 8.42, $P<0.05$) and 15 women (Group B) had significant increase in visfatin during OGTT (69.85 ± 4.49 vs. 99.66 ± 2.59, $P<0.05$). There were no significant difference between Group A and B in BMI (34.85 ± 1.12 vs. 34.65 ± 0.87), age (36.00 ± 4.64 vs. 37.07 ± 1.81), basal glucose (4.91 ± 0.26 vs. 4.73 ± 0.09), basal insulin (14.73 ± 1.82 vs. 17.43 ± 1.67), adiponectin (5.90 ± 3.19 vs. 10.97 ± 2.94), leptin (34.66 ± 6.34 vs. 33.09 ± 3.45), peak glucose (8.96 ± 1.10 vs. 7.85 ± 0.36), insulin at peak glucose (80.54 ± 20.78 vs. 105.97 ± 16.46) neither in insulin sensitivity (5.51 ± 0.87 vs. 4.81 ± 0.64). Our data demonstrate existence of two type of visfatin response during OGTT in obese women. It is still not clear which influence determines different type of visfatin response during OGTT and further studies are necessary to elucidate these mechanisms.

P238

The association of high sensitivity C-reactive protein levels with body fat mass and body fat distribution

Deniz Gokalp[1], Alpaslan Tuzcu[1], Hatice Akay[2], Senay Arikan[1] & Mithat Bahceci[1]
[1]Dicle University School of Medicine Department of Endocrinology, Diyarbakir, Turkey; [2]Dicle University School of Medicine Department of Radiology, Diyarbakir, Turkey.

Background and aim
C-reactive protein (CRP) is a sensitive marker for systemic inflammation. In this study we aimed to investigate the relationship between high-sensitivity C-reactive protein (hs-CRP) levels and BMI, body fat mass and fat distribution in healthy subjects.
Subjects and methods
A total 117 healthy subjects aged with 20–68 yr [normal weight (BMI 18.5–25.0 kg/m^2, *n*:35), overweight (BMI: 25–30 kg/m^2, *n*:27) and obese (BMI $\geq$30.0, *n*: 55)] were included to the study. Body weigt, BMI, waist and hip circumferences, skinfolds (biceps, triceps, suprailiac and subscapular region) with skinfold caliper and ultrasonography and body fat mass with bioelectric impedance of all subjects were measured. Hs-CRP concentrations were measured with immunometric assay. Analysis of variance, post hoc Benferoni test and Pearson correlation test were used for statistical analysis.
Results
Mean serum hs-CRP levels of obese group determined with BMI were higher than overweight and normal weight groups (7.3 ± 5.46, 2.5 ± 3.13, 0.66 ± 1.1, respectively, $P=0.0001$). Mean serum hs-CRP levels of overweight group was not different normal weight groups. In addition hs-CRP levels were positively correlated with BMI, waist and hip circumferences, fat mass and skinfold thickness of all 4 regions. Al data were shown in Table 1.
Conclusions
1-Hs-CRP level is high in obese patients and there was close relationship between BMI and HS-CRP serum levels. 2-Both waist and hip circumference positively correlated with hs-CRP level, these data suggest that not only android obesity but also gineoid obesity increased hs-CRP levels. 3-Skinfold thicknesses were useful methods in clinical practice and they were also positively correlated hs-CRP levels

	BMI (kg/m^2)	Waist crcum. Cm	Hip circum. (cm)	Fatmass (kg)
*Hscrp(mg/*dL	r=0.335	r=0.339	r=0.396	r=0.428
	P=0.0001	P=0.0001	P=0.0001	P=0.0001
		Skinfold	Thickness (cm)	
	Biceps	Triceps	Suprailiac	Subscapular
Hs-crp	r=0.195	r=0.358	r=0.384	r=0.376
	P=0.037	P=0.0001	P=0.0001	P=0.0001
	Biceps(USG)	Triceps(USG)	Suprailiac(USG)	Subscapular(USG)
Hs-crp	r=0.261	r=0.243	r=0.331	r=0.309
	P=0.005	P=0.008	P=0.0001	P=0.001

P239

Innervation of white and brown adipose tissue: dual viral transneuronal tracing study

Ádám Dénes[1], Miklós Darida[1], Zsolt Boldogkoi[2] & Krisztina Kovacs[1]
[1]Institute of Experimental Medicine, Budapest, Hungary; [2]Dept. Biology, University of Szeged, Szeged, Hungary.

Central control of body weight involves coordinated regulation of food intake and energy metabolism. White (WAT) and brown (BAT) adipose tissue represent functionally distinct compartments of lipid storage and fuel consumption, respectively. Both adipose tissues are innervated by the sympathetic nervous system. Tyrosine hydroxylase positive fibers were found in between fat cells. To determine the extent to which the control of different fat compartments is provided by the same pre-autonomic neurons, the central circuit innervating WAT and BAT was compared by dual viral transneuronal tracing using isogenic recombinant strains of the pseudorabies virus. BDL, expressing beta galactosidase was injected to the epididymal WAT and BDG, expressing green fluorescent protein was inoculated into the intrascapular BAT of male rats and virus reporter proteins were revealed by immunocytochemistry. In the spinal cord, BDG infected neurons were found in the intermediolateral and central autonomic nuclei of the upper thoracic segments, while BDL infection appeared in the lower thoracic and lumbar levels. Several brainstem pre-autonomic areas (C1, A5) and the gigantocellular reticular nucleus contained BDG and BDL infected neurons, but relatively few neurons were infected by both viruses. In the dorsal motor nucleus of the vagus, the periaqueductal gray matter, as well as in the dorsomedial, ventromedial, paraventricular hypothalamic nuclei and in the lateral hypothalamic area, anatomically distinct sub-regions were infected by the two recombinant viruses. Following administration of the mixture of BDG and BDL into the WAT, over 70% of the infected neurons contained both recombinant viruses. Our data suggest that neurons involved in the regulation of WAT and BAT coexist in all areas involved in the control of sympathetic outflow, although the relative proportion of these neurons vary across the regions. Double-labeled neurons may represent central command neurons that direct coordinated responses of WAT and BAT to metabolic challenges.

P240

Identification of orexin receptors in brown adipocytes: functional effects of orexin-B

Janet Digby[1], Jing Chen[1], Danijela Markovic[1], Say Viengchareun[2], Marc Lombes[2], Hendrik Lehnert[1] & Harpal Randeva[1]
[1]University of Warwick, Medical School, Coventry, United Kingdom; [2]INSERM, Paris, France.

Objective
Orexin-A and orexin-B and their G-protein coupled receptors (orexin receptor-1 & -2: OX1R, OX2R) have divergent effects on physiological behaviour, cardiovascular regulation, glucocorticoid and insulin release. Furthermore, orexins have been shown to affect both brown adipose tissue energy expenditure and thermogenesis through stimulation of sympathetic nerve activity. Despite *in vivo* studies demonstrating a role for orexins acting centrally on adipose tissue, there are no data on the expression of orexin receptors in brown adipose tissue. We therefore analyzed the expression and localization of OX1R and OX2R in mouse brown adipocytes and in the T37i brown adipocyte cell line. Furthermore, the effects of exposure to orexin-A and orexin-B were measured on the expression of key genes involved in thermoregulation and insulin sensitivity; leptin, uncoupling protein-1 (UCP-1), adipocyte-specific fatty acid binding protein-2 (AP2) and PPARγ.
Methods
Quantitative real time RT-PCR was performed using a Roche Light Cycler™ system, and genes of interest were standardised against the housekeeping gene β-actin. OX1R and OX2R were detected in differentiated T37i brown adipocytes using immunocytochemistry and confocal microscopy.
Results
mRNA expression was detected for OX1R and OX2R in mouse mature interscapular brown adipocytes, as well as in differentiated T37i brown adipocytes *in vitro*. Furthermore, mRNA expression of both receptors increased as a function of the degree of differentiation. Confocal analysis revealed intense localised staining for OX1R around intracellular lipid droplets, whereas more membrane-localised staining was observed for OX2R. T37i brown adipocytes treated with orexin-B (100 nM, 4 h), resulted in significant increases in leptin, UCP-1, AP2 and PPARγ mRNA ($P<0.05$).
Conclusions
These novel findings indicate a direct role for orexin-B in brown adipocyte tissue metabolism and thermogenesis and the potential to affect insulin-sensitivity. Furthermore, the differing cellular receptor localisation suggests divergent roles for orexins in brown adipocytes.

P241

Waist circumference and BMI as predictors of arterial hypertension in childhood and adolescence in Latvia

Inese Madrevica, Juris Krikis & Renate Ligere
Latvian University, Riga, Latvia.

Child and adolescent adiposity is a problem of major concern not only for Europe, but also for the world at large. Increase of waist circumference, BMI and arterial blood pressure are metabolic syndrome risk factors which contribute to the development of cardiovascular disease, hypertension and diabetes mellitus.

To determine whether changes in arterial blood pressure are related to the increase of waist circumference and BMI in childhood and adolescence.

We examined 1049 schoolchildren (aged 7–18), 535 of whom were included in the study. In the risk group 41 schoolchildren were observed. For the study special questionnaires including 25–28 metabolism parameters were used. The obtained data were processed with the SPSS software packages (BMDP and *Systat* 9) adapted for biological and medical studies. We also determined the insulin resistance (Caro *et al.*, 1991) and the insulin resistance index (Dunkan *et al.*, 1995).

In our study elevated arterial blood pressure for boys and girls rather correlated with BMI ($n=532$; $r=0.449$; $P=0.000$) than with the increase of waist circumference ($n=532$; $r=0.427$; $P=0.000$), whereas in the risk group arterial blood pressure for both boys and girls more closely correlated with waist circumference ($n=39$; $r=0.403$; $P<0.05$). In the child and adolescent risk group both waist circumference and BMI have a negative correlation with the blood glucose level ($n=39$; $r=-0.432$; $P=0.000$). BMI also negatively correlates with insulin resistance in the risk group ($n=39$; $r=-0.339$; $P<0.05$).

Elevation of arterial blood pressure in children and adolescents strongly correlates with increase of both waist circumference and BMI. In assessing the metabolic syndrome risk factors for children and adolescents both waist circumference and BMI should be taken into account when working out early metabolic syndrome criteria for children and adolescents.

P242

Uric acid is an important predictor of metabolic disturbances in obese women

Ayse Sertkaya Cikim, Taner Bayraktaroglu, Adil Dogan Azezli & Yusuf Orhan
Istanbul University, Istanbul Faculty of Medicine, Endocrinology and Metabolism, Capa, Fatih/Istanbul, Turkey.

Introduction
It was shown that a relationship between uric acid and cardiovascular diseases, and hyperuricemia is associated with systemic inflammation. But, determination of uric acid is widely available and inexpensive, it has been overlooked as a marker of systemic inflammation and metabolic disturbances. In this study, we aimed to evaluate uric acid value and its association with inflammation and metabolic disturbances in overweight and obese Turkish women.
Material and methods
The study population consisted of 3975 women with BMI of 25 kg/m^2 or greater, classified as overweight (BMI$>=$25 kg/m^2, $n=771$) or obese (BMI$>=$ 30 kg/m2, $n=3204$) by National Institutes of Health and WHO criteria. They divided two groups according to median uric acid levels. Demographic and anthropometric characteristics, blood glucose, insulin and lipid concentrations, and the indices of insulin resistance and inflammation were determined and compared between groups.
Findings
Median uric acid level was 4.40 mg/dl. Therefore, our patients were divided two groups according to median uric acid levels; i.e. 4.40 mg/dl, group 1(women with low uric acid levels; ‹ 4.40 mg/dl) and group 2 (women with high uric acid levels; › 4.40 mg/dl). And metabolic parameters in group 2 having higher uric acid levels were significantly different and disturbed than group 1 with lower uric acid levels.
Conclusion
In this study, we found a significant difference in various metabolic and inflammatory parameters among different uric acid levels groups. The women with high uric acid groups have had high metabolic and inflammatory markers. These findings suggest that the relationship between uric acid and inflammatory markers. However. The nature of such a relationship remains unknown. These findings support the hypothesis that uric acid may negatively impact on metabolic parameters.

P243

Fasting and postprandial plasma obestatin levels are reduced in obesity

Christina Maier[1], Dominik Haider[2], Greisa Vila[1], Michaela Riedl[1], Anton Luger[1] & Michael Wolzt[2]
[1]Department of Internal Medicine III, Division of Endocrinology and Metabolism, Medical University of Vienna, Vienna, Austria; [2]Department of Clinical Pharmacology, Medical University of Vienna, Vienna, Austria.

Obestatin has recently been identified as a peptide derived from pre-proghrelin that opposes ghrelin effects on appetite and body weight in rodents. We studied the effect of food intake on both these hormones in obese and lean subjects and recorded in parallel the subjective sensations of satiety and hunger. Eight obese (two males and six females, BMI = 31–52 kg/m^2) and eight age- and sex-matched lean subjects (BMI = 19–23 kg/m^2) were randomized to 1) take a standard breakfast and 2) time control studies after an overnight fast in a prospective cross-over study design. Obestatin and ghrelin plasma concentrations were quantified by radioimmunoassays, satiety and hunger by visual analogue scales.

Basal circulating obestatin was significantly decreased in obese as compared to lean humans and stable in both study groups during an observation period of 90 minutes. Thirty minutes after food intake, obestatin levels were markly reduced in obese subjects, but increased in lean controls. There was no correlation between ghrelin and obestatin postprandial plasma concentrations. Subjective ratings of satiety and hunger were significantly related to obestatin plasma concentrations only in lean subjects.

We conclude that obestatin concentrations are much lower in obese subjects and inversely regulated by food intake, as compared to lean subjects. Both fasting and postprandial suppression of the anorexigenic obestatin might be of relevance in the pathophysiology of the positive energy balance associated with obesity.

P244

Insulin resistance and insulin secretion in morbidly obese patients before and six months after bariatric surgery

Miriam Promintzer[1], Gerhard Prager[2], Marietta Stadler[3], Christian Anderwald[1], Martina Mandl[1], Soheila Shakeri Manesch[2], Peter Nowotny[1], Martin Georg Bischof[1], Bernhard Ludvik[1], Anton Luger[1] & Michael Krebs[1]
[1]Division of Endocrinology and Metabolism, Department of Internal Medicine III, Medical University of Vienna, Vienna, Austria; [2]Department of Surgery, Medical University of Vienna, Vienna, Austria; [3]Thrid Medical Department of Metabolic Diseases and Nephrology, Lainz Hospital, Ludwig Boltzmann Institute of Metabolic Diseases and Nutrition, Vienna, Vienna, Austria.

Recent case reports describe hyperinsulinemic hypoglycemia after gastric bypass. The aim of this study was the assessment of insulin resistance and insulin secretion in non-diabetic morbidly obese patients before and six months after bariatric surgery.

In 8 non-diabetic morbidly obese patients (OB:6f/2 m, age:42 ± 3a, BMI:47.29 ± 2.2 kg/m^2) and 6 controls (CON:4f/2 m, age:43 ± 0a, BMI:23.8 ± 0.5 kg/m^2) we performed a frequently sampled oral glucose tolerance test (75 g, 3-hOGTT). The OGTT was repeated in 4 patients (3 Roux-en-Y gastric bypass, 1 gastric band) 6 months after surgery. Before bariatric surgery fasting plasma levels of glucose were comparable between OB and CON while fasting insulin and c-peptide were higher in OB (insulin:OB:27.0 ± 5.6/CON:7.0 ± 0.6 μU/ml, $P = 0.01$; c-peptide:OB:4.3 ± 0.7/ CON:1.3 ± 0.1 ng/ml, $P = 0.003$). During the OGTT peak plasma glucose and insulin concentrations were significantly higher in OB (glucose: OB:196.1 ± 15.6/CON:130.5 ± 9.6 mg/dl, $P = 0{,}006$; insulin:OB:119.7 ± 21.6/CON:58.6 ± 9 μU/ml, $P = 0.039$). 6 months after bariatric surgery fasting and early postprandial glucose concentrations were unchanged, while insulin and c-peptide were lower at fasting and higher after glucose load. Insulin resistance, assessed by HOMA-IR and OGIS, improved after bariatric surgery. After glucose load insulin and c-peptide secretion was adapted to insulin resistance prior surgery but was excessively elevated after bariatric surgery (adaptation index: before:119 ± 16/after surgery:228 ± 53, $P < 0.05$, CON:114.5 ± 19.6 total-nmol*m-2, $P = 0.8$, for before surgery vs. CON). Conclusion: Non-diabetic morbidly obese patients exhibit preserved adaptation of insulin secretion to severe insulin resistance. Six months after bariatric surgery elevated fasting insulin and c-peptide were normalized. In the early postprandial state, however, hyperglycemia remained unchanged, while secretion of insulin and c-peptide was excessive and not adapted to improved insulin resistance. Thus, this dissociation between increase of insulin secretion on the one hand and amelioration of insulin resistance on the other hand might put patients at risk for late postprandial hypoglycemia.

P245

Growth hormone Reduces Inflammation in Postmenopausal Women with Abdominal Obesity: a 12-month randomized placebo-controlled trial

Celina Franco[1], Björn Andersson[2], Lars Lönn[3], Bengt-Åke Bengtsson[1], Johan Svensson[1] & Gudmundur Johannsson[1]
[1]Research Center for Endocrinology and Metabolism, Sahlgrenska University Hospital, SE-413 45, Göteborg, Sweden; [2]Department of Medicine, Sahlgrenska University Hospital, SE-413 45, Göteborg, Sweden; [3]Department of Diagnostic Radiology, Sahlgrenska University Hospital, SE-413 45, Göteborg, Sweden.

Context
Abdominally obese individuals have relative hyposomatotropism, elevated serum markers of inflammation, and increased risk of cardiovascular disease (CVD).
Objective
The aim was to study the effect of GH treatment on serum levels of inflammatory markers and vascular adhesion molecules in postmenopausal women with abdominal obesity.
Design
Forty postmenopausal women aged 51–63 yrs with abdominal obesity received GH (0.67 mg/d) in a randomized, double blind, placebo controlled 12-month trial. Measurements of inflammatory markers in serum: interleukin-6 (IL-6), highly sensitive C-reactive protein (CRP), and amyloid polypeptide A (SAA), and markers of endothelial function: selectin, vascular adhesion molecule-1 (VCAM-1), intercellular molecule-1 (ICAM-1) were performed at baseline and after 6 and 12 months of treatment.
Results
The GH and placebo group were comparable at baseline in terms of age, BMI, waist circumference, IGF-1, smoking habits and antihypertensive treatment. After 12 months, mean IGF-I SD score was 0.9 ± 1.5 and − 0.8 ± 0.6 in the GH and placebo groups, respectively. The 12-month GH treatment reduced serum levels of CRP and IL-6 as compared with placebo ($P = 0.03$ and $P = 0.05$, respectively), whereas the markers of endothelial function were unaffected. Within the GH treated group, serum CRP level showed a reduction from 4.3 ± 4 at baseline to 3.0 ± 3 mg/L after 12 months ($P = 0.05$) and serum IL-6 level was reduced from 4.4 ± 2 to 3.3 ± 2 ng/L ($P < 0.01$).
Conclusion
GH treatment in postmenopausal women with abdominal obesity reduced serum levels of inflammatory markers, suggesting that the risk of CVD was reduced. There was no detectable effect of the GH treatment on endothelial function evaluated using measures of vascular adhesion molecule levels in serum.

P246

Hyperactivity of the hypothalamic-pituitary-axis and adrenal hyperandrogenism in polycystic ovary syndrome: a consequence of 5 β-reductase hyperfunction

Alessandra Gambineri, Laura Patton, Giulia Forlani, Alessandra Munarini, Federica Tomassoni, Uberto Pagotto & Renato Pasquali
Endocrinology Unit, Department of Internal Medicine, S. Orsola-Malpighi Hospital, University of Bologna, Bologna, Italy.

Among the uncertainties surrounding the etiology of polycystic ovary syndrome (PCOS) the role of increased peripheral cortisol metabolism has become interesting, particularly in relationship to the pathogenesis of adrenal hyperandrogenism. The patways of cortisol metabolism include irreversible inactivation of cortisol by 5α- and 5β-reductase. To evaluate the association of 5α- and 5β-reductase activity with adrenal hyperandrogenism in PCOS, we recruited 90 PCOS women (age range: 18–45 years) classified into three groups accordingly to the responsiveness of androstenedione (A) and DHEA to $_{1-24}$ACTH: group of low responders (LR) ($n = 27$), defined by A and DHEA responsiveness to $_{1-24}$ACTH within 2 SD of mean of a group of controls; group of medium responders (MR) ($n = 43$), defined by A or DHEA responsiveness to $_{1-24}$ACTH over 2 SD; group of high responders (HR) ($n = 20$), defined by A and DHEA responsiveness to $_{1-24}$ACTH over 2 SD. Excretion of cortisol and its metabolites was measured by electron impact gas chromatography-mass spectrometry in a 24-h urine collection. Relative 5α- and 5β-reduction of cortisol was assessed by 5α-tetrahydrocortisol (5α-THF)/cortisol, and 5β-THF/cortisol and 5β-tetrahydrocortisone (THE)/cortisone, respectively. The three groups were similar for age, body weight and body fat distribution. Testosterone, A and 17OH-progesterone basal levels were also similar among the three groups, whereas DHEA-S was significantly higher in MR ($P < 0.05$) and more in HR ($P < 0.01$) respect to NR. HR presented also basal cortisol levels significantly lower and cortisol responsiveness to $_{1-24}$ACTH significantly higher than MR ($P < 0.01$) and

NR ($P<0.001$, $P<0.05$). 5β-THF/cortisol and 5β-THE/cortisone were significantly higher in HR respect to MR and NR ($P<0.05$). No differences in 5α-THF/cortisol were observed among the three groups. These data open up the intriguing possibility of 5 β-reductase hyperfunction as a new pathogenetic mechanism of adrenal hyperandrogenism in a subgroup of PCOS women.

P247

An examination of the prevalence of IDF and ATPIII defined metabolic syndrome: towards population based screening

Deirdre Waterhouse, Jayant Sharma, Frances Sheehan & Donal O'Shea
St Vincent's University Hospital, Dublin, Ireland.

Introduction
Despite the significant associated cardiovascular morbidity, as well as the significant economic implications, little consideration has been given towards population screening for the Metabolic Syndrome (MetS). Therefore, we wished to estimate the prevalence of MetS, using both the recently published IDF, as well as the previously defined Cholesterol Education Program Adult Treatment Panel III (ATPIII), criteria. Additionally, we hypothesised that simple, inexpensive anthropometric measurements offer an effective means of population based assessment for MetS.
Methods
1716 participants (1026 males, 690 females) underwent full cardiovascular assessment over a twelve month period, including detailed questionnaire, measurement of waist circumference, BMI calculation, sphygomanometry and fasting glucose and lipid profiling. Subsequently, the prevalence of the MetS was defined in accordance with both the IDF and ATPIII definitions.
Results
The prevalence of the MetS was 21.4% ($n=368$) and 13.2% ($n=227$) in accordance with IDF and ATPIII criteria respectively. Subjects identified using IDF criteria had significantly lower waist circumference ($P=0.006$) as well as significantly increased HDL cholesterol ($P=0.008$) when compared to the ATPIII cohort.The prevalence of IDF defined central obesity in our cohort was 56.8% ($n=975$); of these 37.5% ($n=368$) had MetS. The prevalence of MetS within this obese hypertensive cohort was 57.3% ($n=328$). Thus, concurrent central obesity and hypertension would identify 89.1% of the total IDF defined MetS in the population.
Conclusion
When compared to the previous ATPIII criteria, the IDF definition identifies an additional cohort of individuals with metabolic risk factor clustering despite a significantly leaner waist circumference. This leads to a higher prevalence of IDF-defined MetS. Finally, the coexistence of central adiposity and hypertension was noted in the majority of patients with MetS. This simple dysanthropometric phenomenon may potentially be used as an inexpensive means of population assessment for MetS.

P248

Neuroendocrine and genetic aspects of metabolic disturbances in women with simple obesity, polycystic ovary syndrome (PCOS) and eating disorders

Agnieszka Baranowska-Bik[1], Wojciech Bik[1], Ewa Wolinska-Witort[1], Pawel Gaj[2], Michal Mikula[2], Piotr Bragoszewski[2], Jerzy Ostrowski[2] & Boguslawa Baranowska[1]
[1]Medical Centre of Postgraduate Education, Neuroendocrinology Department, Warsaw, Poland; [2]Medical Centre of Postgraduate Education, Department of Gastroenterology, Warsaw, Poland.

Neuropeptides and adipocytokines influence metabolic homeostasis and food intake. Deregulation in their secretion leads to insulin resistance or metabolic syndrome. Adiponectin possesses anti-diabetic and insulin sensitizing properties. Expression of this gene remains under control of nuclear peroxisome proliferator-activated receptor (PPAR)-gamma. Ghrelin, an endogenous ligand for GH secratagogue receptor (GHSR), modulates metabolic homeostasis. A high amino-acid homology and transmembrane localization of G-protein coupled receptor 39 (GPR39) and GHSR suggest that ghrelin secretion can be modified by GPR39. Genetic variation found in genomic DNA sequences is a potentially important factor regulating expression level of mentioned genes. We evaluated the role of genetic factors and relationship between metabolic alterations and plasma adiponectin, ghrelin and leptin levels in women with simple obesity, PCOS (non-obese) and anorexia nervosa (AN).

The study consisted of 142 women (109 patients and 33 healthy lean controls) in similar age and was approved by the Local Ethics Committee. For SNP (single nucleotide polymorphism) analyses we genotyped all women for: (PPAR)-gamma, TNF-alpha, GPR39, GHSR, and ADIPOQ. We compared the distribution of alleles according to different clinical course vs. healthy controls. Our main findings are that in lean PCOS women insulin and HOMA-IR were higher comparing to controls but adiponectin and ghrelin did not differ significantly. Furthermore, in AN adiponectin and ghrelin were higher and leptin was lower compared with controls. The correlations between adiponectin, leptin and metabolic parameters were found. Genetic variant correlation was shown only for (PPAR)-gamma (Pro12Ala-rs1801282) locus comparing AN to healthy controls with a preference of higher level of heterozygosity among these patients. Decreased adiponectin and ghrelin levels in obesity cannot be explained by variations loci we examined. We conclude that lean PCOS women show increased insulin resistance. An evidence of genetic correlation of (PPAR)-gamma (Pro12Ala-rs1801282) locus in the group of AN patients was found.

P249

The effect of body composition and iron status on insulin resistance in hemodialysis patients

Zorica Rasic, Gordana Perunicic, Jelena Tica, Vesna Popovic, Marina Vujovic & Zoran Gluvic
University hospital Zemun, Belgrade, Serbia.

Background/Aims
High level of inflammatory cytokines was present within malnourished and chronic renal failure maintenance hemodialysis (MHD) patients, but there were conflicting data about the role of inflammation on development of insulin resistance (IR) in non-obese and overweight MHD patients.
Methods
We selected 23 well-nourished and 20 middle- to moderate-malnourished, sex and age-matched, stable MHD patients, 23 male and 20 female, with median dialysis duration of 48 months (IQR 24.5–82.0). All patients were treated at the Hemodialysis Unit. To determine the nutritional status, body composition and the presence of inflammation of MHD patients we used: subjective global assessment (SGA), anthropometrics measurements (BMI and waist circumference), bioelectrical impedance analysis (BIA) which was performed to quantify body fat and lean body mass, and biochemical parameters measurements [with serum iron, ferritin, intact parathormone (i-PTH), TNF-alpha, IL-6 and high sensitivity C-reactive protein (hs-CRP)]. All parameters were evaluated by comparisons between HOMA-IR tertiles. By backward multivariate regression analysis we identified independent variables for IR.
Results
As the tetiles of HOMA-IR increased, dialysis duration, systolic blood pressure, serum levels of glucose, insulin, and waist circumference increased, whereas HDL-cholesterol level decreased. Serum iron value was increased also. As we expected, the prevalence of the metabolic syndrome were increased significantly across the tertiles of HOMA-IR. HOMA-IR correlated with the levels of iron, ferritin, adipokine TNF-alpha, waist circumference, and total fat percentages. After adjustment for gender, age, hemodialysis duration, ferritin, BMI and total fat percentages, multivariate regression analysis was performed and the association with HOMA-IR was still strong only for serum levels of iron, TNF-alpha and waist circumference. That explains 17% of the total variation in HOMA-IR (Adjusted $R^2=0.166$, $P=0.04$).
Conclusion
Our study demonstrated that 1) serum iron had participated as independent predictor in the pathogenesis of IR on long-term MHD patients, together with 2) adipokine TNF-alpha and 3) visceral adiposity.

P250

Relationship between obesity, insulin resistance and adipokines in morbidly obese women

Rana Madani[1], Nazar Miheisi[2], Majid Hashemi[1] & Vidya Mohamed-Ali[2]
[1]University College London, Department of Surgery, London, United Kingdom; [2]University College London, Department of Medicine, London, United Kingdom.

Background
Several cytokines and chemokines are released by adipose tissue and associated with insulin resistance. We investigated the systemic and *in vitro*

release of RANTES(Regulated upon Activation Normal T-cell Expressed and Secreted), MCP-1(Monocyte Chemo-attractant Protein-1), adiponectin, leptin and IL-6 from three human adipose tissue depots and their relationship to insulin sensitivity.
Methods
Fasting blood samples were taken from obese female patients undergoing surgery($n=7$, mean age 39 years, mean BMI 46 kg. m^2). Glucose, insulin and lipid profiles and circulating adipokines named above were measured. Subcutaneous(Sc), omental(Om) and Gastric Fat Pad(GFP) adipose tissue organ culture were set up for determining *in vitro* adipokine release. Haemostasis Model Assessment for Resistance(HOMA-R) was calculated. Body fat content was measured using bioelectrical impedance. The study was approved by the hospital ethical committee.
Results
Unlike for leptin no significant correlation was observed between % body fat and other adipokines. Production rates of adipokines *in vitro* per gram adipose tissue per hr were: RANTES(Sc median = 67, Om = 29, GFP = 62 pg/ml), MCP-1(Sc median = 5, Om = 6, GFP = 4 ng/ml), leptin (Sc median = 971, Om = 212, GFP = 447 pg/ml), adiponectin(Sc median = 23, Om = 25 ng/ml) and Il-6(Sc median = 9, Om = 12, GFP = 10 ng/ml). Depot specific differences in adipokine release were not apparent except in leptin which was mainly subcutaneous. There was a direct significant correlation between % body fat and Sc leptin production and an inverse relationship with Om adiponectin. GFP release of RANTES had a negative and MCP-1 a positive relationship with % body fat. Obese subjects were significantly more insulin resistant. Serum MCP-1 was elevated in patients with worsening insulin resistance. There was a negative correlation between HOMA-R and serum adiponectin and HDL.
Conclusion
RANTES, MCP-1 and adiponectin were released *in vivo* from adipose tissue. Local production of adipokines varies between depots. Insulin sensitivity and % body fat can alter local production of the adipokines.

P251

Obesity in pre-school children –new epidemic problem?
Ewa Barg, Chmielarska Joanna, Adamska Barbara, Begar Inna, Konieczna Agnieszka & Skarzynska Malgorzata
Wroclaw medical University, Wroclaw, Poland.

In the opinion of the World Health Organization the obesity will become one of four major epidemics of the 21st century. The most important factor for its occurrence is the lifestyle.

The aim of the research was to analyse the body mass index (BMI) of pre-school children and their parents and the components of their lifestyle, such as diet, sports and free time activities.
Material and Methods
The two-stage study included 537 children (265 boys and 272 girls) aged 3 and 6 (5.63 ± 1.1). The anthropometric measurements of the children were made and the BMI was counted and referred to the percentiles. The parents were questioned about their height and weight and life style.
Results
82 (15%) children fulfilled the criteria of obesity. The fathers' overweight was stated in 54.6%, whereas obesity in 10.7%; mothers' overweight in 14.4%, obesity in 1.34%.
Children eat their first meal around 8.00 a.m., the last at 7.00 p.m.
The average number of main meals is 3.9 ± 0.9. Up to 87% of parents state that their child eats extra food (fruit, yoghurts, sandwiches) between the main meals. A major part in the diet plays the sweets. Up to 48% of children consume sweets everyday, 8.2% of them a few times daily and only 1% once a week.
Only 33% of children regularly do sports. A child spends up to a 100 minutes daily in front of a TV or a computer.
Conclusions
1. The occurrence of obesity in over 15% of pre-school children should keep parents and pediatrists alert because of the possible health consequences.
2. The incorrect nourishment and improper lifestyle may result in obesity becoming an epidemic.
3. It is vital to popularize a healthy lifestyle not only among children but also their parents.

P252

Somatostatin receptor subtype 2 inhibits glucagon secretion and regulates glucose homeostasis
Mathias Strowski, Vandana Singh, Carsten Grötzinger, Sylvia Zacharias, Bertram Wiedenmann & Ursula Plöckinger
Hepatology & Gastroenterology, Berlin, Germany.

Objectives
Somatostatin (SST) inhibits glucagon and insulin secretion. Five receptor subtypes for SST are known (SSTR1-SSTR5), all of which are expressed in the endocrine pancreas. SSTR2 inhibits glucagon secretion in vitro, however its role in vivo is not well understood. Here, we characterize the role of SSTR2 in regulating glucose homeostasis in mice with diet-induced obesity.
Methods
SSTR2-deficient ($SSTR2^{-/-}$) and control mice ($SSTR2^{+/+}$) were fed high-fat diet (HFD) for 14 weeks and the parameters of endocrine pancreas function were determined. Hepatic glycogen and lipid content was evaluated enzymatically and by histomorphology. Expression of enzymes regulating glycogen synthesis and breakdown were measured by a real-time PCR and Western blot. Insulin, somatostatin and glucose tolerance tests were performed. Glucagon secretion from isolated islets was measured by RIA, and glycogenolysis in isolated hepatocytes.
Results
Postprandial glucagon and glucose concentrations were increased in SSTR2-deficient mice. Glucose disappearance rate following administration of glucose, insulin or SST was delayed in $SSTR2^{-/-}$ mice. SSTR2-deficient mice had decreased hepatic glycogen content and decreased glucokinase mRNA. Glycogen synthase of $SSTR2^{-/-}$ mice was decreased while glycogen synthase kinase-3 was increased. Glycogen phosphorylase, phosphorylase-kinase, and CREB were increased. The hepatic lipid content of SSTR2-deficient mice was decreased. Glucose was unable to suppressglucagon secretion from pancreatic islets isolated from SSTR2-deficient mice. Hepatic glycogenolysis was inhibited by an SSTR2-selective agonist.
Conclusions
We demonstrate here that SSTR2 inhibits glucagon secretion in mice with diet-induced obesity. Deletion of SSTR2 accounts for the postprandial hyperglucagonemia. Increased glucose concentration may be due to decreased hepatic glucose utilization, lipid accumulation, and increased glycogen breakdown. SSTR2 may provide a valuable therapeutic target at improving hyperglycemia in patients with peripheral insulin resistance and obesity.

P253

The role of combined treatment of arterial hypertension in patients with obesity
Doliashvili Tamar, Giorgadze Ellen, Asatiani Ketevan & Surmava Arkadi
N4 clinical hospital, Tbilisi, Georgia.

The aim of our study was to assess the advantage of combinative therapy with lisinopril and moxonidin for the treatment of arterial hypertension in group of obese patients in comparison to the single-drug therapy with lisinopril.
Methods
26 obese patients were divided in 2 groups. They underwent a 24-hour monitoring of the arterial pressure and were diagnosed as arterial hypertension II degree (ESH-ESC). In the I group for the purpose of stabilization of arterial hypertension lisinopril was given in the daily dose of 10 mg for 2 times, in the II group we gave a combination of lisinopril in the same dose as in the I group plus moxonidinin the daily dose of 0.4 mg for 1 times. The evaluation of state of health and the ambulatory registration of the arterial pressure data were carried out every week. After 3 weeks from the beginning of the treatment repeatedly the monitoring was done and the data were compared in both of the groups.
Results
At the beginning of the treatment the mean daily indices of the arterial pressure in the groups were 165/100 mmHg and 166/98 mmHg. After 2 weeks from the treatment in both groups the data of the pressure stabilized, however in the group of combinative therapy the decrease of the daily dose of lisinopril was required on 5 mg because of more expressed lowering of the arterial pressure data, and after 3 weeks of the treatment according to repeated monitoring the mean daily indices were 142/87 mmHg and 136/85 mmHg. The state of the health was improved markedly in both of the groups.
Conclusion
Adding the agonist of imidazoline receptor in the standard antihypertension therapy significantly improves the state of the health and tolerance of the therapy, as well as enables the lowering of the other antihypertensive medications.

P254

A registry of GDM in portugal

Luísa Ruas, Tiago Rocha, Jorge Dores & Manuela Carvalheiro
Pregnancy Study Group of the Portuguese Diabetes Society, Coimbra, Portugal.

Introduction
A retrospective study of the year 2003, of 1314 women with GDM, was performed.
Patients and methods
Two groups according to pre-pregnancy BMI: Go - BMI≧30 Kg/m^2; Gno BMI< 30 Kg/m^2. Mean age 32.9±5 years, A1c<6% in both groups. Influence of BMI in different variables was analysed: family history of DM, weight gain during pregnancy; blood pressure, need of insulin, gestation age at the beginning of insulin, time and type of delivery, new-born weight and re-evaluation post-partum.
Results
Mean BMI was 26.7±5.1, 76.3%=BMI<30 and 23.8%=BMI≧30. Family history of DM - BMI 26.93 Kg/m^2, without family history −26.19 Kg/m^2; $P=0.01$. Weight gain was adequate in 41.4%, reduced in 29.9% and excessive in 28.7%. Normal arterial blood pressure −86.5%, hypertension worsened by pregnancy −6.9% and pregnancy induced hypertension −6.6%, BMI in these three groups 26.1, 30.51 and 29.33, respectively ($P<0.05$). There was statistical significant difference ($P<0.05$) between the two groups in these parameters: Insulin therapy 75.2% in Go *vs* 52.5% in Gno and its need earlier in Go −28.83 wks *vs* Gno −30.97 wks; time of delivery 38.1 wks in Go *vs* 38.4−wks in Gno; caesarean section 49.8% in Go *vs* 35% in Gno; new- born weight 3324.8 g in Go *vs* 3167.9 g in Gno; macrosomic babies 8.3% in Go *vs* 4.4% in Gno. In the re-evaluation post-partum higher BMI was related with severe degrees of carbohydrate intolerance ($P<0.05$). We didn't find any difference in the re-evaluation between the women with adequate and excessive weight gain.
Conclusions
Obesity in GDM is a risk factor for maternal and fetal outcomes, with the risk of early development in the mother of glucose intolerance.

P255

The effects of glucocorticoids on the expression of gluconeogenic and lipogenic enzymes in a rodent model of Cushing's syndrome

Francesca Lolli[1], Mirjam Christ-Crain[1], Blerina Kola[1], Csaba Fekete[2], Gábor Wittman[2], Ashley B Grossman[1] & Márta Korbonits[1]
[1]Department of Endocrinology, William Harvey Research Institute, Barts and the London, Queen Mary School of Medicine, London, United Kingdom; [2]Department of Endocrine Neurobiology, Institute of Experimental Medicine, Hungarian Academy of Sciences, Budapest, Hungary.

Background
Cushing's syndrome results from chronic exposure to excessive levels of glucocorticoids (GC). The clinical manifestations associated with hypercortisolaemia are variable and differ widely in severity, including hypertension, apparent obesity and metabolic aberrations such as diabetes, dyslipidaemia, ultimately leading to changes similar to the metabolic syndrome. We hypothesised that GC might influence the expression of the genes involved in lipogenesis and gluconeogenesis in adipose tissue and liver.
Methods
Rats were implanted with corticosterone-containing pellets, and consumed chow and 30% sucrose for two weeks according to a well-established model of glucocorticoid excess. Animals implanted with cholesterol (placebo) pellets consuming sucrose or saline only served as controls. RNA was extracted from mesenteric and subcutaneous adipose tissue and liver. Gene expression was analyzed by reverse transcription followed by real time quantitative PCR with primers specific for phosphoenolpyruvate carboxykinase (PECPK), sterol regulatory element-binding protein (SREBP1c and SREBP2), fatty acid synthase (FAS), glucose-6-phospatase (G6P) and β-actin as housekeeping gene.
Results
In the mesenteric adipose tissue GC significantly increased PEPCK mRNA expression ($P=0.01$), SREBP1c and FAS mRNA expression ($P=0.02$ and $P=0.035$, respectively). No significant changes were observed in subcutaneous fat tissue. In the liver GC significantly increased FAS mRNA expression ($P<0.0001$) and decreased PEPCK mRNA ($P=0.027$), without changes in the expression of G6P or SREBP1c.
Conclusions
GC increase the expression of lipogenic and glyceroneogenic genes in visceral adipose tissue and this could explain the increased fat storage observed in the visceral fat of Cushing's syndrome. The changes in the liver would lead to increased fat deposition with less gluconeogenesis, and this was reflected in the massive fatty liver observed experimentally. We suggest that there may be a common factor leading to these changes secondary to the excess of glucocorticoids.

P256

HRT in treatment of dislipidemia in women with hypogonadotropic hypogonadism

Vita Zektser, Irena Ilovayskaya, Aleksandr Iliyn, Nikolay Goncharov & Galina Melnichenko
Research Centre for Endocrinology, Moscow, Russia.

Hypoestrogenemia is associated with dislipidemia and is an independent risk factor for cardiovascular diseases in postmenopausal women. However, there is a cohort of young women with gonadal steroid deficit caused by the disorders of central regulation. Twenty women with hypogonadotropic hypogonadism (HH) were included in group 1. (median age - 29 years and 3 months, median duration of amenorhea - 5 years 3 months, mean BMI - 24.6±6.05 kg/cm2. Women were examined before and after the 12 months treatment with 2 mg of 17-β-estradiol and 10 mg of dydrogesteron in sequenced manner. Twenty three healthy women were included in the group 2 (control), median age 27 years, Mean BMI - 24.0± 4.37 kg/cm^2.

Dislipidemia was found in all patients with HH before the treatment. The levels of total cholesterol was 5.65±1.26 mmol/l and tryglycerides 1.63±1.0* mmol/l; HDL 1.34±1.0 mmol/l and LDL 3.9±1.1* mmol/l. In the control group total cholesterol was 4.85±0.36 mmol/l, tryglycerides 0.78±0.07 mmol/l, HDL 1.77±0.33 mmol/l and LDL 1.8±0.7 mmol/l ($^*P<0{,}05$). All of the parameters were higher in group 1, but the significant difference was in LDH and tryglyceride levels.

After 12 months treatment BMI didn't change in all of the patientes with HH, there was small but not significant reducing of cholesterol 5.2±1.23 mmol/ l and tryglycerides 1.16±0.78 mmol/l leveles and the LDL 2.96±1.1* mmol/l level reduced significantly ($^*P<0.05$).

It is important to notice that hypoestrogenemia in women of reproductive age with HH leads to dislipidemia and HRT taking can somehowe correct this unpleasant changes.

P257

Rare polimorphism in the intron of human Agouti- related protein gene is associated with obesity

Ivo Kapa[1], Ineta Kalnina[1], Valdis Pirags[1], Helgi Schioth[2] & Janis Klovins[1]
[1]Latvian Biomedical Centre, Riga, Latvia; [2]Uppsala University, Uppsala, Sweden.

Agouti related protein (AGRP) as a endogenous antagonist of melanocortin 4 receptor plays an important role in regulation of food intake and energy balance being one of the most potent orexigenic factors. We have determined complete sequence of AGRP gene and upstream promoter region in 100 patients with severe obesity (BMI>35). Three previously described polymorphisms were identified: silent mutation G538A in second exon, non-synonymous mutation G772A (rs5030980) and C662T located in second intron. Association of C662T mutation with obesity this far has not been studied. We further screened this SNP in the cohort of 1173 patients from Latvian Genome database. Carriers of C662T polymorphism had significantly higher BMI when analyzed in all subjects ($P=0.017$) and in men separately ($P=0.028$). Mean BMI levels were adjusted for other non-genetic factors including age, status of type 2 diabetes cardiovascular disease and other diseases. After adjustment BMI levels remained significantly higher in men carriers of C662T polymorphism ($P=0.035$): mean BMI value (with 95% confidence interval) was 29.768 (26.738–33.572) for CT genotype compared with 26.816 (26.432–27.208) for CC genotype. The association of C662T with higher BMI in women was not significant ($P=0.051$). The present study presents for the first time the association of AGRP polymorphism C662T with obesity in men. The possible functional effects of polymorphism are unclear and may involve splicing defects. Present study has been approved by Latvian Central Committee of Medical Ethics.

P258

The relationship between plasma androgens (testosterone and dehydroepiandrosterone sulfate), insulin resistance and visceral obesity in elderly men

Michal Rabijewski, Lucyna Papierska & Wojciech Zgliczynski
Department of Endocrinology; Medical Center for Postgraduate Education, Warsaw, Poland.

Introduction
In elderly men testosterone and DHEAS deficiency is often observed, also changes of body composition and metabolic disturbances are common disorders.
Objectives
The aim of this study was to analyze the association between testosterone and DHEAS deficiency and waist/hip ratio (WHR) and also levels of glucose, insulin, HOMA and FG/FI ratio in elderly men as well as analysis, whether these sex hormones influent on measured parameters separately.
Material and methods
Together 85 men with age from 60 to 70 years men (mean 66.3 ± 1.5 years) was analyzed. Testosterone levels <4 ng/ml or DHEA levels <2000 ng/ml and BMI <30 kg/m^2 were including criteria. Patients were divided into three groups: 52 with testosterone deficiency (L-T), 32 with DHEA deficiency (L-DHEA-S) and 67 with deficiency of both sex hormones (L-T/DHEA-S).
Results
Testosterone levels in L-T, L-DHEA and L-T/DHEA groups were respectively 3.19 ± 0.23 ng/ml, 4.89 ± 0.45 ng/ml and 3.25 ± 0.34 g/ml ($P < 0.002$). While DHEA-S levels were respectively: 2498 ± 98 ng/ml, 1435 ± 1010 ng/ml and 1501 ± 89 ng/ml). BMI values do not deferent between groups. WHR ratio values were the highest in L-T/DHEA-S group ($P < 0.05$ vs. L-T) group, significant lower in L-T group ($P < 0.005$ vs. L-DHEA-S) and the lowest in L-DHEA-S group. Insulin fasting levels were lowest in L-DHEA-S group, higher in L-T group ($P < 0.01$) and highest in L-T/DHEA-S group ($P < 0.001$ vs, L-T group). FG/FI values were highest in L-DHEA-S group, lower in L-T group (NS) and lowest in L-T/DHEA group ($P < 0.002$ vs. L-T group). HOMA ratio values similarly did not change significantly between L-T (6.6 ± 3.21) and L-DHEA-S group (5.5 ± 2.92), although tendency to higher values in L-T group was noticed, while WHR ratio values were significant higher in L-T/DHEA group (7.3 ± 2.45; $P < 0.002$ vs. L-T group).
Conclusions
DHEAS and testosterone deficiency were independently associated with higher insulin resistance and obesity and also WHR ratio is more sensitive then BMI ratio reflects androgen deficiency influence on obesity and body composition in elderly men.

P259

Prevalence of metabolic syndrome in a cohort of young Mediterranean women with polycystic ovary syndrome and association with clinical and biochemical parameters

Laura Patton, Alessandra Gambineri, Andrea Repaci, Giulia Forlani, Uberto Pagotto & Renato Pasquali
Endocrine Unit, Internal Medicine Department, S. Orsola-Malpighi Hospital, Bologna, Italy.

Aim
The purpose of the study was to evaluate the prevalence of the metabolic syndrome (MS) in a cohort of young Mediterranean women with PCOS in reproductive age and to evaluate the association of the MS with clinical and biochemical parameters.
Setting
Among 200 PCOS (17-31 years) criteria of MS in accordance with the "NCEP-ATPIII" were used to construct 3 groups: no one criteria, 1 or 2 criteria and 3 or more criteria (affected by MS). All patients underwent clinical, hormonal and metabolic assessments.
Results
36 women had no criteria, 101 women had 1 or 2 criteria, 63 women had 3 or more criteria. We found a prevalence of the MS of 31.5%. The women with MS had higher BMI, waist circumference and WHR than the other two groups. Among the 3 groups we found no differences in severity of hirsutism and menses abnormalities. However, the women with more criteria had more frequently acanthosis nigricans and less frequently acne. The group with MS respect the group without any criteria had higher levels of fasting insulin ($P = 0.014$), glucose-stimulated insulin and glucose levels ($P < 0.001$) and HOMA ($P = 0.039$) and lower levels of $HOMA_{OGTT}$ ($P < 0.001$) and QUICKI ($P < 0.001$). Moreover, we found higher levels of cortisol and androstenedione responsiveness to $_{1-24}$ ACTH ($P = 0.004$, $P = 0.040$). There were no differences for the levels of androgens at baseline except for the Free Androgen Index (FAI) which was higher in the group with MS ($P = 0.023$). Finally, the levels of SHBG were lower in patients with the MS respect to patients without any criteria ($P < 0.001$).
Conclusion
Young women of the Mediterranean area present a higher prevalence of the MS respect to the general population. Moreover, the MS is associated with a more severe insulin resistance state and hyperandrogenemia and with a hyperactivity of the hypothalamic-pituitary-adrenal axis.

P260

Obesity: diffusion weighted imaging features of brain

Alpay Alkan[1], Ibrahim Sahin[2], Lezzan Keskin[2], Hakki Muammer Karakas[1], Ayse Sertkaya Cikim[2], Zuhal Karaca[3], Nedim Turhan[3], Ahmet Kemal Firat[1], Ahmet Sigirci[1] & Ozkan Ulutas[3]
[1]Inonu University Faculty of MedicineDepartment Of Radiology, Malatya, Turkey; [2]Inonu University Faculty of Medicine, Department Of Endocrinology and metabolism, Malatya, Turkey; [3]Inonu University Faculty of Medicine, Department Of Internal Medicine, Malatya, Turkey.

Homeostasis of the body weight is maintaining by the interactions between the different sections of the brain and peripheral tissues. Diffusion weighted magnetic resonance imaging (DWI) is a method that investigates the microscopic motion of the water particules in tissues and also depends on measurement of the signal variations in tissues which is connected with the kinetic energy of the molecules called; molecular diffusion. The purpose of this work was to detect brain diffusion abnormalities in obese patients by DWI.
Method
Eighty one obese patients (68 obese (group 1),13 morbid obese (group 2)) and 29 healty control were included. ADC (Appearance Diffusion Coefficience) values were measured with DWI in the hyppocampus, corpus amygdala, insular cortex, orbifrontal cortex,middle temporal cortex and cerebellum, and compared with healty controls.
Results
The ADC values obtained from hypothalamus, hippocampal gyrus, amygdala, insula, cerebellum and midbrain were significantly increased in patients compared to controls.There were statistically significant differences for ADC values that obtained from insula between group 1 (obesity, *n*: 68) and controls.There were statistically significant differences for ADC values that obtained from insula, thalamus, hippocampal gyrus, orbitofrontal cortex, midbrain, and occipital cortex between group 2 (morbid obesity, *n*: 13) and controls. The ADC values were significantly increased in group 2. The ADC values obtained from orbitofrontal and occipital cortex were significantly increased in group 2 (*n*:13) compared to group 1 subjects.The body weight were positively correlated with hippocampal gyrus, insula, orbitofrontal and middle temporal cortex ADC values. The BMI were positively correlated with amygdala, insula, orbitofrontal and middle temporal cortex ADC values.
Conclusion
Increased ADC values in the hypothalamus, hippocampal gyrus, amygdala, insula, cerebellum and midbrain. suggest development of the extracellular water accumulation similar to vasogenic edema in these location.
Key Words: Obesity, Diffusion magnetic resonance imaging.

P261

Hepatic and brain metabolism in young adults with glycogen storage disease type 1

Martina Mandl[1], Daniel Weghuber[2], Martin Krssak[1], Michael Roden[3], Peter Nowotny[1], Atilla Brehm[3], Michael Krebs[1], Kurt Widhalm[2] & Martin Bischof[1]
[1]Division of Endocrinology and Metabolism, Department of Internal Medicine III, Medical University of Vienna, Vienna, Austria; [2]Division of Clinical Nutrition, Department of Pediatrics, Medical University of Vienna, Vienna, Austria; [3]Department of Internal Medicine I, Hanusch Hospital Vienna, Vienna, Austria.

Glycogen storage disease type 1 (GSD1) is a rare inherited defect of endogenous glucose production. While children present with severe hypoglycemia the

propensity for hypoglycemia may decrease with age in these patients. It was the aim of this study to elucidate the mechanisms for milder hypoglycemia symptoms in grown up GSD1 patients. Four patients with GSD1 (BMI: 23.2 ± 6.3 kg/m^2, age: 21 ± 3 yr) and four healthy controls matched for BMI (23.1 ± 3.0 kg/m^2) and age (24 ± 3 yr) were studied. Combined ^{1}H/^{31}P-nuclear-magnetic-resonance-spectroscopy was used to assess brain metabolism. Before and after administration of 1 mg glucagon endogen glucose production (EGP) was measured with D-[6,6-$^2H_2$]glucose while hepatic glucose metabolism was examined by ^{1}H/^{13}C/^{31}P-NMRS. At baseline GSD1 patients exhibited significantly lower rates of EGP (0.53 ± 0.04 vs. 1.74 ± 0.03 mg·kg^{-1}·min^{-1}, $P < 0.01$ vs. control) but an increased intrahepatic glycogen (502 ± 89 vs. 236 ± 11 mmol/l, $P = 0.05$ vs. control) and lipid content (16.3 ± 1.1 vs. 1.4 ± 0.4%, $P < 0.001$ vs. control). After glucagon challenge, EGP did not change in GSD1 patients (0.53 ± 0.04 vs. 0.59 ± 0.24 mg·kg^{-1}·min^{-1}; P=n.s.) but increased in healthy controls (1.74 ± 0.03 vs. 3.95 ± 1.34, $P < 0.0001$). In GSD1 patients we found an exaggerated increase of intrahepatic phosphomonoesters (PME) (0.23 ± 0.08 vs. 0.86 ± 0.19AU, $^P < 0.001$) while inorganic phosphate (P_i) even decreased (0.36 ± 0.08 vs. -0.43 ± 0.17AU, $P < 0.01$). Intracerebral ratios of glucose, glutamate, and myo-inositol:creatine were higher in GSD1 patients (at least $P < 0.05$ vs. control, respectively). Hepatic defects of glucose metabolism persist in grown up GSD1 patients. Upregulation of the glucose and lactate transport at the blood-brain barrier could be responsible for the amelioration of hypoglycemic symptoms.

P262

Body composition and GH status in morbidly obese females before and after laparoscopic silicone adjustable-gastric banding

Silvia Savastano[1], Annamaria Colao[1], Francesca Rota[1], Laura Vuolo[1], Maria Cristina Savanelli[1], Teresa Cascella[1], Francesco Orio[1], Luigi Angrisani[2], Gaetano Lombardi[1] & Carolina Di Somma[1]
[1]Department of Molecular and Clinical Endocrinology, Federico II University, Naples, Italy; [2]Department of Surgery, S.Giovanni Bosco Hospital ASL NA1, Naples, Italy.

The GH/IGF-I axis function are reported to ameliorate after weight-loss. Bariatric surgery leads to a significant weight loss in morbidly obese patients. We investigated the relationships between GH/IGF-I axis and body composition in 20 morbidly obese females (BMI: 44.8 ± 4.7; waist circumference (W) 119.5 ± 7.2 cm, age 33.7 ± 11.7 yrs) with a normal glucose tolerance, before and after laparoscopic silicone adjustable-gastric banding (LASGB). The GH axis was evaluated by GH response after GHRH + arginine test and IGF-I levels. Patients were evaluated 6 months after surgery and a well balanced mildly hypocaloric diet. Fat Mass (FM), Free Fat Mass (FFM) were evaluated by bioimpedance analysis. Before surgery, 8 (40%) subjects were GH deficient (peak GH < 4.2 μg/l), while 7 (35%) had IGF-I levels below the normal values for age and sex. Postoperatively, GH response was persistently impaired in 3 (15%) subjects, while IGF-I levels were still reduced in 9 (45%). After 6 months BMI, W, FM ($P < 0.001$) and FFM ($P = 0.03$) were significantly reduced. The percent decrement of FM was greater than that of FFM (22.4 ± 16% vs 5.6 ± 2.3%; $P < 0.001$). GH response was persistently impaired in 3 (15%) subjects, while IGF-I levels were still reduced in 9 (45%). In addition, a significant correlation was found between the decrement of FFM ($r = 0.81$; $P < 0.001$) and that of FM ($r = 0.47$; $P < 0.04$) and the decrement of IGF-I. At the multiple regression analysis, the percentage of FM and W at baseline were the major determinants of IGF-I. In conclusion, both the nutritional status and a relative malabsorption might affect IGF-I and FFM. After bariatric surgery and after the initial acute negative energy balance, a persistent deficiency in GH/IGF-I axis is present and this particular endocrine profile is also associated to unfavourable body composition changes. The low IGF-I levels might represent a possible marker of an underlying persistent catabolic state in these subjects.

P263

The importance of (TAAAA)n polymorphism of SHBG gene in the metabolic syndrome

Nektaria Xita[1], Charalambos Milionis[2], Ioannis Georgiou[3], Moses Elisaf[2] & Agathocles Tsatsoulis[1]
[1]Department of Endocrinology, University of Ioannina, Ioannina, Greece; [2]Department of Medicine University of Ioannina, Ioannina, Greece; [3]Laboratory of Reproductive Genetics, University of Ioannina, Ioannina, Greece.

Introduction
Sex hormone binding globulin (SHBG) levels have been associated with the development of the metabolic syndrome. In particular, low SHBG levels have been proposed as an indicator of increased risk for metabolic syndrome in men. The (TAAAA)n repeat polymorphism SHBG gene is believed to affect SHBG levels. In vitro experiments have shown that the allele with 6 TAAAA repeats is associated with decreased transcriptional activity of SHBG gene. The aim of this study was to examine the possible role of this polymorphism in the metabolic syndrome.
Subjects and methods
The study population consisted of 44 men with metabolic syndrome aged 51.6 ± 9.9 years and 100 healthy men. The body mass index was recorded and blood samples were obtained after overnight fasting for biochemical and hormonal tests. The fasting glucose to insulin ratio was calculated as an indicator of insulin resistance. The SHBG (TAAAA)n polymorphism was genotyped in peripheral blood leucocytes.
Results
Genotype analysis for the (TAAAA)n polymorphism of the SHBG gene in the patients and controls revealed six alleles having 6–11 TAAAA repeats. The distribution of the alleles between patients and the control group did not show statistically significant differences. However, the 6/6 genotype was more frequent in patients with metabolic syndrome compared to healthy men (22.7% vs 11%, $P = 0.05$). The small number of patients did not allow any association between polymorphism and biochemical parameters.
Conclusion
The (TAAAA)n polymorphism of SHBG gene appears to be associated with metabolic syndrome

P264

Exophthalmos and its relation to adipokines in obese men

Sinan Caglayan[1], Gokhan Ozisik[2], Melih Unal[3], Omer Ozcan[4] & Metin Ozata[2]
[1]Kasimpasa Military Hospital, Division of Endocrinology and Metabolism, Istanbul, Turkey; [2]GATA Haydarpasa Teaching Hospital, Division of Endocrinology and Metabolism, Istanbul, Turkey; [3]GATA Haydarpasa Teaching Hospital, Division of Ophthalmology, Istanbul, Turkey; [4]GATA Haydarpasa Teaching Hospital, Division of Biochemistry, Istanbul, Turkey.

Up to date, many studies were performed regarding the relationship between obesity and inflammation, and exophthalmos which is developed in thyroid-associated ophthalmopathy (Graves' ophthalmopathy). Several studies suggest that transforming orbital preadipocytes into adipocytes may cause exophthalmos because of the inflammation. Therefore, we examined the relationship exophthalmos and obesity which is also called low-grade systemic inflammation. We investigated the relationship between Hertel exophthalmometry values and plasma leptin, adiponectin, TNF-α, IL-6 and IL-1β levels in 52 obese and 34 healthy men who don't smoke and have any systemic illness.

Plasma leptin, adiponectin, TNFα, IL-6 and IL-1β levels were 25.28 ± 8.98 ng/mL, 0.41 ± 0.24 μg/mL, 305.53 ± 153.82 pg/mL, 63.99 ± 20.30 pg/mL ve 95.22 ± 69.54 pg/mL respectively, in obese group, whereas these levels were 2.66 ± 1.81 ng/mL, 1.17 ± 0.98 μg/mL, 69.31 ± 50.22 pg/mL, 18.84 ± 11.12 pg/mL ve 21.77 ± 6.84 pg/mL respectively, in control group. Hertel exophthalmometry values were found as 18.90 ± 1.63 mm in obese group and 16.88 ± 1.69 mm in control group. When obese group's variables compared to control group's variables, plasma adiponectin levels were found significantly lower whereas the other variables were found significantly higher in obese group ($P < 0.05$). In multiple regression models using backwards stepwise regression, we only found that the dependent variable, BMI, was predicted by leptin and TNF-α ($P = 0.004$ ve $P = 0.052$, respectively).

Our results suggest that the inflammation which is resulted by secreted adipokines and cytokines from adipose tissue might be associated with exophthalmos in obesity. Nevertheless, the lack of correlation between Hertel exophthalmometry values and BMI, plasma leptin, adiponectin, TNFα, IL-6 and IL-1β levels shows that there is no directly relation between exophthalmos and adipokines which causes inflammation in obesity.

P265

Influence of orlistat on adiponectin levels in obese women

Marianthi Tzoitou[1], Evridiki Papadopoulou[1], Athanasios Papageorgiou[1], A Galactidou[2], Marina Kita[1], Anargyros Kourtis[1], Pavlos Poulakos[1] & Avraam Avramidis[1]
[1]Endocrinology Clinic Ippokratio General Hospital, Thessaloniki, Greece; [2]Research Department Theagenio Hospital, Thessaloniki, Greece.

Introduction
Adiponectin is secreted by adipocytes and has been linked to glucose and lipid regulation. Obesity, diabetes and atherosclerosis have been associated with reduced adiponectin levels. Orlistat lowers lipids and improves insulin sensitivity but its effect on other metabolic parameters is not known.

The purpose of this study is to evaluate the influence of orlistat on metabolic and hormonal parameters of the adipose tissue.

Materials and methods
Thirty obese female patients with Body Mass Index >30 kg/m2 and mean aged 48.7 ± 12.9yrs and mean weight 92.47 ± 12.5 kg were included. Patients with diabetes and thyroid disorders were excluded. All patients were on a low calorie diet one month before treatment with orlistat.Blood samples for glucose, total cholesterol triglycerides, HDL,LDL,FT4, TSH, insulin and adiponectin were obtained before and three months after orlistat treatment.

Results
19/30 female (63.3%) have lost over five kilos after three months of treatment and diet. Mean body weight was 92.47 ± 12.5 kg and 85.45 ± 11.2 kg $p<0.05$ after treatment. Statistical signigicant differences between glycose triglycerides, cholesterol HDL,LDL were observed after treatment with orlistat(101 ± 31.2 vs 85 ± 14.5 mg/dl $P<0.05$, 207.5 ± 29.8 vs 196.1 ± 25.5 mg/dl $P<0.004$, 127.5 ± 50.9 vs 119.2 ± 41.4 mg/dl $P<0.001$).Insulin levels decreased significantly after three months of treatment (11.3 ± 2.4 μU/ml vs 9.19 ± 2.7 μU/ml $P<0.00$).In contrast adiponectin levels seemed to be increased significantly after treatment with orlistat.($16.284.5\pm21.640.6$ vs $41.798.5\pm64.776.1$ $P<0.00$)

Conclusion
In this study it seems that orlistat could effectively manage obesity. It decreases insulin and increases adiponectin when obese patients reduced caloric intake and lost weight after three months of treatment.

P266

Effect of omega-3 fatty acids on plasma adiponectin levels in Metabolic syndrome subjects

Dimiter Dimitrov
Medical University Varna, Varna, Bulgaria.

Background
Increased consumption of fish and fish oil as a source of *n*-3 long chain polyunsaturated fatty acids ($n-3$ LC-PUFA), mainly eicosapentaenoic acid (EPA, 20:5 *n*-3) and docosahexaenoic acid (DHA, 22:6 *n*-3) is often associated with decreased mortality (as well as morbidity) from cardiovascular disease. Treatment with n-3 LC-PUFA augments circulating adiponectin levels via a PPARγ-dependent mechanism in animal models. Given that adiponectin is known to exert antiinflammatory effects and enhance insulin sensitivity, it is conceivable that *n*-3 LC-PUFA could impede the adipose tissue switch to an inflammatory gene expression profile in response to obesity via a PPARγ- and adiponectindependent mechanism.

Aim
To evaluate the effect of n-3 LC-PUFA on plasma adiponectin levels and components of the Metabolic syndrome (Met-S).

Methods
35 overweight and obese adults ($28<BMI<36$ kg/m^2), aged 18–65 years, having developed the features of Met-S (IDF definition, 2005) were randomized to 2 gr. *n*-3 LC-PUFA daily or placebo for 3 months. All subjects were instructed to follow ad libitum diet without change in dietary lifestyle during that period. Metabolic parameters, plasma adiponectrin, insulin resistance (HOMA-IR) and CRP were measured before and after treatment.

Results
After 3 months, plasma adiponectin concentrations were increased by 44% ($P<0.001$). HDL cholesterol concentrations were increased by 10% ($P<0.001$) .Triglycerides was decreased by 39%, HOMA –IR decreased with 34% and CRP decreased with 20%. There were no significant complications resulting from treatment with n-3 LC-PUFA.

Conclusion
n-3 LC-PUFA may contribute to decreasing the burden of the metabolic syndrome, such as modulating inflammation, lipid abnormalities, endothelial function, and blood pressure via adiponectindependent mechanism.

P267

The polymorphism of PPAR and susceptibility to atherosclerosis in children with low birth weight (below 2500 g)

Ewa Barg, Anna Skoczynska, Beata Wikiera, Dorota Chacka, Ewa Glab, Arleta Lebioda & Barbara Turczyn
Wroclaw Medical University, Wroclaw, Poland.

Children, who are born with low birth weight (less 2500 g) are known to have an increased risk of developing lipid disturbances and atherosclerosis in later live.

PPAR alpha activity could play a regulatory role in the pathogenesis of hyperlipidemia and a modulatory role in the control of inflammatory response.

The aim of this study was to determine whether the presence of polymorphism in gene of peroxisome proliferators-activated receptor(PPAR) alpha is associated with lipid disturbances and susceptibility to apoptosis in children with low birth weight.

Methods
The associations between L162V polymorphism in the gene for PPAR alpha and lipid peroxidation, lipid profile, activity of caspase3 and apoptosis activation was examined in 155 children with low birth weight aged 4–11 years, and in 30 children born with normal weight as a control group.

Results
The frequency of the V allele of the L162 polymorphism gene in PPAR alpha gene in children(0.07) was similar to that in general population(0.06 in controls). In the group with polymorphism gene 4 children with LBW have the 50 Kb domain on the DNA electrophoretic profiles, but 7 children with LBW and control children haven't it.

The effect of the l162V polymorphism within PPAR alpha gene on the serum total HDL levels are observed ($P<0.001$).The levels of HDL and triglycerides and lipid peroxides were statistically higher in children with gene PPAR polymorphism ($P<0.05$) than in those children without polymorphism. Among all the children with the polymorphism, the group born with LBW presented higher level of lipid peroxides ($P<0.05$).

The linear correlations between caspasa 3 and serum cholesterol ($r=-0.999$, $P<0.05$), lipid peroxides and susceptibility of infection ($r=-0.769$, $P<0.05$),

Conclusion
In children more susceptible for atheroscerosis in adulthood due to low birth weight the L162V mutation in PPAR are connected with a protective effect on lipid pattern

P268

Visfatin, adiponectin, leptin and insulin sensitivity in severe obese women with normal and impaired glucose tolerance

Mirjana Sumarac-Dumanovic[1], Dragan Micic[1], Maja Georgiev[1], Danica Stamenkovic-Pejkovic[1], Jagoda Jorga[1], Aleksandra Kenedereski[1], Goran Cvijovic[1], Svetlana Zoric[1], Carlos Dieguez[2] & Felipe Casanueva[2]
[1]Institute of Endocrinology, Diabetes and Diseases of Metabolism, Clinical Center of Serbia, Belgrade, Serbia; [2]Department of Endocrinology, University of Santiago de Compostela, Santiago de Compostela, Spain.

Visfatin, a new adipokine, facilitates adipogenesis and has insulin-mimetic properties. There are data that hyperglycemia causes an increase in plasma visfatin levels in people with T2DM this increase gets more prominent as the glucose intolerance worsens. The aim of the study was to investigate plasma visafatin, leptin and adiponectin in obese women with normal and impaired glucose tolerance. Thirteen obese women (age: 34.50 ± 2.57 yrs; BMI 35.05 ± 0.57 kg/m2) with normal glucose tolerance (NGT) and 11 age and BMI matched obese women (age: 37.0 $2.4734.50\pm2.57$ yrs; BMI 38.20 ± 1.81 kg/m2) with normal fasting and impaired glucose tolerance during oral glucose tolerance test (OGTT) (IGT) were included in the study. Fasting plasma visfatin (EIA Phoenix, ng/ml), adiponectin (Linco RIA, ng/ml), leptin (Linco RIA, ng/ml) and insulin (RIA Inep, mU/l) were measured. OGTT (75 gr of glucose) were performed in all obese women. Insulin sensitivity (M index: mg/kgBW/min) using hyperinsulinemic euglycemic 2hr clamp was measured before and after weight reduction. There was no difference in fasting visfatin between NGT and IGT (68.65 ± 4.78 vs. 73.14 ± 5.22, $P>0.05$), fasting leptin (36.75 ± 3.79 vs. 32.06 ± 3.79, $P>0.05$) fasting adiponectin (6.82 ± 1.84 vs. 10.76 ± 4.14, $P>0.05$) and fasting insulin (17.34 ± 1.44 vs. 19.08 ± 2.65, $P>0.05$). Insulin sensitivity was reduced in obese women with IGT (5.36 ± 0.63 vs. 2.81 ± 0.39, $P<0.05$) while waist circumference were greater in the same subgroup of obese women (101.07 ± 3.12 vs. 113.18 ± 3.60, $P<0.05$). There was significant correlation between M index and waist in obese women ($r=-0.67$, $P<0.05$). In conclusion, decreased insulin sensitivity is confirmed in severe obese women with IGT. Our data suggest that impairment in insulin sensitivity precede change in adipocytokines during development of type 2 diabetes in obesity.

Signal transduction – presented on Sunday

P269

Characterization of the rat homologue of the human neuroendocrine marker secretagogin – new functional implications by *in vitro* studies

Wolfgang Gartner[1], Greisa Vila[4], Teodora Daneva[2], Felic Koc-Saral[1], Otto Majdic[3], Anastasia Nabokih[1], Anton Luger[4] & Ludwig Wagner[1]
[1]Medical University Vienna, Department of Medicine III, Vienna, Austria; [2]Bulgarian Academy of Sciences, Institute of Molecular Immunology, Sofia, Bulgaria; [3]Medical University Vienna, Department of Immunology, Vienna, Austria; [4]Medical University Vienna, Department of Endocrinology and Metabolism, Vienna, Austria.

Objective
Establishment of rodent *in vitro* cell systems for the extension of the functional data about the recently cloned neuroendocrine marker secretagogin.
Methods
1. DNA-cloning; 2. Antibody generation; 3. Immunoblotting and Immunohistochemistry; 4. Cell-transfection; 5. Luciferase Reporter Assays; 6. ELISA.
Results
1. We characterized the rat homologue of human secretagogin (rat secretagogin) and demonstrated the homologous tissue expression pattern of both proteins. 2. Highest rat secretagogin expression levels were found in rat pancreatic islets and in the rat insulinoma cell lines Rin-5F and INS-1. 3. There exists a considerable degree of sequence homology between human and rat secretagogin, indicating comparable functional properties. 4. Overexpression of rat secretagogin in Rin-5F and in INS-1 cells induced an increase in insulin secretion and expression, which is mediated mainly via the promoter elements AP-1 and CRE. 5. Insulin and rat secretagogin are secreted in an inverse ratio by Rin-5F and INS-1 cells upon incubation with dexamethasone and other agents known for influencing the insulin secretion.
Conclusion
We characterized the rat homologue of human secretagogin and present an *in vitro* system for its functional analysis, which emphasize its regulative involvement in insulin secretion and expression.

P270

Tilapia GnRH receptors: signal transduction and internalization rate

Ayelet Avitan & Berta Levavi-Sivan
Department of Animal Sciences, Faculty of Agricultural, Food and Environmental Quality Sciences, The Hebrew University of Jerusalem, Rehovot 76100, Israel.

Multiple subtypes of GnRH receptor (GnRHR) are present in individual vertebrate species. We found two distinct GnRHRs in tilapia, classified as type 1 and 3 (tGnRHR1/3). Amino-acid similarity between the receptors was calculated at 59%, with the different amino acids scattered throughout the receptors. We compared the sequence analysis and signal transduction of the two tGnRHRs, using the human GnRHR type 1 as a control. Sequence analysis revealed that all three receptors exhibit recognition motifs of Galpha q/11, while only tGnRHR3 and the hGnRHR1 revealed also, one recognition motif of Galpha s. We found that both tilapia receptors and the human receptor contain one PKA phosphorylation site. However, tGNRHR3 has five PKC phosphorylation sites whereas both tGnRHR1 and hGnRHR1 have only two sites. This diversity is further supported by the differential signal-transduction pathways: all three receptors activate the PKC pathway (as reflected by measurement of IPs accumulation), but only tGnRHR3 activates the PKA pathway (as reflected by activation of the reporter construct CRE-luciferase). All three receptors were also found to activate the phosphorylation of MAP kinase (ERK-1/2).

tGnRHR3 is highly expressed in the posterior part of the pituitary which contains LH and FSH cells. Hence, we characterized tGnRHR3 in terms of both LH release rate and receptor internalization rate in response to continuous exposure to GnRH.

Constant exposure of tilapia pituitary fragments to sGnRHa resulted in an increased secretion rate for 3 h, followed by a gradual decline to the basal secretion rate which lasted for 22 h. A chimera between tGnRHR3 and green florescence protein (GFP) was prepared and used to observe the changes in receptor distribution and translocation, activated by agonist with time. The receptor is initially localized at the plasma membrane and upon activation by sGnRHa undergoes relatively rapid endocytosis.

P271

The relationship between carotid intima-media thickness metabolic and anthropometric parameters in healthy subjects

Hatice Akay[1], Deniz Gokalp[2], Senay Arikan[2], Alpaslan Tuzcu[2] & Mithat Bahceci[2]
[1]Dicle University School of Medicine Department of Radiology, Diyarbakir, Turkey; [2]Dicle University School of Medicine Department of Endocrinology, Diyarbakir, Turkey.

Background and aim
Carotid intima-media thickness (IMT), as assessed by B-mode ultrasound, is a commonly used clinical marker that reflects systemic burden of atherosclerosis and value of IMT at or above 1 mm at any age is associated with a significantly increased risk of myocardial infarction. On the basis of such findings, we aimed to clarify the relationship between carotid intima-media thickness and anthropometric and metabolic parameters in virtually healthy subjects.
Subjects and methods
A total of 117 apparently healthy subjects were included to the study (age 20–68 year, mean age: 43 ± 12, BMI:30.1 ± 7.99 kg/m^2). Carotid Intima-media thickness (IMT) was measured with ultrasonography. Subjects were divided into two groups according to their IMT higher than 1 mm (group-1) or not (group-2). Total cholesterol, LDL-cholesterol, triglycerides, Hs-CRP, interleukin-1β, interleukin2, interleukin 6, interleukin 8, Tumour necrosis factor α, BMI, body fat mass with bioelectric impedance and body fat distribution (waist and hip circumference) of two groups were compared with independent *t* test.
Results
BMI, body fat mass, hip circumference, plasma LDL cholesterol, Hs-CRP levels of group-1 were higher than group-2 (Table 1) Interleukin-1β, interleukin2, interleukin 6, interleukin 8, Tumour necrosis factor α, triglycerides, waist circumference of the two groups were not show any statistically difference.
Conclusions
1-Carotid intima media thickness are closely related increased BMI, fatmass, hip circumference and LDL-cholesterol levels.
2-Hs-CRP is a useful marker of atherosclerosis.

	Group-1 ($n=17$)	Group-2 ($n=100$)	*P* value
BMI	37.6 ± 7.1	28.7 ± 7.3	$P=0.0001$
Body Fatmass	40.7 ± 15.2	24.9 ± 14.3	$P=0.001$
Hip circumference	122 ± 18	107 ± 14	$P=0.0001$
Hs-CRP	7.23 ± 2.95	3.67 ± 1.50	$P=0.008$
LDL-cholesterol	135.4 ± 29.8	108.8 ± 33.2	$P=0.008$

P272

CRF and the Urocortins activate NFAT and induce catecholamine production in PC12 cells

Erini Dermitzaki, Christos Tsatsanis, Ariadne Androulidaki, Maria Venihaki, Achille Gravanis & Andrew Margioris
University of Crete, School of Medicine, Heraklion, Crete, Greece.

We have previously shown that Corticotropin – releasing factor (CRF) and Urocortins (UCNs) induce the production of catecholamines from normal human and rat adrenal chromaffin and PC12 pheochomocytoma cells via induction of tyrosine hydroxylase, the rate limiting enzyme of catecholamine biosynthesis. We have also shown that CRF induces calcium ion entrance into the cytoplasm from both extracellular sources (influx) and from intracellular stores (mobilization) in PC12 cells. The transcription factor NFAT (Nuclear Factor of Activated T cells) is activated by calcium, is expressed in neuronal tissues and in PC12 cells, and is involved in neuronal cell differentiation. No information is available on its role in chromaffin cells. In the present study we have examined the effect of CRF peptides on NFAT activation, its role on catecholamine production in the PC12 pheochromocytoma cell line and the signaling pathways involved.

Our data demonstrate that: (a) CRF, UCN1 (CRF_1 and CRF_2 receptor agonists), UCN2, UCN3 (preferential CRF_2 receptor agonists) or Cortagine (synthetic CRF_1 receptor agonist) induced NFAT activity in a statistical significant manner in PC12 cells. (b) Cyclosporine A (CsA), a Calcineurin/NFAT inhibitor, abolished UCN2 or Cortagine-induced NFAT transcriptional activity in PC12 cells. (c) The effect of CRF receptor agonists on catecholamine synthesis was abolished by CsA in PC12 cells. In conclusion, our data suggest that CRF and UCNs activate the transcription factor NFAT which appears to be essential for catecholamine synthesis.

P273

Gs-dependent receptor endocytosis of melanocortin-4 receptors

Andreas Breit, Dominik Heling & Thomas Gudermann
Institut of Pharmacology and Toxicology, Philipps University Marburg, Marburg, Germany.

Melanocortin receptors (MCR), which belong to the superfamiliy 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. Recently it has been shown that the MC4R receptor undergoes, similar to most GPCR, GPCR kinase (GRK) and arrestin-mediated ligand-promoted receptor endocytosis. 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 as monitored by the bioluminescence resonance energy transfer technique in living cells, indicating that activation of the GRK/arrestin pathway does not require Gs signalling. However, despite of the key role arrestins play in regulating ligand-promoted receptor endocytosis, arrestin recruitment to the Gs signalling deficient MC4R-D90N variant was not sufficient to induce receptor endocytosis. These data indicate that although arrestin recruitmnet to the MC4R occurs independently of Gs signalling, ligand-promoted MC4R endocytosis requires the activation of Gs proteins, suggesting that so far unknown Gs signalling-dependent mechanism are involved in regulating ligand- promoted MC4R endocytosis.

P274

The endocrine disruptor DDT appears to be an uncompetitive inverse agonist for activating TSHr mutants, FSH receptor and LH receptor

Lucia Montanelli[1], Eleonora Ferrarini[1], Antonio Dimida[1], Mario Rossi[2], Patrizia Agretti[1], Franco Giorgi[2], Giovanni Umberto Corsini[2], Aldo Pinchera[1], Paolo Vitti[1], Roberto Maggio[2] & Massimo Tonacchera[1]
[1]Department of Endocrinology of University of Pisa, Pisa, Italy; [2]Department of Neurosciences of University of Pisa, Pisa, Italy.

The insecticide DDT has been shown to inhibit both the basal and the TSH stimulated accumulation of cAMP in CHO stably transfected with the TSHr (CHO-TSHr). Aim of this study was to evaluate whether the DDT has a similar effect on cells transfected with TSHr mutants displaying a high level of constitutive activity. In addition we investigate the effect of DDT on cells transfected with wtFSHr and wtLHr which share a high degree of amino-acid homology sequence with wtTSHr. In contrast with wtTSHr, wtFSHr and wtLHr do not show constitutive activity. Three TSHr mutants transiently transfected in COS cells were evaluated: S281L located in the ectodomain, I486M in the first extracellular loop and P639S in the sixth helix of the transmembrane domain. After incubation with DDT at increasing concentrations (10, 30 and 100 mcM), basal cAMP of the mutants was measured. Conversely, CHO cells stably expressing the wtFSHr and wtLHr (CHO-FSHr, CHO-LHr) were incubated with increasing concentrations of DDT (0.1, 1, 10 and 100 mcM), in presence of FSH (100 mU/ml) and hCG (1 mU/ml), respectively, and cAMP production was measured. The constitutive activity of the three activating TSHr mutants was inhibited and the maximal inhibition was obtained with the highest concentration of DDT. Similarly, DDT inhibited FSH and hCG induced cAMP activity in the two cell lines. At the highest concentration of DDT the inhibition was of 39% and 92% in CHO-FSHr and CHO-LHr, respectively. In conclusion DDT inhibited the constitutive activity of all activating TSHr mutants and the FSH and hCG stimulated accumulation of cAMP in CHO-FSHr and CHO-LHr. These effects are similar to those displayed by DDT on CHO-TSHr. Our data suggest that DDT might be an uncompetitive inverse agonist.

Steroid receptors – presented on Sunday

P275

Effect of vitamin D replacement on endothelial function and oxidative stress in vitamin D deficient subjects

Ozlem Tarcin[1], Dilek Yavuz[1], Ahmet Toprak[2], Ahu Telli[3], Meral Yuksel[4], Dilek Yazici[1], Seda Sancak[1], Oguzhan Deyneli[1], Goncagul Haklar[3] & Sema Akalin[1]
[1]Marmara University Medical School Section of Endocrinology and Metabolism, Istanbul, Turkey; [2]Marmara University Medical School Department of Internal Medicine, Istanbul, Turkey; [3]Marmara University Medical School Department of Biochemistry, Istanbul, Turkey; [4]Marmara University Vocational School of Health Related Sciences, Istanbul, Turkey.

Introduction
Vitamin D (Vit D) receptors have been shown in extra skeletal tissues. Vit D deficiency plays a role in the development of many malignant, chronic inflammatory, autoimmune and metabolic diseases. Our aim was to evaluate the effect of Vit D replacement therapy on insulin sensitivity, endothelial function and oxidative stress in Vit D deficient subjects.
Material-method
Serum 25(OH) D levels of 74 volunteer-healthy subjects (22.7 ± 2.7) were screened. Twenty subjects (22.6 ± 2.1) with 25(OH) D levels < 20 ng/ml were recruited as deficient group (D) and 20 subjects (23 ± 2.3) with 25(OH) D levels > 40 ng/ml were selected as control group (C). Monthly 300 000 IU Vit D was injected for 3 months to group D. Before and after 3 months, blood samples were collected for serum Ca, P, iPTH, thiobarbituric acid reactive substance (TBARs) and paraoxonase. Endothelial function was evaluated by measuring flow mediated dilatation (FMD) from brachial artery. Insulin sensitivity index was calculated according to 75gr OGTT.
Results
In group D, basal TBARs levels were higher compared to group C and decreased after Vit D therapy (Table 1). Basal FMD of group D were found to be lower than group C and increased after therapy. We found negative correlation between FMD and TBARs ($P=0.001$; $r=-0.51$) in group D. After therapy, 30th sec. insulin level increased during OGTT.

Table 1 Parameters before and after replacement therapy

	Before therapy	After therapy	Control
iPTH(pg/ml)	47.8 ± 22.5*	34 ± 17.6	42.8 ± 12.2
Ca(mg/dl)	9.6 ± 0.7	9.7 ± 0.4	9.8 ± 0.4
P(mg/ml)	3.8 ± 0.4	3.8 ± 0.5	3.7 ± 0.4
FMD(%)	7.2 ± 4*	10.5 ± 4	13 ± 12.6**
TBARs(nmol/mg MDA)	5 ± 1.5*	3 ± 0.7	4 ± 0.8**

*$P<0.05$ before and after therapy; **$P<0.05$ before therapy and control

Discussion
We have shown that vit D deficiency causes endothelial dysfunction. Vit D replacement led to the improvement on endothelial function and decreased lipid peroxidation which made us think that vit D deficiency could have take part in the pathogenesis of atherosclerosis.

P276

Transthyretin is up-regulated by androgens in mice liver and choroid plexus

Cecilia RA Santos[1], C Henrique Alves[1], Maria Joao Saraiva[2], Raquel Abreu[2], Graça Baltazar[1], Silvia Socorro[1], Telma P Quintela[1] & Isabel MT Gonçalves[1]
[1]Centre of Investigation in Health Sciences-CICS, Universidade da Beira Interior, Covilhã, Portugal; [2]Molecular Neurobiology, Instituto de Biologia Molecular e Celular-IBMC and ICBAS, Universidade do Porto, Porto, Portugal.

Transthyretin (TTR) is well documented as a carrier for thyroid hormones. It also binds retinol binding protein preventing its filtration trough the kidneys and therefore is involved in delivering retinol to target cells. Moreover, TTR sequesters amyloid-beta impairing its deposition in nervous tissues and possibly contributing to its removal. Despite its importance in mammalian physiology, there are few studies regarding the regulation of TTR synthesis. *In silico* analysis of the 5′ flanking region of the TTR gene allowed the identification of androgen responsive elements suggesting that androgens may regulate TTR expression in tissues where TTR and androgen receptor (AR) are co-expressed. This could assume particular relevance in the liver and choroid plexus (CP), which are the major sites of TTR synthesis. To test

this hypothesis female and male mice were either ovariectomized ($n=13$) or orchidectomized ($n=12$). Five weeks after surgery, these animals were either implanted with an alzet mini-osmotic pump delivering 419 ug/Kg/day of 5α-dyhidrotestosterone (DHT) or vehicle only, in the subscapular region. Sham operated animals (5 females and 5 males), not implanted, were also included in the experiment. After one week of hormonal stimulation, mice were euthanized and CP, livers, cerebrospinal fluid (CSF) and sera were collected and frozen at −80 °C. The levels of TTR in the CSF and sera were measured by RIA and the expression of TTR in the liver and choroid plexus was analysed by Real-Time PCR. A 3-fold increase of TTR levels in the sera and CSF of females, and a slight but significant increase of TTR levels in the sera of males were observed. As AR is expressed in liver and CP, it is likely that the observed TTR response to DHT is mediated by AR.

P277

Adrenal incidentalomas: aberrant expression of hormone receptors (preliminary results)

Elena Mena, Manel Sahun, Pedro Alià, M Ángel Navarro, Joan Soler & Carles Villabona
Department of Endocrinology and *Hormone Unit, Hospital Universitari Bellvitge, Barcelona, Spain.

Background
In some patients with adrenal tumors cortisol regulation may be under the control of abnormal or ectopic hormone receptors. The objective of this study is investigate the presence of these aberrant receptors in subjects with adrenal incidentaloma and biochemical criteria of subclinical hypercortisolism.
Patients and methods
We studied seventeen patients with adrenal incidentalomas, ten patients with a unilateral tumor (age 48–70, M/F: 4/6) and seven patients with bilateral tumors (age 53–68, M/F: 5/2), and biochemical features of subclinical cortisol hypersecretion. They were studied for plasma cortisol responses to various stimuli: upright posture, meal, terlipressin, cinitapride, combined hypothalamic-hormones (TRH and LHRH) and ACTH. Six normal controls were similarly studied. All subjects were given dexamethasone orally in order to avoid any ACTH-dependent variation of plasma cortisol. Responses to stimulation were classified as negative (increase of cortisol < 25%), partial (25–49%) and positive (≥50%).
Results
Fourteen out of seventeen patients responded to at least one stimulus other than ACTH. The most frequent cortisol response was obtained after terlipressin administration. A positive response to terlipressin was seen in 3/4 patients with bilateral tumors and in all of the patients (5/5) with unilateral incidentaloma. A partial to positive response was seen after the administration the others stimulus except to cinitapride. No response was observed in control subjects. Plasma ACTH remained supressed in all subjects throughout the study.
Conclusions
Aberrant membrane receptors detected by stimulation tests appear to be common in unilateral and bilateral incidentalomas with subclinical autonomous cortisol hypersecretion. The identification of these receptors could provide the novel opportunity to treat some of these patients with pharmacological agents.

P278

SMP30 is expressed in rat mammary gland and down-regulated by estradiol

Cláudio JB Maia, Cecilia RA Santos, Fernando Schmitt & Silvia Socorro
University of Beira Interior, Covilha, Portugal.

The SMP30 (Senescence Marker Protein 30) is involved in the maintenance of intracellular Ca^{2+} homeostasis and in the regulation of various Ca^{2+}- dependent proteins. A suppressive effect on cell proliferation, DNA synthesis and on the expression of oncogenes in rat hepatoma cells overexpressing SMP30 has been reported recently suggesting it may have a role in cancer progression. High levels of SMP30 expression have been found in liver and kidney of rats but no studies have focussed so far on the mammary gland where unbalanced calcium homeostasis and signalling is closely associated with its pathophysiology. The goal of the present study was to determine if SMP30 is expressed in rat mammary gland and to study its regulation by 17β-estradiol (E_2). For this purpose total RNA was extracted from rat mammary glands, reverse transcribed and subjected to PCR using SMP30 highly specific primers. The identity of the PCR product was confirmed by automatic sequencing. The presence of the SMP30 protein was confirmed by Western blotting of total protein extracts, which showed the presence of the protein as an intense band of ∼30 KDa, and by immunohistochemistry showing that SMP30 localizes preferentially in the citosol. To evaluate the responsiveness of SMP30 to E_2, adult females were ovariectomised ($n=10$) and 5 weeks after surgery they were either implanted with an Alzet mini-osmotic pump delivering 400 µg E_2/Kg/day ($n=5$) or vehicle only ($n=5$) for 7 days. Sham operated animals ($n=5$) were also included in the experiment but not implanted. The expression of SMP30 in the mammary gland was analysed by Real-Time PCR and the results showed its downregulation by E_2 in the rat mammary gland ($P<0.05$). These results suggest a likely involvement of SMP30 in breast physiology possibly related to estrogen dependent pathways. Further work to elucidate the SMP30 role in the mammary gland is underway.

Acknowledgements: Portuguese Foundation for Science and Technology (FCT) (SFRH/BD/13388/2003)

P279

Family mutation of PRKRA1A associated with Cushing syndrome from pigmented micronodular adrenal dysplasia

Alfonso Gentil, Tomas Martin, Milagrosa Díaz Galvez, Silvia Maraver & Miguel Lucas
Virgin Macarena University, Sevilla, Spain.

Introduction
Pigmented micronodular adrenal dysplasia (PMNAD) is an infrequent cause of Cushing syndrome ACTH-independent, and can form a part of Carney syndrome (CS). In both, regulatory subunit mutations of the protein kinase A (PRKAR1A) have been demonstrated, but without apparent genotype-phenotype correlation.
Objective
To demonstrate the mutation of PRKAR1A and its functional and clinical expression in a family affected with PMNAD.
Material and method
The index case and nine members of the family at risk were valued to demonstrate mutation of the gene PRKAR1A after diagnosed with PMNAD. DNA was extracted from the index patient and nine family members, primarily to study the segregation and linkage to locus of the PRKRA1A gene. Analysis of microsatellites was done by PCR using 32p-dCTP and autoradiograph of alleles after electrophoresis in acrylamide gel. Afterwards, the sequence was determined. Basal and post dexamethasone plasmatic and urinary cortisol and ACTH were valued.
Results
We describe a deletion of six pared sequence bases of the polypyrimidne tract [exon 7 IVS del (−7→−2)] of PRKRA1A gene in the index case and in four family members, three of them revealing PNMAD. In the remaining two family members (father and aunt of index patient), hypercorticism was not seen, although the father showed paradoxical response to dexamethasone on one occasion. Clinical manifestations of CS were not described. The other family members did not show PRKAR1A mutation.
Conclusions
A small intronic deletion of PRKAR1A gene could cause PMNAD, with a varying grade of penetration and clinical expression. This shows us the first genetic defect of PRKAR1A gene, which is associated to a specific phenotype.

P280

Non-genomic glucocorticoid effects on insulin secretion

Greisa Vila, Ludwig Wagner, Anastasia Nabokikh & Anton Luger
Department of Medicine III, Clinical Division of Endocrinology and Metabolism, Medical University of Vienna, Vienna, Austria.

Glucocorticoids act directly on pancreatic islets suppressing insulin secretion from the beta cells through a genomic mechanism of slow onset. We present here data on immediate actions of dexamethasone on two models of insulin secretion: RINm5F and INS-1 beta cell lines. Under normal glucose concentrations, dexamethasone rapidly (within minutes) decreased insulin secretion about 30%. Under hypoglycaemic conditions (glucose reduced to 50% for 1 hour) dexamethasone increased insulin release. Both these effects were present within 10 minutes and not in longer (up to 1 hour) stimulations. They were completely abolished by preincubation with pertussis toxin, slightly inhibited by the intracellular glucocorticoid receptor (iGR) antagonist mifepristone (RU486) and unaltered by the transcription inhibitor cycloheximide.

Western blotting experiments revealed that serum glucocorticoid kinase 1 (SGK1, a known early transcriptional target of glucocorticoids also known to regulate epithelial ion transport) rapidly translocated to the membrane following Dexamethasone treatment. Rapid changes were also seen in the cellular distribution of the calcium-binding protein secretagogin. Incubation with pertussis toxin 30 minutes prior to Dexamethasone stimulation, abolished not only the above effects, but also the translocation of the iGR to the nucleus and the

increase in SGK1 mRNA levels (starting 30 minutes after stimulation and measured by quantitative RT-PCR). While further mechanisms are still under investigation, we conclude that glucocorticoids act non-genomically on the beta cells affecting insulin secretion, protein distribution and possibly ion exchange. They have a dual role in homeostatic and stress conditions, similar to that seen in the fast feed-back to the HPA axis.

P281

The androgen receptor in the rat choroid plexus

CH Alves, S Socorro, IMT Gonçalves & CRA Santos
Centre of Investigation in Health Sciences-CICS, University of Beira Interior, 6200 001-Covilhã, Portugal.

The choroid plexus (CP) produces cerebrospinal fluid (CSF) and forms the blood-CSF barrier, being involved in the maintenance of the extracellular milieu of the brain and secretion of several neuroprotective factors. There are several experimental evidences showing that androgens enhance cognition and act as potential protective factors against Alzheimer's Disease. It has been shown that testosterone exerts neuroprotective actions against oxidative stress, apoptosis, and against the toxicity of β-amyloid, all via androgen receptor (AR). The AR has been identified in several regions of the central nervous system: the medial preoptic, arcurate, and ventromedial nuclei of the hypothalamus, in the medial nucleus of the amygdala, in the CA-1 hippocampus and the cortex, but not in the CP. In a first approach to study if the neuroprotective effects of CP are mediated by androgens and AR we investigated the presence of AR mRNA and protein in rat CP. Adult animals were euthanized and CPs were collected and frozen at −80 °C or fixed with 4% paraformaldehyde in PBS. The presence and levels of AR protein in the CP were studied by immunohistochemistry and Western blot, and the mRNA expression of AR in the CP was analysed by RT-PCR. The obtained results clearly demonstrate the presence of AR mRNA transcripts and protein in the rat CP with the protein levels in CP slightly higher than those found in prostate, testis, epididymis, and liver. Therefore, it is likely that some of the neuroprotective proteins secreted by the CP may also be regulated by androgens.

Acknowledgements
This work was supported by grant POCI/SAU-NEU/55380/2004 from FCT

P282

Correlation of BclI, N363S and the ER22/23EK polymorphisms of the glucocorticoid receptor gene and bone mineral density in patients with endogenous and exogenous hypercortisolism

Ágnes Szappanos, Judit Toke, Belema Boyle, Judit Majnik, Ibolya Varga, Edit Gláz, Miklós Tóth & Károly Rácz
Semmelweis University, 2nd Department of Medicine, Budapest, Hungary.

Objective
Genetic variation in the glucocorticoid receptor (GR) gene may be related to the clinical heterogenety and severity of the Cushing's syndrome. BclI, N363S and ER22/23EK polymorphisms are the three most investigated polymorphisms within the GR gene, however, the importance and magnitude of their effect in hypercortisolemic states are unclear. The BclI and the N363S variants are associated with increased, while the ER22/23EK variant is associated with reduced glucocorticoid sensitivity.
Methods
The allele frequencies of the BclI, N363S and ER22/23EK polymorphisms were investigated in 74 patients with endogenous or exogenous hypercortisolism and 172 healthy control subjects. The patient population included 31 patients with pituitary ACTH producing adenoma, 24 patients with adrenal Cushing's syndrome, 2 patients with ectopic Cushing's syndrome and 17 patients with glucocorticoid induced osteoporosis (GIO) caused by exogenously administered corticosteroids. DNA was extracted from peripheral blood leucocytes. The BclI and the N363S variants were detected by allele-specific polymerase chain reaction, and PCR-RFLP method was used to determine the ER22/23EK polymorphism. Bone mineral density was measured by DEXA at the lumbar spine and the left femoral neck (FN). This study was approved by local ethics committee, and written informed consent was obtained from all subjects.
Results
The frequency of the N363S polymorphism was significantly higher in patients with GIO than in the healthy control subjects (allele frequency 14.7% vs. 3.8%; $P<0.05$). Patients with the homozygous polymorph variant of the BclI polymorphism had significantly reduced mean FN z-score compared to patients with the wild-type variant (-1.803 ± 0.07 vs. -0.508 ± 0.944; $P<0.001$).
Conclusion
These results suggest that both of the N363S and the BclI polymorphisms of the GR gene may have an impact on the glucocorticoid sensitivity of bones.

P283

***In vitro* effects of 17 βE_2 and raloxifene on desmoid tumour derived cells**

Lucia Picariello[1], Silvia Carbonell Sala[2], Valentina Martineti[2], Alessia Gozzini[2], Maria Luisa Brandi[2] & Francesco Tonelli[1]
[1]Department of Clinical Physiopathology - University of Florence, Florence, Italy; [2]Department of Internal Medicine - University of Florence, Florence, Italy.

Desmoid tumours (DT) are a benign manifestation of familial adenomatous polyposis. The prevalent development in young fertile women, the regression during menopause or with tamoxifen treatment, underlie the potential role of estrogens and Estrogen Receptors (ERs) in the pathogenesis of these tumors. To investigate this hypothesis, the expression of ERs α and β in desmoid tumors derived cell cultures, the effects of $17\beta E_2$ and of raloxifene on DT cell proliferation has been evaluated '*in vitro*'.

Primary cultures from DT tissues obtained from seven patients were developed. RT-PCR and Western blotting analysis revealed that all the cultures expressed ERs α and β. In addition, also the RT-PCR and immunohystochemical analysis on the correspondent tissue samples confirmed the presence of ERs in these tumours. Treatment with $17\beta E_2$ (10^{-12} to 10^{-6} M) induced a dose-dependent cell growth, 10^{-9} M significantly increasing (120% to 250%) cell proliferation of the cell cultures. Raloxifene (10^{-7} to 5×10^{-6} M) reduced cell growth in a dose-dependent manner, with 5×10^{-6} M dose always significantly reducing both cell number (20% to 90%), without affecting apoptosis, as shown by DNA ladder assay. When $17\beta E_2$ and raloxifene effects were evaluated either alone or in combination, raloxifene was capable to significantly reduce (from 40% to 80%) the proliferative effects of $17\beta E_2$.

Although the study was made on a few samples these preliminary results showed that the different effects of $17\beta E_2$ and raloxifene on cell proliferation were probably linked to a different expression of ERα or Erβ in these cells.

These findings support on estrogenic role in the control of DT proliferation providing evidence for raloxifene effect on '*in vitro*' DT cell growth and viability. All together the '*in vitro*' and the previous '*in vivo*' studies encourage future investigation into a possible role of this SERM in the prevention and/or treatment of DT.

P284

ESR2 genotypes are associated with a reduced relative risk for sporadic colorectal cancer

Valentina Martineti[1], Lucia Picariello[2], Francesca Del Monte[1], Rosa Valanzano[2], Debora Strigoli[1], Silvia Carbonell Sala[1], Giuseppe Canonico[2], Francesco Tonelli[2] & Maria Luisa Brandi[1]
[1]Department of Internal Medicine, School of Medicine, University of Florence, Florence, Italy; [2]Department of Clinical Physiopathology, School of Medicine, University of Florence, Florence, Italy.

According to incidence data from Italian cancer registers, colorectal cancer is the third most common cancer in both men and women even considering skin non-melanoma cancers, lung and breast cancers. Moreover it represents the third absolute leading cause of cancer death in women and the fourth in men. Although data on Italian population regarding the role of estrogens in colorectal cancer have not yet been collected, several strands of evidence from international epidemiologic datasets indicate their protective role against the development of colon cancer. The effect of estrogens are mediated by oestrogen receptor (ERs), ERα and ERβ but ERβ has been identified as the predominant ER subtype in human colon, been expressed at higher levels in normal mucosa and significantly decreasing along tumour progression. According to the existence of a genetic predisposition to sporadic colorectal cancer, which is based on the carriage of common, low-penetrance polymorphic alleles, including those of PPARγ, NAT and VDR genes, polymorphism analysis of colorectal cancer has been recently attempted but none of the studies took into consideration the analysis of ERβ polymorphisms, dealing only with the most common estrogen receptor. On the basis of experimental and epidemiological data reported in the literature we thought that polymorphisms of ERβ had to be considered, as well as those of ERα, and indeed we performed an association study on 166 subjects affected by sporadic colorectal cancer and 197 healthy controls matched for age and sex. All enrolled subjects signed the informed consent. No association was ascertained between nor ERα *Pvu*II or *Xba*I polymorphisms, while a significant association emerged between ERβ *Alu*I genotype and colorectal cancer. In particular homozygous AA genotype was associated with a reduced risk (RR 0.57, $P<0.005$) and the homozygous opposite genotype aa with a higher risk (RR 1.59, $P<0.05$) for sporadic colorectal cancer. No further association was detected between ERα or ERβ genotypes and tumour features like Duke's staging, and histopathology.

P285

Frequency of three major glucocorticoid receptor gene polymorphisms in patients with adrenal incidentalomas

Jadranka Antic, Bojana Beleslin Cokic, Sanja Ognjanovic, Jovana Vignjevic, Katarina Mirkovic, Jelena Nikolic, Ivan Paunovic & Svetozar Damjanovic
Institute of Endocrinology, Diabetes and Metabolic Diseases, Belgrade, Serbia.

Background
Several polymorphisms of glucocorticoid receptor (GR) gene, including *Bcl*I, N363S and ER22/23EK, which may have an influence on glucocorticoid sensitivity, have been reported. *Bcl*I and N363S polymorphisms have been associated with clinical characteristics of increased and ER22/23EK of decreased glucocorticoid (GC) effects. On the other hand, metabolic syndrome has been described in patients with adrenal incidentalomas.
Objective and participants
We investigated the relation between *Bcl*I, N363S and ER22/23EK polymorphisms in GR gene in 31 patients with adrenal incidentalomas who underwent unilateral adrenalectomy (26 women; 36–76 yr old) and 117 healthy subjects (38 women; 20–76 yr old). The study was approved by the Institutional Ethical Committee.
Material and method
Several metabolic and anthropometric parameters were determined in order to correlate them to the genotype. Constitutive DNA was isolated from blood leucocytes. Genotyping was performed using PCR-RFLP, allele-specific PCR method and direct DNA sequencing.
Results
The larger allele frequency of the *Bcl*I variant was significantly lower in control subjects than in patients (4.3 vs 41.9%). Similarly, N363S (2.6 vs 16.1%) and ER22/23EK (0.9 vs 3.3%) variants of GR gene were less frequent in controls. Of several variables that were significant in univariate logistic regression analyses including age, gender, BMI, hyperlipidemia, hypertension, and diabetes mellitus, independent predictors of adrenal incidentaloma were *Bcl*I genotype [$P<0.001$, odds ratio (OR) 22.7; 95% confidence interval (CI) 6.7–77.0] and homeostatic model index (R_{HOMA}) ($P=0.028$, OR 1.5; CI 1.1–2.1).
Conclusion
*Bcl*I variant of GR gene is associated not only with metabolic syndrome but also with higher frequency of adrenal incidentalomas in population.

Thyroid – presented on Sunday

P286

An analysis on delays in diagnosis of papillary thyroid cancer

Gianlorenzo Dionigi[1], Renzo Dionigi[1], Luigi Bartalena[2], Maria Laura Tanda[2], Eliana Piantanida[2], Luigi Boni[1] & Francesca Rovera[1]
[1]University of Insubria Department of Surgery, Varese, Italy; [2]University of Insubria Department of Clinical Medicine, Varese, Italy.

Background
Thyroid carcinoma represents the most frequent form of cancer of the endocrine glands. In Italy, temporal trend shows a significant increase of incidence rates. Papillary thyroid cancer is the most common thyroid malignancy. Papillary thyroid carcinoma happens to be a multicentric tumor and trends to spread to the lymph nodes in the early stage of the disease. Thus early diagnosis is vital to improve the outcome for patients with thyroid cancer. The aim of this study was to determine the impact of delays in the diagnosis and treatment of this cancer.
Methods
43 patients [median age 42 (range 19–67), male to female ratio 1:8] with papillary thyroid cancer initially referred by a general practitioner and treated within this Unit from 2002 to 2005 were evaluated. Other histologic type were excluded from the study. Incidental microcarcinomas found in a multinodular goiter were also excluded. Details of referral, investigation and treatment were obtained by patient interview and cross-referenced with the case notes. Subjects completed an utilization questionnaire. The primary outcome variable was the time duration from cancer diagnosis to the time of cancer treatment.
Results
The overall median delay from the onset of symptoms to definite treatment was 13 weeks comprising patient delay in consulting a doctor (59%), delay in referral (19%), delay in diagnosis (10%), and delay in commencing treatment (12%). No significant reduction in total delay vs or change in the stage of disease at diagnosis was identified.
Conclusion
Long-term survival rate for papillary carcinoma is more than 90%, but this varies considerably among subsets of patients. A long delay in initiating this therapy has an adverse and independent effect on prognosis. In our experience the major delay occur prior to referral (patient delay), this has traslated into a significant raise in the overall delay. To achieve this, patient awareness must also be targeted. Patients with symptoms of these diseases should be initially referred for further care or followed up.

P287

Post-treatment effects of maternal hypothyroidism and thyroxin therapy on the subiculum neuronal density of the newborn rats

Morteza Behnam-Rassouli[1], Zahra Delshad[1] & Alireza Fazel[2]
[1]Dept. of Biology, Ferdowsi University, Mashhad, Iran; [2]Dept. of Medicine, Mashhad Medical School, Mashhad, Iran.

Studies in mice and rats suggest that legions of hippocampus interfere with memory for space and context and can have a significant effect on memory storage. The goal of the present study was to investigate the effect of maternal hypothyroidism, and thyroxin therapy on the neuronal density of the subiculum.

Twenty five adult female Wistar rats were divided into experimental groups (Exp) 1 and 2 and control. The Exp groups made hypothyroid (500 mg/l PTU in drinking water). The Exp 2 received levothyroxin as well (1 mg/l in drinking water). The treatment regimes were the same throughout the experimental period. Two 20 days old offspring were randomly selected from each litter, deeply anesthetized (0.2 ml of 2% xylazine), perfused by 10% formaldehyde, their brains processed for histological preparation and the parasagittal sections (9 μm) stained in tuloidin blue. By using the dissector method, the numerical density (N_V) of subicular region of the left hemisphere were estimated and statistically analyzed by JMP software in all groups.

The results show significant differences in subicular N_V in Exp 1 when compared with control and/or Exp 2 ($P<0.0001$). It seems that thyroxin therapy may improve the effects of hypothyroidism on the neuronal growth and extension of dendritic arborization of subicular neurons.

P288

Abstract unavailable

P289

The effects of subclinical hypothyroidism and replacement therapy on paraoxonase-1 (PON-1) and common carotis intima media thickness

Levent Kebapcilar[1], Abdurrahman Comlekci[1], Pinar Tuncel[2], Ahmet Solak[2], Mustafa Secil[3], Omur Gencel[3] & Sena Yesil[1]
[1]Dokuz Eylul University Endocrinology and Metabolism, IZMIR,Inciralti, Turkey; [2]Dokuz Eylul University Biochemistry, IZMIR,Inciralti, Turkey; [3]Dokuz Eylul University Radiology, ZMIR,Inciralti, Turkey.

The mechanism of atherosclerosis in patients with subclinical hypothyroidism (sHT), which has been partly attributed to lipid abnormalities, is still controversial. There is substantial evidence that ox-LDL plays an important role during the atherosclerosis process and paraoxonase-1 (PON-1) significantly inhibits generation of lipid peroxidation and thus plays a role in against atherosclerosis. The aim of the study was evaluate qualitative changes in lipoprotein metabolism, hs-CRP concentrations and PON1 activities with respect to common carotid artery intima-media thickness (CIMT) in 25 sHT (aged

48.96 ± 8.42 yr) patients before and after 4 months of levothyroxine substitution therapy and 24 normolipidemic healthy individuals (aged 42.79 ± 8.12 yr) comprised with the control group. There were no significant differences between controls and patients with sHT for age ($P = 0.05$). At baseline, compared to controls, patients with sHT showed similar levels of total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides levels. PON1 activities, hs-CRP concentrations and mean CIMT were similar between sHT and control group. Levothyroxine treatment had no effect on serum PON-1 activities and hs-CRP concentrations but resulted a significant reduced mean CIMT in the subgroup of patients with TSH levels > 10 mIU/L ($P = 0.017$).

In multiple linear regression analysis, we found the decrement in mean-CIMT was directly related to the decrement of waist circumference ($r = 0.532$, $P = 0.006$). In conclusion, monitoring of PON-1 activities and hs-CRP concentrations did not offer additional arguments for treating patients with sHT. However, the fact that levotiroxine replacement therapy was able to reduced CIMT suggests that beneficial effects of levotiroxine treatment for decreasing the risk of atherosclerosis in the subgroup of patients with TSH levels > 10 mIU/L.

P290

Elevated plasma FABP4 (aP2) levels in hypothyroidism: potential implication for accelerated atherosclerosis

Gokhan Ozisik[1], Sinan Caglayan[1], Mustafa Gultepe[2] & Metin Ozata[1]
[1]GATA H.pasa Teaching Hospital, Div.of Endocrinology and Metabolism, Istanbul, Turkey; [2]GATA H.pasa Teaching Hospital, Div.of Biochemistry, Istanbul, Turkey.

Context
FABP4 (adipocyte-specific fatty acid-binding protein 4, also known as aP2) is a cytoplasmic lipid chaperon involved in lipid metabolism, glucose homeostasis, and the regulation of inflammatory response. Its expression is limited to adipocytes, macrophages, skeletal muscle, and bronchial epithelia. Recently, a polymorphic allele of the *aP2* promoter (−87T→C) has been shown to be associated with decreased FABP4 expression in fat tissue, lowered triglyceride levels, and reduced risk for cardiovascular disease as well as type 2 diabetes (Proc Natl Acad Sci USA, 103:6970, 2006). However, circulating FABP4 levels in various disease states remains to be investigated.
Objective
The aim of this study was to determine circulating FAP4 levels in hypothyroidism.
Design
After having obtained local Ethical Committee approval, circulating FABP4 levels were measured in 38 adult patients with hypothyroidism before and two months after restoration of euthyroid state, and were compared to those levels in 34 age- and sex-matched control subjects.
Main outcome measures
Plasma FABP4 is measured using an ELISA kit (Human FABP4 ELISA, BioVendor-GmbH, Heidelberg). We also measured thyroid hormones, plasma lipids, insulin, and glucose levels. As FABP4 levels were not normally distributed data are given as "median (interquartile range)".
Results
We found that plasma FABP4 levels are elevated in hypothyroidism (0.67 ng/ml vs. 1.23 ng/ml; $P < 0.001$), and restoration of euthyroid state is associated with normalization of FABP4 levels. Hypothyroid state was also associated with elevated LDL-cholesterol, triglycerides, and HOMA-IR all of which decreased significantly following thyroid hormone replacement ($P < 0.001$, $P < 0.01$, and $P = 0.004$; respectively). We did not detect any correlation between plasma FABP4 levels and lipid parameters or HOMA-IR.
Conclusions
This is the first study to report plasma FABP4 levels in hypothyroidism. Our findings suggest that elevated FABP4 levels may be involved in the atherosclerotic process associated with hypothyroidism.

P291

Markers of REDOX system at autoimmune diseases of thyroid gland

Tsotsonava Tea, Adamia Nona & Chitava Irina
Tbilisi State Medical University, Tbilisi, Georgia.

Objective
Oxidative stress is developing by disequilibrium between antioxidative and oxidative mechanisms. In these conditions dysfunction of thyroid gland (TG) has been reported. It is related with deranged biosynthesis of thyroid hormones, in particular, with the absorption of iodine in thyrocytes. The objective of our investigation was to study the impact of oxidative stress on autoimmune diseases (AD) of TG.
Methods
38 patients [group 1 - with diffuse-toxic goiter (DTG, $n = 19$), and group 2 – with chronic autoimmune thyroiditis (ChAT, $n = 19$)] have been investigated. 10 healthy subjects serve as controls. The investigation was approved by the local ethics committees. The parameters of blood redox-system were investigated by electron-paramagnetic resonance. The AD was diagnosed by ultrasonography, function of TG and thyroid autoantibodies.
Results
Ceruloplasmin in group 1 was significantly higher than in controls (18.6 ± 1.3 vs. 16.0 ± 1.1 mm/mg, $P < 0.001$) and lower than in group 2 (18.6 ± 1.3 vs. 20.0 ± 2.0 mm/mg, $P = 0.015$). Fe^{3+}-transferrin in group 1 and 2 was significantly lower than in controls (19.2 ± 1.2 and 18.5 ± 1.3 vs. 22.0 ± 0.9 mm/mg; $P < 0.001$ in both cases). The difference between nitric oxide EPR-signals in groups was not significant. EPR-signals of Mn^{2+}, methemoglobin and lipid peroxyradical ions were appeared in investigated groups. Ceruloplasmin EPR-signals significantly inversely correlated with plasma thyroxine levels in main group and thyroid volume.
Conclusions
The results of our investigation suggest that oxidative stress occurs at AD of TG and expressed: a) by increase of blood ceruloplasmin levels; b) by decrease of blood Fe^{3+}-transferrin levels; c) by appearance of Mn^{2+}, methemoglobin and lipid peroxyradical ions in blood. These changes demonstrate possible association between AD of TG and REDOX-system.

P292

Selenium and its relation to thyroid antibodies, volume and ultrasound texture

Daniel Smutek[1], Ivana Cermakova[2], Jan Jiskra[1], Eliska Potlukova[1] & Ludvik Tesar[3]
[1]1st Medical Faculty, Charles University, Prague, Czech Republic; [2]Institute of Endocrinology, Prague, Czech Republic; [3]Inst. of Information Theory and Automation, Academy of Sciences, Prague, Czech Republic.

Objective
To find a relation between thyroid parameters (thyroxin serum level, thyroid antibodies, thyroid gland volume and ultrasound texture) and serum level of selenium.
Background
Selenium deficiency can lead to a decrease of triiode-thyronine in peripheral tissues. Changes in thyroid hormone production can be reflected in followed thyroid parameters.
Methods
In 33 patients ultrasound examination of thyroid gland was performed, volume was determined and texture features (spatial and second-order co-occurrence texture properties) were computed. Also free thyroxin, anti-thyroglobulin, anti-thyroperoxidase, anti-thyrotropin receptor (TRAK) and selenium serum levels (Se) were measured.
Results
A correlation between TRAK and Se with a very high correlation coefficient 0.95 ($P = 0.01$) was found. Furthermore significant correlation between Se and thyroid volume was found with correlation coefficient −0.54 ($P = 0.001$). Additionally we found several correlations between Se and following texture features: Euclidean distance from standard deviation to the median of original pixel gray levels and their four gray-level transformations ($r = -0.38$, $P < 0.05$), Euclidean distances from average deviation of original pixel grey levels and their four gray-level transformations to their mean and median ($r = -0.38$, $P < 0.05$).
Conclusion
We have found that there is a relation between selenium serum level and volume of thyroid gland. This is in concordance with known fact that selenium deficiency impairs normal thyroid metabolism. Our finding suggests that selenium supplementation, in addition to well-established iodine prophylaxis, may protect against goiter growth and optimize the function of thyroid axis. This is in concordance with other authors' findings. Another interesting finding is that selenium levels were also related to texture features representing thyroid morphological structure and TRAK. This suggests that selenium deficiency might have a role in development of autoimmune thyroid disorders.
The study was supported by of Czech Academy of Sciences (IET101050403)

P293

Which prognosis criteria for thyroid anaplastic carcinoma?

Guillaume Larroumets[1], Frederic Somda[1], Beatrice Roche[1], Catherine Dejax[2], Francoise Desbiez[1], Fabrice Kwiatkowski[2], Philippe Thieblot[1] & Igor Tauveron[1]
[1]CHU Endocrinologie-Groupe Thyroide Auvergne, Clermont-Ferrand, France; [2]Centre Jean Perrin, Clermont-Ferrand, France.

The prognosis of thyroid anaplastic carcinoma is poor. Yet can prognosis criteria change the therapeutic options?
Method
From a series of 26 cases from a single group recorded between 1990 and 2006, we analyzed the outcome after treatment based on surgery, radio and chemotherapy and looked for prognosis criteria.
Results
All but one patients died with a mean survival of 273 days (median survival of 130 days). Over 50% of patients had died within 6 months, and 80% within 12 months. Most deaths are related to loco regional tumour progression ($n=15$), but general dissemination (6) and drug toxicity (2) are also responsible.
Increased age, poor general condition at admission, rapid tumour growth (evaluated by pre-diagnosis duration of symptoms), compressive tracheal or oesophageal symptoms, and metastasis are associated with poorer prognosis while the concomitant presence of another histological thyroid carcinoma seems of better outcome.
Treatment can also influence the prognosis: complete surgery (563 vs 123 days) and multimodal treatment improve survival.
Conclusion
Despite poor overall prognosis, accurate evaluation of TAC at diagnosis is needed to evaluate outcome. Intensive treatment should always be applied.

P294

Clinical-epidemiological characteristics of thyroid cancer (TC) in the Crimea

Olexandr Butyrsky, Oraz Hajiyev & Oleg Bezrukov
Crimean State Medical University, Simferopol, Crimea, Ukraine.

We retrospectively analyzed case reports of patients been operated within 50 yrs. Our investigation demonstrates increasing tendency of TC. Total frequency of TC has increased from 0.76% in 1953–1964 to 8.48% in 2001–2005. Analysis revealed prevalence of TC in women (87.6%), sharp increase of morbidity after 30 years (14.6%), peak of morbidity – after 60 years (27.4%). Frequency of TC among adolescents is not increased (1.6–2.3%) that's associated with relative prosperity on pollution with iodine isotopes. TC is more frequent in town-dwellers (72.2%) due to higher pollution of environment that's a factors of thyroid hyperplasia.

Analysis of CT morphology demonstrates prevalence of differentiated forms: papillary (24.9%), follicular (15.5%), papillary-follicular (20.4%), microcarcinoma is revealed in 32%, medullary - in 4.5%, anapl?stic - in 1.9%, non-epithelial tumors - 0.8%.

We occupy active position for treatment of thyroid nodes, especially in doubtful cytological results, elderly women, children/adolescents, after radiation in the past.

Thyroid surgery isn't indifferent to patients. Baseless thyroidectomy worsens life quality (constant replacement therapy, intensifies accompanying diseases, provides background for other tumors), increases risk of complications. Therefore in differentiated TC we prefer sparing surgery – hemithyroidectomy, resection of isthmus & medial part of another lobe. Thyroidectomy and fat dissection is indicated in non-differentiated TC if tumor is extended out one lobe, multifocal growth in both lobes, distant metastases before iodine-therapy. Crile's operation is performed if TC proliferates into sternocleidomastoid muscle/internal jugular vein.

Conclusions

1. Thyroid surgery must be provided in specialized clinics.
2. Differentiated TC is indication for sparing surgery. Thyroidectomy must be adequately based.

All thyroid nodes should be operated with following histological identification and adequate post-surgery management.

P295

Fine needle aspiration biopsy of the thyroid. Cytohistologic correlation: experience in a central military hospital

Ema Lacerda Nobre, Helena Vilar, Zulmira Jorge, Mafalda Marcelino, Saudade André, Paulo Guerra, Carlos Lopes & João Jácome de Castro
Hospital Militar Principal, Lisboa, Portugal.

Background and aims
Thyroid nodular disease (TND) is a common condition in the general population. Malignant nodules occur in 5% of patients with thyroid nodules. Fine-needle aspiration biopsy (FNAB) is considered to be the most reliable method of differentiating benign and malignant thyroid nodules. The purpose of this study was to assess the accuracy of FNABs performed in our Hospital.
Methods
We retrospectively reviewed the medical records of patients submitted to thyroid surgery in our Hospital between June 1999 and June 2005.
Results
FNABs were performed in our Hospital since 1999. We included in our study 98 patients who had undergone thyroid surgery for TND. To the 98 patients a total of 142 FNABs had been performed. 80% were considered benign, 7% malignant and 13% suspicious. The discrepant cases were: 4 false-negative and 1 false-positive. The 4 false-negative cases had a cytologic diagnosis of nodular hyperplasia and found to be papillary thyroid carcinomas on histologic findings. The false positive case had a cytologic diagnosis of papillary carcinoma that revealed to be an Hürthle cell adenoma on histology. Our results showed a sensitivity of 60% and a specificity of 98.6%.
Discussion
All patients with false-negative results had multiple nodular goitre in which carcinoma was found in non dominant nodules on histology. None of these patients performed FNABs guided by ultrasound, consequently, aspirations were only done on the larger, palpable nodules. We suggest to perform ultrasound-guided FNAB in all supracentimetric nodules, in patients with multinodular goitre.

P296

Thyroid nodules in the elderly: role of ultrasound (US) and ultrasound-guided fine-needle aspiration biopsy (US-FNAB)

Vincenzo Triggiani, Edoardo Guastamacchia, Francesco Resta, Carlo Sabba & Emilio Tafaro
University Of Bari, Bari, Italy.

The prevalence of thyroid nodules is increased in the elderly. The aim of this study was to evaluate the accuracy of US and US-FNAB in the diagnosis of thyroid cancer in a population of elderly subjects, all of whose thyroid nodules were subjected to US-FNAB, with no prior selection based on dimensions or echo-pattern. Over a three-year period, 276 consecutive patients (64 males and 212 [76.8%] females), aged 65 to 87 (mean 70 ± 4.4), underwent US evaluation and US-FNAB of all their thyroid nodules. A total of 507 nodules were analyzed. Diameter: 5 to 70 mm (19.7 ± 12 mm). Solitary in 97 cases (19.1%), more than one in 410 cases (80.9%). Echographic pattern: hypoechoic in 255 cases (50.3%), isoechoic in 147 (29%), hyperechoic in 46 (9.1%), anechoic in 19 (3.7%), mixed in 40 (7.9%). Halo was present in 194 cases (38.3%). Microcalcification in 64 cases (12.6%). Cytology: negative in 448 nodules (88.4%), suspicious or indeterminate in 11 (2.2%), positive in 27 (5.3%), non-diagnostic in 21 (4.1%). Twenty-two patients underwent surgery (8%): 13 carcinomas (10 papillary, 2 anaplastic, 1 medullary), and 9 struma/adenomas. A total of 44 excised nodules were finally examined: 17 hyperplastic nodules (38.6%), 3 adenomas (6.8%), 24 carcinomas (54.5%): 19 papillary, 4 anaplastic and 1 medullary carcinoma. Malignant nodules were solitary in 8.3%; more than one in 91.7%; benign nodules were solitary in 10%, more than one in 90% (NS). Malignant nodules were hypoechoic in 21 (87.5%), isoechoic in 3 cases (12.5%). Benign nodules were hypoechoic in 6 cases (30%), isoechoic in 12 cases (60%), mixed in 2 (10%) ($P<0.0005$). A hypoechoic pattern had 87.5% sensitivity, 70% specificity and 79.5% accuracy in the diagnosis of thyroid cancer. A halo sign was present in 12.5% of malignant nodules vs 40% of benign nodules ($P<0.04$). The absence of a halo sign had 87.5% sensitivity, 40% specificity and 66% accuracy in the diagnosis of cancer. Microcalcifications were present in 12.5% of malignant and 10% of benign nodules (NS) (sensitivity 14%, specificity 90%, accuracy 48%). A hypoechoic pattern, microcalcifications and absence of a halo were simultaneously present in 1/20 benign nodules (5%) and in 3/24 malignant nodules (12.5%) (NS). Diameters were not statistically different (M:21.5 ± 14.3 vs B:19.4 ± 8.8 mm). For positive and suspicious or indeterminate cytological results considered in the same category, US-FNAB had 100% sensitivity, 100% specificity and 100% accuracy in this group of subjects.

P297

Clinical and pathological characteristics of thyroid anaplastic carcinoma: a regional survey in Auvergne

Guillaume Larroumets[1], Igor Tauveron[1], Frederic Somda[1], Beatrice Roche[1], Francoise Desbiez[1], Catherine Dejax[2], Fabrice Kwiatkowski[2], Philippe Thieblot[1] & Groupe Thyroide Auvergne[1]
[1]Endocrinology CHU, Clermont-Ferrand, France; [2]Centre Jean Perrin, Clermont-Ferrand, France.

Thyroid anaplastic carcinoma (TAC) is rare among thyroid cancers. Few surveys on these diseases are available.
Aim
To analyze the clinical and pathological characteristics of TAC in Auvergne, France, from a series of 26 patients among a cohort of over 1500 thyroid carcinomas (i.e. 1.7% of total thyroid cancers).
Results
Mean age was 72.1 years (range 42–91 years), with a sex ratio of 19 women to 7 men. A previous history of thyroid disorder is reported in 77%. 17 patients had goitre (among which 3 previously underwent surgery for nodular disease). 21 patients were euthyroid, 4 hyperthyroid and one presented with hypothyroidism. Recent onset (<6 month) of clinical symptoms is the rule. 92% of patients present with rapidly growing cervical mass. Other common symptoms include dyspnoea (50%), dysphagia (46%), dysphonia (42%). Occasionally pain ((8%), superior vena cava syndrome (19%) or poor general condition is reported. Tumour size is large, 8 cm (range 1–19 cm) with capsular overlap in 69%. Muscular extension occurs in 38%. Lymphadenopathies are reported in 38% and metastasis in 15% at admission. Pathological analysis of TAC reveals spindle cell carcinoma (54%), giant cells (46%) or occasionally squamous cells.In conjunction, 9 patients presented other thyroid carcinomas (7 papillary, 1 follicular and 1 sclerous occult).
Conclusion
TAC remains rare, occurs in the elderly with rapid growth and major compressive disorders. Spindle cell and giant cells are the most common pathologic findings, and association with other thyroid carcinomas appear in over 1/3 patients.

P298

Abstract unavailable

P299

Serum n-terminal pro-b-type natriuretic peptide (NT-proBNP) levels in patients with hyper- and hypothyroidism. Hyperthyroidism may affect NT-proBNP levels as independent of cardiac dysfunction

Senay Arikan, Alpaslan Tuzcu, Deniz Gokalp, Mithat Bahceci & Ramazan Danis
University of Dicle, School of Medicine, Department of Endocrinology, Diyarbakir, Turkey.

Background and aim
It was known that NT-proBNP levels increased in cardiac failure. But, NT-proBNP levels in different thyroid status still unclear. We aimed to evaluate serum NT-proBNP levels in both of hyperthyroid and hypothyroid patients without cardiac insufficiency.
Subjects and methods
Thirty nine patients with hyperthyroidism (43.0±16.5 yr), 25 patients with hypothyroidism (35.4±13.9 yr) and 34 ages matched euthyroid subjects (41.4±13.8 yr) were included to study. After all anthropometric evaluations, body fat analyses were determined with bioelectrical impedance. Electrocardiography and echocardiography were used in cardiac evaluations. Serum NT-proBNP was measured with immunoassay.
Results
Mean serum NT-proBNP levels in hyperthyroid patients was higher than both of control subjects ($P=0.02$) and hypothyroid patients ($P=0.03$). But, mean serum NT-proBNP levels in hypothyroid patients was not different from control subjects. There was a positive correlation between serum NT-proBNP and thyroid hormones (NT-proBNP and fT3: $r=0.316$, $P=0.002$; NT-proBNP and fT4: $r=0.284$, $P=0.006$, respectively). Serum NT-proBNP levels were positively correlated with left ventricle end-diastolic diameters ($r=0.317$, $P=0.006$), interventricular septum thickness ($r=0.395$, $P=0.001$), left ventricle posterior wall thickness (systolic) ($r=0.301$, $P=0.01$), left atrial dimension ($r=0.609$, $P=0.0001$) and negatively correlated with left ventricular ejection fraction ($r=-0.338$, $P=0.003$).
Conclusions
Hyperthyroidism may affect serum NT-pro-BNP levels independent of cardiac insufficiency. NT-proBNP values were increased in hyperthyroidism. Hyperthyroidism may lead to cardiac dysfunction undetermined with conventional echocardiography and these undetermined changes in cardiac functions may lead to elevation of NT-proBNP levels.

P300

Soluble intercellular adhesion molecule-1(sICAM-1) levels and different schemes of Graves' ophthalmopathy (GO) treatment

Elena Bogomazova & Tatjana Mokhort
The Republican Clinical Hospital of Medical Rehabilitation, Minsk Region, Belarus, The State Medical University, Minsk, Belarus.

Aim
To evaluate the base-line serum sICAM-1 levels among patients with GO and levels sICAM-1 at the end of 6 month follow up after different schemes of GO treatment.
Material and methods
72 patients with GO have been surveyed. Patients have been put into 4 groups depending on spent treatment: **1st group** – 26 patients received methylprednisolone per os 1 mg/kg in a combination with plasmapheresis; **2nd group** – 14 patients received pulse-therapy by methylprednisolone; **3rd group** – 18 patients received methylprednisolone per os 1 mg/kg in a combination with plasmapheresis and autogemomagnitotherapy; **4th group** – 14 patients received methylprednisolone per os 1 mg/kg.
We used «Human sICAM ELISA, BMS 201» kits for measured serum sICAM-1 levels.
Results
Serum levels sICAM-1 were 48.13±12.61 in the control group.

	1st group	2nd group	3rd group	4th group
Baseline serum sICAM-1 (M±σ, ng/ml)	81.82±33.3[b]	93.03±33.32[b]	79.04±21.94[b]	90.34±29.53[b]
sICAM-1 (M±σ, ng/ml) after 6 month follow up after GO treatment course	56.16±10.99[a, c]	50.82±13.77[c]	55.02±12.89[a, c]	64.27±29.87[a, c]
Δ sICAM-1 (Me)	17.55[d]	41.16	20.32[d]	23.07[d]

[a]$P<0.05$, [b]– $P<0.00001$ –*vs* control group; [c]$P<0.05$ –*vs* same groups before GO treatment; [d]$P<0.05$ – *vs* 2nd group).

Conclusions
The base-line serum sICAM-1 levels was significantly higher in all groups *vs* control and sICAM-1 levels significantly decreased in all groups at the end of 6 months follow up after the complete course of GO treatment. The treatment of GO with used pulse-therapy by methylprednisolone was better among different schemes of Graves' ophthalmopathy treatment, taking into account that ΔsICAM-1 was significantly higher in this group.

P301

The influence of smoking upon the incidence of Graves' disease and severity of Graves' ophthalmopathy

Eusebie Zbranca, Dumitru Branisteanu, Bogdanel Scurtu & Voichita Mogos
University of Medicine and Pharmacy "Gr.T.Popa", Iasi, Romania.

In order to investigate the relationship between Graves' disease, its associated ophthalmopathy and smoking, we followed retrospectively a group of 270 patients with Graves' disease (233 females and 37 males). Smoking incidence within this group was compared to that found in a control, thyroid disease-free group of 120 patients. The incidence of smokers was significantly higher in the group with Graves' disease (145 out of 270, 54%) when compared to the control group (42 out of 120, 35%, $P<0.01$). The 143 patients with Graves' disease having clinically obvious ophthalmopathy included a higher percentage of smokers than those without significant ophthalmopathy (63% compared to 43%, $P<0.01$). This difference was due mainly to female patients (76 smokers out of 123 female patients with ophthalmopathy – 62%, compared to only 44 smoking ophthalmopathy-free Graves' patients out of 109 −40%, $P<0.001$). Forty-four out of 90 (49%) tobacco users having ophthalmopathy were heavy smokers (i.e. over one pack per day for over 20 years), an incidence significantly higher than that of heavy smokers found in the smoking Graves' patients without ophthalmopathy (19 out of 55, 35%), or in the smoking patients from the control group (13 out of 42, 31%) ($P<0.05$). The data obtained support the hypothesis of tobacco influence upon Graves' disease evolution. Smoking seems to trigger both thyroid disease and ophthalmopathy appearance, especially in females. The risk of ophthalmopathy development and its severity might be dependent of the amount of cigarettes smoked.

P302

Fewer and fewer thyroidectomies in the treatment of Graves' disease

Mihai Radu Diaconescu, Mihai Glod & Ioan Costea
Umf "Gr.T. Popa", Iasi, Romania.

Background
Surgery which was until sixty years ago the only treatment available for Graves' disease (Gd) is now the last recommended therapeutical option, the number of thyroidectomies (Tx) being in continous decrease. American physicians prefere radioiodine use while their European and Japanese colleagues like better long-term administration of antithyroid drugs.
Patients and methods
A retrospective study carried out on 52 consecutive patients with Gd [female/male rate of 46/6 and age range at 28–65 (mean 44 years), representing 38.7% from all cases of thyrotoxicosis surgically treated in our clinic in the last two decennies, the annual number of such interventions is gradually diminished each year from 8 to only one. In all the cases a large subtotal Tx was performed (Dunhill's technique in three patients) conserving less of 5 g of functional tissue. The weight of resected gland varied between 40 to 260 (range 80) g. We had not neither postoperative crisis nor mortality, but permanent recurrent palsy and hypoparathyroidism was noted each in only one case. None of operated patients developed hypothyroidism or recurrent thyrotoxicosis and exophtalmy – present in half of our cases – diminished in 5 patients and was stablished in the rest of them.
Discussion
The better understanding of biologic behavior and natural history of Gd and the availability of effectiveness of another modalities of treatment refined our own philosophy about indications for surgery. So we operated on only patients with failure, major adverse reaction or poor compliance at medical therapy and consumpting clinical syndrome, large size (third or fourth degree) of diffuse goiter eventually with presence of a dominant cold nodule with suspicious FNAB or refusal of radioiodine administration.
Conclusion
In the absence of the golden standard therapy of Gd and in spite of increased worldwide preference for medical and radioiodine treatment with correspondent reduction of number of thyroidectomies, surgery attentive indicated in selected cases proved yet to be a safe and highly efficient solution which quickly restores euthyroidism with minimal risk of anatomic and functional complications.

P303

The prevalence of thyroid cancer in Albania

Agron Ylli[1], Florian Toti[1], Thanas Furreraj[1], Gerond Husi[1], Evis Sinjari[1], Medi Shehu[2] & Edmond Celiku[3]
[1]UHC "Mother Theresa" Service of Endocrinology & Metabolic Diseases, Tirana, Albania; [2]UHC"Mother Theresa" Service of Morphology, Tirana, Albania; [3]UHC"Mother Theresa" Service of Surgery, Tirana, Algeria.

Introduction
The prevalence of thyroid cancer is increasing worldwide as well as in Albania. For the first time we have created the National Thyroid Cancer Register, including the period 2000–2005.
Aim
Define the prevalence of thyroid cancer in Albanian population, the prevalence of different histopathologic forms of cancer and the probable risk factors.
Results
During this period 83 patients were diagnosed of Thyroid cancer. 62 (74.6%) were females. According to histopathologic form we found: papillary form 32 (38.6%), follicular 29 (34.9%), papillo-follicular 6 (7.3%), anaplastic 4 (4.8%), medullary cancer 4 (4.8%), other forms (metastases and lymphoma) 8 (9.6%). The clinical diagnosis at admission was: multinodular goiter 39 cases (46.9%), cold nodule 25 (30.1%), suspected thyroid cancer 11 (13.4%), toxic adenoma 4 (4.8%), benign adenoma 2 (2.4%), Graves' disease 1 (1.2%). According to the age-group: 20–30 yrs old 12 (14.4%), 30–40 yrs 21 (25.3%), 40–50 yrs 18 (21.6%), 50–60 yrs 17 (20.4%), > 60 yrs 15 (18.3%). The papillary form was more frequent in the age group 30–40 yrs old. It was present in M/F 42.8/37%, whilst follicular form was present in M/F 14.2/41.9%.
Conclusions
The thyroid cancer in Albania is more frequent in females than in males, with a 3:1 ratio. The follicular form is more frequent in females, while in general the papillary form is the more frequent one. Almost half of our patients (46.9%) belong to the age group of 30–50 years old. More efforts have to be done for a better and faster diagnosis where the FNA could play an important role.

P304

The prevalence of anti-C1q antibodies is increased in autoimmune thyroid disorders

Eliska Potlukova[1], Jan Jiskra[1], Petra Sandova[2], Zdenka Limanova[1], Daniel Smutek[1], Helena Mareckova[2], Marie Antosova[1] & Marten Trendelenburg[3]
[1]3rd Clinic of Medicine, General Teaching Hospital, 1st Faculty of Medicine, Charles University, Prague, Czech Republic; [2]Laboratory of Clinical Immunology and Allergology, General Teaching Hospital, Prague, Czech Republic; [3]Internal Medicine B and Clinical Immunology laboratory, University Hospital Basel, Basel, Switzerland.

Background
Autoantibodies directed against complement C1q (anti-C1q) have been described in a number of systemic autoimmune disorders. In systemic lupus erythematosus, they are strongly associated with proliferative lupus nephritis. However, no study has focused on the presence of anti-C1q in organ specific autoimmune disorders. The aim of this study was to determine the prevalence of anti-C1q in autoimmune thyroid disorders (AITD).
Methods
Serum levels of anti-C1q were measured using a commercially available ELISA kit (Bühlmann Lab. AG) in 23 patients with Graves' disease (GD) and 51 patients with Hashimoto's thyroiditis (HT). As controls, 16 patients with polynodular goitre and 72 normal blood donors were included. The patients underwent standard endocrinological evaluation.
Results
Positive serum concentrations of anti-C1q (>15 U/ml) were found in significantly more patients with AITD than in controls: 7/23 patients with GD (30.4%; $P<0.005$) and 10/51 patients with HT (19.6%; $P<0.05$), compared to 0/16 with polynodular goitre and 6/72 blood donors (8.3%). In patients with HT, anti-C1q correlated significantly with autoantibodies against thyroglobulin (Spearman $r=0.3312$, $P<0.01$) and against thyroid peroxidase ($r=0.2339$, $P<0.05$). Interestingly, in HT anti-C1q correlated also with thyroid stimulating hormone (TSH) ($r=0.2684$; $P<0.05$). In contrast, in patients with GD we found a negative correlation of anti-C1q with TSH ($r=-0.4169$, $P<0.05$) and a positive correlation with free thyroxine ($r=0.4365$, $P<0.05$).
Conclusions
Anti-C1q antibodies have an increased prevalence in patients with AITD. Their concentration correlates with autoantibodies against thyroid autoantigens and with some of the parameters of thyroid function.
Supported by the grant of the Czech Health Ministry IGA NR 8352-3.

P305

The analgesic efficacy of lidocaine/prilocaine (EMLA) cream during the fine-needle aspiration biopsy of thyroid nodules

Alptekin Gursoy, Derun Ertugrul, Mustafa Sahin, Neslihan Bascil Tutuncu, Asli Nar Demirer & Nilgun Guvener Demirag
Baskent University Faculty of Medicine, Department of Endocrinology and Metabolism, Ankara, Turkey.

Pain is one of the few drawbacks of fine-needle aspiration biopsy (FNAB) in patients with nodular thyroid disease (NTD). Lidocaine/prilocaine cream, an eutectic mixture of local anesthetics (EMLA), is a frequently used topical anesthetic. Despite its well-documented efficacy for the relief of pain associated with other cutaneous procedures that involve needle insertion, the analgesic role of EMLA has not been previously reported in patients with NTD who are undergoing FNAB. The aim of this study was to determine the analgesic efficacy of EMLA for FNAB-associated pain in patients with NTD. This double-blind, placebo-controlled clinical trial was conducted at a thyroid outpatient clinic. We studied 99 patients with NTD. Patients with NTD were allocated to receive either 2.5 g of EMLA ($n=50$) or placebo ($n=49$) 60 minutes before ultrasonographically guided FNAB. A series of 4 biopsies of each nodule was performed. Patients rated pain associated with the procedure according to a 100-mm visual analog scale (VAS), an 11-point numeric rating scale (NRS), and 4-category verbal rating scale (VRS). When the EMLA group was compared with the placebo group, there were no significant differences with respect to age, sex, thyroid volume, nodule size, or nodule site. Significant differences were noted in the pain ratings of those 2 groups according to all 3 pain scales. When the effectiveness of EMLA was compared with that of placebo, the mean VAS score was 25.0 ± 22.3 mm versus 40.0 ± 30.5 mm ($P=.006$) and the mean NRS score was 2.9 ± 2.3 points versus 4.0 ± 2.6 points ($P=.02$). The absolute numbers according to VRS score in each group was also significantly different ($P=.01$). No adverse events from the use of EMLA were reported. To our knowledge, this is the first study demonstrating that a topical anesthetic, EMLA, provides effective and noninvasive analgesia during the FNAB of NTD.

P306

Radioactive iodine in the treatment of type 2 amiodarone-induced thyrotoxicosis

Alptekin Gursoy[1], Neslihan Bascil Tutuncu[1], Arzu Gencoglu[2], Hamiyet Yilmaz[1], Asli Nar Demirer[1] & Nilgun Guvener Demirag[1]
[1]Baskent University Faculty of Medicine, Department of Endocrinology and Metabolism, Ankara, Turkey; [2]Baskent University Faculty of Medicine, Department of Nuclear Medicine, Ankara, Turkey.

Objective
Amiodarone-induced thyrotoxicosis (AIT) is usually classified into 2 types (type 1, in which a high iodine content triggers the autonomous production of thyroid hormone; and type 2, in which destructive thyroiditis causes the release of preformed thyroid hormone). A mixed form of AIT has also been also described. AIT is a difficult management problem that sometimes requires ablative thyroid therapy. The use of radioactive iodine (RAI) therapy in patients with type 1 AIT who had a 24-hour radioactive iodine uptake (RAIU) value of more than 10% has been previously reported. Despite its documented efficacy at usual doses (10–30 mCi) in patients with type 1 AIT, the efficacy of RAI in those with type 2 AIT has never been questioned, because type 2 patients usually have low RAIU. We thought that high adjusted-dose RAI (an adjustment made in accordance with the patient's 24-hour RAIU value and thyroid weight) might be an attractive alternative to thyroid gland ablation in patients with type 2 AIT.
Patients and methods
Four patients with type 2 AIT who required thyroid ablation were included in the study. These individuals were either poor candidates for surgery or had refused surgery. The size of the thyroid gland in all subjects was within normal limits, and each thyroid was characterized by a homogenous echotexture on ultrasonography, the absence of vascularity on Doppler sonography, a low ($<4\%$) 24-hour RAIU value, and the absence of thyroid autoantibodies, all of which are characteristic of type 2 AIT.
Results
The patients were initially treated with thionamides and glucocorticoids. All patients except 1 were euthyroid before RAI therapy. All 4 patients received 1 dose of RAI (range, 29–80 mCi), and followed-up for 12 months. No exacerbation of thyrotoxicosis was noted after RAI therapy. Hypothyroidism (in 3 patients) or euthyroidism (in 1 patient) was achieved in first 6 months.
Conclusions
In patients with type 2 AIT, RAI treatment may be the therapy of choice for thyroid gland ablation.

P307

The prevalence of hyperprolactinaemia in overt and subclinical hypothyroidism

Zeliha Hekimsoy, Sabriye Kafesçiler, Feyzullah Güçlü & Bilgin Özmen
Celal Bayar University Medical Faculty Department of Internal Medicine, Division of Endocrinology and Metabolism, Manisa, Turkey.

Hyperprolactinaemia can occur in patients with primary hypothyroidism. Although its prevalence in overt hypothyroidism varies from 0 to 40%, its prevalence and clinical significance in subclinical hypothyroidism has not been studied. In this prospective, observational study, serum levels of prolactin (PRL) were measured in 167 consecutive patients presenting to our endocrinology clinic for evaluation of hypothyroidism, and correlation of PRL levels with the severity of hypothyroidism (overt or subclinical) was performed. Forty three patients (37 women, 6 men, mean age 46.18 ± 12.98 years) had overt hypothyroidism. One hundred twenty four patients (112 women, 12 men, mean age 44.14 ± 12.19 years) had subclinical hypothyroidism. The other potential causes of the PRL elevation were evaluated. Serum levels of thyrotropin (TSH), free thyroxine, free triiodothyronine and PRL were measured in all patients before L-thyroxine treatment and after TSH normalization. PRL elevation was found in 10 patients (23.25%) with overt hypothyroidism, and in 29 patients (23.39%) with subclinical hypothyroidism. PRL levels decreased to normal levels after thyroid function normalised with L-thyroxin treatment. In both overt and subclinical hypothyroid patients, no relationship was found between TSH and PRL levels. In conclusion, our data confirm that in overt and subclinical hypothyroidism PRL regulation is altered, and that PRL levels return to normal with appropriate L-thyroxine treatment.

P308

The role of parathyroid hormone monitoring after total thyroidectomy in predicting post-thyroidectomy related hypocalcaemia

Evangelos Athanassiou, Gregory Christodoulidis, Constantinos Hatzitheofilou, Constantinos Tepetes, Demetrios Zacharoulis, George Tzovaras & Manthos Euthymiou
University of Thessaly, Larissa, Greece.

Objective
Clinically apparent hypocalcaemia following total thyroidectomy occurs in 20–25% of patients subjected to total thyroidectomy, in 2-4% of these patients the hypocalcaemia is permanent. Treatment by per-os or iv administration of calcium supplements and vitamin D is suggested when the serum calcium concentration falls bellow a critical level, either before or whenever the patients develop clinical symptoms of tetany. The purpose of this study is to evaluate the parathyroid hormone (PTH) serum levels, measured 6, 12, and 24 hours postoperatively, as predictors of hypocalcaemic symptoms in patients undergoing total thyroidectomy.
Materials and methods
In a period of 2 years (2004–2006) 108 patients were subjected to total thyroidectomy for benign or malignant thyroid pathology. Serum PTH, ionized serum calcium, and serum phosphorus levels were measured prior to surgery, 6, 12, and 24 hours postoperatively.
Results
In thirty-one out of 108 patients postoperative hypocalcaemia was observed (28,7%). In four of the 31 patients permanent symptomatic hypocalcaemia occurred, requiring vitamin D and calcium supplements indefinitely (3.7%). Tetany in 22/31 patients with PTH levels lower than 8pg/ml (normal range 8 pg/ml–75 pg/ml). These patients required vitamin D and calcium supplements for a few weeks or months (transient hypocalcaemia). Although 5/31 patients with clinical symptoms of hypocalcaemia (tetany) had the PTH levels recorded postoperatively within the normal range, an abrupt decline of serum PTH of more than 50% of the initial preoperative value was observed. These patients were also treated with vitamin D and calcium supplements for a few weeks postoperatively until the normal function of the parathyroid glands recovered (transient hypocalcaemia). In 77 out of 108 patients with normal calcium, phosphorus and PTH levels no symptoms of hypocalcaemia were noticed.
Conclusion
Following total thyroidectomy, an abrupt decrease in PTH serum levels either within or below the lower value of the normal range, a few hours postoperatively, serves as a reliable predictor of the development of clinically significant hypocalcaemia. Further studies are required however for validation of post-op PTH levels assay, in identification of a group of operated patients requiring prompt early therapy before tetany occurs.

P309

Abstract unavailable

P310

Intraorbital tissues effects of rituximab (RTX) treatment in patients with thyroid-associated ophthalmopathy (TAO)

Guia Vannucchi[1], Irene Campi[1], Stefania Rossi[2], Paola Bonara[3], Claudio Guastella[4], Nicola Currò[5], Simona Simonetta[5], Clara Sina[6], Roberto Ratiglia[5], Paolo Beck-Peccoz[1] & Mario Salvi[1]
[1]Endocrine Unit, Department of Medical Sciences, Fondazione Policlinico IRCCS, University of Milan, Milan, Italy; [2]Pathology Unit, Department of Medicine, Surgery and Dentistry, University of Milan, Ospedale San Paolo, Milan, Italy; [3]Internal Medicine, Fondazione Policlinico IRCCS, University of Milan, Milan, Italy; [4]Otolaryngology, Fondazione Policlinico IRCCS, University of Milan, Milan, Italy; [5]Ophthalmology, Fondazione Policlinico IRCCS, University of Milan, Milan, Italy; [6]Neuroradiology, Fondazione Policlinico IRCCS, University of Milan, Milan, Italy.

We previously described a significant response to RTX treatment in patients with active TAO, with no effect on TRAb and hyperthyroidism. In order to study the effect of RTX in the orbit, we analyzed the orbital tissues of 9 patients with TAO at decompression after RTX (n.2) or other treatments. Decompression was carried out in 2 patients for sight threatening optic neuropathy and in 7 for correction of proptosis. Of the RTX treated patients, one was decompressed after 12 months because of optic neuropathy, while the other after 23 months with burnt out disease. Of the other 7 patients, one was decompressed for the second time because of relapse of optic neuropathy that did not respond to steroids and 6 had burnt out disease of 15-175 months of duration. Immunohistochemistry of orbital fat and muscle showed presence of infiltrating immune cells in all patients. Infiltrates were present independently of the duration and the type of treatment of TAO and of thyroid disease. Interestingly, in the orbital fat of the patient who underwent decompression twice, we observed a typical lymphoid aggregate with CD3+ and CD20+ cells. In patients treated with RTX immunohistochemistry and cytofluorimetry were performed. While no cells were observed in the orbital fat of the patient with burnt out disease, we found persistence of CD3+ cells in the muscle of the patient with optic neuropathy at immunohistochemistry. In this patient, RTX induced peripheral CD20+ depletion, but persistence of 3 and 6% CD19+ after the first and a second cycle of treatment, respectively. Cytofluorimetry showed that almost all of these cells were CD19+5+ both in periphery and the orbital fat, suggestive of autoreactive clones. An increase of the absolute and relative numbers of CD19+5+ was observed in relation to the worsening of optic neuropathy, despite the absence of CD20+. These findings suggest that: 1) immune infiltrates are present in the orbital tissues of TAO patients even in long standing disease; 2) RTX may act by depleting CD20+ in the orbit; 3) persistence of autoreactive CD19+5+ clones in the orbit may correlate with an only temporary and partial response to RTX in TAO patients.

P311

Thyroid and gastric autoimmune diseases

Stéphanie Morel, Agnès Georges, Laurence Bordenave & Jean-Benoît Corcuff
Hôpital Haut-Lévêque, Pessac, France.

Background & aim
Autoimmune thyroid disease (AITD) is frequently accompanied by other organ-specific diseases. The aim of this study was to estimate the frequency of the association AITD-Biermer's disease (pernicious anemia) by investigating the presence of intrinsic factor antibodies (IF-Ab) in the serum of patients with AITD.
Methods
Sera from patients with biological signs of AITD (increased serum TSH levels associated to detectable thyroid peroxydase autoantibodies ($N=55$) or very low serum TSH levels associated to detectable TSHR autoantibodies ($N=58$)) were screened for the presence of type I IF-Ab with an automated chimiluminometric immunoassay based on a competitive method (Access IF Ab). Matched sera from patients with hypothyroidism ($N=66$) or hyperthyroidism ($N=47$) but no detectable peroxydase or TSHR autoantibodies, respectively, were similarly tested.
Results
Sera from 4 patients were tested positive for IF-Ab. All of them suffered from an autoimmune thyroid disease (2 Graves' disease, 2 Hashimoto's thyroiditis). Biermer's disease was previously known for 2 of them. Biermer's disease is strongly suspected in the 2 other patients: for the first, presence of parietal cell autoantibodies, normal serum vitamin B12 concentration and for the second, presence of type I diabetes and vitiligo and low serum B12 concentration. Sera from patients with non autoimmune thyroid dysfunction were all IF-Ab negative.
Conclusion
The incidence of detectable IF-Ab is significantly higher (3.5%) in patients with AITD than in patients with non autoimmune thyroid disease. Testing sera for the other IF-Ab (type 2) should uncover even more patients at risk for vitamin deficiency as the presence of type 2 IF-Ab could occur alone (no type I IF-Ab) in half Biermer's disease (thus potentially doubling the incidence). A prospective study looking for evidence of gastric autoimmunity and vitamin B12 deficiency in patients with AITD should establish whether the need to routinely test the patients is clinically useful or purely academic.

P312

VEGF, FGF and HGF in differentiated thyroid cancer

Elwira Przybylik-Mazurek & Bohdan Huszno
Collegium Medicum of Jagiellonian University in Krakow, Krakow, Poland.

Pathogenesis of thyroid cancer involves a number of biological, and environmental factors. The growth factors have mitogenic, proliferative and dedifferentiating effects. Some of the cytokines: Vascular Endothelial Growth Factor (VEGF), Fibroblast Growth Factor (FGF), Hepatocyte Growth Factor (HGF) are detected in a neoplasmatic tissue. Moreover, there are affected thyroid cancer cell growth and function *in vitro*.
Aim of the study
The aim of the study was to detect if the mitogenic cytokines level is higher in patients with differentiated thyroid cancer (DTC) than in healthy subjects.
Material and methods
There was two groups analysed in this study: 59 patients with DTC (follicular and papillary histotype) age 28 to 68 year and 21 healthy person in the similar age. The personal and familial history of thyroid disease and other chronic diseases was excluded by anamnesis. Blood level of VEGF, FGF and HGF were measured by ELISA kits R&D Systems USA in both groups.
Results
In DTC patients VEGF was significantly higher than in control group: 362.24 pg/ml, vs 198.24 pg/ml. There were no statistic differences between patients wit papillary and follicular histotype. VEGF was highest (413.35 pg/ml) in metastatic patients. FGF was higher in patients (8.37 pg/ml) than in controls (4.10 pg/ml) and in patients with follicular histotype (9.19 pg/ml) than in papillary histoptype (7.85 pg/ml). There were no differences in patients with or without metastases: 7.51 pg/ml vs. 7.37 pg/ml. HGF level in DTC patients was 1434.70 pg/ ml, and in controls 1294.18 pg/ml respectively.
Conclusions
The growth factors: VEGF, and FGF could be sensitive but perhaps not specific peripheral markers of thyroid gland cancer especially in metastatic patients.
Keywords
differentiated thyroid cancer, growth factors, VEGF, HGF, FGF.

P313

Fas and FasL expression on peripheral lymphocytes in patients with autoimmune thyroid disease

Stelios Fountoulakis[1], George Vartholomatis[2], George Philippou[1] & Agathocles Tsatsoulis[1]
[1]Department of Endocrinology, University of Ioannina, Ioannina, Greece; [2]Laboratory of Hematology, Unit of Molecular Biology, University HIospital of Ioannina, Ioannina, Greece.

Objective
The Fas/Fas ligand (FasL) apoptotic pathway is activated in patients with autoimmune thyroid disease (AITD). It is believed that Fas and FasL expression in intrathyroidal T lymphocytes and thyrocytes is regulated in a manner resulting in thyroid cell apoptosis in Hashimoto's thyroiditis (HT) or lymphocyte apoptosis in Graves' disease (GD). The hypothesis that Fas and FasL may be differentially expressed on peripheral lymphocytes in patients with HT and GD was investigated in the present study.
Methods
A total of 45 patients with untreated HT, 30 with subclinical hypothyroidism (mean age 34.9 ± 14.9 years) and 15 with clinical hypothyroidism (mean age 37.0 ± 18.4 years) as well as 13 hyperthyroid patients with untreated GD (mean age 35.8 ± 14 years) were studied and compared with 20 healthy controls (mean age 37.4 ± 15.3

years. Fas and FasL expression on $CD4^+$ and $CD8^+$ peripheral T lymphocytes were evaluated using two- and three-color flow cytometry on FACScan and the appropriate software (CELL Quest, Becton Dickinson).
Results
The proportion of $CD4^+$ T cells expressing Fas was increased in both GD ($64.1\% \pm 14.2$, $P<0.05$) and HT patients ($61.1\% \pm 15.1$ in those with clinical and $61.4\% \pm 13.0$ in those with subclinical hypothyroidism, compared to controls ($49.9\% \pm 7.7$, $P<0.05$). The proportion of CD8+T cells expressing Fas was also increased in patients with HT ($77.4\% \pm 16.6$ in those with clinical and $74.4\% \pm 14.4$ in those with sublclinical hypothyroidism, $P<0.05$) while no significant increase was observed in patients with GD ($67.2\% \pm 10.7$) compared to controls ($59.8\% \pm 14.0$). FasL expression on peripheral $CD4^+$, $CD8^+$ lymphocytes was below 3%.
Conclusion
Fas expression is upregulated in peripheral $CD4^+$ and $CD8^+$ T lymphocytes in patients with untreated AITD with no significant differences between patients with HT and those with GD. This may reflect the activation of the Fas mediated apoptotic pathway in AITD.

P314

A novel pro-migratory action of TGFb in papillary carcinoma

María Elena Rodríguez García-Renueles[1], Susan Belen Bravo López[1], Rafael Seoane[2], Carmen Gil Puig[1], José Cameselle Teijeiro[3], Francisco Barreiro[4] & Clara Álvarez Villamarín[1]
[1]Department of Physiology, School of Medicine, University of Santiago de Compostela, Santiago de Compostela, Spain; [2]Department of Microbiology, School of Medicine, University of Santiago de Compostela, Santiago de Compostela, Spain; [3]Department of Pathology, Hospital Clínico Universitario, Santiago de Compostela, Spain; [4]Department of Surgery, Hospital Clínico Universitario, Santiago de Compostela, Spain.

Neoplastic thyroid diseases (multinodular goiter (MNG), follicular adenoma, differentiated (DTC) and undifferentiated thyroid carcinoma) have a higher incidence in women than in men. In fact, in the last ten years, DTC is the only cancer increasing the frequency in women, with an incidence similar to ovarian carcinoma or lymphomas.

TGFb is a secreted factor important in the normal function of the thyrocyte. It has two independent actions: a fast antiproliferative action, inhibiting cell division through Smads and p15Ink, and an apoptotic action, decreasing p27Kip1 levels and activating Cdk2. In PC2 we have demonstrated that p27Kip1 overexpression blocked TGFb-induced apoptosis and induced a new slow-proliferating action, leading to a slow, but steady cell cycle that increases cell numbers in presence of TGFb.

In this study we have performed microarrays expression study in PC2 cells transiently tranfected with p27Kip1-expressing vectors (or the corresponding empty vector as control), with or without TGFb treatment.

In summary our results show that TGFbeta, apoptotic or anti proliferating genes are increased at the same time that anti-apoptotic genes are decreased in response to TGFb treatment. Interesting, p27Kip1expression reversed this signature causing induction of anti-apoptotic genes and reduction in apoptotic or antiproliferative genes after TGFb treatment. For example, BAX beta is increased in TGFbeta-treated cells but decreased in presence of TGFbeta in p27kip1-overespressin cells. Moreover, we discovered that the experimental condition p27Kip1+TGFbeta induced 12 migration genes and repressed 7 genes whereas mock-transfected cells exposed to TGFbeta increased 2 anti-migration genes and repressed only one.

A new pro-migratory action of TGFbeta in thyroid Papilar Carcinoma suggested by this fingerprint will be discussed.

P315

Significance of accurate serum thyroglobulin cut-off values in the postoperative follow-up of differentiated thyroid carcinoma

András Boér, Borbála Vincze, István Sinkovics, Sándor Keresztes, Bence Kapuvári, Mariann Boldizsár, Éva Remenár & Szabolcs Otto
National Institute of Oncology, Budapest, Hungary.

The present study was undertaken to evaluate the diagnostic performance of a high-sensitive thyroglobulin (Tg) immunoradiometric assay (BRAHMS Tg-S) in the follow-up of papillary and follicular thyroid cancer patients treated with total/nearly total thyreoidectomy and radioiodine ablation therapy. During TSH suppression serum Tg concentration was measured 6 weeks prior to the radioiodine ablation (onT4-Tg before ablation) as well as 3 months following treatment (onT4–Tg after ablation) in 54 tumour-free and 43 metastatic TgAb-negative patients, and accurate cut-off values were calculated. The selectivity and specificity of the measurement were determined by ROC curve analysis (MedCalc statistical software). The cut-off values calculated from the serum Tg levels of 'onT4–Tg before ablation' and 'onT4–Tg after ablation' were 1.9 ng/mL and as low as 0.6 ng/mL respectively. Medical history of 894 patients (differentiated papillary $n=715$ and follicular thyroid carcinoma $n=179$) were compared with the serum levels of Tg, TgAb and TSH at regular intervals. Serum Tg concentrations of clinically tumour-free, TSH-suppressed (TSH <0.3 mIU/L) patients ($N=774$) treated with total/nearly total thyroidectomy was below the threshold level of the kit (<1.9 ng/mL). The sensitivity of Tg determination in TSH-suppressed thyroid cancer patients with local recurrences or lung metastases was 86% and in bone metastases was 100%. The number of false negative data (11/29) was high in patients with papillary cancer and lymph node metastases. The sensitivity of Tg determination could be increased considerably even in case of patients with lymph node metastases by excluding TgAb positive patients. Measuring of Tg and TgAb, with IRMA and RIA methods applied proved to be effective for monitoring differentiated thyroid tumours. The determination of TgAb is highly recommended for the adequate interpretation of serum Tg levels. During the follow-up of patients the most accurate cut-off value should be selected according to the applied therapy.

P316

Thyroid ultrasonography and ultrasonography-guided fine-needle aspiration biopsy of thyroid nodules in correlation with pathological findings

Anna Poplawska-Kita[1], Janusz Dzieciol[2], Urszula Lebkowska[3], Anna Zonenberg[1], Arkadiusz Kita[4], Katarzyna Siewko[1] & Maria Górska[1]
[1]Department of Endocrinology, Diabetology and Internal Medicine, Medical University of Bialsytok, Bialystok, Sklodowskiej 24A, Poland; [2]Department of Human Anatomy, Medical University of Bialystok, Bialystok, Sklodowskiej 24A, Poland; [3]Department of Radiology, Medical University of Bialystok, Bialystok, Sklodowskiej 24A, Poland; [4]Orthopedic Department Regional Hospital, Bialystok, Sklodowskiej 26, Poland.

Introduction
Ultrasonography-guided fine-needle aspiration biopsy (ug-FNAB) is recommended as the first and most important step in the management of nodular thyroid disease.
Material and methods
We compared the results of ultrasonography examination (US) and the ug-FNAB of the thyroid gland with postoperative histpathological findings in 387 patients with thyroidectomy operated on (61 cytological and 326 clinical indication).
Results
Cytological diagnoses included 298 benign nodules (BN) (77%), 40 suspicious of follicular (FN) or 16 of Hurthle cell neoplasm (HCN), 21 papillary carcinoma and 8cysts.The incidence of thyroid carcinomas in the population studied was 8.5%. The size of the nodule was not related to the probability of getting an adequate specimen for cytological diagnosis. All patients were divided into four groups. Group I subjects with BN-97.8% were confirmed on histological results, whereas 6 of them were malignant (4 papillary, 1 follicular, 1 Hurthle cell). Group II histological confirmation of malignancy was 8 (20%) out of 40 patients with a diagnosis of FN (5 follicular, 3 papillary carcinoma). In this group we found also 17 follicular adenoma and 15benign nodules. Group III in the ug-FNAB diagnosed group of HCN after histological verification were 18.7% of carcinoma. GroupIV-in the 21 patients with diagnosis of papillary carcinoma, 16 cases were confirmed, 1 was FN and 4 benign. Correlation of cytology and histology showed that 76.2% ug-FNAB results correlated with the histological diagnoses, whereas 23.8% was discrepant. The smallest papillary carcinoma diagnosed by ug-FNAB had a diameter of 0.4 cm and 30% of all papillary cancer <1 cm displayed stage pT4.
Conclusion
Nodules with non-suspicious ug-FNAB results can be safety followed-up by US and ug-FNAB. However, FN and HCN remain the limitation of ug-FNAB, as the cytology cannot distinguish between benign and malignant nodules. Clinical characteristics, such as gender, age and nodule size, are not useful predictors for the presence of malignancy.

P317

Different prevalence of type 1 and type 2 amiodarone-induced thyrotoxicosis over a 30-year period

Luca Tomisti[1], Enrica Dell'Unto[1], Chiara Cosci[1], Chiara Sardella[1], Luigi Bartalena[2], Fausto Bogazzi[1] & Enio Martino[1]
[1]University of Pisa, Pisa, Italy; [2]University of Insubria, Varese, Italy.

Amiodarone induced thyrotoxicosis (AIT) may develop in patients with either underlying thyroid disorders (type 1) or normal gland (type 2). The latter is

considered a drug-induced destructive thyroditis, usually responding to glucocorticoids. Further treatments after restoring euthyroidism are often not necessary. The former is a true form of iodine-induced hyperthyroidism the management of which includes thionamides, potassium perchlorate and thyrodectomy. The prevalence of the two forms of AIT is unknown.
Objective
To study the prevalence of type 1 and type 2 AIT.
Patients
Two hundred and fifteen consecutive patients with AIT referred to our Department over a 30-year period.
Results
Type 1 AIT was more prevalent at the beginning of the study(67%). During the middle 80's the prevalence of the two AIT forms crossed each other. Thereafter prevalence of type 2 AIT progressively increased (up to 88% in 2006; $P<0.0001$) while that of type 1 AIT decreased. Type 2 AIT patients had a male preponderance, higher serum FT4/FT3 ratio ($P<0.002$), lower thyroidal 3 °h and 24 °h RAIU values($P<0.0001$) and received a higher cumulative dose of amiodarone than type 1 AIT patients ($P<0.0001$).
Conclusions
Over a 30-year period, the prevalence of type 2 AIT progressively increased and that of type 1 decreased. Thus, endocrinologist will face mostly with type 2 AIT patients, who will have a potentially self-limiting destructive thyroiditis, often successfully treated with glucocorticoids. On the other hand, a more aggressive (total thyrodectomy) therapeutic option might be necessary in patients unresponsive to glucocorticoids. Finally, after restoring euthyroidism, patients should be followed for late-developing hypothyroidism.

P318

Carbamazepine and risk of hypothyroidism: a prospective study

Jiri Horacek, Julius Simko & Gerhard Waberzinek
[1]Charles University, Faculty of Medicine and University Hospital, Dept. Internal Medicine, Hradec Kralove, Czech Republic; [2]Charles University, Faculty of Medicine and University Hospital, Dept. Neurology, Hradec Kralove, Czech Republic.

While carbamazepine (CBZ) treatment may affect serum thyroid hormone concentrations it rarely leads to clinically important hypothyroidism. This study was aimed to evaluate an early effect of CBZ on thyroid status in hypothyroid patients with thyroid hormone replacement, as compared with patients without a thyroid disorder.

Twenty-nine patients indicated for CBZ treatment were followed prospectively. Their thyrotropin (TSH), total thyroxine (TT4) and free thyroxine (FT4) serum levels were assayed before the start of CBZ medication (150 mg/d in the 1st week, then 450 mg/d), and then at week intervals for 7 weeks. Nineteen patients had no thyroid disorder before CBZ treatment (control group A), whereas 10 patients were treated with L-thyroxine (median 100 ug/d) for hypothyroidism and were stable before CBZ treatment (group B). The fluctuations of thyroid status after the start of CBZ treatment were compared between the groups.

In the control group, TT4 was significantly decreased by ca. 15 to 25%, starting from the 1st week of treatment (Friedman, $P<0.001$), while FT4 was decreased by only ca. 10 to 15%, and the significance ($P<0.001$) was delayed till the 2nd week. There was a concomitant increase in FT4/TT4 ratio ($P<0.001$) and a mild, non-significant increase in TSH ($P=0.073$) never exceeding normal range. Conversely, in group B with hormonal replacement, a similar TT4 and FT4 decline was followed by significantly increasing TSH levels ($P=0.011$), while the FT4/TT4 ratio was not significantly changed ($P=0.218$). In 3 of 10 patients TSH rose over 5 mU/L in the 3rd and 4th week, and the treatment had to be modified.

In patients with no thyroid disorder, CBZ caused subtle hormonal changes of no clinical relevance, due to adaptive response. In hypothyroid patients with replacement therapy this adaptation is lacking, and CBZ may precipitate subclinical or overt hypothyroidism. In this group, thyroid function monitoring early in the course of CBZ treatment seems advisable.

The study has been supported by research project MZO 00179906

P319

Epidemiology of thyroid cancer in the north eastern region of Poland

Anna Zonenberg[1], Beata Telejko[1], Agnieszka Nikolajuk[1], Mikolaj Rybaczuk[2], Janusz Dzieciol[3], Lech Zimnoch[4], Maciej Kokoszko[5], Jacek Dadan[5], Mieczyslaw Wasielica[6], Jacek Borawski[1], Zbigniew Puchalski[5], Marzena Juczewska[7], Ida Kinalska[1] & Maria Gorska[1]
[1]Department of Endocrinology, Diabetology and Internal Medicine, Medical University, Bialystok, Poland; [2]Department of Business, Informatics and Logistics, Technical University, Bialystok, Poland; [3]Department of Human Anatomy, Medical University, Bialystok, Poland; [4]Department of Clinical Pathomorphology, Medical University, Bialystok, Poland; [5]Department of General Surgery, Medical University, Bialystok, Poland; [6]Voivodship Hospital, Bialystok, Poland; [7]Oncological Hospital, Bialystok, Poland.

Before the introduction of mandatory salt iodination in 1997 the North-Eastern Region of Poland was known to be a moderate iodine deficiency area. Moreover, it was one of the mostly contaminated regions after the Chernobyl accident in 1986. The aim of our study was to evaluate the descriptive epidemiological features of incident thyroid cancers diagnosed among the residents of this area between 1996 and 2005. The Regional Cancer Surveillance Program was used to collect data on 691 newly diagnosed thyroid cancers registered during a 10–year period. The average annual incidence of all types of thyroid cancer per 100 000 residents rose from 3.9 in 1996 to 5.5 in 2005 (mean – 5.8 cases per 100 000 inhabitants). Thyroid cancer was more frequently diagnosed in women (82%) than in men. The majority of all cases was diagnosed in the age group of 46–55 years. There were 12 newly diagnosed cancers in children under 15 years of age (3 cases among children born after the Chernobyl disaster). The commonest histological type was papillary carcinoma (74.5%). Follicular type accounted for 10.9%, oxyphilic – 5.4%, medullar - 4.5%, anaplastic - 3.0% and other types – for 1.7% of cases. Conclusion: The increased incidence of thyroid cancers between 1996 and 2005 is most likely explained by the improvement in diagnostic techniques, but the effect of ionizing radiation after the Chernobyl accident has also to be taken into account. The influence of iodine deficiency seems to be a less probable factor in view of the predominance of the papillary type of carcinoma.

P320

The Na^+/I^- symporter (NIS) transports two of its substrates, I^- and ClO_4^-, with different stoichiometries

Orsolya Dohan[2], Carla Portulano[1], Cecile Basquin[1], L Mario Amzel[3] & Nancy Carrasco[1]
[1]Albert Einstein College of Medicine, Bronx, New York, United States; [2]Institute of Experimental Medicine Hungarian Academy of Sciences and Szent Rokus Hospital and Institutions, Budapest, Hungary; [3]Johns Hopkins School of Medicine, Baltimore, MD, United States.

The sodium/iodide transporter (NIS) mediates active I^- uptake in thyroid, lactating breast, salivary gland, and stomach epithelial cells. NIS-mediated I^- transport is electrogenic with a 2:1 Na^+:I^- stoichiometry, i.e. when NIS activity is measured electrophysiologically in NIS-expressing oocytes, inward positive currents are recorded upon addition of I^- or other anions that are NIS substrates. However, no currents are detected when perchlorate (ClO_4^-), a competitive inhibitor of NIS is used. This suggests that ClO_4^- either blocks NIS function without being transported, is translocated with a 1:1 stochiometry, or is transported at an extremely slow rate. ClO_4^-, which is used in military industry as a component of jet fuel, is a well known environmental contaminant of water supplies. The possible impact of environmental ClO_4^- exposure on the thyroid function of adults and nursing newborns is widely debated.

We report a thorough analysis of NIS-mediated ClO_4^- transport *in vivo* and *in vitro*. When lactating rats received ClO_4^-, both mothers and suckling pups exhibited a ~50% decrease in thyroidal I^- uptake relative to controls. For *in vitro* studies, we used a polarized MDCK epithelial monolayer setup in which NIS is expressed on only one side. Simultaneous addition of I^- and perchlorate markedly slowed NIS translocation of I^- to the opposite side, as compared to the control with I^- alone, because perchlorate was translocated first.

Hill plot analysis of NIS-mediated Na^+-dependent perrhenate transport revealed that perrhenate, an analogue of ClO_4^-, is transported with a 1:1 stoichiometry, explaining the absence of electrical currents observed with perrhenate also. Taken together, these observations provide novel mechanistic information on NIS, i.e. that NIS catalyzes substrate transport with different coupling ratios. In addition, that perchlorate is unequivocally transported by NIS and therefore actively concentrated in the milk, suggests that ClO_4^- water contamination may be more serious than previously thought, particularly for the most susceptible population, pregnant and lactating women and nursing newborns.

P321

Estimation of influence radioiodine treatment on course of Graves' ophthalmopathy

Olga Nechaeva, Alexander Dreval, Tatiyana Shestakova, Yulia Bardeeva, Irina Komerdous, Oksana Perepelova & Irina Chzikh
Moskow Regional Research Clinical Institute, Moskow, Russia.

Aim
To estimate influence of radioiodine treatment of Graves' disease (GD) on course of Graves' ophthalmopathy (GO).
Material and methods
9 patients with GD and mild or moderate GO were included to the study (3 (33%) men and 6 (67%) women); the anamnesis of smoking had 4 persons (44%). Diagnosis of GO was evaluated by determination of severity and activity of disease with CAS, presents of diplopia, orbital ultrasound. CAS before radioiodine treatment (RIT) and glucocorticoid pulse – therapy was 2.7 ± 0.7 points. The thickness of rectal extraocular muscles were (right/left eyes): upper – $5.44 \pm 0.37/5.4 \pm 0.4$ mm, low – $5.6 \pm 0.3/5.5 \pm 0.08$ mm, lateral – $5.1 \pm 0.3/5.1 \pm 0.3$ mm, medial – $5.2 \pm 0.5/5.2 \pm 0.5$ mm.
5 (55.6%) patients were underwent of prevention intravenous pulse therapy with glucocorticoids in a mean doze of 4.4 ± 2.3 gr. This therapy was spent 0.5–1.5 months prior to RIT. CAS in all patients after pulse therapy was 1.5 ± 0.7 points. The median of activity of 131I was 10.4 mCu.
Results
Right after treatment periorbital edema was determined in 2 cases (22%), burning of cornea – 2 (22%). All symptoms were stopped within 10 days. We did not find significant changes of eye muscles thickness.
In 1.5 months after RIT 7 (77.8%) patients were without worsening of GO. There was increasing of CAS to 2.5 points in other cases, but all these patients were hypothyroid. Symptoms of activity were decreased without additional treatment after administration of L-T4. Diplopia was kept in 1 patient without worsening.
Conclusions
After RIT worsening of GO was observed only in hypothyroid patients. In all cases it was not required to special therapy. In some cases symptomatic therapy was appointed.

P322

Influence of a subclinical thyrotoxicosis on heart in various age-grades

Alina Babenko, Elena Grineva & Natalia Vashrameeva
Pavlov Medical State University, St.Petersburg, Russia.

Subclinical thyrotoxicosis (ST) characterized by low serum TSH and normal FT_4 and FT_3 concentrations. ST may cause changes of geometry of heart and developments of diastolic dysfunction. Influence of ST on this evolutions depending on age of patients, duration of S?, effect of TSH level is not clear. In present research the effects of ST on changes of EchoCG at a different age were studied. The present study includes 66 normotensive patients with ST without any CVD (the age of 20–60 years, 6 men and 60 women) The patients were examined echocardiography by standard method. The patients were distributed on 3 age-grades: 1st group (gr1) ($n=20$) – 20–35 years; 2-nd group (gr2) ($n=24$) – 35–45 years and 3-rd group (gr3) ($n=22$) – 45–60 years. The parameters EchoCG were normal in patients of gr1 and gr2: relative wall thickness (RWT) ($0.34+0.009$ and $0.35+0.01$ cm), left atrial diameter (LAD)($3.8+0.09$ and $3.8+0.07$ cm), isovolumic relaxation time (IVRT) ($93.8+1.93$ and $92.7+3.1$ msec) left ventricular mass index (LVMI) ($83.6+3.24$ and $90.5+5.1$ g/m^2). However, the mean RWT ($0.41+0.01$ cm, $P<0.05$), LAD($4.1+0.18$ cm, $P<0.05$), IVRT($100.6+4.1$ msec, $P<0.05$) and LVMI ($103.2+7.3$ g/m^2 $P<0.05$) in patients gr3 was higher than that in gr1 and gr2. The frequency of left ventricular hypertrophy (LVH) was in gr1 – 10%, in gr2 – 8.3%, in gr3 – 36.4%, left atrial enlargement (LAE) was in gr1 – 25%, in gr2 – 20.8%, in gr3 – 35.5%, diastolic dysfunction (DD) was in gr1 – 30%, in gr2 – 31.8%, in gr3 – 47.4%, increase pulmonal pressure >30 (IPP) was in gr1 – 19%, in gr2 – 59%, in gr3 – 19%. The level T3, T4 was highly positive correlated with LAD($r=0.32$, $P<0.05$) and pLA ($r=0.55$, $P<0.01$) and level TSH was highly negative correlated with pLA($r=-0.31$, $P<0.05$). The LVMI and IVRT were positive correlated both with age ($r=0.49$, $P<0.01$ and $r=0.34$, $P<0.05$) and level T3($r=0.32$, $P<0.05$ and $r=0.25$, $P<0.1$). Specific attributes of influence of ST on a heart were appearanced of IPP, LAE and DD, which were meet at any age with high often. The LVH was less characterised at ST and frequency of its development at young age is similar as in a comparable population on age. Frequency of LVH was significantly higher in patients >45 years old.

P323

Thyroid abnormalities during treatment with PEGIFNα-2a and PEGIFNα-2b in patients affected by HCV-related chronic disease: a prospective randomized study

Francesco Scavuzzo[1], Guido Di Monda[1], Domenico Serino[1], Maria Teresa Tartaglione[2], Filippo Lampasi[2], Antonio Ascione[2] & Raffaele Volpe[1]
[1]Endocrinology Unit – Cardarelli Hospital, Naples, Italy; [2]Liver Unit – Cardarelli Hospital, Naples, Italy.

Background
Correlation between therapy with interferons, thyroid autoimmunity and dysfunction is widely reported. Currently we used two different pegilated-interferons (α-2a, α-2b).
Aim
Evaluate a probable different behaviour of two PEG-IFN responsible of thyroid abnormalities.
Patients and methods
We observed 236 consecutive naïve-patients with HCV-related chronic-disease undergoing a treatment with antiviral therapy from June 2003 to June 2005; we enrolled 54 females and 68 males alternatively to α-2a (median age 49.03, chronic hepatitis 98, cirrhosis 24) and α-2b (median age 48.3, chronic hepatitis 106, cirrhosis 16). Thyroid autoimmunity (TgAb, TPOAb) and function (FT_4, FT_3, TSH) were evaluated before, during treatment (3, 6, 9, 12 months) and in follow up (12 months).
Results
At the end of treatment 21 patients (8,6%), median age 48.03, 10 females, all chronic hepatitis without cirrhosis, 16 without preexisting thyroid dysfunction, 5 with low positivity for thyroid autoantibodies (Abs+), developed thyroid disorders:

	pts	hypothyroidism hyperthyroidism in autoimmune thyroiditis		Subacute thyroiditis	Abs+
A-2a	10	4	2	1	3
A-2b	11	9	2	0	0

Therapy was discontinued for thyroid abnormalities in 3 patients: 2 for hyperthyroidism to VI month, (one with α-2a, one with α-2b, Abs+ before therapy), confirmed in the follow up; 1 for subacute thyroiditis to VI month with α-2a, with euthyroidism in the follow up. At the end of follow up 6 patients were Abs−, 3 was Abs+; for 8 patients hypothyroidism, for 4 patients hyperthyroidism remained.
Conclusions
Thyroid disease appear to III,VI and IX month of therapy in: **α-2a** 4,4 and 2 patients (8 females; median age 47); **α-2b** 1,7 and 3 patients (5 females; median age 44.3). Two PEG-IFN don't show significative differences for induced thyroid dysfunction; furthermore none cirrhotic patients developed thyroid abnormalities.

P324

The effect of L-thyroxin theraphy on left ventricular diastolic dysfunction in patients with subclinical hypothyroidism

Tayyibe Saler[1], Zuhal Aydan Saglam[1], Kazim Korkmaz[1], Hayriye Esra Ataoglu[1], Mustafa Yenigun[1] & Berrin Oguzhan[2]
[1]Haseki Training and Research Hospital 4th Clinic of Internal Medicine, Istanbul, Turkey; [2]Brussels Free University, Department of Hormonology, Brussels, Belgium.

Aim
Subclinical Hypothyroidism (SH) is associated with cardiovascular disorders which may include increased risk for atherosclerosis, endothelial dysfunction and myocardial dysfunction.To investigate the prevalence of left ventricular systolic and diastolic dysfunction in patients with subclinical hypothyroidism and the effect of L-thyroxin therapy on myocardial performance using conventional echocardiographic parameters.
Method
The study includes 95 patients (F/M:83/12, age: 40.91 ± 10.07 years) with SH as judged by elevated serum thyroid-stimulating hormone (TSH) levels (>4.2 mIU/l) and FT3 and FT4 within the normal range and 44 healthy controls (F/M: 39/5, mean age 38.77 ± 9.59 years). None of the participants had hypertension or BMI >25 kg/m^2. All patients and the control group underwent standart echocardiography and Doppler imaging. E/A ratio [early (E) and late (A) mitral peak velocities] and the interventricular septum thickness (IVST) were determined. 25 SH patients with E/A ratio <1 were diagnosed as myocardial diastolic dysfunction and received LT4 replacement therapy during 6 months in order to establish euthyroidism. The biochemical and echocardiographic measurements were repeted six months later.
Results
The E/A ratio was significantly different among SH and control group.At the baseline the SH patients showed significantly lower E (0.83 ± 0.25 vs 0.99 ± 0.17, $P<0.0001$), E/A ratio 1.18 ± 0.33 vs 1.33 ± 0.23, $P<0.003$) and IVST (0.98 ± 0.12 vs 0.91 ± 0.08, $P=0.001$). Left ventricular end systolic and diastolic diameters were comparable between the two groups ($P=0.025$ and $P=0.494$ respectively). After 6 months of follow-up with LT4 replacement therapy, 25 patients with SH had significantly higher

E/A ratio (1.09±0.22 vs 0.75±0.23, $P<0.0001$) and reduced (1.05±0.14 vs 0.95±0.10, $P<0.0001$) IVTS measurements. With the comparison of all groups with Pearson test, TSH levels show a parallelism with IVST ($r=0.194$; $P=0.031$).
Conclusions
LT4 replacement therapy may reverse the impairment of left ventricular dysfunction and IVST observed in SH patients and should be advised to prevent the alteration of myocardial function.

P325

Risk factors for thyrotoxic cardiomyopathy

Veronika Shulgina[1], Galina Melnichenko[1], Valentine Fadeev[2] & Sergey Shornikov[2]
[1]National Research Centre of Endocrinology, Moscow, Russia; [2]Sechenov Moscow Medical Academy, Moscow, Russia.

Objective of the study
To examine risk factors for thyrotoxic cardiomyopathy (ThC).
Methods
In retrospective study (1975 to 2003) 272 patients aged 54 [43; 62] years with different forms of toxic goiter in combination with cardiac rhythm disturbances with or without heart failure (HF) were included. Atrial fibrillation (AF) and/or atrial flutter and/or ventricular premature beats accompanied with HF were diagnosed in 80.5% (219/272) patients (group 1), whereas 19.5% (53/272) patients had sinus tachycardia and/or atrial premature beats without HF (group 2).
Results
The prevalence of demographic and clinical characteristics of two groups was compared by use of χ^2-test. The factors associated with ThC $P<0.05$ (age at onset of thyrotoxicosis, age at hospitalization, period from onset of thyrotoxicosis until first treatment, period from onset of thyrotoxicosis until hospitalization, ophthalmopathy, relapse of Graves' disease, familial history of hypertension and coronary heart disease, such cardiovascular characteristics as previous history of rhythm disturbances, angina and HF) were retained as potential confounders. Then, binominal logistic regression was performed to identify those factors most associated with ThC using a probability value of $P<0.05$ and to calculate odds ratios (ORs) and 95% confidence intervals (CIs).
After adjustment for above-mentioned factors period over 1 year from onset of thyrotoxicosis until first treatment (OR=1.8, CI 95% 1.06–3.13; $P=0.02$) and age at hospitalization (OR per 1-year increment=1.1, CI 95% 1.02–1.15; $P=0.01$) remained independently associated with ThC. Weak positive interaction was observed between these two factors ($r=0.16$; $P=0.007$).
Conclusion
The data on natural history of patients with thyrotoxicosis and cardiovascular symptoms allowed us to identify risk factors for ThC. The frequency of ThC is increased in older patients with period from onset of thyrotoxicosis until first treatment over 1 year.

P326

Partial withdrawal of levothyroxine to stimulate serum thyroglobulin (TG) in the follow-up of differentiated thyroid carcinoma (DTC)

Francesca Ianni, Carlo Antonio Rota, Salvatore Maria Corsello, Annapina De Rosa, Francesca Gallo, Rosa Maria Paragliola, Francesca Ingraudo, Maria Pia Ricciato, Maria Ludovica Maussier, Massimo Salvatori & Alfredo Pontecorvi
Catholic University of Sacred Heart, Rome, Italy.

Aim
We compared effectiveness of partial withdrawal of levothyroxine (L-T4) to the use of recombinant human TSH (rhTSH) in preparation for Tg testing. We also evaluated clinical aspects and quality-of-life (QOL) during both regimens.
Materials and methods
Ten consecutive patients, previously treated with total thyroidectomy and radioiodine ablation for DTC, underwent rhTSH protocol and, after 15 days, reduced their L-T4 dose by 50% for 5 weeks. At the fourth week TSH was tested (predictive cut-off >10 μUI/ml), and at the fifth week TSH and Tg were measured (cut-off TSH >25 μUI/ml). Patients who did not reach the last cut-off were asked to continue half-dose protocol and to repeat TSH and Tg dosage at the sixth week.
At baseline and at the end of both rhTSH and "half-dose" protocols, all patients filled out questionnaires for QOL (SF-36) and symptoms and signs of hypothyroidism (Zulewski score). The study was approved by local ethical committee.
Results
Adequate stimulation of Tg was obtained in all patients after rhTSH. At half-dose protocol, 5/10 patients had TSH >25 μUI/ml at the end of the fifth week and 2/10 attained cut-off at the end of the sixth week. One patient left the study, another patient had limited compliance because of depression, and the last one completely withdrew L-T4 to receive radioiodine treatment because of high stimulated-Tg levels although not attaining TSH cut-off.
Tg levels were slightly more sensitive in the partial withdrawal scheme than in the use of rhTSH, but without any statistically significant difference. During the partial withdrawal period 5/7 patients reported no disease-specific morbidity, while 2/7 had just minimal discomfort. On the SF-36 health survey no statistically significant differences were found.
Conclusion
Partial L-T4 withdrawal seems to be an effective, simple, economical and well-tolerated procedure for Tg stimulation during follow-up for DTC.

P327

Selected markers of endothelial dysfunction in patients with subclinical and overt hyperthyroidism

Anna Modzelewska, Malgorzata Szelachowska, Anna Zonenberg & Maria Gorska
Department of Endocrinology, Diabetolygy and Internal Medicine Medical University of Bialystok, Bialystok, Poland.

Objective
The aim of this study was to evaluate the influence of subclinical and overt hyperthyroidism on the chosen markers of endothelial function.
Material and methods
The groups studied consisted of 97 hyperthyroid subjects (51 with subclinical and 46 with overt hyperthyroidism) and 39 healthy controls matched for age, gender and body mass index. The following parameters were measured: TSH, FT3, FT4 (by MEIA), VCAM-1 (vascular cell adhesion molecule 1), ICAM-1 (intercellular adhesion molecule 1), von Willebrand factor (vWF) and PAI-1 (plasminogen activator inhibitor 1) (by ELISA). Statistical analysis was performed using the computer program STATISTICA 6.0. The local ethical committee aproved the study.
Results
Among hyperthyroid patients 71 had toxic goiter (42 with subclinical and 29 with overt hyperthyroidism) and 26 had Graves' disease (9-subclinical, 17-overt hyperthyroidism). Significantly higher VCAM-1 levels were found in patients with overt and subclinical hyperthyroidism in comparison with the control group (1336.5+608.5 and 1168.9+508.4 vs 835.5+302.6 ng/ml, $P<0.001$ and $P<0.001$, respectively); vWF concentration was also significantly higher in patients with overt and subclinical hyperthyroidism than in the controls ($P<0.001$ and $P<0.01$, respectively), and in patients with overt hyperthyroidism in comparison with the subclinical group ($P<0.01$). The highest PAI-1 values were observed in patients with overt hyperthyridism (68.07 ng/ml, $P<0.001$ in comparison with subclinical hyperthyroidism and $P<0.001$ in comparison with the control group). There were not significant differences in ICAM-1 levels between the groups studied.
Conclusion
Our results suggest that endothelial dysfunction occurs in patients with overt as well as subclical hyperthyroidism.

P328

The changes in the incidence of nodular goitre, thyroid cancer and urine excretion of iodine in the inhabitants of north eastern Poland in 1997 and 2005

Anna Zonenberg[1], Wieslaw Zarzycki[1], Beata Telejko[1], Anna Modzelewska[1], Agnieszka Nikolajuk[1], Mikolaj Rybaczuk[2], Janusz Dzieciol[3], Katarzyna Siewko[1], Ida Kinalska[1] & Maria Gorska[1]
[1]Department of Endocrinology, Diabetology and Internal Medicine, Medical University, Bialystok, Poland; [2]Department of Business, Informatics and Logistics, Technical University, Bialystok, Poland; [3]Department of Human Anatomy, Medical University, Bialystok, Poland.

A higher incidence of nodular goitre and less differentiated types of thyroid carcinoma have been observed in iodine deficient regions. North-Eastern Poland was an area with a moderate deficiency of iodine until the introduction of the mandatory salt iodination in 1997 (30±10 mg KJ/kg NaCl). The aim of our study was to compare the incidence of goitre, thyroid carcinoma and urine excretion of iodine in the inhabitants of the North-Eastern Region of Poland in 1997 and 2005. In 1997 816 persons were investigated, 431 (52.8%) of whom reported for follow-up investigation in 2005. The study consisted of a questionnaire. thyroid ultrasonography and the measurement of iodine concentration in random urine sample. Parenchymatous goitre was found in 267 persons (32.7%) in 1997 and in 37 persons (8.6%) in 2005 ($P<0.001$. chi^2-58.165). The incidence of nodular

goitre was 12.75% (104 persons) and 24.59% (106 persons), respectively ($P<0.001$. chi^2-19.557). In 1997 three cases of papillary carcinoma were diagnosed. and in 2005 – 1 case. Decreased iodine excretion was observed in 71.28% subjects in 1997 and in 19.1% in 2005 ($P<0.001$. chi^2-105.748). Conclusion. During the last 8 years, the incidence of parenchymatous goitre in the North–Eastern Poland significantly decreased, whereas the percentage of nodular goitre increased in the period analysed. Prospective analysis did not reveal an increase in thyroid carcinoma incidence. The observed changes may be due to the introduction of the mandatory iodination of table salt in Poland in 1997.

P329

The role of deiodinases in thyronamine biosynthesis

Susanne Piehl[1], Balizs Gabor[2], Heberer Thomas[2] & Josef Köhrle[1]
[1]Institut für Experimentelle Endokrinologie, Charité – Universitätsmedizin, Berlin, Germany; [2]Bundesinstitut für Risikobewertung, Fachgruppe 55, Berlin, Germany.

Deiodinases (5′-D1, 5′-D2, 5-D3) control the systemic and local bioavailability of thyroid hormones by removing iodine from their substrates. Thyronamine (T0AM) and 3-iodothyronamine (3-T1AM) are possible novel metabolites of classical thyroid hormones which have been demonstrated to occur endogenously and to display unique effects such as reducing body temperature in mice and activating the plasma membrane bound G-Protein coupled receptor TAAR1 (Scanlan *et al.*, 2004). As the pathways of thyronamine biosynthesis are still unknown, we reasoned whether deiodinases might be involved.

In preliminary experiments using classical ^{125}I release assays the HepG1 cell line was found to express a specific 5′-D1 activity of $1.2+0.29$ pmol iodide released$\times$ mg$^{-1}\times$min^{-1} but not to exhibit 5′-D2 or 5-D3 activity at all. Thus, HepG2 cells were used to study the ability of 5′-D1 to accept thyronamines as substrates. Cells were homogenized in HEPES buffer containing sucrose, EDTA and DTT. Homogenates were incubated for 2 h at 37 °C in the absence or presence of 1 mM PTU in 100 mM sodium phosphate buffer at pH = 6.8 containing 1 mM EDTA, 20 mM DTT and various concentrations of the following substrates: thyronamine (T0AM), 3-iodothyronamine (3-T1AM), 3,5-diiodothyronamine (3,5-T2AM), 3,5,3′-triiodothyronamine (3,5,3′-T3AM), 3,5,3′,5′-tetraiodothyronamine (T4AM) as well as rT3 and 3′,5′-diiodothyronine (3′,5′-T2) as positive controls. Deiodination products were analysed using a newly established selected reaction monitoring (SRM) based liquid chromatography tandem mass spectrometry (LC-MS/MS) method.

5′-D1 from HepG2 cells did not deiodinate any of the thyronamines at the substrate concentrations tested (50 nM to 20 μM). Thus, a role of 5′-D1 in thyronamine deiodination is rather unlikely. The ability of 5′-D2 and 5-D3 to accept thyronamines as substrates still remains to be tested.

Supported by Graduate College 1208, DFG

P330

Thyroid disease prevalance in Cushing's disease

Tayfur Toptas, Ozay Tiryakioglu, Demet Ozgil Yetkin & Pinar Kadioglu
Istanbul University Cerrahpasa Medical Faculty Division of Endocrinology and Metabolisim, Istanbul, Turkey.

Purpose
To determine the prevalence of nodular thyroid disease, autoimmune thyroid disease, goiter and primary thyroid dysfunction in patients with active Cushing's disease.
Patients and methods
Nineteen patients with active Cushing's disease (17 female, 2 male, mean age 43.16 ± 3.55 years) and forty, age and gender matched healthy volunteers who served as the control group (34 female, 6 male, mean age 47.28 ± 2.31 years) were included in the study. The diagnosis of active Cushing's disease was determined by, 24 hour urine free cortisol levels, 1 mg dexamethasone suppression test and loss of diurnal rhythm. fT3, fT4, TSH, anti TPO, anti TG measurements and thyroid ultrasound were performed in both groups.
Results
Thyroid gland volume was smaller in patients with Cushing's disease (11.84 ± 1.5 ml vs 17.85 ± 1.84 ml). The prevalence of goiter was 2/19 (11%) and 12/40 (30%), the prevalence of nodular thyroid disease was 10/19 (52%), and 20/40 (50%), the prevalence of autoimmune thyroid disease was 7/19 (58%) and 20/40 (50%), the prevalence of primary thyroid disease was 6/19 (27%) and 10/40 (25%) in Cushing's disease and in control group respectively.
Conclusion
The prevalence of nodular thyroid disease, goiter, autoimmune thyroid disease and primary thyroid dysfunction in Cushing's disease was found similar to control group.

P331

From sampling to analytics: experience and diagnostic consequences with some thyroid markers

Erzsebet Toldy[1], Zoltan Locsei[2], Istvan Szabolcs[3], Erzsebet Toldy[4] & Gabor L. Kovacs[5]
[1]Central Laboratory Markusovszky Teaching Hospital of County Vas, Szombathaly, Hungary; [2]1st Department of Medicine, Markusovszky Teaching Hospital of County Vas, Szombathely, Hungary; [3]National Medical Centre, Budapest, Hungary; [4]Department of Practical Diagnostics, Institute of Diagnostics and Management University of Pecs, Szombathely, Hungary; [5]Institute of Laboratory Medicine, University of Pecs, Pecs, Hungary.

In the clinical laboratory practice, endocrine biomolecules are mainly measured by immunoassay. Storage of the samples can not be avoided in many cases. Measurement in the low concentration ranges require exact knowledge on how storage would influence the functional sensitivity of the measurement.
Aim
To evaluate the effect of storage of serum samples on their stability and the functional sensitivity of the applied method.
Methods
The biomolecules parathormone intact (PTHi), thyroglobulin (Tg) and thyroglobulin antibody (TgAb) were studied.The measurements were performed by an electrochemiluminescence immunoassay (Elecsys 2010, Roche). The stability of Tg and TgAb were studied in serum ($N=71$) and that of PTHi in plasma ($N=31$) as well. The parameters were measured in the fresh samples as well as after 4 and 8 hour of storage at room temperature and after 48 hour of storage at 4–10 °C. A longer-term storability test was also performed by keeping the samples for 1–4 weeks in deep freezer. The functional sensitivity of the methods was calculated from the results of deep frozen samples.
Results
In the first 8 hours the immunoreactivity of Tg, TgAb and PTHi changed only marginally (2–8%). During 48 hours storage, the Tg immunoreactivity increased by 23%, the PTHi molecule by 5–12% and the TgAb immunoreactivity decreased by 8–13%. During the long-term deep freezing, the immunoreactivity of all biomarkers decreased by 12–39%. A stronger degradation of molecules was observed in the lower range. PTHi appeared to be more stable in plasma than in serum samples. The functional sensitivity of the PTHi (2.6 pg/ml) and Tg (0.66 ng/ml) methods were excellent, but the TgAb (85 IU/ml) sensitivity makes questionable the application as a tumor marker.
Conclusions
The immunoreactivity of Tg, TgAb and PTHi is not influenced by a short storage at room temperature, but freezing even for longer-term significantly alters the analytic results.

P332

$G_{q/11}$-dependent signaling of the tyhrotropin receptor regulates metallothionein 1 expression in human thyroid carcinoma cells

Thomas Buech[1], Christer Baeck[1], Nicola Nowak[2] & Thomas Gudermann[1]
[1]Department of Pharmacology, Philipps University, Marburg, Germany; [2]CRBA Gynecology & Andrology, Schering AG, Berlin, Germany.

Metallothioneins (MT) are cystein-rich intracellular proteins which exert anti-apoptotic effects by protecting cells against oxidative stress and DNA damage. Previously, expression of MT in normal and neoplastic thyroid tissue has been demonstrated. However, the thyroidal regulation of MT expression is widely unsettled. Thus, we investigated the expression of MT isoform 1 in human thyroid carcinoma cells (FTC-133-TSHR) upon stimulation with thyrotropin (TSH). Using quantitative RT-PCR we found that TSH led to a dose-dependent increase in MT-1 mRNA levels in these cells. To further characterize the signaling pathway involved in MT-1 induction we investigated thyroid carcinoma cells expressing a mutated TSH receptor incapable to couple to $G_{q/11}$ proteins (FTC-133 Y601H cells). In these cells, TSH still led to a marked increase in intracellular cAMP levels wheras an increase in inostiol phosphates was completely absent. Interestingly, TSH did not induce MT-1 in these cells, giving evidence that regulation of MT-1 was cAMP-independent but dependent on $G_{q/11}$-coupling. This finding was further corrobated by the fact that TSH-promoted incuction of MT-1 in FTC-133-TSHR cells was blocked by inhibitors of phospholipase C, whereas treatment with phorbol esters mimicked the effect of TSH. Finally, we investigated changes in MT-1 protein levels. Immunoblots and immunocytochemistry with MT-1 specific antibodies revealed a TSH-induced up-regulation of MT-1 in FTC-133-TSHR cells whereas no effect of TSH occurred in FTC-133 Y601H cells. The finding of $G_{q/11}$-dependent regulation of MT-1 by TSH adds further complexity to possible cAMP-independent functions of the TSH receptor.

P333

Association of cytokine gene polymorphism with Graves' disease in Turkish population

Faruk Kutluturk, Sema Yarman, Fatma Oguz Savran & Cigdem Kekik
Istanbul University, Istanbul, Turkey.

Cytokines play a crucial role in the pathogenesis of autoimmune thyroid disease, and recent studies have demonstrated an association between cytokine gene polymorphisms and Graves' disease (GD) in different ethnic groups.The aim of the present study was to investigate the relationship of IL-6, IL-10, TNF-α, TGF-β, and INF-γ gene polymorphisms with the development of GD in Turkish population. A total of 224 subjects were included in the study comprising of 100 patients with GD (70F/30M; mean age, 43.9 ± 13.8 years) and 124 healty subjects (81F/43M; mean age, 37.8 ± 10.2 years) without antithyroid autoantibodies or family history of autoimmune disorders.Genotyping was done by using PCR and sequence-specific primers. Statistical analysis showed a significant association between high TNF-α −308GA and IL-6 −174CC gene polymorphisms in patients with GD compared to control subjects ($P=0.044$, $P=0.016$, respectively). On the otherhand, the frequency of TNF-α −308GG genotype was significantly increased in control subjects compared to patient ($P=0.049$). However, no differences were observed between GD and control subjects for IL-10, TGF-β, and INF-γ gene polymorphisms. In conclusion, these results suggested that TNF-α −308GA and IL-6 −174CC gene polymorphisms are involved in susceptibility for GD, whereas TNF-α −308GG gene polymorphism has a protective effects against the development of GD in Turkish population.

P334

Hashimoto's encephalitis: role of diagnostic SPECT

Tomas Martin, Alfonso Gentil, Alberto Torres, Silvia Maraver & Milagrosa Diaz-Galvez
University Hospital "Virgen Macarena", Seville, Spain.

In autoimmune thyroid disease some diverse neurological alterations like dementia, psychosis or peripheral neuropathy, are described. Hashimoto's encephalopathy (EH) is a serious form of these neurological alterations. We describe three cases with different presentation and morphologic normal tests where cerebral SPECT was diagnostic.

Case n°1. A 32-year-old male diagnosed of autoimmune hypothyroidism which presents paresthetics and muscular stiffening, what do not improve with oral levotiroxine. The analyses shows a TSH> 200 and T4L of 0.2 ng/dl, with Ac. antiTPO> 4500 U/ml. After substitution, TSH 9, T4 l 1.80 ng/dl. RMN cranial and EEG were no diagnostic, SPECT shows cortical diffuse hypoperfusión, starting therapy with deflazacort 60 mg/24 h with evident improvement, worsening when the was reduced. Treatment was restored by 2 mg/kg. with resolution of the clinic.

Case n°2. 39-year-old female presents migraine, confusion and agitation with hallucinations and fever treated with aciclovir and antibiotics. A normal thyroid function with Ac. antiTPO >3000 U/ml was found and SPECT show patched cortical affectation in temporal lobe. Therapy with prednisona to 1.5 mg/kg was established, with successful results.

Case n°3. 33-year-old male with hiperthyroidisim autoimmune, in treatment with carbimazole, present a convulsive stroke. Increase TSI (TSI> 40 U/ml) and Ac antiTPO: 5850 U/ml, with normal thyroid function was found (TSH: 0,025 mU/ml, T4L 1,90 ng/dl). A treatment with carbamazepine (800 mg/24 h), discharging him. One month later he shows recidivants convulsive attacks again. Normal RMN, slow wave diffuse EEG without epileptic foci. SPECT showed a decrease of cortical perfusión. Therapy with steroids achieved disapparing the convulsions.

Conclusions

EH's diagnosis must be considered in subacute presentation, high levels of antithyroid antibodies (even with thyroid normal function) and absence of another pathology. The practice of cerebral SPECT a and a fast response to steroids are important confirmation signs in this pathology.

P335

Usefulness of Ki-67, PCNA, c-erbB-2 and CK 19 in the diagnosis of some thyroid follicular tumors

Ioana Zosin[1], Marioara Cornianu[2], Ioana Golu[1], Melania Balas[1] & Aurora Milos[1]
[1]University of Medicine and Pharmacy Victor Babes, Clinic of Endocrinology, Timisoara, Romania; [2]University of Medicine and Pharmacy Victor Babes, Department of Morphopathology, Timisoara, Romania.

This study represents a complex evaluation of a group of 26 cases with thyroid nodular disease (TND), using different diagnostic methods. Clinically and by means of ultrasonography, 12 patients were suspected of malignancy. Fine-needle aspiration biopsy (FNAB) detected mainly suspicious (12 cases) and malignant smears (5 cases). The microscopic examination of surgical specimens established the following diagnosis: follicular adenoma – FA (4 cases), papillary hyperplasia – PH (2 cases), papillary carcinoma – PC (10 cases), follicular carcinoma – FC (2 cases) and Hürthle cell tumors – HCT (8 cases). PCs were represented by occult, classic forms and variants, HCT included adenomas, carcinomas and some adenomas showed an uncertain malignant behaviour. Metastases were diagnosed in 6 cases.The expression of Ki-67 antigen, proliferating cell nuclear antigen (PCNA), cytokeratin (CK) 19 and c-erbB-2/neu oncogene was evaluated by IHC (DAKO LSAB method) in all surgical specimens. For IHC we used paraffin-embaded sections and monoclonal antibodies (mAb): MIB-1, PC10, mAb against c-erbB-2 and mAb CK 19. The most interesting conclusions regard the expression of CK 19 and c-erbB-2. CK 19 was diffusely and intense expressed in all cases of PCs, 1 case of Hürthle cell carcinoma (HCC), but never in PH. There was no apparent difference in immunostaining reactivity between tumors with or without metastases. Follicular and oxyphilic cell neoplasia showed at best a focal staining. Regarding the expression of c-erbB-2, 50% of PCs presented a cytoplasmic staining pattern and the rest a mixed one (cytoplasmic and membranous). Some FC and HCC showed also a mixed staining. The epithelial malignant tumors with metastases presented more expressed reactivity versus the cases without metastases.

The used corroborated investigations helped us to obtain an accurate diagnosis in some peculiar epithelial thyroid tumors.

P336

Soluble CTLA-4 is increased in Graves' disease and not related to thyroid status or ophthalmopathy severity

Jacek Daroszewski[1], Edyta Pawlak[2], Marek Bolanowski[1], Miroslaw Slowik[3] & Irena Frydecka[4]
[1]Dept. of Endocrinology, Diabetology and Isotope Treatment, Medical University, Wroclaw, Poland; [2]Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wroclaw, Poland; [3]Dept. of Ophthalmology, Medical University, Wroclaw, Poland; [4]Dept. of Hematology, Blood Neoplastic Diseases and Bone Marrow Transplantation, Medical University, Wroclaw, Poland.

Cytotoxic T-lymphocyte antigen-4 (CTLA-4) is a B7-binding protein that plays an important role in the down-regulation of T-cell activation. CTLA-4 function is closely associated with predisposition to autoimmune diseases. A native soluble form of CTLA-4 (sCTLA-4) is reported to be present in the sera of patients suffering from autoimmune thyroid disease. In this study we report data on sCTLA-4 concentrations in patients with clinical expression of Graves' disease.

The study group consisted of 102 patients with Graves' disease (83 females and 19 males, mean age: 50 ± 11 years). Of these, 47 were euthyroid, 38 were hyper-, and 9 were hypothyroid. Nine patients were without clinical signs and symptoms of ophthalmopathy, while 42 presented mild and 51 severe ophthalmopathy. The control group was 38 apparently healthy volunteers. Study was approved by a local Ethical Committee.

Soluble CTLA-4 was measured in serum by means of ELISA.

sCTLA-4 was not measurable in 13 samples from the control group, while it could be estimated in all the patient serum samples and was higher than in control group (range: 0.02-1983.94 ng/ml, median: 7.48 ng/ml, dispersion: 11.2 ng/ml vs. range: 0.16-35.49 ng/ml, median: 3.2 ng/ml, dispersion: 3.98 ng/ml, respectively, $P=0.03$).

Soluble CTLA-4 concentration was not related to FT4 or to FT3 level ($r=0.026$ and $r=-0.034$, respectively). Regression analysis of factors describing the severity of the course of disease (thyroidectomy, ^{131}I treatment, or methylprednisolone treatment in the past) did not reveal any link with sCTLA-4 concentration ($P=0.15$). Soluble CTLA-4 serum level was also not related to the severity of ophthalmopathy.

In our group of 102 patients with Graves' disease, sCTLA-4 was higher than in the control subjects. Soluble CTLA-4 was a sensitive marker of the disease and appeared to be related neither to metabolic status nor to clinical course of the disease or the severity of eye changes.

P337

The relationship of epicardial fat thickness with carotid intima media thickness and endothelial function in subclinical and overt hypothyroidism

Dilek Yazici[1], Hasan Aydin[1], Beste Özben[2], Ahmet Toprak[2], Dilek Yavuz[1], Ozlem Tarcin[1], Seda Sancak[1], Oguzhan Deyneli[1] & Sema Akalin[1]
[1]Marmara University Medical School, Section of Endocrinology and Metabolism, Istanbul, Turkey; [2]Marmara University Medical School, Deparment of Cardiology, Istanbul, Turkey.

Hypothyroidism is associated with increased cardiovascular mortality. Epicardial fat thickness (EFT) has been found to be correlated with visceral fat accumulation and is thought to be a novel cardiovascular risk factor. The aim of this study was to determine EFT and its relationship with carotid intima media thickness (CIMT) and flow-mediated vasodilation (FMD) in subclinical and overt hypothyroid patients.

Ten patients with overt (Group H) (42.2±15.1 y; F/M:9/1) and 18 patients with subclinical hypothyroidism (Group SH) (34.7±10.3 y; F/M:16/1) and without any other systemic disease were included. 28 healthy volunteers were recruited as controls. EFT was determined by M-mode echocardiography and FMD and CIMT were evaluated by Doppler echocardiography. The study was approved by local Ethical Committee.

EFT, FMD and CIMT results and the comparisons between the groups are shown in the table. EFT was weakly correlated with CIMT ($r=0.33$; $P=0.11$) and FMD ($r=-0.26$; $P=0.22$). TSH was also weakly correlated with CIMT ($r=0.33$, $P=0.11$) and FMD ($r=-0.38$; $P=0.06$).

	GROUP H ($n=10$)	GROUP SH ($n=18$)	CONTROLS ($n=27$)	P
Epicardial fat thickness (mm)	4.42±2.41[a]	2.41±1.49	3.28±0.31	P<0.05
FMD (%)	6.63±4.05[b]	11.33±6.07	9.99±5.44	NS
CIMT (mm)	0.60±0.18[c]	0.51±0.05	0.52±0.07	NS

[a]$P<0.05$; [b]$P=0.06$; [c]$P=0.05$ compared to group SH

Epicardial fat accumulation is greater in subclinical and overt hypothyroid patients than healthy controls. This finding is more prominent in overt hypothyroid patients. Although larger studies are needed to confirm this preliminary finding, EFT seems to be a promising marker for early atherosclerotic changes in this group of patients.

P338

Prevalence of thyroid antibodies in gestational diabetes mellitus

Luísa Ruas[1], Sandra Paiva[1], Maria Campos[1], Elvira Marta[2], António Lobo[2], Annette Cravo[2] & Manuela Carvalheiro[1]
[1]Endocrinology Dept – University Hospital of Coimbra, Coimbra, Portugal; [2]Obstetrical Dept – University Hospital of Coimbra, Coimbra, Portugal.

Background
Pregnancy alters the natural history of autoimmune thyroid disorders. The incidence rate of positive thyroid antibodies (T-abs +) in asymptomatic women during pregnancy has been reported to be between 6 and 19.6%.
Aim
To determine the prevalence of thyroid antibodies (T-abs) in Gestational Diabetes Mellitus (GDM).
Subjects & Method
In 408 women, at the time of diagnose of GDM, TSH, free thyroxine, free tri-iodothyronine and anti-thyroid antibodies (T-abs) (thyroperoxidase and thyroglobulin) were measured. In these women we evaluated: previous thyroid disease, maternal age, BMI, spontaneous abortion, first degree relatives with D.M., Sullivan and OGTT values, insulin needed for diabetes control, new-born weight, gestational age at the time of GD diagnose and at delivery, evaluation of glucose tolerance after delivery. Statistical analysis involved SPSS (Statistical Analysis for Social Sciences); $P<0.05$ was considered to indicate statistical significance.
Results
From the women (408) who were enrolled in the study 21(5.1%) had positive T-abs. Only 20 women had thyroid disease (2%), with no direct relation with the presence of T-abs. The presence of T-abs + had a positive correlation with type 1 DM abs ($r=0.202$, $P<0.001$). There was no correlation between T-abs + and TSH, free thyroxine and free tri-iodothyronine values, as well as with the other maternal and fetal variables.
Conclusion
The results revealed a prevalence of autoimmune thyroid disease of 5.1% in women with GDM, identical to normal pregnant women, thus this measurement should not be systematic in women with GDM during prenancy. However, in the sub-group of GDM with type 1 DM positive abs, the positive correlation founded, suggests a systematic screening for T-abs. These data reinforce the importance of screening of latent pluri glandular auto immune disorders during pregnancy in women prone for those.

P339

Thyroid investigation profile in patients with Hashimoto's thyroiditis associated with other autoimmune disorders

Catalina Poiana[1], Dan Peretianu[2] & Mara Carsote[3]
[1]"Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania; [2]SCM "Povernei", Bucharest, Romania; [3]"C.I.Parhon" Institute of Endocrinology, Bucharest, Romania.

Introduction
The prevalence of autoimmune disorders (AID) is more frequent in patients with Hashimoto's thyroiditis (HT).
Aim
To see if the clinical and biochemical aspects are different among the patients with HT and if they change related to the systemic or organ specific AID.
Material and method
A. HT was diagnosed on antithyroperoxidase antibodies (ATPO) over 34 UI/ml. **B**. 491 patients with HT were investigated; 67 (15.8%) of them associated other known AID. **C.** AID were also searched in 404 patients with ATPO less than 34UI/ml, as control group; 21 (5.19%) of them had at least one AID. **D.** TSH, antithyreoglobulin antibodies (ATG) and the thyroid echographic pattern – spilt into 7 subtypes, according to our original classification, were also investigated. **E.** Statistical analysis was performed using student' t test and χ^2 test, as appropriately.
Results
1. Prevalence of AID in HT patients is higher than in control group ($P<0.001$, $\chi^2=17.82$, 56 degrees of freedom). **2**. The most frequent AID were vitiligo, immune hepatitis, rheumatoid arthritis, drugs allergies and premature ovarian failure. **3.** The mean age at diagnosis was not statistically different between patients with HT and AID and patients with HT, but without AID, respectively 50.97 years vs. 50.06 years, $P=0.6$. **4**. The sex ratio in HT-AID patients and HT-nonAID patients was the same (96% women). **5**. Average of ATPO levels in HT-AID patients was statistically significant higher than in HT-nonAID patients (respectively 964.47 UI/ml vs. 587.44 UI/ml, $P=0.054$). **6**. The mean values of TSH were not different between the two subgroups (8.81 μUI/ml vs. 9.76 μUI/ml, $P=0.75$). **7**. The difference between mean ATG levels was small and non significant ($P=0.34$). **8**. There was a certain difference between echographic patterns ($P=0.025$, $\chi^2=16.06$, 7 degrees of freedom), but without the predominance of a specific subtype.
Conclusions
1. In HT, AID are more frequent than in control group. **2**. Vitiligo is by far the most frequently AID associated with HT. **3**. Higher ATPO levels are found in patients with HT associated with other AID.

Thyroid – presented on Monday

P340

Ultrasound patterns in patients with autoimmune thyroiditis

Dan Peretianu[1], Mara Carsote[2] & Catalina Poiana[3]
[1]SCM "Povernei", Bucharest, Romania; [2]"C.I.Parhon" Institute of Endocrinology, Bucharest, Romania; [3]Carol Davila University of Medicine and Pharmacy, Bucharest, Romania.

Objectives
To analyze the conceptual frame for a correlation between thyroid echographic description and antithyroid peroxydase antibodies (ATPO) levels in Hashimoto's thyroiditis (HT).
Material-methods
A. 783 patients: 396 with HT (ATPO > 34 UI/ml), 386-control (ATPO < 34 UI/ml). **B**. Ultrasound aspects were described in 8 patterns: 0-thyroid lack; 1 – hypoechogenic and pseudonodular; 2 – hypoechogenic and homogenous; 3 – hypoechogenic micronodular; 4 – macronodular (>10 mm), 5 – inhomogeneous hypoechogenic and pseudonodular; 6 – anechogenic micronodular; 7 – diffuse hyperechogenic (normal). **C**. ATPO was split into 9

intervals as: 0 – 34, 35 – 100, 101 – 350, 351 – 550, 551 – 800, 801 – 999, 1000 – 3000, 3001 – 5000, > 5000 UI/ml.
Results
A. In HT cases, pattern - number: 0 – 2; 1 – 186; 2 – 53; 3 – 25; 4 – 75; 5 – 39; 6 – 10; 7 –6. In controls: 0 - 1, 1 – 21; 2 –46; 3 –55; 4 –150; 5 –20; 6 – 21 7 – 73. **B**. Sensibility, specificity and positive predictive value (PPV): pattern 0: 51%, 99.74%, 66.67%; pattern 1: 46.97%, 94.56%, 89.86%; pattern 2: 12.28%, 88.08%, 53.54%; pattern 3: 6.31%, 85.55%, 31.25%; pattern 4: 18.04%, 61.14%, 34.44%; pattern 5: 9.85%, 24.82%, 66.1%; pattern 6: 2.53%, 94.56%, 33.26%; pattern 7: 1.52%, 81.09%, 7.54%. **C.** Correlation between serum ATPO and ultrasonographic patterns: χ^2 test (54 degrees of freedom) = 100.94; $P = 0{,}0002$.
Conclusions
1. There are differences in sensitivity, specificity and positive predictive values for the 7 patterns. **2**. When PPV is near 90%, as in pattern 1, the test may be very suggestive for HT. Therefore, "hypoechogenic-pseudonodular" type means HT in 90% cases. **3.** PPV around 30% as in 3, 4, 6 patterns reveals low probably of HT. **4**. In type 7, PPV of 7.54% reflects a very low possibility of HT.

P341

Iodine intake in Portugal: preliminary results in pregnant women
Edward Limbert[1], Susana Prazeres[1], Ana Miranda[1], João Castro[2], Francisco Carrilho[3], Henrique Reguengo[4] & Fatima Borges[4]
[1]Portuguese Cancer Institute, Lisbon, Portugal, [2]Military Hospital, Lisbon, Portugal, [3]University Hospital, Coimbra, Portugal, [4]Sto Antonio Hospital, Porto, Portugal.

Introduction
Iodine is the key element for the synthesis of thyroid hormones and its intake modulates the physiology and physiopathology of thyroid gland. In Portugal, endemic goiter has disappeared, but some data make us consider that iodine intake, as in other European areas, is far from being sufficient. Taking into account the potential harmful effects of moderate iodine deficiency during pregnancy, when needs are increased, and the absence of recent data on iodine intake in Portugal, a countrywide study on urine iodine was undertaken. Preliminary results of this on going study from pregnant women are presented
Material and Methods
Target Population-Pregnant women from maternity hospitals and school children from strategic geographical areas (coast line and inland);1911 urines from 8 maternity hospitals were analysed.
Urinary iodide-A fast colorimetric method (Gnat *et al*, Clin Chem 2003) is being used
Statistical methods-Central methods and proportional comparison tests
Global Results
Median urinary iodide concentration was 88.9 μg/L, being 21.3% below 50 μg/L.19% had values above. 150 μg/L
Results by Hospital
Median urinary iodine varied from 78 to 124 μg/L; 13.9% to 29.6% of women had values below 50 μg/L and 12.5 to 34% had values above 150 μg/L In South Portugal the proportion of women with values below 50 μg/L was significantly lower in Greater Lisbon than in other cities.
Conclusions
Although this results are preliminary they point out to an inadequate iodine intake in pregnant women, from most Portuguese regions. Considering these preliminary results the on going study needs to be completed (data from pregnant women and also from school children) and more detailed analysis is warranted in order to explain the observed differences between regions. Taking into account the potential deleterious effects of inadequate iodine supply during pregnancy, iodine supplementation is recommended in this period of life.

P342

"False negative" results of Tg in patients with DTC (differentiated thyroid carcinoma)
Vassilios Voliotopoulos[1], Vassilios Gianakopoulos[2], Ioannis Valais[1] & Isaia Charalabidou[1]
[1]Iatroplis Magnitiki Tomografia, Athens, Greece, [2]Sotiria Hospital, Athens, Greece.

Purpose
The long-term monitoring of patients with differentiated thyroid carcinoma is essential throughout the patient's life after total or near total thyroidectomy followed by I-131 ablation after thyroid suppression using recombinant TSH (Thyrogen).
As known the administration of rhTSH increase the sensitivity of Tg concentrations measurements. Antithyroglobulin antibodies are common clinical problem in patients with differentiated thyroid carcinoma. Because the presence of these antibodies usually interferes with serum globulin.
Methods and materials
We used the recombinant human TSH in 20 patients one year after the ablation therapy. All patients underwent WBS I-131 scan and thyroglobulin (Tg) and antithyroglobulin antibodies (ATG) were measured using eclia assay technique.
Results
8 patients had positive anti-Tg antibodies and in these patients the result was confirmed using the Tg confirmatory test (Roche Cobas 6000 eclia method).
In 3 patients the percentage recovery wasn't in our laboratory's expected values (a finding of 70–130% indicates correct recovery). In these patients we suggest another I-131 therapy.
Conclusion
Our data suggest that ATG determination and the following recovery test may determine some additional information to the follow-up of patients with DTC. We have to improve our ability to predict and monitor wich patients are likely to be harmed by their disease or oppose to those who will live unaffected by theirs.

P343

The incidence of Hashimoto's thyroiditis in the differentiated thyroid carcinoma
Vassilios Voliotopoulos[1], Vassilios Gianakopoulos[2] & Ioannis Valais[1]
Iatropolis Magnitiki Tomografia, Athens, Greece, [2]Sotiria General Hospital, Athens, Greece.

Purpose
Hashimoto's thyroiditis is medical disease witch affects more than 5% of the population and represents the most common cause of hypothyroidism. The possibility of an immunological and autoimmune mechanism in the pathogenia of the disease has been suggested.
Methods and Materials
In 200 patients, who received iodine 131 therapy after total or near total thyroidectomy for one or more cold nodules,in our department last year (71% with papillary and the rest with follicular carcinoma) 50 (25%) had Hashimoto's thyroiditis, based in the cytological analysis of the surgical resects thyroid gland. In 25 patients the diagnosis of Hashimoto's thyroiditis was not reached before the surgery.
Conclusion
An adequate follow-up of the patients with Hashimoto's thyroiditis may permit an early diagnosis of the differentiated thyroid cancer and its appropriate management, because the increased incidence of DTC and HT may indicate that HT is a precursor of thyroid cancer.

P344

Thyroid cancer radioidine therapy using recombinant human TSH
Vassilios Voliotopoulos[1], Vassilios Gianakopoulos[2] & Ioannis Valais[1]
[1]Iatropolis Magnitiki Tomografia, Athens, Greece, [2]Sotiria General Hospital, Athens, Greece.

Purpose
The use of recombinant TSH (Thyrogen) has already entered in the clinical routine in order to avoid the discomfort and the morbidity associated with the withdraw of the thyroid hormone.
Methods and Materials
We used the recombinant human TSH in 20 patients (age > 50 years) totally or near totally thyroidectomized who came in our clinic to receive radioiodine therapy for locally invasive differentiated thyroid cancer. All patients were treated, while euthyroid on L-4, after rhTSH administration with to consecutive daily injections (0.9 mg) of rhTSH. Half of them underwent diagnostic –before therapy diagnostic whole body scan using again rhTSH administration and after that iodine therapy using an identical second course of rhTSH.
Results
Administration of Thyrogen promoted I-131 therapy uptake in all patients as demonstrated with the post-therapeutic whole body scan. As known the administration of rhTSH increase the sensitivity of the Tg (thyrogobulin) concentrations measurements. About 12 months after therapy we performed whole body I-131 scan and we show a complete remission of the residual sites and in two patients reduction in one metastatic site.
Conclusion
Administration of rhTSH is safe and a very useful tool for inducing I-131 uptake in local or metastatic differentiated thyroid cancer avoiding L-T4 withdrawal.

P345

Characterization of facilitative glucose transporters (GLUT) in human thyroid carcinoma cell lines

Raffaele Ciampi[1], Agnese Vivaldi[1], Pamela Piampiani[1], Laura Agate[1], Piero Salvadori[2], Alberto Del Guerra[3], Aldo Pinchera[1] & Rossella Elisei[1]
[1]Department of Endocrinology, University of Pisa, Pisa, Italy, [2]Institute of Clinical Physiology (C.N.R.), Pisa, Italy, [3]Department of Physics "E. Fermi", University of Pisa, Pisa, Italy.

18FDG-PET is based on the capability of tumor cells to take up glucose. An increment in expression of the glucose transporter 1 (GLUT1) has been observed in thyroid tumors with poor prognosis but very few data are available about the expression of other glucose transporters in thyroid. Here, we study the expression and function of GLUT isoforms 1, 2, 3, 4, 6, 8, 10, 12 in human thyroid carcinoma cell lines ARO and FRO (anaplastic carcinoma), NPA (poorly-differentiated papillary carcinoma), WRO (follicular carcinoma) and TT (medullary carcinoma). We studied expression of GLUTs by conventional and quantitative RT-PCR, we evaluated cell 2-Deoxy-D-[^{3}H]-glucose uptake and we studied GLUT1 protein on cell membrane fractions. We confirmed that GLUT1 is the predominant isoform in thyroid carcinoma with higher expression in ARO and FRO. By contrast, GLUT3 expression is lower in these two cell lines but comparable to GLUT1 in WRO, NPA and TT. GLUT4 and GLUT10 are barely expressed in all cell lines. We also observed GLUT6 and GLUT8 expression in all cell lines and GLUT12 in ARO, TT and FRO. Western blot shows GLUT1 protein in ARO and FRO membrane fractions. All lines studied but TT display different levels of glucose uptake; surprisingly, NPA and WRO uptake is higher than in ARO and FRO although these latter show higher levels of GLUT1 expression. In conclusion, we confirm that GLUT1 is the predominant form in thyroid tumors but other isoforms can be present and its protein is abundant in anaplastic carcinoma cell membranes. Medullary carcinoma cell line TT, despite the expression of some GLUT isoforms, is not able to take up glucose. Finally, the high rate of glucose uptake observed in NPA and WRO could be justified by presence of other forms of GLUT not considered in this study.

P346

Genotype/phenotype relation for toxic thyroid nodules with or without TSH receptor mutations

Hulya Gozu[1], Sandra Mueller[2], Rifat Bircan[3], Knut Krohn[4], Mehmet Sargin[1], Haluk Sargin[1], Cem Gezen[5], Turgay Erginer[5], Nimet Karadayi[6] & Ralf Paschke[1]
[1]Dr. Lutfi Kirdar Kartal Education and Research Hospital, Section of Endocrinology and Metabolisim, Istanbul, Turkey; [2]Leipzig University, Department of Internal Medicine III, Leipzig, Germany; [3]Marmara University, School of Medicine, Department of Medical Biology, Istanbul, Turkey; [4]Leipzig University, Interdisciplinary Center of Clinical Research, Leipzig, Germany; [5]Dr. Lutfi Kirdar Kartal Education and Research Hospital, Section of 1.General Surgery, Istanbul, Turkey; [6]Dr. Lutfi Kirdar Kartal Education and Research Hospital, Department of Pathology, Istanbul, Turkey.

Constitutive activation of the cAMP pathway by activating TSHR mutation stimulates both thyrocyte proliferation and function. Thus they lead to formation of toxic thyroid nodules (TTNs) and ultimately hyperthyroidism. The *in vitro* activity of the various TSH-receptor mutation varies from 2–7 fold cAMP increase over the wild type TSH receptor. One previous study invrestigated a possible genotype to phenotype relation in TTNs with somatic TSHR mutation with a negative result.

TSHR mutations have been identified in 52(70.2%) of 74 TTNs in a recent study. In order to investigate the genotype-phenotype relation in TTNs we compared the clinical and laboratory findings of these patient (nodules) with or without TSHR mutation.

Most strikingly, nodule volume was found significantly higher in the mutation + groups (Z:-2,803;*P*:0,005). No significant difference between iodine sufficient and deficient regions of Turkey was established for all of the clinical and laboratory findings. Genotype-phenotype relation was also evaluated for the different *in vitro* basal cAMP fold increases of the somatic TSH receptor mutations over the wild type TSH-receptor. No statistical difference was noticed for the clinical (age, time for euthyroidism, cumulative dose of propylthiouracil (PTU), nodule and thyorid volume) and laboratory (TSH, FT4, FT3)) findings between groups of different basal cAMP fold (basal fold ≤ 2, $n=5$, fold 2–5, $n=15$ fold ≥ 5, $n=8$).

TSH at the start of PTU treatment was found significantly lower in the mutation (+) group (Z:-2.058; *P*: 0,040). FT3 level was also found higher in the mutation positive groups, but it was not significant (Z:-1.755; *P*: 0,079). No significant difference was found between mutation +/− groups for serum level of FT4, age, gender, thyroid volume, time to euthyroidism until the end of PTU treatment and cumulative dose PTU for establishment of euthyroidism.

No significant difference was found between TSHR D727E variant +/− nodules for thyroid and nodule volume, time to euthyroidism after begin of PTU treatment, cumulative dose of PTU for establishment of euthyroidism and serum levels of FT3, FT4. Serum level of TSH was found significantly lower in the variant positive groups (Z:-2,385; *P*:0,017).

Our results suggest that hot nodules with a somatic TSHR mutation are larger than those without a TSHR mutation.

P347

High prevalence of ER22/23EK polymorphism of the glucocorticoid receptor gene in patients with Graves' orbitopathy

Belema Boyle, Katalin Korányi, Rita Bertalan, Károly Rácz & Csaba Balázs Semmelweis University, Budapest, Hungary.

Objective

To investigate whether three polymorphisms of the glucocorticoid receptor gene known to influence the sensitivity to glucocorticoids could be implicated in the pathomechanism of Graves' orbitopathy.

Methods

Allelic frequencies of the ER22/23EK, Bcl I and N363S polymorphisms of the glucocorticoid receptor gene were investigated in 99 patients with Graves' orbitopathy (mean age, 47.8 ± 13.4 years) and in 175 healthy individuals (mean age 54.4 ± 14.2 years). DNA was isolated from whole blood. Genotypes for the N363S and the Bcl I variants were determined by allele-specific polymerase chain reaction (PCR) and the ER22/23EK polymorphism was genotyped by PCR-RFLP analysis. The study was approved by local ethics committee, and written informed consent was obtained from all subjects.

Results

A significantly higher frequency of the ER22/23EK polymorphic allele was detected in patients with Graves' orbitopathy compared to that found in healthy control subjects (allelic frequency 5.05% *vs.* 2.0%, $P<0,05$), whereas the allelic frequencies of the Bcl I and N363S polymorphisms were similar in the two groups.

Conclusion

In this study we found that the ER22/23EK polymorphic allele of the glucocorticoid receptor is significantly overrepresented in patients with Graves' orbitopathy compared to healthy individuals. This polymorphism is known to be associated with a decreased sensitivity to glucocorticoids and, therefore, its high prevalence could increase the risk for the development of tissue-specific autoimmune inflammation underlying Graves' orbitopathy.

P348

Removal of tick box for TFT in pathology request forms reduces TFT performed during acute medical admission

Milan Piya, Biparna Belbase, Meenakshi Thapa, Anu Koppada, Martin Myers, Susan Baxter & Satyan Rajbhandari
Lancashire Teaching Hospitals NHS Trust, Lancashire, United Kingdom

Joint UK guideline (2006) recommends that routine testing of thyroid function (TFT) in patients admitted acutely to hospital is not warranted unless specific clinical indications exist. Despite this, TFT is frequently requested during acute medical admission. In our previous audit in 2002, during a 1 month period from 18th September, 458 subjects were admitted to medical assessment unit (MAU) and 183 (40%) were offered TFT. 39 (29%) results were beyond the laboratory reference range but this changed management only in 2 (1.1%) subjects. We recommended that TFT during acute medical admission should not be checked routinely as it known to alter transiently during acute illness. To implement this, the tick box for TFT was removed from the standard pathology request form but could be requested in the free text box without restriction. We re-audited the effect of the change in 2005 in the same MAU over the same month starting 18th September, and found that there had been a 55% reduction ($P<0.0001$) in request for TFT during acute medical admission. Out of 698 subjects admitted to MAU during this period, only 127 (18.2%) had TFT checked and 30 (23.6%) had results beyond the laboratory reference range. When these notes were reviewed, 7 (5.5%) had their management changed ($P=0.03$). In comparison to the previous audit, the removal of TFT tick box from the standard pathology form reduced routine testing by 3 fold (odds ratio 3.0 & 95% Confidence Interval 2.3 to 3.9), and improved efficiency by 5 fold (OR 5.3 & 95% CI 1.1 to 25.9). Our audit suggests that it is possible to reduce unnecessary TFT request during acute medical admission simply by removing tick boxes from the standard pathology request form. This helped reduce unnecessary TFT requests, in keeping with the 2006 UK guidelines for thyroid function tests.

P349

Thyroid hormones in serum and cerebrospinal fluid in patients with brain tumor and acute stroke

Darko Katalinic, Martina Matovinovic, Miljenko Solter, Andreja Maric, Jure Murgic, Milan Vrkljan & Karolina Dobrovic
Sestre milosrdnice University Hospital, Zagreb, Croatia.

We studied levels of T_3, T_4, FT_3, FT_4, rT_3 and TSH concentrations in serum and FT_3, FT_4, rT_3 and TSH concentrations in cerebrospinal fluid (CSF) in 10 patients with brain tumor and 20 patients with acute stroke and compared them to 7 patients in control group (further clinical evaluation in control group did not show brain lesions). All patients were euthyroid. The study was approved by local Ethical Committee. Serum T_3 and T_4 levels were similar in all three group. The values of FT_3, FT_4 and TSH did not significantly differ to control group neither in serum nor in CSF. On the contrary, significantly elevated rT_3 was found in serum and CSF, at both group of patients. The rT_3/FT_3 ratio were the highest in patients suffering from brain tumor and were significantly elevated compared to control group (serum, CSF), as well as compared to the patients with acute stroke. The values were particularly high in CSF (4 times higher) which would suggest that changes connected with "low T_3 syndrome" in patients with brain lesion are more obvious in CSF than in serum and identify brain tumor as a prototype of serious "local" nonthyroid illness. Serum and CSF test showed positive correlation for FT_4 and FT_3 in patients with acute stroke and for rT_3 in patients with brain tumor. This suggests that hormones are passing through still functional blood-brain barrier. The study did not show correlation between elevated rT_3 or rT_3/FT_3 ratio and poor prognosis. Thyroid hormones are present in CSF at concentration lower than in serum. There are probably two mechanisms responsible: hormones are partly crossing the blood-brain barrier from serum, but also T_3 and rT_3 may derive from local conversion of T_4 within the central nervous system. The impairment of this conversion which occurs in different brain lesions could be responsible for the changes in hormones level known as "low T_3 syndrome", which are particularly evident in CSF.

P350

The influence of universal salt-iodization on the iodine status of County Mures, detected through TSH determinations in newborns between 2001–2006

Zsuzsanna Szanto[1], Ildiko Kun[2], I. Kun[1], Ligia Coros[3] & Manuela Cucerea[4]
[1]Endocrinology Clinic, UMPh, Tg.Mures, Romania; [2]Clinic of Internal Medicine No. II, UMPh, Tg.Mures, Romania; [3]Central Laboratory of Emergency Clinical Hospital County Mures, Tg.Mures, Romania; [4]Clinic of Obstetrics & Gynecology No. I, UMPh, Tg.Mures, Romania.

Iodine deficiency in a geographical area can be quantified not only by urinary iodine excretion, but by the frequency of elevated TSH-levels in newborns, too. We compared the TSH-levels obtained between 2001–2003 with those collected after extension of universal salt-iodization with increased iodine-content (KIO_3 34 ± 8.5 mg-/kg) in the whole country (2004–2006). The governmental decision was adopted in 2002, implemented in practice in December 2003, and extended only in 2004 (the iodized salt was used in 96% of households). We observed TSH-levels (10 μIU/mL (WHO-criteria) at 8.23% of 2454 newborns tested between 2001–2003, in comparison with the 9.91% from 555 subjects borned between 2004–2006. Accordingly to the upper normal TSH-level (12 μIU/mL) used at the Central Laboratory of Emergency Clinical Hospital County Mures, 6.07% and 6.31% of the newborns seen between 2001–2003, and 2004–2006, respectively, had elevated TSH-levels. The difference between the two periods was not significant. Based upon these results, County Mures can be characterized at present as a moderate/mild iodine-deficient area.

However, we observed an important change: the mean TSH-level obtained in the period of 2001–2003 ($19.81 + 12.63$ μIU/mL) was reduced significantly in the second period ($15.63 + 7.35$ μUI-/mL), i.e. a decrease with 4.18 μIU/mL ($P = 0.02$). In conclusion, after increasing the iodine-content of the alimentary salt and applying the measures for the universal iodization, the incidence of elevated TSH-level did not decrease, but its mean value was reduced statistically significant, showing an impro-ve-ment of iodine supple-mentation.

While the moderate increased TSH-levels (10–12 μIU/mL) are considered as indicators of the iodine deficiency, the higher concentrations ((20 μIU/mL) usually indicate the coexis-ten-ce of hypothyroidism due to reduced iodine supply. We observed an important reduction of the hypothyroidism induced by iodine-deficiency: if in the first period its incidence was 2.49%, in the second it decreased to 1.46%.

P351

The evolution of hypothyroidism in pregnant women in County Mures between 2001–2006

Ildiko Kun[1], Zsuzsanna Szanto[2], I Kun[2], B Szabo[3] & Ligia Coros[4]
[1]Clinic of Internal Medicine No. II, UMPh, Tg.Mures, Romania; [2]Endocrinology Clinic, UMPh, Tg.Mures, Romania; [3]Clinic of Obstetrics & Gynecology No. I, UMPh, Tg.Mures, Romania; [4]Central Laboratory of Emergency Hospital County Mures, Tg.Mures, Romania.

County Mures is a moderate/mild iodine-deficient area, the iodine-prophylaxis having an important role in prevention of IDD. Between 2001–2003 we made a partial screening at 320 pregnant women to detect thy-roid dysfunctions, and in 13% (43 cases) we observed hypo-thy-roidism, the majority being subclinical forms. The most frequent compli-ca-tions were threatened abortion or premature birth, and dys-gra-vidia. We found that even the subclinical hypothyroidism can cause se-ve-re complica-tions in preg-nan-cy or may contribute to their development.

The governmental decision from 2002 regarding the universal iodization of alimentary salt was put in practice from December 2003, while in 2004 was decided the obligatory iodization of the salt used in the baking industry. Consequently, in 2004 the iodized salt was used in 96% of households, according to some authors. Our aim was to evaluate the influence of these new measures on the thyroid function of pregnant women, so we restarted the TSH- and FT_4-determinations between 2004–2006, and compared the results with those obtained bet-ween 2001–2003. In the period of 2004–2006 from the 205 pregnant women 7.3% (15) presented hypothyroidism (increased TSH-levels and/or decreased FT_4-values), a much more reduced percentage as in the first period (13%). Thus, between 2004–2006 the frequency of hypo-thyro-idism decreased significantly comparing with 2001–2003 ($P < 0.05$). However, the values of urinary iodine excretion of the two periods did not differ significantly, in concordance with the similar data obtained in whole country in 2004. So, other factors could contribute to the better results, i.e. a more rigurous follow-up of the thyroid function and a more adequate treatment of hypothyroidism in pregnancy, taking into account that this dysfunction can be determined besides the IDD by other thyroid disorders (especially by chronic thyroidites), too, or can be a consequence of an inadequately treated thyroid ablation.

P352

The use of perchlorates in the treatment of some special forms of hyperthyroidism (report of two cases)

I Kun, Zsuzsanna Szanto & Emilia Let
Endocrinology Clinic, UMPh, Tg.Mures, Romania.

The perchlorates block the enzyme NIS, inhibiting iodine accumulation in the thyroid and favour the elimination of intrathyroidal iodine unused for hormone synthesis. Their therapeutical utilisation actually is limited due to the toxicity. In the literature there are different opinions regarding the adverse effects (nephrotic syndrome, irreversible aplastic anemia etc.), but several authors sustain that these appear only after high doses, and after the development of therapeutical actions.

Perchlorates are used rarely in the treatment of hyperthyroidism, mainly in iodine-, especially amiodarone-induced forms. They are indicated also to prevent these forms, using perchlorates pre- and postinterventionally with iodine-containing substances (e.g.contrast agents). In hyper-thyroidism induced by amiodarone, perchlorates are usually associated with thioamides. Similarly, these drugs can be attempted in cases of intolerance to other antithyroid drugs, e.g. thioamides, when can not be applied ablative measures.

We report two cases of hyperthyroidism treated with perchlorates, obtaining good therapeutical results. In both cases perchlorates were introduced after (hema-to-lo-gic and CNS) adverse effects produced by methimazole, alone and associated with lithium carbonate. Taking into account the recommended short duration of the therapy with perchlorates (not excee-ding 1 month) and lacking the possibilities for other efficient and durable conservative treatment (both pa-tients presented Hashitoxicosis aggravated through iodine intake, and had thioamide-intolerance), we indicated thyroidectomy after obtaining euthyroidism with perchlorates. At 7–10 days after surgery their thyroid status evolu-a-ted to hypothyroidism, so now they are receiving thyroxine substitution under longitudinal follow-up.

P353

Iodine deficiency detected through urinary iodine excretion in school-children living in goiter prevalent regions of County Mures (2005–2006)

I Kun[1], J Balazs[1], Anisie Nasalean[1], Camelia Gliga[1], Gabriela Detesan[2], Mihaela Simescu[3], Ligia Coros[4], Anca Ionescu[1], Gabriela Madaras[1], Zsuzsanna Szanto[1] & C Macarie[1]
[1]Endocrinology Clinic, UMPh, Tg.Mures, Romania; [2]Municipal Hospital, Reghin, Romania; [3]Institute of Endocrinology C. I. Parhon, Bucuresti, Romania; [4]Central Laboratory of Emergency Clinical Hospital County Mures, Tg.Mures, Romania.

Our previous studies made between 1999–2003 demonstrated that County Mures is a moderate/mild iodine-deficient geogra-phical area. In 2002 a governmental decision was given for universal iodization of alimentary salt with increased iodine-content, realized during 2004. The aim of our study was to investigate the effect of in-creased iodine-supplementation at school children living in different iodine-deficient areas in County Mures, through urinary iodine excretion (UIE). In December 2005 were tested 50 school-children from a rural mountain area, while in October 2006 other 133 children from surrounding villages: 55 from Casva, 28 from Glajarie and 50 from Ibanesti.

The group tested in 2005 had mean UIE of 56.00±38.07 μg/L, only 6% of children having normal values. The group studied in October 2006 had mean UIE of 85.37±60.05 μg/L, only 30.8% having normal values, 38.3% between 50–99 μg/L, 22.6% between 20–49 μg/L (mild and moderate dec-re-a-se), and 8.3% under 20 μg/L (very low levels). Thus, 69.2% of child-ren had subnormal levels, and the percentage of UIE <50 μg/L reached 30.8%, which is above 20%, the upper admitted limit for an adequate iodine-intake. Our results from 2005 are similar with those obtained by Balazs *et al.* in 1999 in the superior and middle hydrographic basin of the river Mures (mean value 59.95±30.22 μg/L, normal UIE in 6.9%) at a group of 58 school-children from zone of locality Deda. At the same time, our recent results (October 2006) are much better: the mean value rose to 85.37±60.05 μg/L and 30.8% of children had normal UIE. Ana-lysing separately the groups of villages, the results are somehow different: 72.90±48.63 μg/L in Casva, 75.42±60.30 μg/L in Glajarie and 109.83±73.22 μg/L in Ibanesti.

In conclusion, the rural mountain zones of County Mures known before as moderate/mild iodine-deficient areas, became mild deficient, due to the new measures of iodine prophylaxis. In these areas is necessary to apply permanently special prophylactic measures, too.

P354

Endothelial function and hemostasis factors in hypothyroidism and subclinical hyperthyroidism

Annamaria Gazdag[1], Endre Nagy V[1], Emma Varga[1], Laszlo Samson[1], Annamaria Erdei[1] & Zoltan Jenei[2]
[1]Division of Endocrinology, Department of Medicine, Health and Science Centre, Universitiy of Debrecen, Debrecen, Hungary; [2]Division of Nephrology, Department of Medicine, Health and Science Centre, Universitiy of Debrecen, Debrecen, Hungary.

Long-term follow-up of differentiated thyroid cancer (DTC) patients requires yearly measurements of serum thyroglobulin (Tg) levels is endogenous (4 weeks off-thyroxin) or exogenous (human recombinant TSH injection) high TSH state. Subclinical hyperthyroidism is maintained in the patients throughout the rest of the year. We examined the endothelial function, hemostasis factors and metabolic parameters in short-term iatrogenic hypothyroidism (HO) and in subclinical hyperthyroidism (SH) in patients with DTC. In seventeen patients who had undergone total thyroidectomy and radioiodine ablation, blood pressure (RR), Tg, thyroid function, lipid parameters, homocystein, CRP, fibrinogen, von Willebrandt factor activity (vWF), flow-mediated vasodilatation (FMD) and nitroglycerin-mediated vasodilatation of the brachial artery in HO (TSH=89.82±29.36 mU/L) and in SH (TSH=0.24±0.11 mU/L) were measured. The study protocol has been approved by the institutional ethics committee. In HO the FMD was markedly lower than in SH (6.79±4.44 vs.14.37±8.33%, $P<0.001$), whereas the vasodilatation in response to nitroglycerine was not different between HO and SH (28.20±8.33 vs. 29.27±14.19%, ns). RR did not significantly differ in HO and SH (128.62±7.17/82.29±3.98 vs. 125.8±7.05/85.2±5.8 Hgmm, ns). Total cholesterol (7.34±1.23 vs. 4.75±/1.24 mmol/L, $P<0.002$), LDL-cholesterol (4.55±1.10 vs. 2.70±0.89 mmol/L, $P<0.001$) and homocystein (12.95±4.49 vs. 9.62±2.3 μmol/L, $P<0.01$) were significantly higher in HO than in SH. Triglyceride (1.79±1.12 vs. 1.03±0.73 mmol/L) and HDL-cholesterol (1.95±0.47 vs. 1.58±0.42 mmol/L) were similar in HO and SH. Fibrinogen (3.23±0.50 vs. 4.38±0.84 g/L, $P<0.01$), vWF activity (90.09±25.92 vs.130.62±29.97%, $P<0.001$) and CRP (4.12±4.67 vs. 5.32±5.15 mg/L, $P<0.05$) were lower in HO. In conclusion, FMD, fibrinogen and vWF activity was found to be lower in HO than in SH. Thyroxin normalizes the low FMD in HO patients.

P355

The relevance of thyroid echography in diagnostic of subclinical autoimmune thyroiditis during pregnancy and postpartum

Gorduza Carmne[1], Mogos Voichita[2], Gorduza Eusebiu Vlad[2] & Zbranca Eusebie[2]
[1]St. Spiridon Hospital Iasi, Iasi, Romania; [2]University of Medicine and Pharmacy Iasi, Iasi, Romania.

Thyroid diseases are more frequent in women. We made a longitudinal study at 112 healthy pregnant women from Iasi county, Romania. The aim of study was to determine incidence and evolution of autoimmune thyroiditis in pregnancy and postpartum. The proceedings of study imposed to verify clinical aspect of thyroid, the volume of thyroid measured echographic, echostructure of thyroid, function of thyroid (TSH, FT4) and autoimmune modification of thyroid (anti-thyroid peroxidase autoantibodies - AAT-anti-TPO). Knowing that in postpartum it exists a risk to develop a subclinical autoimmune thyroiditis, even in normal women, we tried to find a specific element that can be use like "signal" to identify such disease risk during pregnancy. In these conditions we followed in progress the echostructure of thyroid during pregnancy and in the first 3 months after delivery. We observed an hypoechogenity of thyroid correlated with the levels of AAT-anti-TPO, TSH and FT4. The prevalence of hypoechogenity was 12.5% in I trimester, 16% in 2nd trimester, 23% in the end of pregnancy and 25% after delivery. Majority of cases with thyroid hypoechogenity (accentuated in 3rd trimester) presented some degree of autoimmunity, despite of reduction of AAT anti-TPO level in the end of pregnancy. The conclusion of our study is that the echography of thyroid can represent a screening method for detection of subclinical autoimmune thyroiditis during the pregnancy.

P356

Outcomes of a fixed dose of 370 MBq of radioiodine in hyperthyroidism

Isabel Howat, Hilary Sawers & Stewart Sawers
Cumberland Infirmary, Carlisle, United Kingdom.

In 1995 the Royal College of Physicians issued guidelines for the use of radioiodine in hyperthyroidism. They recommended administration of enough radioiodine to achieve euthyroidism, with acceptance of a moderate rate of hypothyroidism e.g. 15–20% at 2 years and 1–3% per annum thereafter. Guide activity was 400–550 MBq for standard hyperthyroidism (mainly Graves' disease), at least 550 MBq for toxic multinodular goitre, and 300–500 MBq in toxic adenoma.

We wished to see if we were achieving the recommended outcomes. We conducted a retrospective audit over 5 years from January 2000 to December 2004. During that time we used a fixed dose of 370 MBq. 351 patients received 390 doses of radioiodine. Mean follow-up was 35 months (1–66). We reviewed the outcomes of patients who had a diagnosis documented in their case records.

114 patients had documented Graves' disease. During follow-up 75 (65.78%) became hypothyroid, 73 (64.03%) within 2 years, 2 (1.75%) within 3 years. 17 (14.91%) remained euthyroid at follow-up. 18 (15.78%) remained hyperthyroid or required up to 2 further doses of radioiodine. 4 patients were lost to follow-up.

57 patients had multinodular goitre. During follow-up 8 (14.03%) became hypothyroid, all within 2 years, 39 (68.42%) remained euthyroid, 10 (17.54%) remained hyperthyroid or required 1–3 further doses of radioiodine.

16 patients had toxic adenoma, 6 (37.5%) became hypothyroid, all within 2 years, 7 (43.75%) remained euthyroid, 3 (18.75%) required 1 further dose of radioiodine.

Despite using a dose less than that stipulated in the guidelines, our rate of hypothyroidism was higher than recommended for patients with standard hyperthyroidism and within the recommendation for toxic multinodular goitre. Our rate of hypothyroidism was also high for toxic adenoma although there were only 16 patients.

P357

The lymphocyte interactions in thyroid tissue in graves' disease

Iwona Ben-Skowronek[1], Jadwiga Sierocinska-Sawa[2], Leszek Szewczyk[1] & Elzbieta Korobowicz[2]
[1]Dpt. Pediatrics Rndocrinology and Neurology Medical Academy of Lublin, Lublin, Poland; [2]Dpt. Pathomorphology Medical Academy of Lublin, Lublin, Poland.

Introduction

The T,B and antigen presenting cells play a key role in the pathogenesis of autoimmune diseases.

The aim of the studies was to analyse different regulatory cells subsets interaction in patients with Graves' disease.

Material and methods
We have studied paraffin thyroid specimens obtained from 10 children with Graves' disease after thiamazole treatment. The thyroid tissue was stained with hematoksylin-eosin (HE). The mononuclear T-cells were detected by CD3+, CD4+, CD8+ antibodies and the presenting antigen's dendritic cells with CD1a and CD35 and antibodies (DakoCytomation Denmark).
Results
Thyroid tissue infiltrates were observed in HE staining. Intensivity of infiltrates was correlated with time of thiamazole treatment.
The B cell CD3+ and T suppressor/cytotoxic cell CD8+ between thyroid follicles. In the 4 patients with thiamazole short treatment (<6 mc) the lymphocytes have formed the lymphatic follicles in thyroid tissue. We have observed dendritic cells presenting antigen (APC) CD1a+ in reproduction centre. On the edges of lymphatic follicles were present lymphocytes T-helper CD4+, T-suppressor CD8+ and B-cells CD79+. In 6 patients after long thiamazole therapy the B and T cells were rarely observed in interstitium. It was interesting, that thyrocytes revealed positive reaction with CD1a monoclonaly antibody, which detected transmembrane α-chain connected with β-l microglobulin.
Conclusions
In the active states of Graves' disease, lymphocytes T, B and antigen presenting cells are present in big amount in interstitium and in lymphatic follicles. Thiamazole treatment leads to reduction of their amount.
Thyrocytes can have in their structure components similar to α-chains connected with β-microglobulins, which are characteristic for APS.
Grant 2P05E04327 Min. Science and Inform. Poland

P358

Water purification technology reduces iodine content of drinking water and contributes to iodine deficiency

Laszlo Samson, Ildiko Czegeny, Endre V Nagy, Emese Mezosi & Annamaria Erdei
Division of Endocrinology, Department of Medicine, University of Debrecen Medical and Health Science Center, Debrecen, Hungary.

Drinking water is the major natural source of iodine in many European countries. In the present study, we examined possible sites of iodine loss during the usual water purification process. Water samples from 6 sites during the technological process were taken and analyzed for iodine content. Under laboratory circumstances, prepared iodine in water solution has been used as a model to test the effect of the presence of chlorine. Samples from the purification sites revealed that in the presence of chlorine there is a progressive loss of iodine from the water. In the chlorine concentrations employed in the purification process, twenty four hour chlorine exposure eliminated more than 50% of iodine when the initial iodine concentration was 250 μg/L or less. Iodine was completely eliminated if the starting concentration was 16 μg/L. We conclude that chlorine used during water purification may be a major contributor to iodine deficiency in European communities.

P359

The ultrastructural changes of thyroid tissue in recipient of bone marrow graft with Graves' disease

Iwona Ben-Skowronek[1], Jadwiga Sierocinska-Sawa[2], Leszek Szewczyk[1], Elzbieta Korobowicz[2] & Zofia Karpinska[2]
[1]Dpt. Pediatrics Endicrinology and Neurology Medical Academy of Lublin, Lublin, Poland; [2]Dpt. Pathomorphology Medical Academy of Lublin, Lublin, Poland.

Introduction
In connection with usage of allogeneic bone marrow transplantations (BMT) there rises the problem of transfer of lymphocytes capable of induction autoimmunological reactions in recipient.
The aim of our study
Is a presentation of ultrastructural changes in thyroid tissue as the pathogenesis of autoimmunological thyroid disease in a recipient following BMT from donor with Graves' disease after total surgical treatment.
Material and methods
The thyroid gland tissue removed during surgery was routinely fixed and stained with hematoxylin and eosin. The immunohistochemical investigation of lymphocyte subsets was performed using DAKO monoclonal antibodies. Fluorescence *in situ* hybridization studies (FISH) was performed using a commercially available CEP X/Y DNA Probe (Vysis). Histological specimens were routine estimated and investigated in electron microscope.
Case report
The 14 -year boy who underwent bone marrow transplantation (BMT) for severe aplastic anemia from his HLA matched sister, who had been diagnosed with Graves' disease 5 years before transplantation. After 2 years of BMT, the same disease was diagnosed in the recipient. Thyreoidectomy was performed after achieving a euthyroid state. The thyroid gland contained interstitial lymphocytic infiltrates: T, B and antigen presenting cells. FISH showed that at least some of the lymphocytes were of donor origin and these could be seen among the recipient's thyroid cells. In the ultrastructural investigations were noticed numerous lymphocytes such as plasmocytes between thyroid cells in contact with thyrocytes. It was observed the lymphocytes in contact with plasmocytes and the lymphoblasts and lymphocytes in lymphatic follicules. The thyrocytes were very active and in numerous places were proliferated.
Conclusions
In thyroid were ultrastructural changes typical for AITD observed. The transfer of donor immunocompetent cells to the recipient of hematopoetic stem cells has been proposed as a mechanism of inducing autoimmune thyroiditis post BMT.
Grant 2P05E04327 Min. Science and Inform. Poland

P360

Do patients and clinicians agree about which aspects of quality of life are relevant when evaluating the impact of thyroid diseases?

Torquil Watt[1], Laszlo Hegedüs[2], Åse Krogh Rasmussen[1], Mogens Groenvold[3], Steen Joop Bonnema[2], Jakob Bjorner[4] & Ulla Feldt-Rasmussen[1]
[1]Copenhagen University Hospital Rigshospitalet, Copenhagen, Denmark; [2]Odense University Hospital, Odense, Denmark; [3]Institute of Public Health, Copenhagen, Denmark; [4]Quality Metric Inc., Lincoln, RI, United States.

Objective
During the development of a thyroid-specific quality of life (QoL) questionnaire, patients and clinicians rated the relative relevance of a list of possibly relevant QoL issues. In this study we compare the patient and clinician ratings.
Methods
Fifteen thyroid experts and 80 thyroid outpatients (14 with non-toxic goitre, 12 nodular toxic goitre, 21 Graves' disease, 17 thyroid associated ophthalmopathy (TAO) and 16 primary hypothyroidism) were interviewed, using semi-structured interviews.
The relevance of 138 thyroid disease related issues was rated. Patients' rating of importance was combined with prevalence of the issue in question to calculate a mean relevance rank for each patient category. Experts rated the relevance directly. Patient and expert relevance ratings were compared using nonparametric correlation. To explore the (dis-)agreement in greater detail, the 15 issues considered most relevant by the patients were compared to the 15 issues considered most relevant by the clinicians.
Results
The Spearman correlations between patient and expert ratings were: Graves' disease 0.69, TAO 0.48, toxic nodular goitre 0.60, non-toxic goitre 0.35 and primary hypothyroidism 0.46 ($P < 0.0001$ for all coefficients). This corresponds to substantial agreement regarding Graves' disease, moderate agreement about TAO, toxic nodular goitre and primary hypothyroidism and only fair agreement in non-toxic goiter.
For most disease categories, less than half of the 15 issues considered most relevant by the patients were also among the 15 most relevant to clinicians. Generally, issues among the 15 most relevant according to clinicians only were physical symptoms characteristic of the diagnosis in question. Issues among the 15 most relevant according to patients only were generally non-physical aspects of HRQL such as emotional susceptibility and nervousness as well as general physical symptoms.
Conclusions
When evaluating possibly relevant QoL-issues, clinicians focused more on specific symptoms, whereas patients focused more on emotional, mental and social aspects of QoL.

P361

ret/PTC oncogene expression in papillary thyroid carcinoma

Dilek Arpaci[1], Yersu Kapran[2], Ayse Kubat Uzum[3], Ferihan Aral[3], Nese Ozbey[3] & Faruk Alagol[1]
[1]Istanbul University, Istanbul Faculty of Medicine, Department of Internal Medicine, Istanbul, Turkey; [2]Istanbul University, Istanbul Faculty of Medicine, Department of Pathology, Istanbul, Turkey; [3]Istanbul University, Istanbul Faculty of Medicine, Department of Internal Medicine, Division of Endocrinology and Metabolism, Istanbul, Turkey.

Objective
We aimed to investigate the expression of ret/PTC oncogene in PTC and to determine the relationship of this expression with clinical parameters and the prognosis.

Materials and methods
Surgical specimens of 45 PTC and adjacent normal thyroid tissues were obtained from the files of the Department of Pathology at Istanbul Faculty of Medicine, Istanbul University. All of the patients had definite diagnosis of PTC between 1995–2003 and had adequate clinical information and a continous follow-up. ret/PTC expression was studied with the immunohistochemistry method. Correlation between ret/PTC expression positivity and the pathologic parameters at initial diagnose and during the follow up were examined.
Results
Study group consisted of 39 (86.7%) female, six (13.3%) male patients. Mean age was 44.4 ± 11.28 years, follow-up time was 59 ± 25 (24–120). Mean tumor size was 18.13 ± 15.75 mm (3–80 mm). According to TNM staging % 22 (n = 10), %13.3 (n = 6), %8.9 (n = 4) and %55.6 (n = 25) of the tumors were T1, T2, T3 and T4 respectively. Lymph node metastasis, capsule invasion, vascular invasion, soft tissue invasion, multicentricity, and relaps rates were 24.4% (n = 11), 71.1% (n = 32), 40% (n = 18), 51.1% (n = 23), 42% (n = 19) and %6.8 (n = 3) respectively. In 17 (37.8%) of the 45 specimens, ret/PTC was found positive immunohistochemically. There was no significant difference in ret/PTC expression rate according to gender, stage of tumor, invasion of lymph node, capsule, soft tissue and vascular invasion, multicentricity and relaps ($P > 0.05$). Ret/PTC expression positivity was not different between patient < 40 and ≥ 40 years old. No correlation was found between ret/PTC positivity and tumor size (< 10 mm and ≥ 10 mm) ($P = 0.160$) as well as between the histologycal subtypes ($P = 0.60$).
Discussion
In our study, ret/PTC expression had no influence on initial clinicopathological findings and the prognosis.
(The present work was supported by the Research Fund of Istanbul University. Project No:950,T-601/170320)

P362

Prognostic value of polymorphisms and somatic RET proto-oncogene mutations in sporadic medullary thyroid carcinoma (MTC)
Barbara Cosci[1], Cristina Romei[1], Pamela Piampiani[1], Mariangela Sculli[1], Paolo Miccoli[2], Clara Ugolini[3], Michele Minuto[2], Aldo Pinchera[1] & Rossella Elisei[1]
[1]Department of Endocrinology, University of Pisa, Pisa, Italy; [2]Department of Surgery, University of Pisa, Pisa, Italy; [3]Department of Oncology, University of Pisa, Pisa, Italy.

Germline point mutations of the RET proto-oncogene are causative of hereditary MTC. Somatic mutations are described in 40% of sporadic MTCs. Although a relationship between somatic mutations and bad prognosis has been described, data are controversial.No date on the prognostic value of RET polymorphisms are available.
Aim of the work was to verify if the presence of a somatic RET mutation and or a polymorphisms can influence the prognosis of MTC. This study was performed in a large series of MTC with a mean follow-up of 10 years.
Seventy MTC cases, known to be sporadic on the basis of genetic analysis, were studied. RET point mutations and polymorphisms were analysed by direct sequencing.
We identified a total of 28 somatic RET mutations (40%). In particular 1 (3%) 48 bp deletion in exon 10, 1 (3%) 883 mutation in exon 15, 3 (10.7%) 634 mutations in exon 11 and 23 (82%) 918 mutations in exon 16 were described. RET mutations were correlated with TNM and outcome. Among 28 mutated patients, 6 were free of disease and 22 were affected by MTC or dead. On the contrary among the 42 not mutated patients, 23 were free of disease and 19 were affected by MTC or dead ($P = 0.006$). In the group of mutated tumors we found 16 patients (57%) with lymph-node metastasis. On the contrary only 12 (28.5%) cases of lymph-node metastasis were identified among not mutated patients ($P = 0.004$). No statistically significant correlation between RET mutation, the size of the tumour and the presence of distant metastasis was found. RET polymorphisms did not show any correlation with clinico-pathological features of the tumor.
In conclusion our study show that RET somatic mutation is a negative prognostic factor for MTC and is significantly correlated with lymph-node metastasis. Although Met918Thr mutation is the most frequent, somatic RET mutations can be found in different exons.

P363

Partial redifferentiation of thyroid carcinoma cell lines treated with decitabine and retinoic acid
Agnese Vivaldi[1], Miasaki Fabiola[1], Raffaele Ciampi[1], Laura Agate[1], Valeria Bottici[1], Alessandra Capodanno[2], Paola Collecchi[2], Aldo Pinchera[1] & Rossella Elisei[1]
[1]Department of Endocrinology and Metabolism, University of Pisa, Pisa, Italy; [2]Division of Pathology, University of Pisa, Pisa, Italy.

In a previous study we demonstrated that retinoic acid (RA) decreased the growth only of thyroid carcinoma cell lines expressing RA receptor β (RAR β) and that decitabine (5-Aza-CdR) re-induced RARβ expression. The aim of this study was to analyze the effects induced by the combined treatment with RA and 5-Aza-CdR in the same thyroid cancer cell lines.
We studied the effect of 5-Aza-CdR 800 nM and RA 1 μM on the expression of thyroid specific genes and RARα, β and γ by quantitative RT-PCR and the effect of the two drugs on cell growth by cell counting, cytotoxicity, bromodeoxyuridine, apoptosis assays and FACS analysis.
After the combined treatment, we observed the induction of the RARβ mRNA expression in all cell lines, of NIS mRNA in ARO, FRO, WRO and TT, of TTF-1 in ARO, Tg in FRO and Pax-8 in WRO and TT. However, no cell line was able to actively take up ^{125}I despite of NIS mRNA re-expression. Accordingly, immunofluorescence showed NIS protein expression only in the cytoplasm.
The combined treatment determined an inhibition of the growth curve in all cell lines: after 24 h in FRO and NPA, after 48 h in WRO, after 72 h in ARO and after a week in TT. We observed inhibition of DNA synthesis in NPA and WRO and apoptosis in ARO and, NPA and TT. Finally, FACS analysis showed a G0/G1 increase in FRO and WRO.
In conclusion, the combined treatment with 5-Aza-CdR and RA reduces the tumoral growth speed *in vitro* by means of apoptosis in ARO, NPA and TT and of inhibition of DNA synthesis in NPA and WRO. The combined treatment can also partially re-differentiate the analyzed thyroid cancer cell lines, inducing NIS mRNA expression. The cytoplasmic localization of NIS protein explains the inability of cells to take up radioiodine.

P364

Comparison between serum calcitonin (CT) levels following Pentagastrin (Pg) and Calcium (CA) stimulus
Valeria Bottici, Mariangela Sculli, Giulia Renzini, Paolo Passannanti, Fabiana Lucchetti, Lucia Grasso, Aldo Pinchera & Rossella Elisei
Department of Endocrinology, University of Pisa, Pisa, Italy.

Serum CT is the most specific marker of Medullary Thyroid carcinoma (MTC). Although Pg test is the most frequently used to induce CT secretion, the poor availability of Pg makes it necessary to look for different agents. Aim of this work was to compare the induction of CT secretion following 2 different stimuli in the same patient. We studied 25 patients (14 females, 11 males; mean age 50 ± 15 yrs; range 12–77 yrs). All patients were subjected to both tests by injection of 0.5 μg/kg of Pg and 2 mg/kg of CA in 5 fminutes. Thirteen/25 patients showed undetectable basal CT (< 10 pg/ml); these cases were already treated with total thyroidectomy. Six/13 patients showed undetectable CT levels both after Pg and CA stimulation and were disease-free. In 2 patients CT was elevated both after Pg (mean 37 pg/ml, range 11–63) and CA (mean 22 pg/ml, range 21–23). Imaging was negative (biochemical persistence of disease, BP). In 5/13 patients CT was undectable after CA but not after Pg (mean 33 pg/ml, range 11–114); all of them were BP. In12 patients basal CT was detectable (mean 980 ± 1782 pg/ml, range 62-4590 pg/ml). In all patients CT peak after Pg and CA was higher than basal CT (mean 3196 pg/ml, range 65-17990; mean 1522 pg/ml, range 60-9650, respectively). Six/12 patients had a metastatic disease, 3/12 showed a BP, 3/12 were under presurgical investigation for MTC. In summary, we demonstrated that Pg and CA test give similar results in 20/25 cases, although CT levels after CA injection are lower than after Pg. In 5 cases the CA test was negative while Pg test was positive with moderate levels of CT. These patients were already been subjected to thyroidectomy for MTC and they would be considered erroneously as disease free on the basis of CA test. In conclusion, Pg test is more sensitive than CA test in patients with basal undectable CT levels. It has a similar sensitivity in patient with elevated basal CT. Although CA stimulation induces a lower secretion of CT than Pg, we propose that CA test is useful in the diagnosis and follow-up of these patients. Patients, already treated by surgery, showing a negative CA test should repeat this test before declaring them as disease-free.

P365

Usefulness of calcitonin (CT) measurement in wash-out fluid from fine needle aspiration biopsy in thyroid nodules of patients with dectectable serum CT

Giulia Renzini, Teresa Rago, Paolo Passannanti, David Viola, Barbara Cosci, Eleonora Molinaro, Lucia Grasso, Fabiana Lucchetti, Paolo Vitti, Aldo Pinchera & Rossella Elisei
Department of Endocrinology, University of Pisa, Pisa, Italy.

Up to now there isn't any study to validate the CT measurement in wash-out-fluid from FNAB in the diagnosis of medullary thyroid cancer (MTC). To demonstrate the usefulness of CT measurement in wash-out fluid from FNAB in thyroid nodules, we have retrospectively analyzed 25 cases with detectable serum CT in which CT measurement in wash-out-fluid from FNAB, citology and histological examination were available. In 7 cases CT level was <10 pg/ml: cytology was negative in 4 cases and not diagnostic in 3 cases. In 6 cases C cell iperplasia (ICC) or MTC was identified at histology but in a different nodule and in one case a focus of MTC was found in the punctured nodule. In 6 cases the CT level was $10<CT<1000$ pg/ml: an MTC was found in 5 cases at histology; in one case a papillary thyroid carcinoma (PTC) was found both at histology and citology. Citology described a MTC in 2 cases and was not diagnostic in 3 cases. In 6 cases CT level was $1000<CT<10000$ pg/ml. In all cases the histology described a MTC with the exception of one case in which there was a PTC. Citology found 4 cases of MTC, but it was not diagnostic in 2 cases. In 6 cases CT levels was >10000 pg/ml: in all cases a MTC was described both at histology and citology.
In conclusion CT level <10 pg/ml in wash-out-fluid from FNAB was indicative of absence of cancer in 86% of cases. The citology identifies only 57% of benign nodules. CT level >10 pg/ml in FNAB was indicative of presence of malignant or premalignant in 100% of cases (15 MTC; 1 ICC; 2 PTC), while cytology only in 72% of cases. We conclude that CT measurement in wash-out-fluid from FNAB increases diagnostic sensibility of citology from 65% to 95% and it represents an useful diagnostic tool to associate with citology when an MTC is suspected.

P366

Prognostic significance of BRAF mutation in patients affected by papillary thyroid carcinoma with a follow up of 20 years

David Viola[1], Cristina Romei[1], Agnese Biagini[1], Laura Agate[1], Eleonora Molinaro[1], Raffaele Ciampi[1], Giulia Renzini[1], Riccardo Giannini[2], Clara Ugolini[1], Piero Berti[3], Aldo Pinchera[1] & Rossella Elisei[1]
[1]Department of Endocrinology, University of Pisa, Pisa, Italy; [2]Department of Oncology, University of Pisa, Pisa, Italy; [3]Department of Surgery, University of Pisa, P, Italy.

$BRAF^{V600E}$ is the most common mutation in papillary thyroid carcinoma (PTC). Anatomo-patology and clinical features of PTC with $BRAF^{V600E}$ are well described in literature.
Aim of this study was to examine the prognostic significance of $BRAF^{V600E}$ in patients with PTC and a follow-up of 15-20 years.
Genomic DNA was purified from 67 paraffin-embedded tumoral tissue. A PCR-SSCP analysis of exon 15 of BRAF was performed. Direct sequencing of SSCP positive cases was made. $BRAF^{V600E}$ was found in 23/67 cases (34%): 18 females (78%) and 5 males (22%), with a mean age of 48.9 ± 16.2 yrs (median: 50 yrs). Ten were in class 1 (43.6%), 6 in class 2 (26%), 4 in class 3 (17.4%) and 3 in class 4 (13%). Among the 44 patients without $BRAF^{V600E}$ 37 were females (84%) and 7 were males (16%), with a mean age of 42.2 ± 15.36 (median: 39.5 yrs). Twenty-seven were in class 1 (61.4%), 12 in class 2 (27.3%) and 5 in class 3 (11.3%). At the end of the study 54 patients (80.5%) were free of disease, 9 (13.5%) had persistent disease and 4 (6%) died of thyroid carcinoma. Among the 44 patients without $BRAF^{V600E}$ mutation, 41 (93.2%) were free of disease, 2 (4.5%) had persistent disease and only 1 (2.3%) died for PTC at the end of follow-up. Between the 23 patients $BRAF^{V600E}$, 13 (56.5%) were free of disease, 7 (30.5%) had persistent disease and 3 (13%) died for thyroid carcinoma. The statistical analysis showed a positive correlation between $BRAF^{V600E}$ mutation and the outcome of the patients ($p=0.0003$). Older age, male sex, advanced tumoral class, loco-regional and/or distant metastasis were more frequent in the patients with $BRAF^{V600E}$ without statistically significant correlation.
In conclusion our data suggest that $BRAF^{V600E}$ is an unfavorable prognostic factor in patients affected by PTC.

P367

Expression of folate receptor is down-regulated in somatotropinomas

Jadwiga Zebracka[1], Adam Rudnik[2], Kornelia Hasse Lazar[1], Dawid Larysz[2], Michal Jarzab[3], Beata Lubieniecka[1], Grzegorz Gala[1] & Barbara Jarzab[1]
[1]Department of Nuclear Medicine and Endocrine Oncology; MSC Cancer Center and Institute of Oncology, Gliwice Branch, Gliwice, Poland; [2]Department of Neurosurgery, Silesian University School of Medicine, Katowice, Poland; [3]Department of Tumor Biology; MSC Cancer Center and Institute of Oncology, Gliwice Branch,, Gliwice, Poland.

Introduction
Pathogenesis of pituitary adenomas is largely unknown thus, identification of genes specific for various types of pituitary tumors should enable better understanding of their biology.
The aim of our study was to analyze differences in gene expression between functional (FA) and non-functional (NFA) pituitary adenomas. For this goal, we considered folate receptor (*FOLR1*) shown by previous study (Evans *et al.* 2003) to be overexpressed in NFA, as well as some other genes reported for its changed expression.
Material and methods
Analysis of gene expression was performed by real-time quantitative PCR (QPCR) with the use of fluorescent probes, based on 5′-nuclease assay (TaqMan). Within the 54 pituitary adenomas collected there were 16 nonfunctioning and 38 functioning ones, among them 7 GH and 13 PRL- secreting adenomas. Expression of the examined genes was normalized to the reference index, obtained by calculation of geometric mean of reference genes expression: *GUS-B, B2M, ACTB, EIF3S10, UBE2D2 and ATP6V1E.*
Results
Folate receptor gene (*FOLR1*) was not significantly overexpressed in NFA compared with FA but was significantly overexpressed when NFA were compared to GH (but not PRL) adenomas. Also, we observed a 3-fold decrease of *CCND1* expression in GH adenomas compared with NFA. Again, the change in expression was not significant at the comparison PRL/NFA. *hPTTG1* and *MEN1* expression was similar in all tumors analyzed.
Conclusions
Folate receptor expression and cyclin D1 expression are down-regulated in somatotropinomas when compared to non-functioning pituitary tumors while prolactinomas do not show such a distinct change in their expression.

P368

Initiating mutations of BRAF gene in papillary thyroid carcinoma and their relation to gene expression profile

Dagmara Rusinek[1], Malgorzata Wiench[1], Malgorzata Oczko-Wojciechowska[1], Jadwiga Zebracka[1], Malgorzata Kowalska[1], Daria Handkiewicz-Junak[1], Sylwia Szpak-Ulczok[1], Ewa Chmielik[2], Agnieszka Czarniecka[3], Michal Jarzab[4], Grzegorz Gala[1], Elzbieta Gubala[1] & Barbara Jarzab[1]
[1]Department of Nuclear Medicine and Endocrine Oncology; Maria Sklodowska-Curie Memorial Cancer Center and Institute of Oncology, Gliwice, Poland; [2]Department of Tumor Pathology; Maria Sklodowska-Curie Memorial Cancer Center and Institute of Oncology, Gliwice, Poland; [3]Department of Oncological Surgery; Maria Sklodowska-Curie Memorial Cancer Center and Institute of Oncology, Gliwice, Poland; [4]Department of Tumor Biology; Maria Sklodowska-Curie Memorial Cancer Center and Institute of Oncology, Gliwice, Poland.

Introduction
Discovery of V600E ($BRAF^{T1799A}$) mutation in papillary thyroid carcinoma (PTC) widened our knowledge about mechanisms of its molecular initiation. It has been revealed that activating mutations of the BRAF kinase are much more frequent in PTC than *RET* rearrangements.
Aim of the study
Estimation of V600E *BRAF* mutation frequency in PTC and analysis of differences in gene expression profile between papillary thyroid carcinomas activated by various molecular events with particular consideration of age of the patients.
Material and methods
The analysis of frequency of *BRAF* mutation was carried out in 77 PTC tumors. In the collection of 45 of these tissues *RET/PTC* rearrangements were analyzed and gene expression profiles were previously obtained (Genechip, Affymetrix). Total RNA was extracted from postoperative tumor tissues, cDNA was synthesized by gene-specific primers. Exon 15 of the *BRAF* gene was amplified by PCR and analyzed by automated sequencing.

Results
The V600E mutation was detected in 54.5% cases of PTC whereas *RET/PTC* rearrangements were identified in 11/42 cases (we identified $BRAF^{T1799A}$ mutation in two patients with previously detected *RET/PTC* rearrangement). The frequency of the V600E mutation was the highest in patients older than 40 years (67% of cases). Patients below 21 years harbouring $BRAF^{T1799A}$ mutation constituted only 7%, in contrast to *RET* rearrangements which were more often found in young patients. Meta-analysis of our own microarray data and these published by Giordano et al., 2005, showed significant differences in gene expression profiles dependent on the type of initiating mutation in PTC. Genes specified by this analysis were subsequently validated by QPCR.
Conclusions
The frequency of *BRAF* mutation in PTC is almost two times higher than of *RET* rearrangements. The occurrence of these genetic alterations is age-dependent. The meta-analysis of PTC gene expression profiles indicates a distinct difference between BRAF-induced and RET-induced papillary thyroid cancers.

P369

CRABP2 expression is up-regulated in parathyroid adenomas in correlation with HRPT2 down-regulation

Jadwiga Zebracka[1], Janusz Waler[2], Malgorzata Kowalska[1], Grzegorz Gala[1], Jacek Gawrychowski[2] & Barbara Jarzab[1]
[1]Department of Nuclear Medicine and Endocrine Oncology; MSC Cancer Center and Institute of Oncology, Gliwice Branch, Gliwice, Poland; [2]Department of General Surgery, Silesian University School of Medicine, Bytom, Poland.

Introduction
The molecular events involved in the formation of parathyroid adenomas are not well understood. Two genes, cyclin D1 (*CCND1*) and *MEN-1*, have been established as having major roles in parathyroid tumorigenesis. Tumor suppressor gene *HRPT2* is frequently mutated in parathyroid carcinoma. The aim of our study was to analyze *HRPT2* expression in parathyroid adenomas and in residual normal/atrophic parathyroid tissue and to relate it to other molecular markers – *CCND1* (cyclin D1) and *MEN-1* expression. We also put the question whether *CRABP2* (cellular retinoic acid binding protein 2), a gene selected on the basis of the microarray study by Forsberg *et al.*, 2005, does show the change in expression in parathyroid adenomas when analyzed by Q-PCR.
Material and methods
The analysis of *HRPT2, CRABP2, c-JUN, CCND1* and *MEN-1* was carried out in 19 parathyroid adenomas taken intraoperatively, and 56 normal/atrophic parathyroid samples. Analysis of gene expression was performed by real-time quantitative PCR (QPCR) with the use of fluorescent probes, based on 5′-nuclease assay (TaqMan). Expression of the examined genes was normalized to the reference index, obtained by calculation of geometric mean of reference genes expression: *EIF3S10, UBE2D2 and ATP6V1E.*
Results
We observed a 1.5-fold, non significant decrease of *HRPT2* expression in adenomas in comparison to normal/atrophic parathyroids. The expression of the gene was significantly correlated with *c-JUN* expression but not with *CCND1* and *MEN-1*. *CRABP2* expression was significantly increased ($P<0.05$) in adenomas and the change in expression (mean: 1.3-fold) was correlated with *HRPT2* expression.
Conclusion
CRABP2 expression is up-regulated in parathyroid adenomas in correlation with *HRPT2* down-regulation.

P370

Hypothyroid Graves' ophthalmopathy: a case report

Jasmina Ciric, Milos Zarkovic, Biljana Beleslin, Mirjana Stojkovic, Slavica Savic & Bozo Trbojevic
Institute of Endocrinology, Belgrade, Serbia.

Background
Hypothyroid Graves' ophthalmopathy is a rare condition, present in about 3% of all cases. Since thyroid-stimulating antibodies could be detected in a majority of euthyroid and hypothyroid Graves' patients, the most probable explanation for unincreased thyroid function is a reduction of tissue capability to response to stimulation.
Case report
A 57-yr-old man visited the hospital with signs and symptoms typical of hypothyroidism. Since TSH was 77 IU/ml, FT4 6.8 pmol/l and TPO Ab 4828 IU/ml, the treatment with 100 mcg/day T4 was started. Three months later, when euthyroid, he developed Graves' ophthalmopathy with slight proptosis, moderate palpebral edema, conjuctival injection and chemosis, reduction of visual acuity to 0.7, diplopia and secondary glaucoma. He had no palpable goiter and ultrasound revealed small (V 5 cm^3), diffuse hypoechoic thyroid. Orbital computed tomography (CT) showed a pronounced enlargement of all extraocular muscles (9–15 mm). TSH receptor antibodies were 65 U/l. Patient was treated with two doses of 0.5 g intravenous methylprednisolone during three days, followed by oral prednisone 40 mg/day tapered to 10 mg/day in four weeks. Six courses of therapy were performed. There were no significant side effects during the treatment. A prompt improvement of visual acuity, intraocular pressure and inflammatory signs was noticed, but diplopia became permanent. Orbital CT revealed a significant reduction of all rectus muscles (2–10 mm). TSH receptor antibodies were 10 U/l, TPO Ab 8603 IU/ml. He developed cataract on his left eye and refused extraocular muscle surgery since he lost diplopia.
Conclusion
Hypothyroid Graves' disease reflects a subtle relation between destructive changes in the thyroid gland and autoimmune mechanisms involved in thyroid pathology.

P371

Increased risk of cardiovascular events in subclinical hyperthyroidism

Marianne Schultz[1], Caroline Kistorp[2], Ilan Raymond[1] & Jens Faber[2]
[1]Dep. of Cardiology & Endocrinology E, Frederiksberg University Hospital, Frederiksberg, Denmark; [2]Dep. of Endocrinology J, Herlev University Hospital, Herlev, Denmark.

Objective
Untreated overt hyperthyroidism is known to predispose the patient to cardiovascular diseases, while predisposition in subclinical hyperthyroidism has been debated. The1 cut-off point of TSH for initiating treatment in subclinical hyperthyroidism is still undefined.
Method
A community-based prospective study including non-hospitalised participants, aged 51 to 91 years, living in Copenhagen, Denmark were examined between September 1998 and January 2000 and provided blood and urinary samples on inclusion. All participants had normal left ventricular ejection fraction (LVEF > 50%), estimated by echocardiography, and were without heart or renal failure. The follow-up period was up to 5 years (to December, 2003). The local ethical committee approved the study.
Results
609 participants were included in the study, 549 (90.1%) were euthyroid (TSH 0.4–4.0 mU/L), 34 (5.6%) had TSH >4.0 mU/L and 26 (4.3%) had TSH <0.4 mU/L. Three were overt hypothyroid and one overt hyperthyroid. Of the participants having TSH ≤4.0 mU/L, 86 died and 59 had first major cardiovascular event during follow-up. In the subclinical hyperthyroid group, the mean value of TSH was 0.2 mU/L (range 0.0–0.4 mU/L). The incidence of major cardiovascular events incl. cardiovascular dead ($r=0.8$, $P=0.04$), as well as the incidence of stroke ($r=1.4$, $P=0.01$) was increased among the subclinical hyperthyroid participants. The TSH < 0.4 mU/L were independently associated with the risk of stroke ($r=1.2$, $P=0.03$), hazard ratio 3.28, even after adjusting for sex, age and atrial fibrillation.
Conclusion
Subclinical hyperthyroidism was a risk factor for developing major cardiovascular events including cardiovascular dead, in particular stroke, in a group of 575 non-hospitalised individuals with TSH ≤4.0 mU/L, aged 51 to 91 years. On this perspective, we recommend the condition subclinical hyperthyroidism to be treated as a disease instead of a condition to be observed.

P372

Predictive factors for thyroid function abnormalities in patients with chronic hepatitis C treated with pegylated interferon and ribavirin

Cristina Cijevschi[1], Catalina Mihai[1], Eusebie Zbranca[2] & Paul Gogalniceanu[1]
[1]Institute of Gastroenterology and Hepatology, Iasi, Romania; [2]Endocrinological Clinic, University Hospital 'St.Spiridon', Iasi, Romania.

Introduction
Chronic hepatitis C has a high incidence in our country being a major public health issue.
Aims and methods
To establish prognostic factors for developing thyroid dysfunction in patients with chronic hepatitis C receiving pegylated interferon and ribavirin therapy. A prospective study of patients with chronic hepatitis C on antiviral therapy was undertaken. 68 patients started on antiviral therapy in the period 1st January 2003 – 1st January 2005 were enrolled in the study. Patient with pre-existing thyroid pathology were excluded from the study. Patient follow-up occurred at 3, 6, 8 and 12 months after commencement of treatment. Follow-up consisted of thyroid echography, TSH, fT3 and fT4 measurement, as well as anti-thyroid anti-body (anti-thyroglobulin and anti-peroxidase) and anti-TSH receptor antibodies assessment. The patients were divided into two groups: group A – patients who developed thyroid dysfunction; group B – patients who did not develop thyroid dysfunction. The following parameters were recorded: age, gender, family history of thyroid disease, initial viral load, cytolysis, histology, early viral response and type of interferon used. Viral genotyping was not performed, as Hepatitis C genotype 1b is present in over 90% of cases diagnosed in our country.
Results
11 patients (16.7%) developed thyroid dysfunction (7 hypothyroid, 4 hyperthyroid), forming group A. The remaining patients (57) formed group B. Statistically significant factors associated with thyroid dysfunction were: female gender (8 patients group A, 29 group B), family history of thyroid disease (6 patients group A, 13 group B), severe hepatic fibrosis (6 patients group A, 19 group B).
Conclusions
Thyroid dysfunction in more common in elderly patients, being associated with female gender, family history of thyroid disease and degree of hepatic fibrosis. Thyroid dysfunction is not associated with initial viraemia, cytolysis, early viral response, type of pegylated interferon used.

P373

Aetiology and therapeutic guideines in thyrid dysfunction at the patients with chronic hepatitis C treated with pegylated interferon and ribavirin

Cristina Cijevschi[1], Catalina Mihai[1], Eusebie Zbranca[2], Andreea Brumaru[1] & Paul Gogalniceanu[1]
[1]Institute of Gastroenterology and Hepatology, Iasi, Romania;
[2]Endocrinological Clinic, University Hospital'St.Spiridon', Iasi, Romania.

Introduction
Chronic Hepatitis C is highly prevalent in our country.
Management involves combination treatment with pegylated interferon and ribavirin. Thyroid disease in affected patients can be caused by the hepatitis C virus or by the interferon therapy.
Aims and method
The study aims to investigate thyroid dysfunction and optimal management strategies for patients with chronic hepatitis C treated with pegylated interferon and ribavirin. A prospective study of 68 patients with chronic hepatitis C was undertaken. Patients commenced treatment between1st January 2003 – 1st January 2005. Patients with previous thyroid pathology were excluded from the study. All patients were followed up at 3, 6, 8 and 12 months from starting therapy. Patients were investigated using thyroid echography, TSH, fT3 and fT4 measurement, as well as anti-thyroid anti-body (anti-thyroglobulin and anti-peroxidase) and anti-TSH receptor antibodies assessment.
Results
11 patients (16.17%) developed thyroid pathology: 7 patients developed hypothyroidism and 4 developed hyperthyroidism. Of the latter, 3 developed destructive thyrotoxicosis and one developed Graves' disease.6 patients (54.54%) were asymptomatic (especially those with hypothyroidsim), whilst 75% of those with hyperthyroidism were symptomatic. 3 out of 7 patients with hypothyroidism developed antithyroid antibodies, probably due to an undiagnosed destructive thyroiditis. Only 2 patients (18.18% of those with thyroid pathology and 2.94% of all patients) stoped peginterferon treatment due to the thyroid related side effects.
Conclusion
The prevalence of thyroid dysfunction in chronic hepatitis C treated with pegylated interferon and ribavirin is 16.17%, mostly manifesting as hypothyroidism. The majority of patients are asymptomatic. Few patients required cessation of antiviral treatment. Monitoring of thyroid function during antiviral therapy is compulsory.

P374

Thyroid function in pregnancy

Emma Varga[1], Zita Molnar[2], Zoltan Szabo[1], Annamaria Gazdag[1], Katalin Jenei[3], Attila Jakab[3], Emese Mezosi[4], Judit Boda[1], Zsolt Karanyi[1] & Endre Nagy V[1]
[1]University of Debrecen Medical and Health Science Center 1st Department fo Medicine, Debrecen, Hungary; [2]BAZ County Hospital, Miskolc, Hungary; [3]University of Debrecen Medical and Health Science Center Department of Obstetrics and Gynecology, Debrecen, Hungary; [4]Pécs University Medical School 2nd Department of Medicine, Pécs, Hungary.

Pregnancy induces hormonal and metabolic changes that result in profound alterations of thyroid hormone economy and regulation. Adaptation of the pituitary-thyroid axis may be influenced by the iodine supply, especially iodine deficiency.

The aim of the study was to define characteristics of changes in certain biochemical parameters and regulation of thyroid function during pregnancy in a mildly iodine-deficient region of Hungary. Thirty-eight healthy pregnant women were enrolled in the study. The local ethical committee approved the study. Serial TSH, free thyroid hormone, total thyroid hormone, chorionic gonadotropin (hCG) and thyroid autoantibody levels were determined 5 times during gestation and 6 months after delivery. Data of 19 individuals were analyzed. To study the influence of pregnancy on the results of free thyroxin measurement, kits of five manufacturers were compared on 40 samples of women with varying gestational ages.

An increase of total T3 and T4 levels was observed parallel with changes of TBG concentration during the first 4 months of gestation. Serum TSH time-curve showed a transient fall in the first trimester, thereafter it returned to the non-pregnant values. Curves of serum TSH and hCG created clear mirror images. Free T4 concentrations elevated in line with the hCG peak at the beginning of gestation, thereafter it clearly followed the course of serum TSH. Free T3 levels gradually decreased throughout pregnancy.

The negative correlation between hCG and TSH levels, and the clear identity of the hCG+TSH and free T4 curves, suggest that thyroid function in pregnancy is the result of the two glycoprotein hormones, TSH and hCG. In pregnancy, total T3 may not be substituted for free T3 in thyroid function estimation, as total and free T3 levels do not correlate. Manufacturers' non-pregnant reference ranges do not apply to pregnancy.

P375

Painful Hashimoto's thyroiditis – 2 cases report

Carmen Vulpoi, Delia Ciobanu, Cristina Preda, Dumitru Branisteanu, Maria-Christina Ungureanu, Elena Livadaru, Roxana Dinu & Eusebie Zbranca
University of Medicine and Pharmacy 'Gr.T.Popa', Iasi, Romania.

Hashimoto's thyroiditis (HT) is usually characterized by goiter and/or hypothyroidism. Thyroid pain and tenderness are rare and suggest an alternative diagnostic of subacute thyroiditis (SAT).

We present two cases of painful HT, who had temporary relief with corticosteroids and required surgical intervention for persistent pain. Both patients were middle-aged women with painful goiter, fever, and inflammatory syndrome. Thyroid function was normal, and ultrasonography showed a hypoechoic inhomogeneous pattern. Corticosteroid treatment was started with rapid amelioration of both pain and inflammatory syndrome, but with relapse in about two months. First patient (MR, 52 y) had moderate hypothyroidism and restarted the corticosteroid treatment in association with l-thyroxine, with a new amelioration. Six months later, she presented relapse of intense pain with inflammatory syndrome, with no response to corticoids, and she was operated. Pathology confirmed lymphocytic thyroiditis, with diffuse fibrosis. She had a favourable evolution for the next 10 years. On her second episode, second patient (MD, 50 y) had high antibodies titre with normal thyroid function. Corticosteroids induced a new amelioration but with relapse at smaller doses. Ultrasonography showed a left thyroid nodule with suspicious cytology after FNAB. She was

operated, with favourable evolution until nowadays. Pathology found a rare association of lymphocytic thyroiditis with giant cells, suggesting the association of subacute thyroiditis.

The overlapping of the symptoms may lead to confusion between painful HT and SAT. Thyroid function is variable and antibodies titre are not always elevated. There are few small series of painful HT published in the literature, in which surgery was imposed by the evolution of the disease. In front of a clinical picture of SAT with no or little response to anti inflammatory treatment, painful HT must be considered. Thyroidectomy seams to be the best option, with relief of the symptoms.

P376

Increase of L-thyroxine requirement during pregnancy

Laura Russo, Massimo Tonacchera, Elena Gianetti, Paolo Vitti & Aldo Pinchera
Department of Endocrinology of Pisa, Pisa, Italy.

In pregnant women with a thyroid disease 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 138 women treated with LT4 divided in two groups: 47 euthyroid (E) (affected by nodular goiter (NG) under LT4 suppressive therapy) and 91 hypothyroid (H). This last group was divided in two subgroups: women with a residual functioning thyroid tissue (R-H) and women without residual thyroid tissue (NR-H). 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. 21 E and 48 R-H and 19 NR-H pregnant women respected these criteria during the entire pregnancy.

Only 11 out of 21 (52%) E had to increase LT4 in order to maintain serum TSH in the appropriate range. The mean increase was 125% at 3rd trimester with respect to pre-gravidic dose. In 32 out of 48 (66%) R-H and in 14/19 (74%) NR-H an increase of L-T4 was necessary to maintain serum TSH in the appropriate range. The mean increase was 134% in R-H and 140% in NR-H 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 and 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 is higher in hypothyroid with respect to NG pregnant women.

P377

Safety of pharmacological treatment of thyroid diseases during pregnancy

Elena Gianetti, Massimo Tonacchera, Laura Russo, Paolo Vitti & Aldo Pinchera
Department of Endocrinology of Pisa, Pisa, Italy.

Pregnant women may require treatment of hypo- or hyperthyroidism, L-thyroxine (LT4), propylthiouralcil (PTU) and methimazole (MMI) being the most frequently used drugs. Aim of this study was to verify the consequences of pharmacological treatment during pregnancy. We retrospectively evaluated 379 pregnancies: 124 patients under MMI treatment, 35 of whom still hyperthyroid in spite of treatment (H-MMI) and 89 euthyroid (E-MMI); 52 GD patients under PTU, 20 of whom still hyperthyroid (H-PTU) and 32 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 nodular goiter or autoimmune thyroid disease. 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: 9/89 (10.1%) E-MMI, 3/35 (8.5%) H-MMI, 4/32 (12.5%) 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. In 2 H-PTU newborns a fetal goiter and a hypertrophic pyloric stenosis 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 that currently there are not contraindications for the use of LT4, MMI and PTU treatment during pregnancy.

P378

Fine-needle aspiration biopsy – possibilities and limitations

Delia Ciobanu[1], Carmen Vulpoi[1], Niculina Florea[2], Lidia Andriescu[1], Alexandru Grigorovici[1], Voichita Mogos[1], Bogdan Galusca[1], Cristina Cristea[1], Letitia Leustean[1] & Eusebie Zbranca[1]
[1]University of Medicine and Pharmacy 'Gr.T.Popa', Iasi, Romania; [2]University Hospital "Sf.Spiridon", Iasi, Romania.

The critical problem of thyroid nodules is to identify the malignant ones Fine needle aspiration biopsy (FNAB) plays a crucial role in this diagnosis and enables the number of surgical operations to be reduced. We have evaluated the performances of FNAB in comparison with the histological examination in 1971 consecutive patients who suffered both fine-needle puncture and surgery in a 5 years interval at a University Hospital. FNAB was malignant or suspicious in 8.4% patients, and the histology confirmed thyroid cancer in 8.7% (confirming all those diagnosed by FNAB). Statistical analysis revealed a sensitivity of 77% and a specificity of 95%, better than the admitted inferior limit of the literature data (71% respectively 72%). Papillary thyroid carcinoma was the easiest to diangose by the cytology, the efficacy of the method being 97%. For anaplastic and medullary carcinoma, FNAB is a good method to diagnose the malignancy (concordance of 97%) but has not the capacity to confirm the type of the neoplasia. In the follicular carcinoma, the positive predictive value is lower than for the other forms (27% vs 99%) although the efficacy is not significantly modified (94%). These data justify the introduction of morphometric methods and of the cytochemistry, able to enhance the accuracy of FNAB. These methods are time-consuming and we were using them only in controversial cases. With a very good sensitivity and specificity, FNAB is a reliable method of diagnosis in thyroid nodules, easy to perform and permitting to avoid unnecessary surgery.

P379

Association of p53 codon 72 polymorphism with thyroid cancer

Sinan Caglayan[1], Cenk Aral[2], Sirin Massaumilary[2], Gokhan Ozisik[3], Huseyin Baloglu[4], Mustafa Akkiprik[2], Ayse Ozer[2] & Metin Ozata[3]
[1]Kasimpasa Military Hospital, Division of Endocrinology and Metabolism, Istanbul, Turkey; [2]Marmara University, School of Medicine, Department of Medical Biology, Istanbul, Turkey; [3]GATA Haydarpasa Teaching Hospital, Division of Endocrinology and Metabolism, Istanbul, Turkey; [4]GATA Haydarpasa Teaching Hospital, Department of Pathology, Istanbul, Turkey.

Tumors of thyroid gland are one of the most prevalent forms of human cancers. Despite the various molecular mechanisms, mutations or polymorphisms of p53 have a potential role in the development and/or progression of human malignancies including thyroid. A common variation in p53 that results in adenine to proline change in codon 72 has been identified as a predisposing factor for various cancers since controversial results have been reported. In this study, we investigated codon 72 polymorphism in 58 thyroid cancer patients and 115 healthy individuals. Genomic DNAs were extracted from paraffin embedded tumor tissues of patients and blood samples of healthy individuals. PCR-RFLP method was applied for determination of codon 72 polymorphism. Genotype frequencies of arg/arg, arg/pro and pro/pro were 0.293, 0.483, 0.224 for patients and 0.461, 0.452, 0.087 for healthy controls, respectively. Proline allele frequencies of patients and healthy controls were 0.466 and 0.313, respectively. A significant difference was found between genotypes of patients and controls ($P=0.006$). Also, proline allele frequency was significantly higher in patients group than healthy control ($P=0.005$) (Odds ratio=0.527, 95% CI=0.341–0.817). No difference was found between 16 follicular adenoma and 18 papillary carcinoma patients ($P>0.05$). Additionally, no significant difference was found for TNM classification of papillary carcinoma patients for codon 72 status ($P>0.05$). In conclusion, p53 codon 72 polymorphism is a significant contributor of thyroid malign and benign lesions and proline allele is significantly increasing the risk of thyroid cancer.

P380

Adiponectin in patients with Graves' ophthalmopathy (GO)

Helena Jastrzebska[1], Malgorzata Gietka-Czernel[1], Jadwiga Janik[1], Izabella Czajka-Oraniec[1], Wojciech Jeske[1], Regina Karczmarzyk[2] & Wojciech Zgliczynski[1]
[1]Medical Centre of Postgraduate Education, Department of Endocrinology, Warsaw, Poland; [2]Institute of Oncology; Department of Teleradiotherapy, Warsaw, Poland.

Adiponectin is a soluble protein produced solely by matured adipocytes. Adipogenesis contributes to the pathogenesis of GO in many ways including a direct effect on proptosis due to increased volume of mature adipocytes.

The aim of the study was to estimate an influence of immunosupresive and anti-inflammatory treatment using systemic corticotherapy combined with orbital radiotherapy on serum adiponectin level in GO patients. The study was accepted by Ethical Commitee.

Material consisted of eight previously untreated euthyroid women aged 53.62±4.89 yrs. Corticotherapy was aplicated once a week, intravenously following a protocol: methylprednisolone in a dose of 0.5 g for the first 6 weeks, therafter the dose was reduced to 0.25 g for another 6 weeks, and from the third week was combined with weekly orbital irradiation (2 Gy) over 10 weeks.

Clinical examination with estimation of clinical activity score (CAS), proptosis, ophthalmopathy index (OI), BMI as well as blood sampling for adiponectin estimation were performed before therapy, after second methylprednisolone injection and after last orbital irradiation. Adiponectin was measured using RIA kits (Linco Research). Treatment resulted with significant clinical improvement and decrease in CAS of 3 points ($P<0.01$), reduction of proptosis >2 mm ($P<0.01$) and OI from 6.5 points±1.19 to 4.0±0.53 ($P<0.01$). BMI did not change during the study (mean 26.64±3.90 kg/m^2 vs. 26.43±3.37 kg/m^2). Serum levels of adiponectin were in normal range in all patients: before therapy mean 16.10±6.10 mcg/ml, during therapy mean 16.42±6.03 mcg/ml and after therapy mean 17.08±7.48 mcg/ml.

No significances were observed in adiponectin concentration during the treatment in all subjects.

Our results may suggest that changes in proptosis in GO patients during anti-inflammatory and immunosupressive therapy are not associated with any significant changes in serum adiponectin level.

P381

Resistin levels in hypothyroid patients before and after treatment with thyroxin

Marianthi Tzoitou[1], Evridiki Papadopoulou[1], A Galactidou[2], Athanasios Papageorgiou[1], Anargyros Kourtis[1], Marina Kita[1], louiza ventzi[1] & Avraam Avramidis[1]
[1]Endocrinology Department, Hippokratio General Hospital, Thessaloniki, Greece; [2]Research Department of Theagenio Hospital, Thessaloniki, Greece.

Introduction
Resistin is a peptide hormone that is secreted from fat cells and its secretion is regulated from hormonal and dietary factors. In hypothyroid patients its levels are decreased.

The purpose of this study is to evaluate the levels of circulating resistin in hypothyroid patients before and after thyroid function is normalized with thyroxin therapy.

Materials and methods
Twenty (20) hypothyroid patients (2M, 18F) mean aged 49.9±12.4 and mean weight 75.1±19.4 Kgr) were studied.

FT4, TSH, AMA, ATA, Resistin were measured before and three months after thyroxin therapy.

Results
Resistin levels do not change significantly (5.8±4.1 vs 5.1±3.4 μg/lt).All patients became euthyroid after three months of treatment and TSH, FT4, AMA, ATA levels were changed significantly(16.7±3.4 miu/lt vs1.2±0.2 miu/lt, 0.8±0.07 ng/dl vs1.3±0.07 ng/dl and 1579.6±653 vs 412±219. 441.8±205 vs 264.8±111). The body weight of the patients was not change significantly during therapy(75.1±19.4 vs 74.1±17.2 Kgr).

Conclusions
Normalization of thyroid function did not affect resistin levels significantly. Possibly this is because there was no change IN the patients weight during treatment.

P382

Increased need for oral thyroxine in total thyroidectomized patients: a prospective analysis

Lucilla Gargano, Camilla Virili, Nicola Viceconti, Maria Giulia Santaguida, Serena Nardo & Marco Centanni
Endocrinology Unit, University of Rome 'La Sapienza', Latina, Italy.

Increasing evidence suggests a relevant role for thyroid gland in maintaining hypophysis-thyroid homeostasis even in patients treated with oral thyroxine. Aim of the study was to compare the daily dose of thyroxine required to attain subnormal serum TSH levels in patients with nontoxic goitre before and following total thyroidectomy. To address this question we have studied: a) 15 patients (8 women and 7 men; median age=53 years) with nontoxic goitre (NTG) and no evidence of autonomous functioning nodule, prospectively analyzed before and after total thyroidectomy for differentiated thyroid carcinoma and b) a cohort of 45 randomly selected patients (35 women and 10 men; median age=51 years) with similar characteristics submitted to total thyroidectomy. Thirty-nine randomly selected T4-treated patients with NTG (33F, 6M; median age=46 years) represented the reference group. In all these patients we compared the dose of thyroxine (normalized by Kg body weight/day) required to stably attain plasma TSH levels to within 0.1–0.2 mU/l. No patients were taking drugs or had evidence of other diseases, known to interfere with the absorption of thyroxine. In the patients prospectively studied the median dose of thyroxine required to obtain low TSH (median=0.11 mU/l) was 1.41 μg/Kg/day. Following thyroid removal, being the thyroxine dose maintained to pre-surgical levels, median TSH significantly rose to 2.94 mU/l ($P=0.031$). Low serum TSH (median=0.16 mU/l) was restored in all patients by increasing the median dose by 37% (1.94 μg/Kg/day; $P=0.0013$). Similarly, in the randomly selected patients the median dose of thyroxine required was higher in thyroidectomized patients (1.83 μg/Kg/day) than in those with nontoxic goitre (1.50 μg/Kg/day; $P<0.0001$). These data indicate that, both in the same patient and in different groups of patients, the daily dose of thyroxine required to lower plasma TSH is 1/3 higher when the thyroid is absent.

P383

Efficacy and safety of radiofrequency thermal ablation in the treatment of thyroid nodules with pressure symptoms in elderly patients

Antongiulio Faggiano[1], Carolina Di Somma[1], Francesco Milone[1], Valeria Ramundo[1], Gaetano Lombardi[1], Annamaria Coloa[1] & Stefano Spiezia[2]
[1]Dept. Of Molecular And Clinical Endocrinology And Oncology, 'Federico Ii' University Of Naples, Naples, Italy; [2]Unit Of Ultrasound-Guided And Neck Pathologies Surgery, 'Sm Del Popolo Degli Incurabili' Hospital, Naples, Italy.

Background
Nodular goiter is one of the commonest endocrinopathy. Its incidence increases with age accounting for more than 50% of subjects older than 60 years. Elderly subjects more frequently suffer from pressure symptoms. Loco-regional treatments, like laser photocoagulation and percutaneous ethanol injection, are a potentially useful tool to treat TNs but their efficacy is still debated. Radiofrequency thermal ablation (RTA) has been applied to several benign and malignant tumors proving to be a safe procedure, potentially helpful to stabilize or decrease tumor growth. Recently, RTA proved to be safe and to induce short-time effects in the treatment of patients with thyroid nodules.
Objective
The aim of this study is to evaluate safety and efficacy of RTA in elderly patients with compressive thyroid nodules followed-up for 1 year.
Patients and methods
Thirty-nine elderly patients with cytologically benign compressive TNs were enrolled in the study. Twenty-seven of them were affected with nontoxic goiter, five with pre-toxic goiter, four with toxic goiter, three with toxic adenoma. Thyroid surgery was contraindicated in 22 and refused in 17 cases. RTA was performed by using a RITA © Starbust needle inserted under ultrasonographic real time guide. Efficacy and safety of RTA were followed-up at 1, 3, 6, 12 month.
Results
After treatment, all TNs showed a significant decrease during the follow-up. Mean TN volume decreased from 24.3±2.6 to 6.4±1.6 ml ($P<0.001$) with a mean percent decrease of 78.6±2.5% 12 months after RTA. Compressive symptoms improved in all cases and disappeared in 82%. The treatment was well tolerated by all patients. No major complications were observed.
Conclusions
RTA seems to be a valid and safe approach in the treatment of benign thyroid nodules with pressure symptoms. RTA may be of great benefit in elderly patients in whom surgery or radio-iodine therapy are contraindicated or refused.

P384

The effect of nodule size on diagnostic efficacy in fine needle aspiration biopsy of thyroid nodules

Dimitrios Thomas[1], Ifigenia Kostoglou-Athanassiou[1], Emmanouil Vassiliou[1], Vassilios Liakos[1], Fotini Chatzimarkou[1], Anastasios Pappas[1], Panagiotis Athanassiou[2] & Philippos Kaldrymidis[1]
[1]Department of Endocrinology, Metaxa Hospital, Piraeus, Greece; [2]Department of Rheumatology, Asclepeion Hospital, Athens, Greece.

Fine needle aspiration is used extensively in the diagnostic evaluation of thyroid nodules. However, its diagnostic efficacy may be reduced by non-diagnostic findings. The aim was to study the effect of nodule size on the diagnostic efficacy of ultrasound-guided fine needle aspiration biopsy in thyroid nodules.

Ultrasound-guided fine needle aspiration biopsy was performed in 210 patients with thyroid nodules. Cytology results were compared to nodule size. Patients were stratified in 5 groups according to nodule size, group A ($n=41$) nodule size 0.1–0.426 cm^3, group B ($n=43$) nodule size 0.427–0.816 cm^3, group C ($n=42$) nodule size 0.817–1.593 cm^3, group D ($n=43$) nodule size 1.594–3.382 cm^3 and group E ($n=41$) nodule size >3.39 cm^3. Ultrasound guidance of the fine needle aspiration biopsy was performed in all patients using the same linear modifier (6–12 MHz) attached in a Toshiba ultrasound apparatus (SSA–550A). Statistical evaluation of the results was performed using x^2 test and ANOVA.

In group A thyroid nodule fine needle aspiration biopsy was successful in 43.9%, in group B 79.1%, in group C 76.2%, in group D 69.8% and in group E 58.5% ($P=0.004$, x^2 test). The number of cystic nodules and the pattern of vascularization (central, peripheral or both) differed significantly between the groups studied.

Diagnostic efficacy of fine needle aspiration biopsy seems to increase in parallel to nodule size. However, this relationship was not apparent in very big nodules, nodule size >3.38 cm^3, possibly due to confounding factors, such as the presence of cystic areas and increased vascularization within the very large thyroid nodules.

P385

The effect of thyroxine suppression therapy on the diagnostic efficacy of fine needle aspiration biopsy in thyroid nodules

Dimitrios Thomas[1], Ifigenia Kostoglou-Athanassiou[1], Emmanouil Vassiliou[1], Vassilios Liakos[1], Fotini Chatzimarkou[1], Anastasios Pappas[1], Panagiotis Athanassiou[2] & Philippos Kaldrymidis[1]
[1]Department of Endocrinology, Metaxa Hospital, Piraeus, Greece; [2]Department of Rheumatology, Asclepeion Hospital, Athens, Greece.

Ultrasound-guided fine needle aspiration biopsy is used extensively in the diagnostic evaluation of thyroid nodules. However, its diagnostic efficacy is hampered by the presence of non-diagnostic cytology results.

The aim was to study the effect of previous thyroxine suppression therapy on the diagnostic efficacy of fine needle aspiration biopsy in thyroid nodules.

Ultrasound-guided aspiration biopsy was performed in 45 patients, 31 patients on thyroxine suppression therapy and 14 patients without current or previous thyroxine therapy. Ultrasound guidance of the fine needle aspiration biopsy was performed in all patients using the same linear modifier (6–12 MHz) attached in a Toshiba ultrasound apparatus (SSA-550A). Statistical evaluation of the results was performed using Student's t test and x^2 test.

Patient characteristics did not differ significantly between the 2 groups studied. In 13 of 14 (92.9%) patients without current or previous thyroxine therapy the cytology result of fine needle aspiration biopsy was diagnostic, whereas the cytology result of the biopsy was diagnostic in 20 of 31 (64.5%) patients on thyroxine suppression therapy ($P=0.046$). The diagnostic efficacy was not found to differ according to the duration of thyroxine therapy, possibly due to the small number of patients studied.

It appears that thyroxine suppression therapy in patients with thyroid nodules is related to lower diagnostic efficacy of ultrasound-guided fine needle aspiration biopsy. Thyroxine suppression therapy may induce changes in thyroid cell structure and size, thus modulating the diagnostic efficacy of fine needle aspiration biopsy in thyroid nodules.

P386

Congenital hypothyroidism – results of a protocol implemented 1993–2006

Miguel Melo[1] & Charles Buchanan[2]
[1]Endocrinology Department, University Hospital of Coimbra, Coimbra, Portugal; [2]King's College Hospital, London, United Kingdom.

Introduction
A new Regional Paediatric Endocrine Service was established in 1993 which implemented a protocol for Congenital Hypothyroidism (CH) management. Its aim is to confirm the diagnosis, establish aetiology and start treatment within 48 hours after the result of the screening test.
Objective
To audit the results of this protocol.
Methods
Case note and laboratory data review for all Neonates referred since 1993 after a positive TSH screening test. The following issues were considered: age at screening, time to lab. Receipt and processed the blood sample, child's age when results known, time from referral to first appointment at, clinical presentation, presence of associated disorders, family history of thyroid diseases, presence of thyroid auto-antibodies in mother's blood, child's age when treatment was started, starting dose of L-T4, time to normality of TSH, diagnostic group – agenesis, dysgenesis, dyshormonogenesis, transient or other; presence of learning difficulties, assessed with Griffiths scale.
Results
A total of 28 patients were included; 7 (36.8%) were premature. Median age at screening was 9 days. Medians of time to laboratory for sample and processing were 6 and 3 days, respectively. By the time the screening test results' were known, children had a median age of 16 days. Median time from referral to first visit was 1 day (mean age 22.0 ± 18.2 days). Median age start treatment was 18 days; mean starting dose 8.8 ± 3.6 mcg/kg/day. At presentation, 15 (54%) babies had jaundice. A ^{99m}Tc scan was done in the first visit in 19 (68%) patients. 22% had thyroid agenesis, 39% dysgenesis, 30% dyshormonogenesis (all normal hearing tests) and 9% were transient 3 patients had Down's syndrome and 1 a CNS malformation. 3 mothers had thyroid antibodies. Median time to normal TSH was 91 days and there was no a statistically significant difference between the aetiological groups. 2 patients had learning difficulties.
Conclusions
The objectives of this protocol were largely achieved, since most of the patients had a full aetiological workup and started treatment in the first 24 hours after the screening test is known.

P387

Relationship of treated maternal hyperthyroidism and perinatal outcome

Amjed Khamis[1], Sharon Murray[1], Sean Daly[2], Richard Firth[1] & Brendan Kinsley[1]
[1]Diabetes and Endocrine department, Mater Misercordea University Hospital, Dublin, Ireland; [2]Coomb's women Hospital, Dublin, Ireland.

Hyperthyroidism in pregnancy is associated with increased foetal and maternal morbidity. Hyperthyroidism occurs in 0.2–0.6% of pregnancies. This suggests that based on 23,000 deliveries in the 3 major Dublin maternity hospitals that 40–60 cases per year would be expected to be at risk of a poor outcome from hyperthyroidism.

To clarify those factors associated with poor outcome in hyperthyroidism in pregnancy we undertook an audit of 53 cases of hyperthyroidism in pregnancy attending from 2004–2005. Women with hyperthyroxinaemia secondary to hyperemesis were excluded. Demographic data, maternal thyroid function tests, doses of anti-thyroid medications were noted. Pregnancy outcomes, birth weight and neonatal TFTs were noted. Cases were divided according to those Delivering pre 37 weeks (Group A, $n=11$) and at term, post 37 weeks (Group B, $n=42$).
Results
Mean age was 31 ± 5 years. Mean booking to OPD at 13 ± 5 weeks gestation. Mean delivery gestation was 39 ± 1 weeks in-group A, 35 ± 3 weeks in group B ($P<00.1$). Mean birth weight 3.3 ± 0.7 kg. One neonatal death occurred in-group A.
In Group A, baseline TSH was 0.09 ± 0.1, $P<0.05$ vs Group B (1.1 ± 1.3). By end of the second trimester, TSH in Group A was 0.17 ± 0.2, $P<0.05$ vs Group B (0.88 ± 1.0). By end of third trimester TSH was 0.34 ± 0.5 (GROUPA), $P<0.05$ vs. Group (0.98 ± 0.9). Average BW in Group A was 2.5 ± 0.9 kg, $P<0.01$ vs Group B (3.44 ± 0.6 kg)

Conclusion
TSH levels were significantly lower in those women with pre-term delivery. This suggests that sub-optimal control of hyperthyroidism during pregnancy is associated with increased infant morbidity or mortality

P388

Characteristics of locally advanced differentiated thyroid cancer in a cohort of patients surgically treated at one oncological institution

R Baldelli, G Pasimeni, A Barnabei & M Appetecchia
Regina Elena Cancer Institute, Rome, Italy.

Differentiated thyroid carcinomas (DTC) (papillary, follicular and follicular type of papillary) are characterized by a favourable prognosis, but a part of patients can develop recurrences and eventually die of the disease. We retrospectively reviewed 108 DTC patients affected by locally advanced thyroid carcinoma (77 females, 31 males) (49 ± 15 years), in order to evaluate validate known prognostic factors that enable them to be recognised as having a low or a high risk of death related to the tumor, by reference to the staging classifications systems. The TNM classification was as followed: T2b (0.9%), T3 (62%), T3b (30%), T4a (5.5%), T4B (1.8%). The mean diameter of tumor was 24 ± 1 mm. In particular the histology was papillary (62%), follicular (8%), follicular type of papillary (28%), Hurtle (1%), Hurtle+ papillary (1%). Lymph nodes status was N0 (9.2%), N1a (13.8%), N1b (26.8%), Nx (50%) while metastases were present in 3.7% of patients. With the regards of stage patients were stage I (50%), stage III (33.3%), stage 4a (12%), stage IV b (3.7%). Seven of them (6.4%) had local or distant recurrences. Thyroiditis was found in 30% at the histology. No deaths were reported regarding our group of patients. Papillary and follicular thyroid carcinoma, referred to as differentiated thyroid carcinoma (DTC) cover the majority of thyroid carcinoma cases. The prognosis for DTC is usually excellent, but even so a proportion of the patients develop recurrences and eventually die of the disease. In particular the majority of our patients (50%) were in the stage I explaining the good prognosis of this group of patients. These previous data show that age at the time of diagnosis, histological type, tumour size and extrathyroidal invasion are associated with a good clinical outcome.

Bone/calcium – presented on Monday

P389

Abnormal calcium metabolism as shown by the Ellsworth-Howard test and its relation to pseudohypoparathyroidism II

Levent Kebapcilar, Sena Yesil & Tevfik Demir
Dokuz Eylul University, IZMIR, Inciralti, Turkey.

Peudohypoparathyroidism is a heterogenous group of disorders characterized by hypocalcemia, hyperphosphatemia, increased serum concentration of parathormone, and insensivity to biological activity of parathormone.

A 45-year-old female was admitted to the regional hospital for check up and routine laboratory studies revealed slightly decreased level of calcium. Her neurologic examination was negative for Chvostek's and Trousseau' signs. Laboratory testing revealed low calcium (8.1 mg/dL; reference: 9.5–10.5 mg/dL) with elevated PTH (388 pg/mL; reference: 12–72 pg/mL) and phosphate levels. 25 hydroxycholecalciferol (56 ng/mL; reference: 7.6–75 ng/mL), and 1.25 dihydroxycholecalciferol levels were normal (40 pg/mL; reference: 30–60 pg/mL). This laboratory tests indicative of PTH resistance and suggested PHP. We therefore applied Ellswoth- Howard (EH) test, which shows receptor function and the presence of intracellular signal transduction disorder in renal tubular cells and to determine the type of PHP.

Both the phosphaturic (Δ) and urinary c AMP (Uc AMP) responses were estimated. The Δ P responses in the patient was significantly lower than normal response (18 mg/2 h) but its UcAMP response did not differ (Δ c AMP ≥7.9 μmol/h and after/ before c AMP ratio: 13.2) from normal subjects. This was suggested us that the patient had PHP type II. We started treatment with calcium (2000 mg daily) and 1.25- vitamin D3 (0.5 μg daily).

Many individuals affected by pseudohypoparathyroidism type II (PHP-II) have no apparent clinical symptoms and may show only a mild PTH elevation as evidence of PTH resistance. Patients with pseudohypoparathyroidism type II lack the features of Albright's hereditary osteodystrophy and may manifest hormonal resistance limited to target tissues.

Peudohypoparathyroidism is a heterogenous group of disorders characterized by hypocalcemia, hyperphosphatemia, increased serum concentration of parathormone, and insensivity to biological activity of parathormone.

A 45-year-old female was admitted to the regional hospital for check up and routine laboratory studies revealed slightly decreased level of calcium. Her neurologic examination was negative for Chvostek's and Trousseau' signs. Laboratory testing revealed low calcium (8.1 mg/dL; reference: 9.5–10.5 mg/dL) with elevated PTH (388 pg/mL; reference: 12–72 pg/mL) and phosphate levels. 25 hydroxycholecalciferol (56 ng/mL; reference: 7.6–75 ng/mL), and 1.25 dihydroxycholecalciferol levels were normal (40 pg/mL; reference: 30–60 pg/mL). This laboratory tests indicative of PTH resistance and suggested PHP. We therefore applied Ellswoth- Howard (EH) test, which shows receptor function and the presence of intracellular signal transduction disorder in renal tubular cells and to determine the type of PHP.

Both the phosphaturic (Δ) and urinary c AMP (Uc AMP) responses were estimated. The Δ P responses in the patient was significantly lower than normal response (18 mg/2 h) but its UcAMP response did not differ (Δ c AMP ≥7.9 μmol/h and after/ before c AMP ratio: 13.2) from normal subjects. This was suggested us that the patient had PHP type II. We started treatment with calcium (2000 mg daily) and 1.25- vitamin D3 (0.5 μg daily).

Many individuals affected by pseudohypoparathyroidism type II (PHP-II) have no apparent clinical symptoms and may show only a mild PTH elevation as evidence of PTH resistance. Patients with pseudohypoparathyroidism type II lack the features of Albright's hereditary osteodystrophy and may manifest hormonal resistance limited to target tissues.

P390

The role of non-calcemic analogs of vitamin D in differentiation of cultured rat bone marrow into osteoblast-like cells: age and sex differences

Dalia Somjen[1], Alvin M Kaye[3], Michal Ofer[2] & Ilan Bleiberg[2]
[1]Tel-Aviv Sourasky Medical Center, Tel-Aviv, Israel; [2]The Sackler Faculty of Medicine, Tel-Aviv Univ, Tel-Aviv, Israel; [3]The Weizmann Institute of Science, Rehovot, Israel.

We have previously demonstrated that rat bone cells *in vivo* and *in vitro*, responded sex-specifically to gonadal steroids in stimulation of the specific activity of creatine kinase (CK). Pretreatment with vitamin D analogs upregulated the sex-specific responsiveness and sensitivity to gonadal steroids. We also found that mice cultured femoral bone marrow (BM) in the presence of dexamethasone (DEX), 1.25(OH)2D3 (1.25D) or both, differentiated into osteoblast-like cells (Obs), acquiring sex-specific responsiveness to gonadal steroids. We now examined the effect of age, sex and vitamin D non-hypercalcemic analogs on differentiation of rat femoral BM into Obs. In female or male, BM from intact but not gonadectomized rats DEX and DEX + 1.25D increased the constitutive levels of CK. BM from old females showed lower stimulation of CK than BM from young females by estradiol-17β (E2) or raloxifene (Ral) in the presence of both DEX and 1.25D. The non-hypercalcemic analogs of vitamin D: CB 1093 (CB), EB 1089 (EB) and MC 1288 (MC) were more effective than 1.25D in both age groups in stimulating CK in the absence of DEX. In the presence of DEX, CK was further increased with the same differential effectiveness. BM from gonadectomized male or female rats, lost the sex-specific response namely responding to both E2 and dihydrotestosterone (DHT). BM derived from intact and gonadectomized males and females, growing with DEX or DEX + 1.25D showed increased activity of basal alkaline phodphatase (AP) with no stimulation by gonadal steroids. These findings suggest that manipulation of the hormonal milieu in early stages of differentiation into Obs determines the subsequent selective responsiveness of the developing bone tissue to sex steroids. Non-calcemic vitamin D analogs were more effective than 1.25D and showed activity even in the absence of DEX and may be applied for bone tissue engineering.

P391

Mutual modulation of the vitamin D system and estrogen receptors in human bone cells in culture

Dalia Somjen[1], Sara Katzburg[1], Orly Sharon[1], Rona Limor[1], David Hendel[3] & Gary H Posner[2]
[1]Tel- Aviv Sourasky Medical Center, Tel-Aviv, Israel; [2]The Johns Hopkins University, Baltimore, United States; [3]Sharei-Zedec Med. Ctr., Jerusalem, Israel.

Vitamin D receptors are widely expressed in the skeletal system, and vitamin D and its metabolites and analogs, exert a variety of biological activities such as regulation of cellular proliferation and differentiation, cell calcium transients and energy metabolism in vitro. The latter is exerted through the control of the brain type isozyme of creatine kinase specific activity (CK), which serves to provide a readily available reservoir for ATP generation under increased workload.

We have previously reported that pretreatment with the less-calcemic analog of vitamin D JKF 1624F_2-2 (JKF) upregulated the responsiveness to estrogenic compounds via modulation of the expression of mRNA for ERs. In the present study we analyzed the mutual modulation of the vitamin D system and estrogens in human cultured female bone cells (hObs). We compared the effects of the different hormones on the expression of mRNA for both ERα and ERβ and 1α 25 vitamin D hydroxylase in hObs. In pre-menopausal hObs all hormones tested increased 1α 25 vitamin D hydroxylase mRNA expression whereas in post-menopausal hObs biochainin A had no effect and genistein is decreasing this mRNA expression. All these compounds increased the expression of mRNA for ERα in pre-menopausal hObs whereas in post-menopausal hObs biochainin A had no effect and estradiol and raloxifene decreased this mRNA expression. ERβ in both hObs was increased only by carboxy-biochainin A and raloxifene and all other hormones decreased ERβ. In conclusion vitamin D analogs and estrogens modulate each other's activity in hObs. The different hormones modulate the response to estradiol by direct modulation of ERs mRNA expression and by indirect modulation via increasing vitamin D in bone cells leading to modulation of responsiveness by this system as well. Whether or not this property can be utilized to achieve better bone protection remains subject to further studies.

P392

Less-calcemic vitamin D analogs enhance biological responses and modulate responsiveness to gonadal steroids in skeletal tissues

Dalia Somjen[1], Gary H Posner[2], Yosef Weisman[1] & Alvin M Kaye[3]
[1]Tel- Aviv Sourasky Medical Center, Tel-Aviv, Israel; [2]The Johns Hopkins University, Baltimore, MD., United States; [3]The Weizmann Institute of Science, Rehovot, Israel.

Vitamin D metabolites modulate creatine kinase specific activity (CK) in cultured skeletal cells. In this study we assess the effect of vitamin D metabolites on CK in rat epiphyseal cartilage (Ep) and diaphyseal bone (Di). Female or male Wistar-derived rats were used either as intact or after gonadectomy (Ovx or cast respectively), and treatments started 2 weeks post surgery. Rats were injected daily for 1, 2 or 8 weeks with the less-calcemic vitamin D analogs CB 1093 (CB), JKF 1624F_2-2 (JKF) or QW 1624F_2-2 (QW) and 24hrs after the last analog injection, rats were injected with E_2, raloxifene (Ral) or tamoxifen (TAM) or both in females or dihydrotestosterone (DHT) in males, and organs were collected for CK measurements and western blot analysis for estradiol receptor ((ERα) 24hrs after last injection. CK was lower in Ep and Di from vitamin D- depleted than in vitamin D- replete rats. Moreover E_2 or DHT, which increases CK in Ep and Di of intact female or male rats, stimulated CK to a much lower extent in vitamin D-depleted rats. Treatment of intact female rats for 2 or 8 weeks with JKF or QW, upregulated the E_2 – or DHT- response of CK in Ep and Di, without affecting constituent levels. All vitamin D analogs enhanced the CK response to Ral and TAM in these organs, but the inhibitory effect of Ral or TAM on E_2-induced CK was lost. CB induced also ER(protein in Ep and Di from intact and Ovx female rats. In conclusion, vitamin D induces CK and upregulates the response and sensitivity of CK to E_2 and SERMs, possibly via increased ER(protein. These results corroborate our *in vitro* studies in human bone cells and provide evidence that vitamin D is crucial to maintain normal skeletal energy metabolism.

P393

The predictive role of body mass and composition upon bone mineral content: differences between premenopausal and postmenopausal women

Dumitru Branisteanu, Anca Piciorea, Eusebie Zbranca & Voichita Mogos
University of Medicine and Pharmacy 'Gr.T.Popa', Iasi, Romania.

Gravitational stress influences bone mass. Adipose tissue represents a supplementary source of estrogens at postmenopausal women, *via* aromatization of androgens. We evaluated the importance of weight and fat tissue on bone mass at premenopausal or postmenopausal women in a cross-sectional study upon a group of 138 women between 25 and 77 ys old and with a BMI between 17.1 and 44.3 kg/m^2. Fifty six women were menstruating, 15 were perimenopausal and 68 were postmenopausal. We assessed the correlation between lumbar bone mineral content (Z and T scores, measured by dual X ray absorptiometry) and: body mass, adipose and lean tissue mass (measured by electric impedance). Postmenopausal women had a significantly lower bone mass than premenopausal women (mean T score of $-1.87+/-0.14$ vs $-0.91+/-0.16$, $P<0.05$). Lean (BMI <24 kg/m^2) postmenopausal women had an even lower mineral content (T score $=-2.17+/-1.23$, $P<0.01$ when compared to premenopausal women), whereas overweight postmenopausal women (BMI >26 kg/m^2) had an intermediate T score between premenopausal and postmenopausal lean women ($-1.63+/-0.19$, $P<0.05$). Total body mass, lean and fat mass were all correlated to bone mineral content, having comparable predictive powers in premenopausal women. When applied to postmenopausal women, correlation significance of fat mass with the Z score augmented ($R^2=0.329$ vs $R^2=0.253$ for premenopausal women), whereas correlation significance between total or lean body mass and Z score decreased (in the case of total body mass - $R^2=0.148$ vs $R^2=0.28$ for premenopausal women). Adipose tissue mass seems therefore to be an important BMD predictive factor. Its predictive value increases in postmenopausal women, whereas total and lean body mass are correlated to BMD especially in premenopausal women, which are not yet submitted to estrogenic depletion.

P394

Increased cortisol level in type 1 diabetic patient may lead decreasing of bone mineral density

Alpaslan Tuzcu[1], Semir Bayer[1], Senay Arikan[1], Deniz Gokalp[1], Mithat Bahceci[1] & Naime Canoruc[2]
[1]Dicle University School of Medicine Department of Endocrinology, Diyarbakir, Turkey; [2]Dicle University School of Medicine Department of Biochemistry, Diyarbakir, Turkey.

Objective
In this study we aim to investigate the association of osteoporosis and type 1 diabetes in 43 type 1 diabetic subjects and 41 control subjects.
Subjects and methods
Bone mineral density of both groups was measured by DEXA. Age, BMI, waist/hip ratio, daily calcium consumption of both groups were determined. Twenty-four hours urinary calcium, phosphorus, deoxipyridinoline and pyridinoline were measured. Osteocalcin ALP, IGF-1, IBF-BP3. HbA1c, cortisol, albumin, LDL and triglyceride were measured in both groups. Independent t-test and chi- square test were used to the groups.
Results
Age, body weight, BMI, waist/hip ratio, daily calcium consumption of diabetics were not different from the control group ($P>0.05$). Total lumbal BMD (0.88 ± 0.1; 0.93 ± 0.1 g/cm^2 respectively; $P<0.05$) total femur BMD (0.93 ± 0.14, 0.99 ± 0.1 g/cm^2 respectively; $P<0.05$) and total femur Z- score (-0.16 ± 1, 0.53 ± 0.7 respectively; $P<0.005$) of the diabetic group were statically lower than control group. Urine DPD/ creatinine level (7.6 ± 6.1, 4.9 ± 3.8 pmol/μmol respectively; $P<0.05$), serum ALP level (113 ± 62, 74 ± 18 U/L respectively; $P<0.001$), IGF-BP3 level (5.4 ± 0.9, 4.7 ± 1 μg/ml respectively; $P<0.001$) of diabetic groups were statically higher than control group. Serum cortisol levels in diabetic group were statically higher than control group (14.7 ± 3, 12.8 ± 2.7 μgr/dl respectively; $P<0,005$).
Conclusions
Bone mineral density of type 1 diabetic patient were decreased due to increased bone turnover.

P395

Body fat concentration is a poor predictor of bone mineral content in hyperthyroid women

Carmen Vulpoi, Dumitru Branisteanu & Eusebie Zbranca
University of Medicine and Pharmacy 'Gr.T.Popa', Iasi, Romania.

Hyperthyroidism has significant impact upon both bone turnover and body composition. The present study was designed to investigate whether there is a connection between changes in body composition and bone mineral content at female patients with perturbed thyroid function. Sixty-seven long standing (over 6 months) overt hyperthyroid women had significantly lower bone mineral content as expressed by the Z score measured by quantitative ultrasonography ($-0.86+/-0.69$ compared to $-0.08+/-0.37$ in the age- and BMI- matched euthyroid control group of 82 women, $P=0.01$) and a modified body composition (evaluated by the bioelectrical impedance technique), with lower body fat percentage ($39+/-2\%$ compared to $44+/-1.9\%$ in controls, $P=0.01$). Bone mineral content of hyperthyroid women was significantly correlated to serum alkaline phosphatase ($R^2=0.545$, $P<0.001$), but not to the percentage of body fat ($R^2=0.0069$, NS). Body fat percentage was however a good predictor for the bone mineral content of control euthyroid women ($R^2=0.176$, $P=0.027$). We conclude that loss of bone mass in hyperthyroid women is caused rather by an increase in bone turnover, under the direct action of thyroid hormones, than by a thyroid hormone-induced decrease of body fat mass.

P396

Vitamin D receptor gene polymorphisms: influence on bone metabolism in type 1 diabetic patients

Dilek Gogas Yavuz[1], Dilek Yazici[1], Meral Yüksel[2], Sinem Kiyici[3], Murat Sert[4], Ibrahim Sahin[5], Lerzan Keskin[5], Oguzhan Deyneli[1], Hasan Aydin[1], Ercan Tuncel[4] & Sema Akalin[1]
[1]Marmara University Medical School, Istanbul, Turkey; [2]Marmara University School of vocational, Istanbul, Turkey; [3]Uludag University Medical School, Bursa, Turkey; [4]Cukurova University Medical School, Adana, Turkey; [5]Inonu University Medical School, Malatya, Turkey.

Patients with type 1diabetes mellitus are at higher risk of developing osteoporosis. Among the genetic factors related to the development of osteoporosis, a possible association between vitamin D receptor (VDR) gene polymorphism and bone mineral density (BMD) has been described in some populations.

The aim of this study is to investigate the distribution of vitamin D receptor (VDR) polymorphisms and relation to bone turnover parameters and bone densitometry in Turkish type 1 diabetic patients/

One hundred nine type 1 diabetic patients (M/F 59/50, 30±7 yrs) and 109 healthy controls (M/F 62/47, 29±8 yrs) were included in the study. Duration of diabetes was 8.1±6.3 yrs in patients. Bone mineral density (BMD) of the lumbar spine (L2–L4) and femoral neck were evaluated by DEXA scans. VDR genotype was assessed by polymerase chain reaction amplification followed by BsmI, Apa, Taq and Fok digestion on DNA isolated from peripheral blood leukocytes. Serum levels of calcium, osteocalcin, parathyroid hormone, ctx, 25-OH-vitamin D levels, and A1c, urinary deoxypyridinoline levels were measured Data were analyzed using the chi.2-test and students *T* test where appropriate.

Genotypes FF, Ff and ff were 55.9%, 36.6%,7.3% vs 37.6%, 32.6%, 8.4%; BB Bb and bb were 20.1%, 39.4%, 40.3% vs 15.5%, 53%, 31.5%; TT, Tt, tt were 33.9%, 58.6%,18.4% vs 28.4%, 55.9%, 15.5% for diabetic and control groups respectively. And distributions did not differ between the groups. Genotype AA, Aa, aa were 32.1%, 47.7%, 20.1% for diabetics and 24.7%, 62.5%,12.8% for controls and significantly different ($P=0.04$). Type 1 diabetic group had a lower BMD at femoral and lomber areas compared with the control group. BMD at the head of femur and serum osteocalcin levels tend to be lower at ff genotype in diabetic patients compared to controls.

These findings suggest a small influence of VDR gene polymorphism on BMD in our group of type 1 diabetic patients. Fok1 polimorfisms may have interaction on bone metabolism and requires further studies of larger cohorts.

P397

Change in bone mass due to hyperthyroidism

Florian Toti[1], Dorina Ruci[2], Vilson Ruci[2], Artur Kollcaku[2], Argjend Tafaj[2] & Ergita Nelaj[1]
[1]UHC'Mother Theresa' Service of Endocrinology & Metabolic Diseases, Tirana, Albania, [2]UHC'Mother Theresa' Service of Reumatolgy, Tirana, Albania.

Objectives
To evaluate the presence and the degree of osteoporosis and osteopenia in a group of patients with established hyperthyroidism from at least six months.
Material and methods
This study was based on a quantitative measurement of bone mineral density with and heel ultrasound densitometer (Type Pegassus). Each selected patient was recorded for the weight, height, BMI, age, gender. No patient was previously treated for osteoporosis or osteopenia. A control group with similar data was selected form the general population, with no personal or familiar history of hyperthyroidism. None of them had a known history for osteoporosis or had received any medication for this condition. The criteria for osteoporosis were those recommended from the WHO. Osteoporosis =T-score <-2.5.
Results
We studied 64 patients with confirmed hyperthyroidism from at least six months. There were 22 males and 42 females (33/67%). Mean age 59.9±12.1 years, weight 74±2 kg, BMI 27.8 kg/m^2. For the control group: 38 patients (18/20 M/F), age 60.5±11.1 years, weight 69.9±12.3 kg, BMI 26.2 kg/m^2. The mean values of T-score for the hyperthyroid patients were -3.7 ± 1.4 and -2.0 for the control group. 55% of patients with hyperthyroidism had severe osteoporosis, compared with only 9.5% of control group ($P<0.001$). The gender itself was no significant.
Conclusions
The silent osteoporosis and osteopenia is relatively frequent in hyperthyroidism, significantly more than in normal population. The stimulation of osteoclasts more than osteoblasts and alteration of remodeling cycling from thyroid hormones is believed to be the causative factor.

P398

Correlation between bone minerale density and bone turnover in delayed puberty

Iulia Bistriceanu, Marian Bistriceanu, Elena Neacsu, Magda Preda & Alida Pruna
Craiova University of Medicine and Pharmacy, CRAIOVA / DOLJ, Romania.

It is established that the delayed puberty is the lack of development of sexual maturation in boys and girls at a chronological age that is 2.5 standard deviation above the mean age. Some possible causes of delayed puberty are: hypothalamic defects, pituitary defects or the gonads.
Objectives
Early diagnosis of the gonadal insufficiency; identification of the bone mass and the bone turnover at the pacients with delayed puberty; prophylaxis measures of the bone modification still in pre, puberal and postpuberal stage which lead to a maximal bone mass in correlation between sex and age.
Materials and methods
The study group includes 23 patients with age under 17–22 years with next forms of delayed puberty: Turner syndrome (8), gonadotropin deficiency (8), growth hormon deficiency with gonadal defects (5), nonsecreting pituitary tumors - the chromophobe adenoma (2). The diagnosis of osteoporosis was besed on BMD measurement using dual energy X-ray absorptiometry (DEXA). The cases were evaluated and diagnosed using the determination of levels seric of bone resorption represented by C-terminal telopeptide of tip I procollagen (CrossLaps) and as marker of bone formation represented by osteocalcine.
Results
Osteoporosis was found in 9 (T-score between -2.73 and -3.50), 7 presents osteopenia (T-score between -1.70 and -2.30) and 7 have normal BMD. The Crosslaps (1.054–2.1 ng/ml) and the calcitonina (47–149 ng/ml) were increased in osteoporosis and the results are comparative with postmenopausal women value, the pacients with osteopenia had identical results with premenopausal women value (osteocalcine 22.91–24.94 ng/ml, Crosslaps 0.179–0.250 ng/ml).
Conclusion
Early diagnosis of gonadic failure in order to stabilize/increase the bone mass and to reduce the fractures' incidents, osteoporosis/osteopenia therapy associates estroprogestative/androgenic substitution with specific means of the bone remineralization (biphosphonates, calcium formulas and vitamin D derivates)
Keywords: delayed puberty, BMD, osteocalcine, Crosslaps.

P399

Prevalence of primary hyperparatiroidism in treated and untreated breast cancer

Claudio Giani, Emilio Fiore, Elisa Giustarini, Ilaria Muller, Edda Vignali, Paola Fierabracci & Aldo Pinchera
Department of Endocrinology and Metabolism, University of Pisa, Pisa, Italy.

Hypercalcemia is a frequent metabolic disorder in metastatic breast cancer (BC). Aim of this study was to evaluate the incidence of hypercalcemia due to PHPT in BC patients. The study group included 271 consecutive BC, mean age±S.D. 57.7± 11.96 yrs. 100/271(36.9%) evaluated at different times after mastectomy (A) and 171(63.1%) before surgery (B), with no distant metastases. Age matched control group included 108 healthy women (Co) and 70 women with thyroid cancer(TC) before thyroidectomy. PTH and total serum calcium were measured in BC, Co and TC. The increment of serum calcium and PTH at the initial observation, indicated PHPT. Subjects with PHPT were selected for parathyroid surgery according to NIH consensus conference. PHPT was diagnosed in 12/271 BC(4.42%) and in none Co or TC. PHPT incidence in A was 7/100(7%). 2/7(28.6%) were submitted to adjuvant radiotherapy, 2/7(28.6%) to adjuvant chemotherapy two years before, and 4/7(57.1%) were on Tamoxifene therapy. A parathyroid adenoma was histologically confirmed in all 7 BC at surgery. The prevalence of PHPT in BC was significantly higher than in Co and TC ($P=0.005$, $P=0.004$ respectively). In the remaining 93 patients with no evidence of PHPT mean values of serum calcium (9.6±0.5 mg/dl) and PTH (38±16.4 pg/ml) were significantly greater than in both Co (PTH 27.9± 10.6 pg/ml, $P=0.0001$; calcium 9.3±0.5 mg/dl, $P=0.001$) and TC (PTH 26.2± 11.0 pg/ml, $P=0.003$; calcium 9.2±0.6 mg/dl, $P=0.001$). PHPT incidence in B was 5/171(2.92%), and in 2/5(40%) a parathyroid adenoma was histologically confirmed. In B mean serum PTH and calcium were similar to Co and TC. This study indicates an increased prevalence of PHPT in BC. The highest frequency of PHPT in A may be explained by the interferences of Tamoxifene or previous X-Ray adjuvant treatment on parathyroid cells activity. The significant increase of mean serum PTH and calcium levels in treated BC patients with no evidence of PHPT seems to confirm this hypothesis.

P400

Relationship between antropometric and metabolic parameters and parathyroid hormone levels in women

Ayse Sertkaya Cikim, Taner Bayraktaroglu, Adil Dogan Azezli & Yusuf Orhan
Istanbul University, Istanbul Faculty of Medicine, Endocrinology and Metabolism, Istanbul, Turkey.

Aim
Increased levels of fasting parathyroid hormone(PTH) have been hypothesized to influence increased levels of body fat mass. Preliminary studies show that serum PTH levels are higher in obese than in non-obese young adults and decline with weight loss. In the present study, it was aimed to evaluate relationship between antropometric and metabolic risk parameters and PTH levels in Turkish women.
Materials and methods
Analyses were performed on 710 Turkish women without hyperparathyroidism. They were enrolled to tertils of PTH levels (group I, <42 pg/ml; group II, 42–62 pg/ml; group III, >63 pg/ml and above) to the study. Body compositions, plasma lipids and lipoprotein levels, glucose homeostasis were determined and compared between groups.
Results
There were 227 patients in group I, 246 in group II and 237 in group III. Mean body mass index (BMI), body fat mass, waist circumferences were highest in group III, and increased with PTH. Mean PTH levels were significantly highest in patients having high BMI (48.6 ± 22.1 pg/ml in patients with <25 kg/m^2, 56.3 ± 35.1 pg/ml within 25–30 kg/m^2, 61.8 ± 30.3 pg/ml within 30–35 kg/m^2, 63.8 ± 29.9 pg/ml within 35–40 kg/m^2)($P < 0.05$). Mean values of total cholesterol, triglycerides, fasting glucose, insulin, HDL-cholesterol, LDL-cholesterol and HOMA were not different between groups ($P > 0.05$). Mean systolic and diastolic blood pressure in group II and III were significantly higher than group I ($P < 0.05$).
Conclusion
Preliminary studies suggest that PTH excess may promote weight gain by impeding catecholamine-induced lipolysis. Our data support a relationship between fasting serum PTH and fat mass in women. Fasting PTH is associated with increased fat mass and BMI.

P401

Serum calcium levels and metabolic disturbances in obese women

Taner Bayraktarogl, Ayse Sertkaya Cikim, Adil Dogan Azezli & Yusuf Orhan
Istanbul University, Istanbul Faculty of Medicine, Endocrinology and Metabolism, Istanbul, Turkey.

Aim
Serum calcium levels have a relation glucose homeostasis and weight management, and controversies in findings. This study carried out relationship between serum calcium levels and various metabolic parameters in obese Turkish Women.
Materials and methods
Subjects for this study were 3544 overweight or obese Turkish women with mean level of serum calcium 9.4 md/dl. According to mean calcium level, they divided group I having >9.4 mg/dl and group II having ≤ 9.4 mg/dl. Thereafter, we determined and compared body compositions (body mass index, abdominal fat mass), resting blood pressures, plasma lipids and lipoprotein levels, glucose homeostasis.
Results
A total of 715 (20.2%) patients were identified as overweight (BMI 25–30 kg/m^2) and 2831 (79.8%) were identified obese (BMI ≥ 30 kg/m^2). Mean fasting glucose, total cholesterol, triglycerides levels, systolic and diastolic blood pressures were significantly different in high calcium group ($n = 1710$, 48.2%) than low calcium group ($n = 1834$, 51.8%). Fasting glucose, total cholesterol,, HDL-cholesterol, triglycerides, insulin levels, HOMA values, systolic and diastolic blood pressures were positively correlated with calcium levels, not correlated with age, body mass index, waist and hip circumferences.
Conclusion
Our data showed that there was no relation between serum calcium levels and body fat distribution. Altough there was no effect on obesity, different metabolic parameters such as fasting glucose, total cholesterol, triglycerides levels and blood pressures were affected and correlated with serum calcium levels. It should be careful during a slimming program with included high calcium diet in obese or overweight women.

P402

Marginal periodontal pathology in patients with pituitary disorders

Ileana Duncea[1], Carmen Georgescu[1] & Ioana Duncea[2]
[1]Clinic of Endocrinology, University of Medicine and Pharmacy Cluj-Napoca, Cluj-Napoca, Romania; [2]Faculty of Dental Medicine, University of Medicine and Pharmacy Cluj-Napoca, Cluj-Napoca, Romania.

Periodontal changes are induced by hormonal alterations through soft tissue oedema, inflammation, demineralization or abnormal periosteal development of the alveolar bones.
Objectives
The purpose of the present work was to evaluate the frequency, severity and type of periodontal disease in subjects with previously diagnosed and non-treated pituitary disorders.
Patients and methods
Twenty-five subjects (21 women and 4 men) aged 44.7 years old were enrolled in the present study. Of the 25 patients, 10 suffered from clinical non-functional pituitary adenoma, 9 were diagnosed with acromegaly, 3 had a prolactinoma and in 3 a severe pituitary insufficiency was diagnosed. The endocrine disorder was diagnosed based on basal and dynamic hormonal tests, nuclear magnetic resonance image and visual champ evaluation. In addition, all patients were subjected to a thorough dental examination completed by orto-pan-tomography imaging. Following oral hygiene indices were calculated: the Green Vermillon index (OHIS), the Russel periodontal index (PI) and the OMS index of periodontal therapy request (CPITN).
Results
All subjects presented different forms of chronic periodontal pathology. Dystrophic periodontal disease was the most prevalent form, followed by superficial chronic periodontal disease. Severe periodontal disease including marked gingival retraction and periodontal pockets with purulent secretion was diagnosed in three patients. Patients had a mean OHIS of 3.54 suggesting an unsatisfactory oral hygiene; correspondingly, 12% of patients were advised to improve the oral hygiene, 72% needed professional dental care and antibacterial therapy. In 16%, surgical periodontal therapy was advised.
Conclusions
All 25 patients with pituitary diseases had periodontal pathology suggesting that this endocrine pathology may represent a risk factor for periodontal disease. Prevention and therapy of periodontal changes in these patients need careful oral personal hygiene and regular professional dental care.

P403

Tumor induced osteomalacia caused by haemangioma of the acetabular surface

Habib Bilen[1], Gungor Akcay[1], Pinar Polat[2], Mufide Akcay[3] & Omer Yildirim[4]
[1]Ataturk University, School of Medicine, Department of Internal Medicine, Division of Endocrinology, Erzurum, Turkey; [2]Ataturk University, School of Medicine, Department of Radiology, Erzurum, Turkey; [3]Ataturk University, School of Medicine, Department of General Surgery, Erzurum, Turkey; [4]Ataturk University, School of Medicine, Department of Orthopaedic Surgery, Erzurum, Turkey.

Objective
Tumor induced osteomalacia (TIO) belongs to the group of hypophosphatemic osteomalacias and is induced by a tumor. It is not common disease.
Case report
We describe a 34-year-old caucasian man who presented with a 5 year history of diffuse bone pain and muscle weakness. Laboratory investigations showed normal calcium level, low phosphate level between 1.01 mg/dl, and 1.6 mg/dl (reference range: 2.6–5.5 mg/dl), raised alkaline phosphatase of 238 and 885 IU/l (reference range: 30–120 IU/l) and high urinary phosphate level. Intact parathyroid hormone was within normal range (50.61 pg/ml; normal reference range 15–65 pg/ml). The plasma concentration of 1–25(OH) 2D was at the lower limit of the normal range (20 pg/ml; reference range 20–30 pg/ml). The tubular reabsorbtion of phosphate (TRP) was 65% (normal range 85–95%). Chest radiograph showed decreased bone mineralization and multiple fractured ribs. Conventional radiographs also showed fractures at the femoral necks bilaterally. There was no etiology of hypophosphatemia. The clinical, biological and radiological findings were compatible with osteomalacia, possibly related to the tumor. The patient was then further evaluated by magnetic resonance imaging (MRI), which showed marked intensity changes at the vertebrae corpuses due to osteoporosis, decreased signal intensities at the femoral necks due to fractures bilaterally, multiple transverse fractures at the proximal and distal metaphysis of tibias bilaterally. However this MRI images didn't show possible finding which associated tumor.

But on pelvic MRI, we detected a hypointense lesion at the superior and posterior surface of the acetabulum measuring 13 mm in diameter. An excision of the mass was performed and histological diagnosis of hemangioma was established. Upon removal of the tumor, laboratory data returned to a normal range within one month.

P404

Fracture risk in diabetic patients

Anna Gusova[1], Maria Pavlova[1], Aleksey Alekseenko[2], Galina Melnitchenko[1], Leonid Silin[1], Natalia Zaytseva[1] & Vyacheslav Pronin[1]
[1]Sechenov Moscow Medical Academy, Moscow, Russia; [2]Local trauma clinic N56, Moscow, Russia.

Diabetes mellitus (DM) is accompanied with a variety of metabolic changes in different systems including bone. In several previous studies it was shown that DM type 1 is associated with a decreased mineral density, whereas the data regarding DM type 2 are still controversial.

In the present study we examined the risk of different bone fractures in diabetic patients visiting local trauma clinic during one year (total area population 50.500). The incidence of fractures in general population was 1.9%, whereas in diabetic population it was twice higher (4.4%; $\chi^2=27.4$; $P<0.001$). Fracture of distal forearm was the most prevalent type of fractures in diabetic patients (32.5%), followed by fractures of the phalanges (27%), proximal humerus (15%) and tibial bone (12.5%). Fractures of distal forearm and humeral fractures were less prevalent in a general population (20.2% and 12.8%, correspondingly), compared to diabetic group, although the any statistical significant difference was found only for fracture of distal forearm ($\chi^2=2.8$; $0.05<P<0.10$). The incidence of fractures in other locations did not differ between two groups.

In conclusion, our data indicate that patients with diabetes have an increased total fracture risk, mainly due to higher incidence of the fractures of distal forearm. There is no difference in risk of fractures of other locations in diabetic patients compared to general population.

P405

Predictors of bone mineral density in women with primary hyperparathyroidism

Laura Gianotti, Francesco Tassone, Anna Pia, Paola Razzore, Flora Cesario, Giampaolo Magro & Giorgio Borretta
S. Croce & Carle Hospital, Division of Endocrinology & Metabolism, Cuneo, Italy.

Aim
Osteoporosis is common in postmenopausal women and in primary hyperparathyroidism (PHPT). PHPT is more prevalent in postmenopausal women. Aim of the present study was to investigate possible predictors of bone mineral density (BMD) in women with PHPT.
Methods
166 consecutive women with PHPT [age: 59.5±13.5 years; Asymptomatic/-Symptomatic: 84/82; premenopausal/postmenopausal: 31/135; BMI: 25.6±4.8 kg/m^2; PTH: 234.2±287.3 pg/ml; Calcium: 11.2±1.2 mg/dl] were studied. Serum levels of calcium, phosphate, intact parathyroid hormone (PTH), 25 hydroxy-vitamin D (25OHD3), creatinine and creatinine clearance (Ccr) were analyzed and bone densitometry was performed at lumbar spine, hip and forearm.
Results
In univariate analysis, age and menopausal status were negatively related with BMD and T-score at any site. BMI was positively associated with BMD and T-score at femur. PTH levels were negatively associated with T-score and BMD at forearm and lumbar spine, whereas ionized calcium at all the three sites. 25OHD3 was positively associated with BMD at lumbar spine and forearm. Ccr was positively associated with BMD and T-score at all the three sites. In multivariate regression analysis, menopausal status resulted an independent predictor of T-score at any site (forearm: ß=−0.31, $P<0.00001$; femur: ß=−0.21, $P<0.006$; lumbar: ß=−0.17, $P=0.025$), while PTH was an independent predictor of T-score at forearm (ß=−0.33, $P=0.010$) and lumbar spine (ß=−0.30, $P=0.037$). Ionized calcium also independently associated with forearm T-score (ß=−0.23, $P=0.0025$) while Ccr with T-score at forearm (ß=0.15, $P=0.035$, respectively) and femur (ß=0.24, $P=0.0016$).
Conclusions
In women with PHPT, menopausal status represents one of the most important predictors of bone mass. However, other factors related to the disease such as PTH, calcium levels or renal function, can each other influence independently bone mass, mainly at cortical level.

P406

Carotid intima media thickness and bone turnover in type 2 diabetic patients

Seda Sancak[1], Dilek Yavuz[1], Ahmet Toprak[2], Oguzhan Deyneli[1], Ozlem Tarcin[1], Dilek Yazici[1], Hasan Aydin[1] & Sema Akalin[1]
[1]Marmara University Medical School Section of Endocrinology and Metabolism, Istanbul, Turkey; [2]Marmara University Medical School Depatment of Internal Medicine, Istanbul, Turkey.

Recent studies indicate that atherosclerotic process is associated with bone metabolism. The aim of this study was to evaluate carotid intima media thickness (CIMT) and its association with bone mineral density and bone turnover markers as well as inflammation markers in type 2 diabetic male patients.
Material-method
184 type 2 diabetic males (56±8 y) and 85 non-diabetic control subjects (52±7 y) were recruited. Bone mineral density was measured by dual X-ray absorptiometry at lumbar spine and proximal femoral areas. Carotid intima media thickness was evaluated by Doppler ultrasound. Serum osteocalcin, CTX, intact parathyroid hormone (iPTH), hsCRP and HbA1c were measured.
Results are shown in table 1

	Diabetic subjects	Control subjects	*P*
Serum calcium (mg/dl)	9.91±0.4	9.8±05	0.03
Serum phosphorus (mg/dl)	3.59±0.57	3.39±0.52	0.02
Serum PTH (pg/mL)	49.7±24	53±22	Ns
Osteocalcin (ng/ml)	6.8±3.4	9.5±4	0.0001
CTX (ng/ml)	0.27±0.16	0.38±0.1	0.004
hsCRP (mg/L)	3.66±4	2.11±1	0.01
HbA1c (%)	7.02±1.7	5.1±0.32	0.0001
Fasting blood glucose (mg/dl)	151±65	92.62±7.9	0.0001
Density of Proximal Femur (gr/cm^2)	0.91±0.1	0.93±0.1	0.4
CIMT (mm)	0.70±0.16	0.61±0.23	0.008

There was a negative correlation between bone mineral content of femur neck and CIMT in diabetic patients ($r=-0$, 22 $P=0.008$).
Conclusion
Atherosclerosis and bone mineral density (BMD) may be related through similar or common pathophysiological mechanisms in type 2 diabetics. Low-grade inflammation may be one of the pathologic mechanisms that depressed bone turnover in diabetic patients.

P407

Primary hyperparathyroidism is associated with an increased risk of vertebral fracture assessed by morphometric x-ray absorptiometry

Giuseppe Viccica[1], Edda Vignali[1], Daniele Diacinti[2], Romano Del Fiacco[2], Tamara Giacomelli[1], Luisella Cianferotti[1], Filomena Cetani[1], Antonietta Picone[1], Chiara Banti[1], Aldo Pinchera[1] & Claudio Marcocci[1]
[1]University of Pisa, Pisa, Italy; [2]University La Sapienza, Roma, Italy.

Primary hyperparathyroidism (PHPT) is a frequent cause of secondary osteoporosis, but its role about the fracture is still controversial. We evaluated 157 consecutive postmenopausal patients with PHPT compared with two control subjects (C), each one matched for age and month-since-menopause (MSM). We measured ionized calcium (Ca^{2+}), parathyroid hormone (PTH), 25-OH-vitamin D (25-OH vit D), osteocalcin (OC), bone alkaline phosphatase (B-ALP) and serum and urinary cross-laps (S-CTX and U-CTX, respectively). Bone mineral density (BMD) were measured at spine (anterior-posterior, L1-L4) (BMD-V), femur (neck and total) (BMD-N and BMD-T, respectively) and radius (1/3 distal)

(BMD-R) by dual energy X-ray absorptiometry (DXA) technique using a QDR-4500 (Hologic, Inc., Bedford, MA, USA). We also acquired lateral scan of the spine from T_4 to L_4 using the DXA machine. Morphometric X-ray absorptiometry (MXA) was performed by a trained operator on the lateral DXA images, using the software supplied by the manufacturer. Reduction of anterior, middle or posterior vertebral height were classified as mild (20–25%), moderate (25–40%) or severe (>40%) vertebral fracture, according to visual semiquantitative Genant's method. No difference was found between PHPT and C groups in age, weight, height, body mass index (BMI) and MSM. The prevalence of vertebral fracture was higher in PHPT (26.7%, $n=42$) than C (5.4%; $n=17$) ($P<0.0001$) [odds ratio (OD) between PHPT and C was 6.38 (CI = 1.66–14.31)]. BMD-N and BMD-R of PHPT fractured patients were significantly lower than unfractured ones ($P<0.002$ and $P<0.0005$, respectively). In the PHPT group, no difference was found in Ca^{2+}, PTH, 25-OH vit D, OC, B-ALP, S-CTX and U-CTX between fractued and unfractured patients. In conclusion, BMD reduction observed in PHPT patients might account for the increased prevalence of vertebral fracture, but other factors may be involved in bone quality and fracture risk.

P408

Abstract unavailable

P409

Bone mineral density and bone markers in patients on long-term suppressive levothyroxine therapy for differentiated thyroid carcinoma

F. Mouton[1], M. D'Herbomez[2], C. Do Cao[1], F. Tison-Muchery[2], X. Marchandise[2] & J.-L. Wemeau[1]
[1]Clinique d'Enocrinologie Marc Linquette, CHRU Lille, Lille, France; [2]Service de Médecine Nuclaire, Hopital Roger Salengro, CHRU Lille, Lille, France.

Treatment with high doses of thyroid hormone was associated with higher risk of osteoporosis.

Aim of the study

To evaluate the predictive value of serum bone remodelling markers, and osteodensity in patients treated for differentiated thyroid carcinoma (DTC).

Methods

In a prospective longitudinal study, 156 determinations of osteocalcine (OC) as a marker of bone formation, and of C-telopeptide of type-1 collagene (CTX and ICTP) as markers of bone resorption were performed in 103 patients {20 men (median age 50 years), 83 females (median age 56 years – 58% with age >50 years)} treated with suppressive levothyroxine therapy for DTC. Bone mineral density (BMD) of the hip was measured by dual X-ray absorptiometry (DXA) and lateral DXA pictures of the lumbar and thoracic vertebrae were performed ($n=16$ for 13 patients). The mean of follow-up was 9 years (range 0.5–36 years).

Results

All OC results, except three, were in the normal range. Thirsty one ICTP and 36 CTX levels were increased (together 13% of the evaluations). A positive significant correlation was found between the ICTP concentrations and the duration of the follow up ($n=146$, $r=0.15$, $0.02<P<0.05$). BMD and resorption markers were concordant for 69% of the evaluations (7 with both normal, 4 with increased resorption markers and decreased BMD). For the discordant results, BMD were low in ostepenia for 4 patients with resorption markers in normal range, one isolated high ICTP concentration has been found.

Conclusion

1) Only the resorption markers are increased in patients on long term LT4 therapy for DTC 2) prevalence of high CTX and ITP is the same for men and females > 50 years (26%), lower (18%) for women <50 years 3) bone resorption markers could be used for screening patients at risk of osteopenia, when treated with suppressive levothyroxine therapy for DTC.

P410

Bone mineral density in end-stage chronic kidney disease patients

Natalia Karlovich[1], Tatiana Mokhort[1], Natalia Vasiljeva[2], Kirill Komissarov[3], Victor Gromyko[3] & Valery Pilotovich[3]
[1]Belarussian State Medical University, Minsk, Belarus; [2]State Rehabilitation Center, Minsk, Belarus; [3]State Nephrology Center, Minsk, Belarus.

The usefulness of bone mineral density (BMD) and bone turnover markers measurements to assess the renal osteodystrophy in patients with chronic kidney disease, stage 5 (CKD5) are not well determined.

The aim was to analyze BMD, serum levels of parathyroid hormone (PTH) and bone turnover markers in dialysis patients. We examined 45 patients (20 f,25 m; age 45.1 ± 10.8 yrs; age at dialysis onset 40.3 ± 12.3 yrs; dialysis duration 5.0 ± 4.0 yrs). BMD of the lumbar spine (LS), femoral neck (FN) were estimated by DEXA (Lunar). Serum PTH, osteocalcin (OC), C-terminal telopeptide of type I collagen (beta-CTx), alkaline phosphotase (ALP), calcium and phosphates were measured.

Median levels of PTH, OC, beta-CTx were significantly higher, than normal values (688.2 pg/ml; 321.7 pg/ml; 1.66 pg/ml, respectively). We found significant correlation of PTH level and age ($r=-0.51$), age at dialysis onset ($r=-0.57$), serum OC ($r=0.54$), beta-CTx ($r=0.72$) and ALP ($r=0.65$). Median BMD, T- and Z-scores in LS (1.15 g/sm^2; −0.40;0.07) and FN (0.94 g/sm^2; −0.62; −0.27) were normal. Osteopenia and osteoporosis were diagnosed in 20(44.4%) and 5 pts (11.1%), respectively. Comparison of subgroups with low and normal BMD didn't revealed significant differences in age, age at dialysis onset, dialysis duration, BMI, levels of PTH and bone turnover markers. CaxPO4-product was higher in patients with normal BMD 7.24 ± 1.98 vs 5.32 ± 1.73 in ones with low BMD ($P=0.025$). In LS Z-score correlated with PTH ($r=-0.48$; $P=0.011$), BMD – with CaxPO4-product ($r=0.51$; $P=0.038$). In FN we found significant correlation of BMD, Z-score and PTH ($r=-0.54$; −0.56); Z-score and age, age at dialysis onset ($r=0.34$;0.31) and serum Ca ($r=0.40$).

We can assume that low BMD is highly prevalent in CKD5 and associated with high PTH, younger age, and younger age at dialysis onset. Serum OC, beta-CTx, ALP positively correlates with PTH, but similar in patients with different BMD. High CaxPO4-product is well known as an important predictor of cardiovascular morbidity and mortality, but seems to preserve bone loss.

P411

Vitamin D receptor gene Bsm1 and Fok1 polymorphisms and indices of bone mass and bone turnover in healty young Turkish men and women

Dilek Gogas Yavuz[1], Meral Yuksel[2], Ozlem Tarcin[1], Dilek Yazici[1], Ahu Telli[3], Seda Sancak[1], Oguzhan Deyneli[1], Hasan Aydin[1], Goncagul Haklar[3] & Sema Akalin[1]
[1]Marmara University Medical School Section of Endocrinology and Metabolism, Istanbul, Turkey; [2]Marmara University Vocasionla school of health, Istanbul, Turkey; [3]Marmara University Medical School, Department of Biochemistry, Istanbul, Turkey.

Aim

Peak bone mass is a major determinant of osteoporosis risk in later life. It is under strong genetic control; In the present study, we investigated the relationship between polymorphisms in the gene encoding the vitamin D receptor (VDR) (FokI and Bsm 1) and bone mineral density (BMD), bone mineral content (BMC), and markers of bone turnover in 106 young Turkish women (19–23 yrs) and 100 man (19–23 yrs). Methods: BMD and BMC were measured by dual-energy X-ray absorptiometry (Lunar). Serum osteocalcin, C-teleopeptide (Ctx) and iPTH, calcium, phosphor and serum 25(OH)D levels were measured. Physical activity, dietary calcium and coffee consumption were assessed by questionnaire. Muscle strength was measured with hand dynamometer. PCR-RFLP methods were used for genotyping.

Results

Calcium, phosphor, PTH and 25(OH)D levels (43 ± 20 ng/ml) were in normal range not different between man and women. BMD (lomber and femur area), muscle strength, calcium intake (680 mg/d vs 571 mg/d in women $P=0.001$), serum osteocalcin and CTx levels were significantly higher in man compared to women.

Frequencies of FF, Ff and ff genotypes were 44.3%, 47.1% and 8.4% in women, and 41.8%, 52.3% and 4.6% in man. Frequencies for BB, Bb were 15%, 52% and 33% in women, and 9.3%, 60.4% and 30.2% in man. Frequency distribution for Bsm and Fok polimorfisms were not different between man and women. Femur BMC was significantly low in "bb" genotype in women ($P=0.01$). Femur and lomber (L1-4) BMC were low in "ff" genotype in women. Serum calcium levels were found to be lower in ff genotype in women. Bone turnover markers were similar among genotypes both man and women.

Conclusion

VDR gene may influence on attainment and maintenance of peak bone mass. "bb" and "ff" genotypes may have a effect on bone metabolism during accumulation of peak bone mass in women.

P412

Parathyroid sonography in patients with normocalcemic primary hyperparathyroidism

Avraham Ishay[1], Leonid Chervinsky[2] & Rafael Luboshitzky[1]
[1]Endocrine Institute, Haemek Medical Center, Afula, Israel; [2]Department of Radiology, Haemek medical Center, Afula, Israel.

Background
Primary hyperparathyroidism (PHPT) is nowadays an asymptomatic disease characterized by mild hypercalcemia and elevated parathormone (PTH) levels. A non-typical form of the disease distinguished by high PTH levels, normal serum calcium concentrations, and no evidence of secondary hyperparathyroidism was recently identified. The data about parathyroid imaging findings in the normocalcemic type of the disease are lacking. Ultrasonography (US) is the most convenient imaging modality for localization of parathyroid adenoma. The purpose of our study was to investigate whether normocalcemic patients harbor abnormal parathyroid glands on high-resolution ultrasonography.
Methods
We studied 14 patients (aged 53.2±10.3 years) with normocalcemic primary hyperparathyroidism. High-resolution ultrasonography was performed to locate parathyroid adenomas. Ten patients with positive sonography underwent a parathyroid ^{99}technetium sestamibi scintigraphy (MIBI).
The following variables were measured: serum total calcium, PTH, creatinine, phosphate, alkaline phosphatase, 25 hydroxyvitamin D and 1.25 dihydroxyvitamin D. A 24-hour urine collection was obtained for assessment of calcium and creatinine excretion rates. Corrected serum calcium level was used as an indirect assessment of ionized calcium.
The local Institutional Review Board approved the study, and all patients gave informed consent.
Results
All patients had high PTH levels (112±33.1 pg/ml), normal corrected serum calcium (9.6±0.3 mg/dl) and 25 hydroxyvitamin D (27.5±5.3 ng/ml) levels and normal creatinine clearance (97±18.6 ml/mn). Ten out of 14 patients (71%) exhibited a total of 12 single or double typical parathyroid adenomas on sonography. Sestamibi imaging correctly localized 8 of them.
Conclusion
The high prevalence of parathyroid adenomas on sonography indicates that normocalcemic primary hyperparathyroidism is characterized by the same morphologic derangement as the hypercalcemic form of the disease. Thus, NPHP is probably an early manifestation of PHPT.

P413

Diagnostic role of GNAS1 mutation screening in patients with pseudohypoparathyroidism

Miriam Ladsous[1], Virginie Vlaeminck-Guillem[1], Anne-Sophie Balavoine[1], Marie-Laure Kottler[2] & Jean-Louis Wémeau[1]
[1]Clinique Linquette-CHRU, Lille, France; [2]CHU Hôpital Clémenceau, Caen, France.

Pseudohypoparathyroidism (PHP) defines a group of disorders characterized by resistance to PTH. They are classified in type Ia, Ib, Ic and type II according to their clinical and biological characteristics. PHP-Ia is caused by heterozygous mutations in the *GNAS1* gene, encoding the alpha subunit of protein Gs. The aim of our study was to describe the diagnostic role of *GNAS1* mutation screening in a large group of patients, and to define the intrafamilial transmission pattern and parental imprinting profile.

Fourteen patients were studied. Eleven patients, from 5 unrelated families, had PHP-Ia, associating Albright's Hereditary Osteodystrophy (AHO), a decreased erythrocyte Gs-alpha protein activity, and other associated hormonal resistances. Two had PHP-Ib, with isolated PTH resistance, normal Gs-alpha activity and absent AHO. One patient had probable PHP-Ic, exhibiting AHO but normal Gs-alpha activity. *GNAS1* mutations were identified in all the patients with PHP-Ia. Six different mutations, not previously described, were observed. In 4 families, mutations were transmitted by mothers. In one family, the mutation was *de novo*. In one family, affected patients had 2 heterozygous *GNAS1* mutations, both located on the maternal allele. The 3 studied transmitting mothers had pseudopseudohypoparathyroidism, a condition associating AHO, decreased Gs-alpha activity but normal hormonal profile. We identified the familial *GNAS1* mutation in an asymptomatic boy whose father had typical PHP-Ia. Finally, isolated subcutaneous calcifications were identified in 2 related subjects who did not have the familial *GNAS1* mutation. We did not identify *GNAS1* mutations in PHP-Ib and PHP-Ic subjects.

In conclusion, our study confirms 1) the usefulness of *GNAS1* mutation screening in ascertaining PHP-Ia diagnostic, 2) the previously described maternal transmission of PHP-Ia, consistent with paternal imprinting of *GNAS1* gene, 3) the need for mutation screening in PHP-Ia related subjects to identify mutation carriers and provide an appropriate genetic counselling.

P414

CYP3A7*1C polymorphism, serum dehydroepiandrosterone sulphate level and bone mineral density in postmenopausal women

Gábor Speer, Krisztián Bácsi, János P. Kósa & Péter Lakatos
Semmelweis University 1st. Department of Medicine, Budapest, Hungary.

Objective
The CYP3A7 enzyme metabolizes some steroid hormones including dehydroepiandrosterone sulphate (DHEAS). Its expression silenced after birth. Previous study has shown that in case of CYP3A7*1C polymorphism, CYP3A7 enzyme activity persisted a higher level, resulting lower levels of DHEAS in men. The age-related decline of serum DHEAS levels is believed to contribute to osteoporosis. We hypothesized that CYP3A7*1C contribute bone loss through decreased level of serum DHEAS in postmenopausal women.
Patients and methods
319 postmenopausal women were admitted to study and divided into two subgroups: 217 women with osteoporosis and 102 aged-matched women, without osteoporosis. The CYP3A7*1C polymorphism was genotyped. Serum DHEAS levels and bone mineral density (BMD) were measured.
Results
Homozygous CYP3A7*1C carriers had significantly lower BMD at lumbar spine than that of wild type (T-score with CYP3A7*1C mutant type: −3.27±1.02, T-score with wild type: −1.35±1.53, $P=0.041$), after a correction of age and DHEAS levels. We did not find significant association between CYP3A7*1C variant and serum DHEAS level in postmenopausal women. Serum DHEAS levels correlated positively with BMD at both lumbar spine ($P<0.005$) and at femoral neck ($P<0.005$) in the whole study population.
Conclusion
We have shown the CYP3A7*1C may be associated with decreased bone mass at the lumbar spine independently of serum DHEAS concentrations. This finding and the lack of association between CYP3A7*1C polymorphism and serum DHEAS level in women support the hypothesis that this genetic variation might lead to reduced bone mass through other CYP3A7 hormonal substrates, than DHEAS.

P415

Evaluation of diastolic function and its relationship with carotis intima media thickness and endothelial function in asymtomatic hyperparathyroid patients

Dilek Yazici[1], Ozlem Tarcin[1], Beste Ozben[2], Ahmet Toprak[2], Oguzhan Deyneli[1], Seda Sancak[1], Dilek Yavuz[1] & Sema Akalin[1]
[1]Marmara University Medical School, Section of Endocrinology and Metabolism, Istanbul, Turkey; [2]Marmara University Medical School, Department of Cardiology, Istanbul, Turkey.

Symptomatic hyperparathyroid patients are under risk of increased cardiovascular mortality, associated with left ventricular hypertrophy, diastolic dysfunction and accelerated atherosclerosis. Data on asymptomatic hyperparathyroid patients are conflicting. This study aimed to determine diastolic dysfunction and its association with carotis intima media thickness (CIMT) and flow-mediated vasodilation (FMD) in a group of asymptomatic hyperparathyroid patients.

Twenty six patients with asymptomatic hyperparathyroidism (HP) (23.4±3.9 y; F/M:17/9) and 25 healthy controls (24.4±3.6 y; K/E:18/7) were recruited. Left ventricular mass index (LVMI), isovolumetric relaxation time (IVRT), early (E) and late (A) atrial peak filling velocity were measured by conventional and Doppler echocardiography. Tissue Doppler imaging, a method with better results in determining diastolic dysfunction, was used to determine mitral annular early (E') and late (A') peak diastolic filling rate. FMD and CIMT were determined by Doppler echocardiography. The study was approved by the local Ethical Committee.

	ASYMTOMATIC HP ($n=26$)	CONTROL ($n=25$)	P
Calcium (mg/dL)	9.72±0.41	9.69±0.76	NS
Phophorus (mg/dL)	3.98±0.66	3.90±0.40	NS
PTH (pg/mL)	91.53±24.50	42.98±10.69	$P<0.0001$
FMD (%)	9.62±3.74	9.52±3.13	NS
CIMT (mm)	0.46±0.05	0.47±0.04	NS

LVED, LVMI, IVRT, E/A, E'/A' and E/E' ratios were comparable between groups. PTH was weakly correlated with CIMT ($r=-0.26$; $P=0.23$), but not with echocardiographic parameters and FMD.

Diastolic dysfunction was not observed in asymptomatic hyperparathyroid patients. It is evident from this preliminary data that cardiac manifestations do not start at this stage of disease, but further studies with larger groups are needed to confirm this finding.

P416

Cinacalcet (Mimpara®, Amgen) is more effective than bisphosphonates at controlling hypercalcemia in patients with parathyroid carcinoma: a case study

Waldemar Misiorowski
Endocrinology Clinic, Medical Centre for Postgraduate Education, Warsaw, Poland.

Parathyroid carcinoma is an uncommon cause of PTH-dependent hypercalcemia. The clinical features of parathyroid carcinoma are due primarily to the effects of excessive secretion of PTH. Thus, signs and symptoms of hypercalcemia often dominate the clinical picture. The therapeutic goal at this point is to control the hypercalcemia. We describe two cases of parathyroid carcinoma, effectively treated with calcimimetic Cinacalcet (Mimpara®), the first of a new class of compounds with activity at the calcium-sensing receptor: 55-years old women with parathyroid carcinoma, and with persistent hypercalcemia after four consecutive surgical attempts with wide excision of the involved area, and 53–yr-old man presented with diffuse lytic changes in the bones and a tumor in mediastinum (eventually diagnosed as parathyroid carcinoma). In both cases severe hypercalcemia (ranged 15–17 mg/dL) and high levels of intact PTH (1176 pg/mL and 546 pg/mL respectively) had been found. Symptomatic treatment: hydratation with iv sodium chloride and iv pamidronate and zolendronante had been installed, however without effects, and eventually cinacalcet, 60–90 mg/day, orally, has been used to treat. After first week of the treatment, in both cases calcium and PTH significantly decreased (to 10.8–11.3 mg/dL and 332–113 pg/mL respectively).Cinacalcet appears to have been more effective at controlling hypercalcemia than bisphosphonates in patients with parathyroid carcinoma.

P417

Is there any relationship between the BsmI polymorphism in the vitamin D receptor gene and the occurrence of glucocorticoid-induced osteoporosis in asthmatic patients with long-term glucocorticoid treatment?

Urszula Tworowska[1], Bozena Bidzinska-Speichert[1], Andrzej Obojski[2], Marek Demissie[1], Ryszard Slezak[3], Agnieszka Lenarcik[1] & Andrzej Milewicz[1]
[1]Departament of Endocrinology and Diabetology, Medical University of Wroclaw, Wroclaw, Poland; [2]Departament of Internal Medicine and Allergology, Medical University of Wroclaw, Wroclaw, Poland; [3]Departament of Genetics, Medical University of Wroclaw, Wroclaw, Poland.

Introduction
Results of many studies indicate that BsmI polymorphism in VDR gene may influence bone tissue metabolism and may be useful in identifying patients at greater risk of glucocorticoid-induced osteoporosis.
Aim of the study
To determine frequency of polymorphic variants of VDR gene (BsmI) and its relationship to phenotypic features characterizing bone status (BMD and metabolic bone turnover).
Material and methods
Following groups were studied: 1. asthmatic patients – no 85; divided into the subgroups: group OS – 38 patients treated with oral steroids: 27 women and 11 men (47.8 ± 10.7 years, 74 ± 13.8 kg), group IS – 34 patients treated with inhaled steroids: 29 women and 5 men (45.4 ± 11.0 years, 73.7 ± 13.9 kg), group NS – 13 patients treated with other drugs than glucocorticoids: 9 women and 4 men (38.8 ± 15.1 years, 66.7 ± 17.9 kg), 2. control group – 31 healthy volunteers, 17 women and 14 men (39.8 ± 9.8 years, 75.1 ± 16.1 kg). Serum levels of PTH, VD3, osteocalcin, ICTP, Ca and phosphates were measured. VDR gene genotypes were determined using PCR-RFLP method. BMD was measured using DXA method.
Results
Genotype bb was found in 34.3%, BB in 8.8%, and bB in 56.9%. Allelic frequency for allele B was 37.2%, and b – 62.8%. There were no significant differences regarding BMD, biochemical and hormonal parameters between any of genotypes.
Conclusions
The data suggest that the VDR genotypes do not seem to be useful for identifying patients at greater risk of glucocorticoid-induced osteoporosis, however it awaits to be confirmed by a population-based study.

P418

The relationship between the increased body mass index and the bone fracture prevalence in postmenopausal pollen allergic women

Viktoria Ferencz[1], Szilvia Meszaros[1], Emoke Csupor[2], Edit Toth[3], Katalin Bors[4], Andras Falus[5] & Csaba Horvath[1]
[1]1st Department of Internal Medicine, Faculty of Medicine, Semmelweis University, Budapest, Hungary; [2]The Health Service, Budavar Local Authorities, Budapest, Hungary; [3]Department of Reumatology, Flor Ferenc Country Hospital, Kerepestarcsa, Hungary; [4]The Health Sevice, Ferencvaros Local Authorities, Budapest, Hungary; [5]Department of Genetics, Cell- and Immunobiology, Faculty of Medicine, Semmelweis University, Budapest, Hungary.

Our aim was to investigate whether pollen-allergy can affect fractures in postmenopausal women. A total of 125 postmenopausal pollen-allergic women (mean age 61.26 yr) were split into four groups: treated neither with H1 histamine receptor (H1R) antagonist nor with inhaled corticosteroid ($n=43$), treated only with H1R antagonist ($n=53$), treated both with H1R antagonist and inhaled corticosteroid ($n=17$), treated only with inhaled corticosteroid ($n=12$) for at least 5 years, seasonally. One hundred non-allergic postmenopausal subjects matched for age, body mass index (BMI) and age at menopause served as controls. Overweight and obesity ($25\ kg/m^2 \leq BMI$) were common among allergic women (76%). Untreated allergic had almost triple the rate of prevalent low-energy fractures (distal forearm, hip and clinical vertebral fractures: 34.9%) compared to non-allergic women (13%, chi^2 $P=0.003$). Bone fracture occurred more often in H1R-only treated patients (30.19%) than in controls (chi^2 $P=0.01$), however, clinical vertebral or hip fractures developed neither in those treated only with H1R antagonist nor in those who received both H1R antagonist and inhaled corticosteroid. Bone fractures were more frequent among patients with inhaled steroid treatment than among patients with a combined treatment of inhaled steroid and antihistamine (50% vs. 29.4%). BMI predicted prevalent fractures at 1.278 (95% CI, 1.047 to 1.559, $P=0.016$) for $1\ kg/m^2$ increase among untreated allergic patients. In conclusion we found a high prevalence of low-energy fractures among pollen-allergic postmenopausal women, which was associated with obesity. It is possible that the H1R antagonists compensate for the negative effect of pollen-allergy and the adverse effect of inhaled corticosteroid treatment on bone fracture risk.

P419

Decreased bone resorption in H1 histamine receptor antagonist treated allergic children

Szilvia Meszaros[1], Viktoria Ferencz[1], Agnes Bojszko[2], Eva Pallinger[3], Andras Falus[3], Eva Hosszu[4] & Csaba Horvath[1]
[1]1st Department of Internal Medicine, Faculty of Medicine, Semmelweis University, Budapest, Hungary; [2]Heim Pal Paediatric Hospital, Budapest, Hungary; [3]Department of Genetics, Cell- and Immunobiology, Faculty of Medicine, Semmelweis University, Budapest, Hungary; [4]2nd Department of Paediatric, Faculty of Medicine, Semmelweis University, Budapest, Hungary.

Histamine receptor antagonists seem to have effect on bone metabolism according to previous studies. We investigated the bone turnover in allergic children who were treated with H1 hisztamin receptor (H1R) antagonists.

The biochemical bone turnover markers [β-CrossLapps (β-CTx), osteocalcin (OCN), β-CTx/OCN ratio], parathyroid hormone (PTH) and the 25-OH-vitamin D_3 were determined in 37 H1R antagonist treated multiplex allergic children and

in 21 age and gender matched healthy children. The intracytoplasmatic histidine decarboxylase (HDC), hisztamin, and surface H1 and H2 receptors expression were assessed by flow cytometry on peripheral leukocytes. The distribution of lymphocyte subpopulation were also determined.

The serum OCN, PTH and 25-OH-vitamin D_3 levels did not differ between the healthy and the allergic groups. However, the β-CTx was lower in the H1R antagonists treated allergic children (1090.82 ± 80.25 pg/ml) in comparison with controls (1456.58 ± 95.81 pg/ml; $P=0.006$). The β-CTx/OCN ratio was found to be lower in the H1R antagonists treated allergic patients than in the controls (9.24 ± 0.608 vs 12.65 ± 0.53; $P=0.001$). β-CTx serum level correlated with OCN in the controls ($r=0.845$, $P<0.001$) and in the H1R antagonist treated allergic, too ($r=0.519$, $P=0.005$). Higher HDC expression and H1 receptor down regulation was found in allergic children. The CD3+/CD16-56+T cells were in higher rate in children of control group.

Decreased bone resorption was found among H1 receptor antagonist treated allergic children, which is indicated by serum markers. Therefore, bone turnover is shifted toward bone formation in the H1R antagonist treated allergic subjects.

P420

Changes of bone metabolism at the onset of puberty

Violetta Csákváry[1], Erzsébet Toldy[2], Tamás Puskás[3], Gábor L. Kovács[4] & György Oroszlán[1]
[1]Markusovszky Teaching Hospital, Children's Department, Szombathely, Hungary; [2]Department of Practical Diagnostics of Institute of Diagnostics and Management, University of Pécs, Szombathely, Hungary; [3]Markusovszky Teaching Hospital, Radiological Department, Szombathely, Hungary; [4]Institute of Laboratory Medicine, University of Pécs, Pécs, Hungary.

Object
Adolescence is the period during which the greatest accrual of bone mineral occurs. During puberty, changes of bone metabolism primarily depend on maturity. Diagnosis and therapy of childhood bone diseases is difficult due to the lack of reference ranges of metabolic bone markers. Our aim was to establish the reference values of bone markers in primary school students (mean age: 13.2 ± 1.2 years; 65 girls, 56 boys).
Methods
The children were divided into two groups: prepubertal (boys:22, girls:38) and pubertal (boys:34, girls:27). This classification was based on the Tanner stage and levels of serum sexual steroids (testosteron, estradiol). Physical activity, dietary habits, calcium intake, consumption of soft drinks and body mass index (BMI) was established. Bone mineral density (BMD), bone mineral content (BMC), vertebral Z-score (DEXA Medical Systems Prodigy), and serum biochemical markers (osteocalcin: OC; beta-crosslaps: βCL; procollagen type I N-terminal propeptid: P1NP) were measured by an electrochemiluminescence immunoassay system (ECLIA, Elecsys 2010, Roche). The data were analysed in terms of sexual maturation by one way ANOVA.
Results
The Tanner stage (3.14 ± 0.78) and BMD (0.99 ± 0.14) values of girls were significantly higher than those in boys (Tanner stage: 2.75 ± 0.61, BMD:0.87 ± 0.12). A significant ($P<0.001$) positive correlation ($r=0.4$–0.5) was observed between the Tanner stage and the parameters of mineral density (BMD, BMC, Z-score). Significantly ($P<0.001$) higher OC (190 ± 66 vs. 139 ± 61 ng/ml) P1NP (838 ± 280 vs 569 ± 360 ng/ml), βCL (2.03 ± 0.65 vs. 1.50 ± 0.60 ng/ml) values were measured in boys than in girls. Boys not consuming soft-drinks regularly exhibited significantly higher ($P<0.05$) prepubertal Z-score values ($+0.28 \pm 0.77$) that regular soft-drink-consumer boys (-0.72 ± 1.02). iPTH levels in soft drink-consuming prepubertal girls (47.7 ± 13.6) were significantly higher ($P<0,01$) than in the non-consuming prepubertal girls (32.8 ± 9.4 ng/ml).
Conclusion
The results call the attention on the significance of appropriate reference ranges. It is advisable that boys and girls are evaluated separately with the sexual maturity taken into consideration. The assessment of dietary habits strongly suggests insufficient spontaneous calcium intake among children.

P421

Hypophosphatemic rickets and mutations in the PHEX gene

Frédérique Faivre-Defrance, Benoit Soudan, Sophie Marcelli-Tourvieille, Jean-Louis Wémeau & Marie-Christine Vantyghem
Lille University Hospital, Lille, France.

Hypophosphatemic rickets is associated with mutations in the PHEX gene leaving phosphaturic peptides such as FGF23 uncleaved, enabling them to exert their phosphaturic potential in the kidney. Other forms are caused by mutations in the proteolytic processing site of FGF23 itself, while in tumour-induced Hp, overproduction of FGF23 causes the processing capacity to be exceeded, resulting in phosphaturic Hp. The aim of this work was to assess blood FGF23 levels in Hp patients and in normophosphatemic controls. Methods: 17 patients suffering from chronic Hp without HPT were compared to an age-matched control group of 18 patients. Blood levels of calcium, phosphate, PTH, 25-OH vitamin D (Nichols Diagnostics) and FGF 23 (ELISA Immunotopics) were determined. Results: FGF23 levels were higher in Hp: 46.3 ± 614.9 vs. controls: 20.3 ± 1.6 pg/ml, $P<0.05$, in regard of phosphate levels of 20.2 ± 0.7 (Hp) vs 34.5 ± 1.2 mg/l (controls). Vitamin D and calcium levels were normal and similar in both groups. PTH levels were higher in Hp: 70.5 ± 15.5 vs. controls: 31.5 ± 2.6 pg/l; $P<0.05$. FGF 23 correlated neither to phosphate nor vitamin D, nor calcium levels in the whole population and in Hp and control groups.
Conclusion
The lack of correlation between FGF23 levels and Hp suggests an heterogeneity of hypophosphatemic patients despite their higher FGF23 levels than controls. New genes regulating FGF 23 such as the recently discovered DMP1 could explain this heterogeneity (Nat Genet 2006 Nov).

P422

Changes of serum bone marker concentrations after effective therapy of patients with Cushing's syndrome

Miklós Tóth[1], Judit Toke[1], Dóra Lippai[1], Márta Sereg[1], Ágnes Szappanos[1], László Füto[2], Ibolya Varga[1], Nikolette Szücs[1], Róbert Kiss[1], Edit Gláz[1] & Károly Rácz[1]
[1]Semmelweis University, 2nd Department of Medicine, Budapest, Hungary; [2]Makhot Ferenc Hospital, Department of Medicine, Eger, Hungary.

Introduction
The most important feature of bone metabolism in patients with Cushing's syndrome is the uncoupling of osteoblast and osteoclast activity resulting in suppressed bone formation.
Objective
The aim of the present study was to investigate the altered bone turnover in patients with various forms of Cushing's syndrome in the active phase of the disease as well as after successful normalization of cortisol overproduction.
Patients and methods
This retrospective study included 63 patients with Cushing's syndrome (38 Cushing's disease, 6 ectopic ACTH syndrome, 19 ACTH-independent adrenal disease).The patients were monitored over a period of up to 48 months after treatment or up to the recurrence of their disease. Patients with known metabolic bone disease, or with medication affecting bone mineral content were excluded. 148 blood samples were evaluated (67 samples from active and 81 samples from inactive phase of Cushing's syndrome).Serum osteocalcin (OC) and type I collagen breakdown products (beta-CrossLaps, β-CL) were measured with standard test kits. SPSS v13.0 software package was used for statistical analysis.
Results
OC concentration which was suppressed in the active phase of the disease (mean, 12.1 ± 8.0 ng/ml) increased to 38.0 ± 26.0 ng/ml within the first month after the effective therapy, reached the maxiumum level after 6 months (52.3 ± 33.6 ng/ml) and became normal after the second year. There were no significant changes in β-CL concentrations. Using ROC analysis, 17.2 ng/ml serum OC concentration was found as the best cutoff value in differentiating between active and inactive phase of bone disease related to Cushing's syndrome. The sensitivity and specificity of OC at this concentration were 87.1% and 82.1%, respectively.
Conclusion
Our results indicate that the suppressed serum OC concentration increases rapidly and elevates above the normal range after treatment of Cushing's syndrome. Markers of bone turnover are normal after the second year of the cure of Cushing's syndrome.

P423

Percutaneous ethanol injection therapy in patients with primary and secondary hyperparathyroidism

Spyros Stratigis, Kostas Stylianou, Evangelia Mamalaki, Eleftheria vardaki, Nikolaos Tzenakis, Eirini Katsipi, Paraskevi Tzoutzouraki & Eugenios Dafnis
University Hospital of Crete, Iraklio, Crete, Greece.

Introduction
Recent years PEIT has been introduced as an alternative to parathyroidectomy. We evaluated the results of PEIT in patients with primary (pHPT) or secondary hyperparathyroidism (sHPT).
Patients and methods
18 patients (6M/12F) with pHPT and 20 patients (7M/13F) with sHPT underwent PEIT between 2001 and 2005 and had a mean follow up of 24.3±9 and 27±10 months respectively. The PTGs were identified and blood supply to the gland was examined by power Doppler ultrasonography pre and post infusion. 95% ethanol was injected at a volume 85% of the PTG volume. pHTP patients underwent a total of 51 ethanol infusions. sHPT patients underwent a total of 76 infusions in 30 adenomas The volume of the PTGs, serum iPTH, calcium, phosphate, albumin and alkaline phosphatase were measured at the beginning and after each infusion. The patients were divided to responders and non responders based on the normalization of iPTH levels at.
Results
In the pHPT group, 11 patients (61.1%) normalized iPTH levels, 5 (27.8%) had a significant (>50%) reduction of iPTH levels and 2 (11.1%) had a modest response (<50% reduction of PTH) and were referred for surgery. In all patients calcium levels decreased significantly (10.96±0.84 mg/dl to 9.81±0.6)($P<0.001$). Phosphorus increased from 2.52±0.38 mg/dl to 2.96±0.5 mg/dl ($P=0.05$).
In the sHPT group PTH decreased significantly (1280.9±447 pg/ml to 770.5±465, $P<0.001$) in all patients; however it was normal in only 3 patients (15%). Phosphorus decreased from 5.57±0.47 mg/dl to 4.93±0.42 mg/dl ($P=0.03$).
Conclusions
PEIT is a safe and easy to perform technique for the treatment of HPT. In patients with pHPT may be a considerable alternative to surgical PTx with a curative rate of 61% in our series. In patients with sHPT appears a significant adjunct to medical therapy since it reduced iPTH levels by 42%.

P424

Juvenile osteoporosis in untreated GH-deficient patient – is treatment with GH replacement indicated? A clinical case report

Catarina Coelho, Catarina Saraiva, Isabel Manita, Andreia Velosa, Dolores Passos, Maria Carlos, Luisa Raimundo & Jorge Portugal
Hospital Garcia De Orta EPE, Almada, Portugal.

Treatment for osteoporosis in children/adolescents is extremely important not only to improve bone quality but also because, if left untreated, could lead to severe and precocious loss of bone mass. Studies in growth hormone (GH) deficient adults, in turn, have shown that treatment with GH produce bone mass gain and improve the occurrence of both bone formation and reabsorption.

The authors present the following case report of a 16 year old Caucasian female with congenital blindness. Suspected of having short stature at the age of 14 she was referred to the endocrinology department for further examination. Eutocic delivery at the gestational age of 39 weaks, A.I 8/9, W=2850 gr, L=47 cm; PC=35 cm. Breast-fead during the first 3 months. Food diversification from the 4th month, without intolerance. Growth retardation detected at the age of 2 (−3 sds) and delayed psycho-motor development. Puberty arousal at 12, with menarche at 14. oligoamenorrhea since then. Physical examination: bilateral blindness, W=25.7 Kg (−3 sds), H=128 cm (−3 sds); BMI- 15.6 Kg/m2. Bone age exam showed closed cartilage. Laboratory findings revealed: IGF1<20 ng/mL (163–972); GH< 0.1 ng/mL, TSH 4.3 mU/L (0.1–4.0); PRL 9.8; urine density - 1014; CRH test - basal/pick - ACTH 16.6/51 pg/mL and cortisol 10.6/22.8 ug/dL; LHRH test – basal/pick – LH 9.8/89 UI/L and FSH 9.2/20.4 UI/L). The MRI showed hypophysis and pituitary stalk hypoplasia with ectopic location of the posterior lobe; along with bone malformation of the cranial – vertebral gynglymus. The osteodensitometry of the lombar spine revealed severe osteoporosis (Z score of −4.3). Etthynylestradiol 15 mcg/ gestodene 60 mcg and alendronate 70 mg/weekly were started. Reevaluation of bone density after one year showed stable density. Although in Portugal GH treatment is not available for use in adults we ask…

Should this patient be further considered for GH treatment?

P425

Biomarkers of hypercoagulability and inflammation in primary hyperparathyroidism

Elena Chertok- Shacham[1], Avraham Ishay[1], Idit Lavi[2], Naomi Lanir[3] & Rafael Luboshitzky[1]
[1]Endocrine Institute, Haemek Medical Center, Afula, Israel; [2]Department of Epidemiology and Community Medicine, Carmel Medical Center, Haifa, Israel; [3]Department of Hematology, Hematology Laboratory, Rambam Medical Center, Haifa, Israel.

Background
The association between primary hyperparathyroidism (PHPT) and cardiovascular disease (CVD) morbidity and mortality is well known in symptomatic PHPT patients. Atherosclerosis is considered nowadays as an inflammatory process. Elevated serum levels of acute phase proteins, C-reactive protein (CRP) and the proinflammatory cytokines tumor necrosis factor alpha (TNF α), Interleukin-6 (IL-6), as well as insulin resistance, indicating chronic subclinical inflammation, have been associated with cardiovascular disease. The aim of this study was to evaluate CVD- related biomarkers of hypercoagulability and inflammation in PHPT patients.
Methods
Thirty-five PHPT patients (aged 57.5±10.8 years) without known CVD were evaluated. Results were compared with those obtained in 25 weight and gender matched controls of similar age. According to disease severity, patients were subdivided into two groups, symptomatic and asymptomatic hyperparathyroidism (SPHP and APHP, respectively).Local Helsinki committee approved the study, and all participants gave their informed written consent. Plasma levels of plasminogen activator inhibitor 1 (PAI-1), fibrinogen, d-dimers, interleukin 6 (IL-6), C-reactive protein (CRP), white blood cells (WBC) were determined in all participants.
Results
PAI-1 was significantly higher in symptomatic PHPT patients (41.4 mg/ml±20) versus APHP and control groups (25.0±12.8 and 32.5 mg/ml±13.0, respectively; $P=0.009$). Levels of fibrinogen, d-dimers, IL-6, CRP and leukocytes were similar in PHPT and controls. Across all subjects PAI-1 was significantly correlated with PTH levels ($r=8.44$; $P=0.005$). After multivariate regression analysis, a significant correlation between Il-6 and PTH was maintained ($r=0.43$, $P=0.008$). No significant correlations were found between PTH or calcium levels and values of fibrinogen, d-dimers, CRP, leukocytes.
Conclusions
Our results suggest that PAI-1 as a marker of hypercoagulability is increased in symptomatic PHPT patients. Elevated plasma levels of PAI-1 in PHPT and the significant correlation with PTH levels, suggest that hypercoagulability mechanisms may be operating in the development of CVD in these patients.

P426

Dehydroepiandrosterone and bone mineral density in elderly women

Lucyna Papierska, Michal Rabijewski, Agnieszka Wiktorowicz-Dudek & Wojciech Zgliczynski
Center For Postgraduate Midical Education, Warsaw, Poland.

Dehydroepiandrosterone (DHEA) and its sulphate (DHEAS) are weak adrenal androgens, which may exert anabolic effect on bone tissue.

We have measured serum DHEAS levels and bone mineral density in lumbar spine and femoral neck in 131 healthy, agile, postmenopausal women aged 59–89. There were no diseases which aggravate bone loss and no hormonal replacement therapy in medical history of participants.

Women were divided into groups:

1 according to DHEAS concentrations:
a. With extremely low (<500 ng/ml) versus
b. moderate-low (>500 ng/ml) serum DHEAS concentrations
2. according to BMD:
a. "Low lumbar spine BMD", with T-score L2/L4 <−2 versus
b. T-score L2/L4 >−2.0

In 76 women with very low serum DHEAS (DHEAS = 258 ± 89 ng/ml) femoral neck BMD was significantly lower than in 55 women with moderate-low serum DHEAS (T-score = −1.15 ± 0.51 vs. −0,89 ± 0,6 $P < 0.05$). There was no significant difference in L2/L4 BMD (T-score = −0.68 ± 1.17 vs. T-score = −0.45 ± 1.38 ns).

In 30 women with low lumbar BMD (Tscore = −2.71 ± 0.44) serum DHEAS was significantly lower than in other women (432 ± 89 ng/ml vs. 498 ± 92 ng/ml $P < 0.05$).

There was also significant difference between femoral neck BMD in these groups (T-score = −1.56 ± 0.61 vs. T-score = 0.84 ± 0.69 $P < 0.05$)

We have concluded, that women with low DHEAS concentrations have lower femoral neck BMD and women with low lumbar and femoral neck BMD have lower DHEAS concentrations. These findings confirm possible role of adrenal androgens in maintenance bone mass in elderly women.

P427

Bone mineral density and calcium deficiencies in adult patients with celiac disease

Jadwiga Szymczak[1], Anna Bohdanowicz-Pawlak[1], Ewa Waszczuk[2] & Andrzej Milewicz[1]
[1]Wroclaw Medical University, Department of Endocrinology and Diabetology, Wroclaw, Poland; [2]Wroclaw Medical University, Department of Gastroenterology and Hepatology, Wroclaw, Poland.

Background/Aims
Calcium and vitamin D malabsorption in celiac disease predispose to skeletal demineralization. The aim of this study was to evaluate the prevalence of bone mineral density (BMD) and calcium deficiencies in adult patients with CD and assess whether a gluten-free diet is sufficiently effective for BMD restoration or whether calcium and vitamin D should be applied.
Methods
BMD and biochemical parameters of bone and mineral metabolism were measured in 35 adult CD patients receiving (19) or not receiving (16) a gluten-free diet (GFD) and in 36 controls. Then the CD patients were treated with a GFD and calcium (1.0 g/day) plus alfacalcidol (0.25–1 μg/day) for one year.
Results
Reduced BMD was diagnosed in 57–77% of the patients. Mean calcemia, calciuria, and 25(OH) Vitamin D were lower, but serum PTH and bone-turnover markers (ALP, osteocalcin, ICTP) were significantly higher in CD patients than in controls. In the patients on the diet (GFD(+)), BMD was higher than in GFD(−) patients, but lower than in controls. Biochemical parameters were normal in GFD(+) patients except for diminished calciuria. Mean BMD after one year of treatment significantly increased ($P < 0.05$), mostly in the lumbar spine (mean: 7.3%), but decreased in five patients who did not strictly adhere to the GFD.
Conclusions
Impaired calcium and vitamin D intestinal absorption and low BMD are very common in adult CD patients. Gluten avoidance increased BMD, although the values still remained markedly lower in several patients. Because of chronic calcium deficiency despite GFD, we propose calcium and vitamin D supplementation in most adult CD patients.
The Local Ethical Committee approved the study.

P428

Implication of magnesium in calcium metabolism – a case report

Carmen Moldovanu, Carmen Vulpoi, Roxana Dinu, Elena Livadariu, Lidia Andriescu & Eusebie Zbranca
University of Medicine and Pharmacy "Gr.T.Popa", Iasi, Romania.

Magnesium (Mg), the second most abundant intracellular cation of the human body, plays a crucial role in nerve and muscle function. Although a frequent electrolyte abnormality, hypomagnesaemia is one of the most underdiagnosed one, symptoms being present only when Mg levels decrease bellow 0.5 mmol/l, Among the various causes of Mg deficiency endocrine disorders are neither the most frequent nor the most studied. An exception is the implication of Mg in bone and calcium metabolism. Mg deficiency can interfere with the recovery after parathyroidectomy, or from vitamin D deficiency. We present the evolution of postsurgical parathyroidism in the case of a 43 years old woman who has suffered near-total thyroidectomy for Graves' disease. She developed overt signs of tetany, with very low calcium values (1.6 mmol/l) and hyperphosphoremia (2.3 mmol/l). She received high calcium doses (3–4 g/day) associated with vitamin D but the improvement was only temporary and Ca values remained low. Although Mg values were only to the inferior limit of the normal (0.65 mmol/l) we have associated oral sustained preparations (300 mg of mg/day). The Mg supplementation helped to improve patient's state, biologically (Ca = 2.10 g/l) and clinically. The etiology of hypocalcaemia in the setting of hypomagnesaemia is multi-factorial. Hypomagnesaemia has a suppressive effect on PTH secretion and induces PTH resistance by interfering with G protein activation, but in the case of PTH deficiency, the main feature seems to be vitamin D resistance. The correlation between low Mg and low vitamin D levels is not clearly established. Since our patient associated osteoporosis (T score −3.6), dietary calcium supplementation is also necessary to improve bone turnover. Although calcium remains the star of bone remodeling, Mg have also an important contribution. Concomitant Mg intake will prevent the Ca/Mg imbalance and improve bone mineralization.

P429

Abstract unavailable

Clinical case reports – presented on Monday

P430

The effect of surgical cure of acromegaly on glycemic control in an elderly female patient suffering from type 2 diabetes – a case report

Koviljka Milenkovic Vulovic & Snezana Polovina
General Hospital, Cacak, United States.

Introduction
Insulin resistance occurs in 80% of patients with acromegaly. This report illustrates the case of a female patient with unsatisfactory control of type 2 diabetes and a beneficial effect on glycaemic control after the somatotropic pituitary adenoma surgery.
Case report
A 76-year-old female patient with diabetes diagnosed more than twenty years ago was treated with oral hypoglycaemic agents for a long time. In the past ten years she has been taking insulin and has had extremely poor glycaemic control for a long time. She presented with an average daily level of blood glucose 11.2 mmol/l (measured by the device for self-monitoring of blood glucose before and two hours after the main meals) and HbA_{1c} 9%, while taking 62 units of insulin as a total daily dose. On that occasion the body mass index (BMI) was 23.8 kg/m^2, since the patient weighed 61 kg and was 1.6 m tall.
The patient had slightly visible signs of acromegaly. Therefore she underwent IGF-1 tests which showed high levels on two occasions, 380 and 369 μg/l (standard levels being 59–177 μg/l for the patient's age). An MRI scans showed sellar and infra-sellar macroadenoma and the patient underwent a transphenoidal surgery. Two months postoperatively the IGF-1 test showed 94.5 μg/l, the average daily level of blood glucose was 7.6 mmol/l, HbA_{1c} 7.2%, and the daily dose of insulin was 16 units.
Conclusion
This case confirms the significance of an analytical approach to each patient with unsatisfactory glycemic control. The significant reduction of the daily dose of insulin after the somatotropic pituitary adenoma surgery as well as attaining satisfactory glycemic control proves that growth hormone significantly affects insulin resistance.

P431

Endocrine function in a 48,XXYY adult

Séverine Dubois[1], Fréderic Illouz[1], Sandrine Laboureau[1], Catherine Draunet[1], Vincent Rohmer[1] & Agnès Guichet[2]
[1]Pôle d'Endocrinologie, Diabètes et Maladies Métaboliques, Centre Hospitalier et Universitaire, Angers, France; [2]Service de génétique, Centre Hospitalier et Universitaire, Angers, France.

Within the group of gonosomal aneuploidy, the 47,XXY Klinefelter syndrome is a well-known chromosomal anomaly with a clearly delineated phenotype. Since the 48,XXYY polysomy is rather rare and associated with hypogenitalism, it has often been considered as a variant of the Klinefelter syndrome. Nevertheless, several differences have been reported, in particular the greater severity and prevalence of mental retardation and psychiatric illness in patients with a 48,XXYY syndrome. Although the 48,XXYY is now considered to be a distinct clinical and genetic entity, there is very little data available in the literature, especially about adults. Moreover, endocrine studies are rarely performed.

To our knowledge, this is the first report of a case of an adult with the 48,XXYY syndrome concomitant with type 2 diabetes. The diabetes is probably related to a metabolic syndrome associated with the truncular obesity, a common feature in this X/Y polysomy. The physiopathology of abdominal obesity in the 48,XXYY syndrome is unknown.

Endocrine assays in our patient showed normal pituitary function in spite of hypergonadotrophic hypogonadism. The endocrine findings suggest dysfunction of the Leydig as well as the Sertoli cells, probably explained by the lengthy duration of the disorder. Other adult cases will be required to confirm these anomalies since very few accurate endocrine studies on the 48,XXYY syndrome have been published so far. We make a literature review.

Borgaonkar *et al.* reviewed the published data on the height of the 53 patients and they concluded that 48,XXYY boys are taller from an earlier age, compared to the general population. Our patient reached only his genetic target height and GH level was normal. Bertelloni *et al.* reported a central precocious puberty in the 48,XXYY syndrome. We have no indication of this pathology in our case.

P432

Simultaneous occurrence of multicentric medullary and papillary thyroid cancer: a case report

Gianlorenzo Dionigi[1], Patrizia Castano[1], Valentina Bertolini[2], Maria Laura Tanda[3] & Luigi Bartalena[3]
[1]Department of Surgical Sciences, University of Insubria, Varese, Italy; [2]Anatomic Pathology Unit, Department of Human Morphology, University of Insubria, Varese, Italy; [3]Department of Clinical Medicine, University of Insubria, Varese, Italy.

Background
Papillary thyroid cancer is a well-differentiated neoplasm and is the most common, accounting for 65–85% of all thyroid cancer. On the other hand, medullary carcinoma represents only 3–12%. The concurrence of distinct medullary and papillary carcinoma within the same thyroid has been sporadically described.
Case presentation
We report a rare case of simultaneous sporadic both multicentric medullary and papillary thyroid cancer with lymph node metastases in a 65 years old man patient. He presented with a one-month history of solitary right lobe thyroid node and watery diarrhea. He was biochemically euthyroid. Basal serum calcitonin levels was high. Diagnosis of medullary carcinoma was confirmed by positive aspirate immunohistochemical staining for calcitonin and negative thyroglobulin staining. Pheochromocytoma was excluded before operation. Patient was screened for the presence of the specific ret mutations. After total thyroidectomy and dissection of central lymph nodes, histopathological definitive examination of the speciment revealed medullary carcinoma in right lobe (4 cm), two distinct nodules of medullary (0.4 cm) and papillary (0.5 cm, with follicular components) carcinoma in the isthmus, papillary microcarcinoma (0.5 cm) in the left lobe and lymph node metastases of medullary cancer. All tumors were clearly separated from each other, representing the pure entity of each type. The postoperative course was uneventfull. Six months after operation he has no signs of progression of the tumour.
Conclusion
Medullary carcinoma derives from parafollicular cells or C cells of the thyroid. C cells have a neuroendocrine origin, being derived from ectodermal neural crest precursors. Papillary carcinoma derives from the follicular cells. This patient developed distinct histologic type tumors in separate lobes of the thyroid. This is the first report in which different synchronous both multicentric tumors type have been identified in one case. Although the simultaneous occurrence of papillary and medullary carcinoma may have been a simple coincidence, a common oncogenic stimulus may have been involved.

P433

Simultaneous bilateral transperitoneal laparoscopic adrenalectomy (SBTLA)

Ferenc Juhasz, Zsolt Kanyari, Ferenc Gyory & Géza Lukacs
University of Debrecen, Surgical Department, Debrecen, Hungary.

Background and Purpose
Laparoscopic adrenalectomy has become the preferred surgical approach to manage adrenal disorders. Bilateral adrenalectomy is performed for diseases that are unresponsive to medical management and, frequently, for neoplastic disease. The aim of this study was to review our experience with laparoscopic bilateral adrenalectomy and to evaluate its safety, efficacy, and outcomes.
Patients and Methods
Between May 1999 and May 2005, four male and four female patients with a mean age of 37 years (range 24–55 years) presented for bilateral adrenalectomy (pheochromocytoma [$N=4$], Cushing's disease [$N=2$], malignant neuroendocrine tumor [$N=1$] and incidentaloma [$N=1$]. All procedures were performed using a simultan bilateral transperitoneal approach (SBTLA).
Results
Laparoscopic bilateral transperitoneal adrenalectomy was completed simultaneously in eight patients, while in one case the operation was converted due to the neuroendocrine carcinoma localised just behind the confluence of the right renal vein and I.V.C. One tripple tumor was operated by the staged procedure because there was no agreement on a one stage (simultan) operation between the chest surgeon consultant and us. The mean operative time was 189 minutes (range 165–240 minutes), and the mean estimated blood loss was 76 mL (range 55–90 mL). There were no postoperative complications. All patients have been treated postoperatively with daily hydrocortisone and fludrocortisone replacement. After a mean follow-up of 33 months (range 2–45 months), all of the eight patients are alive.
Conclusion
Simultan bilateral transperitoneal laparoscopic adrenalectomy is a safe and effective procedure. Patients are discharged postoperatively in a relatively short time with few complications. Appropriate steroid replacement (if its necessary) and close follow-up allows these patients to return to their regular life style. The meticulous adrenal preserving technic of the LA makes possible to avoid unnecessary hormone supplementation.

P434

Amyloid goitre: report of a case

Gianlorenzo Dionigi[1], Maurizio Salvadore[2], Maria Laura Tanda[3], Eliana Piantanida[3] & Francesca Villa[1]
[1]Department of Surgical Sciences, University of Insubria, Varese, Italy; [2]Department of Surgical Pathology, Anatomia Patologica, Ospedale di Circolo-Universita dell'Insubria, Varese, Italy; [3]Department of Clinical Medicine, University of Insubria, Varese, Italy.

Background
Amyloidosis is an important etiological factor of end stage renal disease. Apart from major target organs as cardiovascular, respiratory and gastrointestinal

system, endocrine organs can also be involved. Amyloid goitre was described for the first time by Beckmann in 1858. Approximately 200 cases of amyloid goiter have been reported in English literature.
Case presentation
The patient is a 67-year-old woman. The patient referred in 1989 the presence of a thyroid nodule of the left lobe investigated by scintigraphy and fine needle aspiration cytology (compatible with goitre). 3 months prior to her admission, the patient noticed a progressive enlargement in the anterior region of the neck associated with dyspnea dysphagia and hoarsness. Preoperative ultrasound showed an enlargement thyroid with US stimated gland volume of 105 mL, a 3 cm nodule in the left lobe and micronodularity in the right lobe. Chest X-ray revealed a deviation of the trachea. She was biochemically euthyroid. Because of the obstructive symptoms the patient underwent thyroidectomy. Histologic examination confirmed diffuse amyloid deposition surrounding thyroid follicles. Moreover, a nodular pattern of amyloid deposition was seen resulting in compression and distortion of the follicular architecture. Confirmation of amyloid was made by the presence of congophilia and apple-green birefringence under polarized-light microscopy. No Immunoreactivity was seen with calcitonin or thyroglobulin. One year after primary surgery, the patient was admitted to the Nephrology Department because of acute renal failure.
Conclusion
Amyloid goitre as the initial manifestation of systemic amyloidosis is an exceedingly rare condition associated with clinically apparent enlargement of the thyroid gland due to massive amyloid infiltration. We describe the clinical and pathological features of amyloid goitre and the difficulties in making a pre-operative diagnosis. In this case, amyloid goitre had no significant influence on thyroid function even when extensive parenchyma replacement was present. A plan of management for this rare thyroid condition must be suggested.

P435

Autoimmune polyglandular syndrome type I associated with motor focal epilepsy – a case report

Alina Maria Suta & Carmen Burloiu
Clinical Hospital Al Obregia, Bucharest, Romania.

Autoimmune polyglandular syndrome type I is a very rare disorder. We present the case of a six-year-old girl admitted to our hospital in September 1999 for recurring seizures and a history of muscle cramps and carpal spasms. Neurological examination showed congenital partial palsy of cranial nerves III and VI, EEG revealed abnormal electric activity and cerebral CT was normal. Laboratory findings (hypocalcemia-5 mg/dl, hyperphosphatemia-10.3 mg/dl and low serum PTH level-4.72 pg/ml; serum cortisol, electrolytes, TSH – in normal range) sustained the diagnosis of motor focal epilepsy and idiopathic hypoparathyroidism and the child was treated with calcitriol, calcium salts and antiseizure drug (carbamazepine). She was followed up for two years and lost after that.

In May 2005 the patient was hospitalized again for symptoms of adrenal crisis preceded by skin hyperpigmentation. New laboratory findings: blood sugar-40 mg/dl, blood ureea-63.8 mg/dl, hyponatremia-120 mEq/l, hypochloremia-80 mEq/l and hyperkaliemia-10.6 mEq/l; random cortisol level-3.13 μg/dl; hypocalcemia-5.9 mg/dl. This time cerebral CT showed calcification of basal ganglia, frontoparietal cerebral cortex and cerebellum. After emergency treatment of adrenal crisis, the maintenance therapy of chronic primary adrenal insufficiency has been initiated: replacement of glucocorticoids and mineralocorticoids with prednisone, respective fludrocortisone. The therapy with calcitriol and calcium salts has been resumed. After two months the patient presented candidiasis of the mouth with a good answer to therapy with fluconazol.

The patient's mother was diagnosed with Hashimoto's thyroiditis at the age of 37 years, in July 2005.

This is a case of an unusual sequence of development of the three major component of PGA1 (hypoparathyroidism, adrenal insufficiency and chronic mucocutaneous candidiasis). Till now, we didn't find other autoimmune or ectodermal disorders, but there is a neurological pathology unrelated to hypoparathyroidism with special problems of management.

P436

Postpartum autoimmune hypophysitis, autoimmune hyperthyroidism and reversible hepatitis at a patient with partial empty sella

Ionut Repede, Ioana Asiminoaiei, Roxana Acasandrei,
Loredana Ungureanu, Mihai Grigoras & Dumitru Branisteanu
University of Medicine and Pharmacy "Gr.T.Popa", Iasi, Romania.

The postpartum period is accompanied by an increased risk for autoimmune diseases. SN, 29 years of age, developed subsequent to her second pregnancy a polymorphic syndrome, characterized by fatigue, paleness, amenorrhea, agalactia, palpitations, weight loss. Hormonal investigations suggested corticotrophic, somatotrophic (basal morning plasma cortisol – 35 ng/dl, basal GH – 0.1 mIU/l, insulin-induced hypoglycemia test: plasma cortisol – 58 ng/dl, GH – 0.1 mIU/l) gonadotrophic (FSH = 0.3 IU/l, LH = 0.2 IU/l, oestradiol = 22 pg/ml), and prolactinic insufficiency (prolactin = 3.5 ng/dl), but measured high levels of thyroid hormones (fT_4 = 3.4 ng/dl) in the presence of low TSH (0.1 mIU/l), setting the diagnosis of autoimmune postpartum thyroiditis in the clinical, immune (positive antibodies vs TPO) and imagistic (thyroid ultrasound) context. NMR investigation of the pituitary region showed partially empty sella and glandular parenchyma with diffusely reduced contrast. Clinical evolution (the appearance of hypopituitarism in the postpartum period, after uncomplicated labor and associated with other autoimmune pathology) chose the diagnosis of autoimmune postpartum hypophysitis the most probable, and glucocorticoid and oestrogenic substitution were started accordingly. During her admission in our department, the patient complained of nausea and lack of appetite. Liver enzymes were increased (TGO = 97 U/l, TGP = 89 U/l) before the onset of antithyroid therapy, but spontaneously got normalised after one week. Subsequent to the therapy with antithyroid drugs, the patient developed a clinically suggestive episode of transient hypothyroidism with low fT_4 values (0.8 ng/dl), but unaccompanied with a correspondant TSH increase, fact certifying the existence of a thyrotrophic defficiency accompanying the autoimmune hypophysitis. This is the first case of association between reversible hepatitis and multiple endocrine immunopathy. The aetiology of hepatitis, although not proven, might have also been autoimmune. Another rare particularity was the tricky co-existence of hyperthyroidism and pituitary insufficiency.

P437

One case of sellar and suprasellar chordoma

Corina Galesanu, Ioana Asiminoaiei, Elena Saviuc, Tamara Bostaca,
Dragos Negru & Dumitru Branisteanu
University of Medicine and Pharmacy "Gr.T.Popa", Iasi, Romania.

Chordomas are slow growing neoplasms arising from notochordal remnants of the axial skeleton. The second most common site for chordomas, after the sacrococcygeal region, is the base of the skull. We describe one case of sellar and suprasellar chordoma found in a 44 year old female, with tumoral syndrome, bitemporal hemianopsia and secondary amenorrhea. Skull X-ray showed an enlarged sella turcica with destruction of the dorsum and impressive intra- and suprasellar calcifications, reason why a craniopharyngeoma was initially suspected. MRI depicted a voluminous and expansive solid tumor mass, accompanied by destruction of the sellar base and temporal bone on the left side. The lesion was compressing the optical chiasm and the third ventricle on the left side. Hormonal investigations showed corticotroph and somatotroph deficiency (morning plasma cortisol of 45 ng/ml, basal GH of 0.2 ng/ml both insufficiently stimulated by insulin-induced hypoglycemia test – to 56 ng/ml for cortisol and 1.1 ng/ml for GH) as well as thyrotroph (basal TSH of 0.19 mIU/l, stimulated only to 1.66 mIU/l at TRH test – 500 microg iv in the context of low total T_4 – 5.2 mg/l) and gonadotroph deficiency (low basal FSH, of 1.5 mIU/ml, in the context of low plasma oestradiol, of 29 pg/ml). Basal prolactin was moderately increased (79.6 ng/ml) and further stimulated by the TRH test (to 117.4 ng/ml), suggesting pituitary stalk disjunction rather than tumoral secretion. The patient was submitted to transfrontal surgery under intravenous glucocorticoid protection. The anatomopathological investigation set the final diagnosis of chordoma, due to the presence of physalyphorous cells. After surgery the visual field broadened, but the patient developed irreversible diabetes insipidus. The patient was hormonally substituted and submitted two years later to telecobaltotherapy due to the growth of tumor reminiscence and re-narrowing of the visual field. Her evolution eight years after the diagnosis is stable.

P438

Cutaneous modifications suggestive for Cushing's syndrome induced by topical corticoid application

Simona Mogos, Dumitru Branisteanu, Anca Zbranca, Eusebie Zbranca & Voichita Mogos
University of Medicine and Pharmacy "Gr.T.Popa", Iasi, Romania.

Topical application of corticosteroids is frequently used in the therapy of various dermatological diseases due to their antiinflammatory and immunsuppressive effects. Systemic pharmacological levels of glucocorticoids lead, on the other hand, to Cushing syndrome, i.e. significant modifications of intermediary metabolism, body composition, bone mass, haematolymphopoietic system and, last but not least, to skin modifications: purple striae, petechiae, infections. We describe a clinical case of cutaneous changes suggestive for Cushing syndrome of pre-existent axillary striae at an obese male using topic corticoid administration, limited to the surface of application. Although transcutaneous corticoid absorption may lead to overt Cushing syndrome through exceeding the physiological level of plasma glucocorticoids, causing at the same time an inhibition of endogenous corticotroph function, the corticotropic axis of our patient was functioning normally at the moment of the admission (morning plasma cortisol of 11.2 microg/dl, 24 hour urinary cortisol excretion of 76 microg/24 h).The patient equally had normal blood pressure, normal electrolytes, normal blood cell count, absence of osteopenia by DXA-assessed bone mineral density. Abdominal ultrasound investigation showed adrenal glands within normal range and the absence of adrenal or extraadrenal tumors. Skin lesions suggestive for glucocorticoid excess, but unaccompanied by other features of Cushing syndrome, should determine the physician to proceed to a thorough anamnesis. Endogenous or exogenous systemic Cushing syndrome should be nevertheless ruled out.

P439

Clinical presentation of a patient with giant prolactinoma

Ekaterina N. Giniatullina, Larisa K. Dzeranova, Andrey Y. Grigoriev & Alexandr V. Voronzov
Research Centre for Endocrinology, Moscow, Russia.

The aim of said presentation was to direct attention to possibility of misdiagnosis of patients with a giant prolactinomas.

A 36 years-old man was admitted to our Centre with complaints of headaches, decline of left eye vision, absence of erection, accompanied with a decline in libido and galactorrhea. These symptoms began since August 2001. At October 2004 reduction of the vision on both eyes was revealed as well as contraction of vision fields. At the presentation the patient had excess weight, muscle weakness, body hair reduction, along the following with lab data (prolactin level was 48527 mU/l, Testosterone level – 2.1 nmol/l, DHA-S 14 mcmol/l) and data of MRI inspection (macroadenoma with endo-ante-supra-infra-laterocellaric expansion). This led us to suspect the diagnosis of giant prolactinomas, secondary hypogonadism, galactorrhea. The treatment of cabergoline (0.5 mg a week with gradual increase until dosage of 3,5 mg a week was reached) was recommended. ?t control examination at March 2005 the decline in frequency of headaches, vision disturbances, galactorrhea and also the recovery of erection was noted. The PRL level decreased to 990 mU/l. Data of MRI – reduction of the tumor size by 2,3 times was noted. During the period of the treatment the patient's wife become pregnant.

The diagnostics of male prolactinomas is a complicated task, because clinical signs of the disease can vary broadly and thus, by their subjective character, can prevent the timely medical attention. But in presence of a primary medicament treatment the positive dynamic, recovery of reproductive function, reduction of the tumor sizes can be observed in most cases.

P440

Thyrotropin-producing pituitary adenoma discovered because of galactorrhea

M Teresa Herrera[1], Cristina Abreu[1], Marion Hemmersbach-Miller[2], Ricardo Darias[1], Pilar Olvera[1] & Enrique Palacios[1]
[1]Hospital Universitario Nuestra Señora De Candelaria, Santa Cruz De Tenerife, Spain; [2]Hospital San Roque Maspalomas, Maspalomas, Gran Canaria, Spain.

Introduction
Thyrotropin-producing adenomas (TSH-omas) constitute about 1% of pituitary adenomas. TSH-omas are a rare cause of hyperthyroidism. In conjunction with biochemical parameters and dynamic endocrine testing, image evaluation of the pituitary gland and sella turcica is mandatory for establishing a correct diagnosis. TSH-omas are usually large tumors and tend to be invasive. Greater amounts of invasion correlate with incomplete surgical removal of the tumor and, thus, continued hormonal secretion. Therefore, an early diagnosis and a complete surgical removal are essential.
Case report
A 29-year-old female was referred to the endocrinology outpatient unit because of a 5 months history of bilateral galactorrhea and amenorrhea. She also complained about symptoms of hyperthyroidism (13 Kg weight loss in 10 months, palpitations, hand tremors, heat intolerance and nervousness). On physical a grade I goiter was observed. Pituitary hormone levels were determined; abnormal values are shown in table 1 – the rest was normal. In order to rule out the thyroid hormone resistance syndrome, TRH testing and a MRI of the pituitary gland was performed. TRH testing was compatible with a TSH-oma (Basal TSH 7.63 μUI/mL; after 20 minutes 7.99 μUI/mL; after 60 minutes 6.97 μUI/mL). Pituitary MRI showed a macroadenoma.
The patient was started on a long-acting somatostatin analog (Octreotide) and is currently awaiting surgery.

Table 1 Results of hormone determinations

	TSH (μUI/mL)	FT4 (ng/dl)	PRL (ng/mL)	FSH (mUI/mL)	LH (mUI/mL)	17-β-estradiol (pg/mL)
28/07/2006	6.63	2.56	61.52	6.36	3.88	<10
10/07/2006	5.64	2.77	43.74	5.09	3.04	13

Discussion
1- Signs and symptoms of TSH-oma vary and are unspecific. Galactorrhea and amenorrhea are present in 30% of these patients.
2- In case of hyperthyroidism without TSH suppression and abnormal pituitary hormone values, a TSH producing pituitary adenoma should be suspected.

P441

An 8-year-old boy with seizures and hypokalemia due to a paraganglioma

Vassilios Petrou[3], Kolofotis George[3], Eleni Georgouli[2], Asteroula Papathanasiou[3], Tertipi Athanasia[3], Constantinos Stefanidis[1] & Charalambos Hadjiathanasiou[3]
[1]Children's Hospital "P. & A. Kyriacou" Department of Nefrology, Athens, Greece; [2]Children's Hospital "P. & A. Kyriacou" B' Pediatric clinic of Athens University, Athens, Greece; [3]Children's Hospital "P. & A. Kyriacou" Department of Endocrinology, Athens, Greece.

Pheochromocytomas and paragangliomas, tumors originating from the chromaffin cells, are rare in children.

We report an 8-year-old boy who was admitted to the intensive care unit with seizures for which the child had to be intubated, severe hypokalemia (1.8 mEq/l), hyponatremia (127 mEq/l) and fever. Parents reported that several months before admission the boy had nocturnal sweating. Brain MRI revealed areas of increased sign intensity in the parietal lobes bilaterally between the cortex and the subcortical region. Blood thyroid hormone levels were normal. He was initially treated as encephalitis and several boluses of potassium chloride were administered and the serum levels of sodium and potassium returned to normal, without fluid restriction. The child showed remarkable improvement in 48 hours. During hospitalization hypertension was diagnosed (180/95 mmHg) and the child complained for headaches, palpitations, polydipsia, polyouria and nocturnal sweatings. He was treated with combination of dihydralazine, felodipine, enalaprile and propranolole but without blood pressure control. Urinary 24-hour catecholamines (6440microg, normal range 14–108) and normetanephrines (19222microg normal range 88–444) were markedly elevated. Serum levels of renin (49.4 microIU/ml, normal range 3.3–41) and aldosterone (37.7 ng/dl, normal range 3–28) were elevated. Abdomen MRI showed a mass (4×4.5×3 cm) in the left paraspinal area pushing down left kidney. Whole body MIBG I-131 scan was negative. The antihypertensive therapy was modified to phenoxybenzamine followed by propranolole with normalization of

blood pressure. A laparotomy with removal of the retroperitoneal mass was perfomed. The intraoperative course was uneventful. Histologically the mass proved a well-demarcated paraganglioma. No infiltration of nearby structures or other malignant features were noted. Postoperatively, the child was asymptomatic, blood pressure and urinary catecholamines returned to normal. Genetic testing of VHL, SDHB, SDHD and RET genes was recommended.

P442

Neuropsychiatric manifestations in patients of primary hyperparathyroidism and outcome following surgery

Alexandra Raceala, Paul Ivanov, Vasiliu Octavian & Diana Paun
[1]Universitary Endocrinology Hospital "Dr. C.I. Parhon", Bucharest, Romania; [2]Emergency Military Hospital "Dr. Carol Davila", Department of Psychiatry, Bucharest, Romania.

Background
Primary hyperparathyroidism (PHPT) associates many psychiatric symptoms and is therefore important to find out if surgery can alleviates the psychiatric symptoms and improve the quality of patient's life.
Objectives
To study the nature and severity of neuropsychiatric manifestations in patients diagnosed with hyperparathyroidism before and after surgery, as well as to evaluate the correlation of such symptoms with levels of serum calcium.
Methods
During this study we monitored the psychiatric symptoms occurrence and their correlation with serum calcium among 24 patients with primary hyperparathyroidism (group I), using a control group with 20 patients that were surgicaly treated with total thyroidectomy (group II). We assessed these patients using Brief Psychiatric Rating Scale (BPRS), Clinical Global Impression (CGI) and Global Assessment of Functioning (GAF) before surgery and at 1, 8, 12 and 24 weeks after surgery.
Results
The PHTP patients had significantly higher levels of total serum calcium and PTH preoperatively, with biochemical normalization after surgery. The baseline BPRS score were higher in PHTP group, mean score 76.5, before surgery, compared to the control group with a mean score of 51.2. The CGI and GAF scores were also different between groups: 3.4 and 68.4 (group I before surgery), compared to 2.1 and 77.2 (group II). The improvement in neuropsychiatric symptoms was significant at 8 weeks after surgery as reflected in BPRS decreasing to 45.3, while CGI and GAF improved also, to 1.7 and 87.2. No correlation was found between the serum calcium levels and the psychiatric manifestations.
Conclusions
The PHTP associated psychopathology is very complex and symptoms significantly improved by 8 weeks post-parathyroidectomy. The evaluation of surgical interventions over the patients status is useful using clinical psychiatric rating scales but there was recorded no correlation of clinical mental status with serum calcium level.

P443

Hyperthyroidism in molar pregnancy: rapid preoperative preparation by plasmapheresis and complete after evacuation

Adil Dogan Azezli[1], Taner Bayraktaroglu[1], Samet Topuz[2], Sevgi Kalayoglu Besisik[3] & Yusuf Orhan[1]
[1]Istanbul University, Istanbul Faculty of Medicine, Endocrinology and Metabolism, Istanbul, Capa/Fatih, Turkey; [2]Istanbul University, Istanbul Faculty of Medicine, Obstetrics and Gynaecology, Istanbul, Capa/Fatih, Turkey; [3]Istanbul University, Istanbul Faculty of Medicine, Haematology, Istanbul, Capa/Fatih, Turkey.

Human chorionic gonadotropin bears structural homology to pituitary thyrotropin. The extremely elevated levels of human chorionic gonadotropin in patients with molar pregnancy or other trophoblastic diseases can lead to hyperthyroidism. We describe a patient with molar pregnancy who had secondary hyperthyroidism prepared rapidly by plasmapheresis for surgery. After first plasmapheresis the clinical picture improved dramatically. Three subsequent plasmapheresis provided a 75.1% decrease in serum free T3 concentrations and 63.9% free T4 concentrations and recovered after evacuation. This is the first using of the plasmapheresis in rapidly preparation of the patient who had secondary hyperthyroidism due to molar pregnancy.

P444

Finasteride treatment of premature androgenetic alopecia

Michaela Duskova, Luboslav Starka, Martin Hill & Ivana Cermakova
Institute of Endocrinology, Prague, Czech Republic.

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 prostate hyperplasia and prostate carcinoma.
Methods
A group of 26 men (mean age: 31 years, mean BMI 25.58), in which premature hair loss begun before 30 years of age was involved in the present study. In all individuals, their hormonal profile involving total testosterone, androstenedione, dehydroepiandrosterone, epitestosterone, dihydrotestosterone, cortisol, estradiol, SHBG, prolactin, TSH, LH, FSH and index of free testosterone was determined and insulin tolerance test before the treatment with finasterid was carried out. Finasteride in the daily dose of 1 mg was administered for 3 months. After the treatment hormonal profile was determined again. Wilcoxon robust test was used for statistic comparison of pre- and post-treatment results.
Results
The hormonal levels before and after the finasteride treatment were compared. The ratios of dihydrotestosterone/testosterone before and after treatment differed significantly while in the other hormonal levels no significant differences were found. Among 26 men examined and treated 17 subjects described the amelioration of hair quality and the stop of hair loss and no side effects during the treatment period. They were satisfied with treatment asking for the treatment to continue. Eight men have observed no treatment effect after the 3 months of finasteride administration. One man has shown the discrete sign of gynecomastia, and interrupted the treatment. No other side effects have been recorded. The insuline tolerance test before treatment was normal.
Conclusions
Finasteride in dose of 1 mg can present safe eventuality of the androgenetic alopecia control experiencing discrete amelioration of problems with hair loss in prematurely balding men.
The study was supported by grant No. NR/8525 – 5 of the IGA MZCR

P445

Secondary adrenal failure and secondary amenorrhea due to hydromorphone treatment

Karsten Müssig[1], Daniele Knaus-Dittmann[1], Helmut Schmidt[2], Klaus Mörike[3] & Hans-Ulrich Häring[1]
[1]Department of Endocrinology, Metabolism and Pathobiochemistry, University Hospital of Internal Medicine, University of Tübingen, Tübingen, Germany; [2]Pharmazentrum frankfurt/ZAFES, Institute of Clinical Pharmacology, Johann Wolfgang Goethe-University, Frankfurt am Main, Frankfurt am Main, Germany; [3]Division of Clinical Pharmacology, University Hospital Tübingen, Tübingen, Germany.

Objective
Opioids are among the most commonly used symptomatic treatments of somatoform pain disorder. Human and animal studies suggest that chronic exposure to opioids suppresses the hypothalamo-pituitary-adrenal (HPA) and the hypothalamic-pituitary-gonadal axis. We report on a rare case of secondary adrenal failure and secondary amenorrhea due to hydromorphone treatment.
Case report
A 32-year-old female patient presented with fatigue, weakness, orthostatic dysregulation, dizziness, and secondary amenorrhea for three months. The patient's past medical history revealed chronic pain syndrome (DSM-III-R) lasting two years. Four months before presentation, analgesic treatment had been changed to hydromorphone 32 mg BID and up to four times daily hydromorphone 2.6 mg as single dosages by a pain clinic. Decreased basal concentrations of plasma ACTH, serum cortisol, as well as mean 24-h urinary free cortisol excretion, and reduced peak responses of cortisol to ACTH 250 µg, to corticotrophin releasing hormone 100 µg, and during an insulin tolerance test with 0.5 IU insulin per kg body weight were consistent with secondary adrenal insufficiency. Estradiol levels were diminished with luteinizing hormone and follicle-stimulating hormone concentrations within the normal range, indicating secondary amenorrhea due to hypogonadotropic hypogonadism. Magnetic resonance imaging of the pituitary gland revealed no abnormal findings. The patient denied traumatic brain injury as well as skull radiation. After tapering from the benzodiazepine treatment we observed a stable increase to normal levels of the serum and urinary concentrations of cortisol as well as of ACTH, estradiol, FSH, and LH levels. The patient tolerated the treatment conversion very well. At the end of the tapering period she reported a clear improvement in vitality.
Conclusion
Clinicians should be alerted to the, though rare, endocrine side effects of hydromorphone treatment.

P446

Pseudophaeochromocytoma in Parkinson's disease

Karsten Müssig[1], Albrecht Pfäfflin[1], Daniele Knaus-Dittmann[1], Marius Horger[2], Klaus Mörike[3], Hans-Ulrich Häring[1] & Erwin D Schleicher[1]
[1]Division of Endocrinology, Diabetology, Nephrology, Angiology, and Clinical Chemistry, Department of Internal Medicine, University Hospital of Tübingen, Tübingen, Germany; [2]Department of Diagnostic Radiology, University Hospital of Tübingen, Tübingen, Germany; [3]Division of Clinical Pharmacology, University Hospital of Tübingen, Tübingen, Germany.

Objective
Despite combination with peripheral decarboxylase inhibitors significant amounts of L-dopa are peripherally metabolised. In patients with Parkinson's disease (PD) treated with L-dopa and a dopa decarboxylase inhibitor, urinary dopamine concentrations are markedly elevated. We describe here a L-dopa treated PD patient presenting with a clinical and biochemical picture suspicious of phaeochromocytoma.
Case report
A 73-year-old female patient diagnosed with dopamine-secreting phaeochromocytoma was referred to the Department of Internal Medicine for preoperative pharmacological treatment of severe and symptomatic paroxysmal hypertension. Endocrine evaluation of an adrenal mass had revealed markedly increased urinary dopamine levels and urinary epinephrine and norepinephrine levels within the normal range. On admission the patient reported that she had been diagnosed three years ago with PD. Medication comprised L-dopa 100 mg/benserazide 25 mg qid and pramipexole 0.7 mg tid. Endocrine evaluation confirmed markedly elevated urinary dopamine and homovanillic acid levels as well as plasma dopamine levels. Cortisol diurnal rhythm was normal. Plasma aldosterone concentration and plasma renin activity were within the normal range. Iodine-131-meta-iodobenzylguanidine (MIBG) scintigraphy proved negative. L-dopa/benserazide treatment was discontinued for three days and replaced by amantadine 200 mg qd. Twelve hours after discontinuation we observed a normalisation of the elevated urinary and plasma dopamine levels as well as the increased urinary homovanillic acid levels, indicating that increased dopamine levels were not due to phaeochromocytoma but due to PD therapy. Radiological follow-up of the adrenal incidentaloma was advised.
Conclusions
Clinicians should be alerted to increased urinary dopamine levels in patients treated with L-dopa. Unawareness of this association may lead to the misdiagnosis of phaeochromocytoma.

P447

Kallmann syndrome – deletion of the short arm of chromosome 8

Joana Guimarães, Margarida Bastos, Leonor Gomes, Miguel Melo & Manuela Carvalheiro
Hospitais da Universidade de Coimbra, Coimbra, Portugal.

Introduction
Kallmann Syndrome (KS) consists of hypogonadotropic hypogonadism and anosmia, and is 5 fold more prevalent in males. There is a considerable clinical and genetic heterogeneity and a crescent interest in autosomal genes. The FGFR1 gene, located on the short arm of chromossome 8, encodes a glycoprotein fibroblast growth factor receptor and FGFR1 mutations has been identified in 10% of KS patients. The clinical picture include typical KS and associated features.
Case study
A female, 30 years old, with primary amenorrhea, short statue (P5–P10), cleft palate, hyposmia, mental retardation and right hearing loss. Laboratory evaluation showed hypoganodotropic hypogonadism, an GnRH stimulation test showed a probable hypothalamic origin of the hypogonadism (IGF-1, GH, FT3, FT4, TSH and cortisol were normal). The pelvic ultrasonography was normal and MRI showed a lipoma of the III ventricle and agenesis of the corpus callosum. Analysis of G-banded prometaphase chromosomes from lymphocyte cultures showed a deletion on the short arm of chromosome 8: 46,XX,del(8)(p12-pter).
Conclusion
We present a patient with an 8p12-pter deletion, agenesis of the corpus callosum, cleft palate, mental retardation, right hearing loss in association with Kallmann syndrome phenotype. There are rare cases describe in literature with this associations. These findings suggest that autosomal genes are important for KS and we have to pay attention to other features associated with KS phenotype.

P448

Hyperprolactinemia in post-acute phase after severe TBI or SAH is mostly iatrogenic or due to physical stress

Ingo Kilimann[1], Günter Karl Stalla[2] & Friedrich von Rosen[1]
[1]Neurological Hospital Bad Aibling, Bad Aibling, Germany; [2]Max Planck Institute of Psychiatry, Munich, Germany.

Background
Recent studies demonstrated partial or complete hypopituitarism in 30–70% of survivors of traumatic brain injury (TBI) and subarachnoid hemorrhage (SAH). Hyperprolactinemia may indicate damage of the pituitary stalk or the hypothalamus. Physical and psychological stress and a considerable list of medications can also lead to increased prolactin values.
Methods
Prolactin was measured in 103 male and 54 female patients aged 14 to 89 years after severe TBI or SAH in the post-acute or chronic state (mean 4 month after onset) as part of a hormone screening also including cortisol, fT4, testosterone, estradiol und IGF1. Cut-off levels for normal prolactin was 18.0 ng/ml in male and 25.0 ng/ml in female patients. Medication, body temperature, serum glucose and C-reactive protein were registered.
Results
23% of the screened patients had increased levels of prolactin. Significantly more male were found to have hyperprolactinemia (25% of males vs. 8% of females). All patients with hyperprolactinemia had common hyperprolactinemic factors such as infection ($n=16$), hypoglycemia (blood glucose below 70 mg/dl) ($n=2$) or medications known to increase prolactin levels such as dopamin antagonists ($n=29$), central catecholamine depletors ($n=8$), GABA agonists ($n=6$) or opiats ($n=4$).
Hyperprolactinemia was not correlated with deficiency of other hormones.

Conclusion
Hyperprolactinemia in patients after severe TBI or SAH is usually secondary to medication or physical stress and does not indicate damage to the hypothalamus or pituitary gland.

P449

Rapid normalization of highly elevated serum chromogranin A after cessation of proton pump inhibitor therapy

Peter Igaz[1], Katalin Müllner[1], Beáta Hargitai[2], Károly Rácz[1] & Zsolt Tulassay[1]
[1]2nd Department of Medicine, Faculty of Medicine, Semmelweis University, Budapest, Hungary; [2]1st Department of Pathology, Faculty of Medicine, Semmelweis University, Budapest, Hungary.

Introduction
Proton pump inhibitors (PPIs) are widely used for treating various upper gastrointestinal disorders. A well-known side effect of PPI therapy that may cause serious differential diagnostic problems is the elevation of serum chromogranin A (CgA).
Objective
We report a case with highly elevated serum CgA in a patient with bilateral adrenal adenomas that was clearly associated with PPI therapy. Suspension of PPI intake for a few days resulted in the normalization of serum CgA.
Results
The 73-year-old woman with a history of hypertension, gastroesophageal reflux disease was found to have bilateral adrenal incidentalomas revealed by routine abdominal ultrasonography and CT. Detailed endocrinological examination including cortisol rhythm, low dose dexamethason suppression, mineralocorticoid activity, urinary catecholamine excretion did not suggest hormonal activity. ^{131}I-MIBG scintigraphy did not show pathologic isotope accumulation either. MRI indicated adrenal cortex-related adenomes. CgA measured by radioimmunoassay (CIS Bio International) was 7-fold higher than the upper normal value (728 ng/ml v.s. 98.1 ng/ml). No clinical or biochemical signs of pheochromocytoma, other neuroendocrine or carcinoid tumours, or renal insufficiency were observed. As the patient took high doses (2×30 mg) of the PPI lansoprazole, iatrogenic elevation of CgA was suspected. Immunohistochemical analysis of biopsy samples from the gastric mucosa did not indicate enterochromaffin-like (ECL) cell hyperplasia. After replacing lansoprazole with sucralphate, CgA fell rapidly, with levels normalizing within five days (84.6 ng/ml). Following the intake of a single dose of lansoprazole, serum CgA again slightly surpassed the upper normal range (132.4 ng/ml).
Conclusions
This case demonstrates that by suspending PPI therapy for a few days, highly elevated CgA can be normalized. It can thus be suggested that for the correct interpretation of results, the suspension of PPI therapy for 5 days before CgA measurement may be sufficient.

P450

Case of primary bilateral adrenal lymphoma

Timur Britvin, Oleg Bogatyriov, Svetlana Vorontsova, Galina Polyakova, Larisa Gurevich, Irina Kazantseva & Arian Kalinin
M.F. Vladimirsky Moscow Regional Clinical and Research Institute, Moscow, Russia.

Primary bilateral adrenal lymphoma is extremely rare. We report a case of bilateral adrenal lymphoma developing in a 61-year-old woman. The patient presented with weakness, fever, anorexia, nausea, and weight loss. Her vital signs were as follows: body temperature 37.2°C, pulse rate 98 beats per minute, and blood pressure 125/70 mmHg. Examination of head and neck was unremarkable. Lymphadenopathy and skin lesion weren't found. The chest X-ray was normal, without evidence of hilar lymphadenopathy. US and CT-scan revealed bilateral adrenal masses: to the right – $90\times36\times78$ mm, to the left – $70\times35\times70$ mm. Endocrine studies didn't show adrenal insuffiency – the serum cortisol (8AM) was 374 nmol/l (normal range: 180–650), serum aldosterone was 0.4 nmol/l (normal range: 0.14–1.24), and the plasma ACTH (8AM) increased to 13.5 pmol/l (normal range: 2.2–13.2). The level of 24-hour urine epinephrine was 24 nmol (normal range: 11–44), norepinephrine 59 nmol (normal range: 47–236), and free cortisol 108 nmol (normal range: 80–250). Ultrasound-guided needle biopsy was performed at the right adrenal mass. Cytologic examination showed adrenal cortical carcinoma. We performed right adrenalectomy. Microscopically, the tumor was composed of large, markedly atypical cells showing high mitotic activity. Complete substitution of tumor tissue for adrenal gland was noted as well as the tumor spread through capsule and invasion of surrounding fat. Immunohistochemical staining revealed positive reaction of tumor cells with LCA and B-lymphocyte antigen. But the cells were negative for CD30, cytokeratin A1/A3, vimentin, chromogranin A, synaptophysin and antigen of T-lymphocytes that allowed to diagnose large diffuse B-cell lymphoma. The patient refused chemotherapy and died 6 months later.

P451

Familial hypocalciuric hypercalcemia: mutation in the calcium sensing receptor gene

Sandra Paiva[1], Cristina Ribeiro[1], Luisa Barros[1], Leonor Gomes[1], Miguel Melo[1], Joana Guimarães[1], Margarida Venâncio[2], Jorge Saraiva[2] & Manuela Carvalheiro[1]
[1]Serviço de Endocrinologia, Diabetes e Metabolismo dos Hospitais da Universidade de Coimbra, Coimbra, Portugal; [2]Serviço de Genática Médica do Hospital Pediátrico de Coimbra, Coimbra, Portugal.

Familial hypocalciuria hypercalcemia (FHH) is an autossomal dominant condition caused by mutations in the calcium sensing receptor gene. It is characterized by moderate hypercalcemia, with normal or slightly elevated PTH levels and hypocalciuria secondary to the increased calcium reabsorption at the distal tubule level.
We present a case report of a 16 year old patient, who was referred to our department at the age of 14 because of obesity (BMI: 36.9 K/m^2). Initial biochemical evaluation revealed hypercalcemia (11.1 mg/dL – Normal range: 8.4–10.4) and normal albumin levels. These findings prompted further evaluation, with the following results: PTH: 98/92 pg/ml (*N*: 9–72), urine 24 hour calcium levels: 92 mg/24 h (*N*: 100–300) and cervical ultrassonography revealed a small 5 mm nodular structure. One could however not exclude that this was in fact parathyroidal tissue. Cintigraphy with Sestamibi did not show abnormal fixation. Given these results, further study was pursued in 1st degree relatives, and it was found that the father and one of the siblings had slight hypercalcemia and hypocalciuria.
Genetical analysis of the *propositus* undercovered a heterozygous mutation in R648X of CASR gene (located in the long arm of chromossome 3).
This case underscores the relevance of genetical characterization in disturbances of calcium metabolism, in particular in differential diagnosis of hyperparathyroidism and FHH, which is often difficult in light of conventional assessment. Accurate diagnosis is essential for correct therapeutic management, which stresses the need for genetical analysis in current clinical practice.

P452

Structured assessment of neuroendocrine dysfunction following traumatic brain injury and aneurysmal subarachnoid hemorrhage – the interdisciplinary German database

Ilonka Kreitschmann-Andermahr[1], Harald Schneider[2], Michael Buchfelder[3], Bernhard Saller[4], Eberhard Koenig[5], Martina Jordan[1] & Günter Stalla[2]
[1]RWTH Aachen University, Aachen, Germany; [2]Max-Planck-Institute for Psychiatry, Internal Medicine/Endocrinology and Clinical Chemistry, Munich, Germany; [3]Erlangen University, Erlangen, Germany; [4]Endoscience GmbH, Munich, Germany; [5]Neurological Rehabilitation Clinic, Bad Aibling, Germany, ClinSupport GmbH, Erlangen, Germany.

The results of recent studies indicate that traumatic brain injury (TBI) and aneurysmal subarachnoid hemorrhage (SAH) must be considered as frequent causes of long-term disturbances of hypothalamo-pituitary function. Indeed, partial hypopituitarism has been established with a pooled frequency of 33% in TBI and of 48% in SAH survivors. Nevertheless, still little is known about risk factors and clinical characteristics of pituitary impairment after these two types of brain damage.

In order to address these questions on a larger scale, a multi-center, structured data assessment to create a national registry of these patients has been established in 2005. It is coordinated by an endocrinological department in the south of Germany and is financed by an independent investigator grant. At present, 10 active neurosurgical, rehabilitation and endocrinological centers in all of Germany participate in the database. Ethical committee approval has been obtained for the project. Data are collected using a structured, internet-based study sheet, obtaining information on clinical, radiological and hormonal parameters. The database aims to connect clinical information on trauma and presence and type of hypopituitarism. At the first data close, which is due in November 2006 more than 500 patients with TBI ($n=322$) or SAH ($n=178$) have been included of whom clinical data and basal hormone values are available. In 112 TBI patients (34.8%) and 46 SAH patients (25.4%) additional endocrine function testing has been performed. This conference contribution aims to present the scientific results of the first data close and to introduce this epidemiological tool which is open to all disciplines treating patients with brain injury in Germany to the European scientific community. The authors present this database on behalf of all participating centers.

P453

Normal age-dependent values of serum insulin growth factor (IGF)–I: results from a healthy Italian population

V Gasco[1], G Aimaretti[2], M Boschetti[3], G Corneli[2], D Valle[4], M Borsotti[5], A Rossi[4], S Rovere[1], L Fazzuoli[3], D Ferone[3], E Ghigo[1] & F Minuto[3]
[1]Division of Endocrinology and Metabolism, Department of Internal Medicine, University of Turin, Torino, Italy; [2]Endocrinology, Department of Clinical and Experimental Medicine, University of Piemonte Orientale, Novara, Italy; [3]Endocrinology, DISEM, University of Genova, Genova, Italy; [4]Eli Lilly Italia SpA, Sesto Fiorentino, FI, Italy; [5]SOD Safety and Quality, Careggi Hospital, Firenze, Italy.

Serum IGF-I levels were measured in 547 non-hypopituitaric, non acromegalic healthy subjects of both sexes in Italy to develop reference values in relation to age and gender. Participant subjects were stratified in three age classes (25–39, 40–59 and ≥ 60 years) and IGF-I assay was carried out by double-antibody radio immunoassay. The Pearson's correlation coefficient between age and IGF-I values was calculated by sex and pre-defined age ranges. IGF-I levels significantly decreased with age ($P<0.001$, Kruskal-Wallis test) while age was not a significant factor. The median IGF-I levels were 206 ng/ml in the range 25–39 years, 147 ng/ml in the range 40–59 years and 103 ng/ml in the range ≥ 60 years. The Pearson's correlation coefficient confirmed the negative correlation between age and IGF-I levels in the total sample of subjects ($r=-0.529$), with no sex-effect ($r=-0.570$ in males and $r=-0.529$ in females). No correlations were also found in the 25–39 years ($r=-0.036$) and in the 40–59 years range ($r=-0.080$), while in subjects aged >60 years, IGF-I levels tended to further decrease with increased age ($r=0.389$). Ranges of normal values set at the 2.5th–97.5th percentile in the 3 age ranges were 95.6–366.7 ng/ml between 25–39 years, 60.8–297.7 ng/ml between 40–59 years and 34.5–219.8 ng/ml in subjects aged ≥ 60 years. This study may contribute in the development of age-specific reference ranges for IGF-I determination in serum of normal subjects of either sex, irrespective of the used method of assay.

P454

Four cases of propylthiouracil–induced antineutrophil cytoplasmatic antibody-associated autoimmune syndrome

Zsuzsanna Nagy, Laszlo Bajnok, Beata Bodis, Zsuzsanna Keszthelyi, Orsolya Nemes, Karoly Rucz, Beata Ruzsa, Csaba Gyorgy Kiss, Gabor Kumanovics, Gabor Suto, Timea Berki & Emese Mezosi
University of Pecs, Pecs, Hungary.

Drug-induced vasculitis or lupus-like syndromes can complicate the clinical course of PTU-treated patients. The clinical manifestations of four patients treated with PTU for Graves' disease are presented.

A 37-year-old woman was treated with PTU for six years. She had severe thyrotoxicosis, high fever and polyarthralgia. Elevated doses of PTU resulted in normalization of thyroid function, but the fever and arthralgia persisted even after steroid administration. ANA, a-MPO, a-PR3 and a-cardiolipin IgM positivities were detected. The patient underwent thyroidectomy. Eight months after the withdrawal of PTU she was asymptomatic with negative serology.

A 34-year old woman was previously treated with PTU for two years. Four years later hyperthyroidism recurred. After PTU therapy she presented with urticaria vasculitis and thrombocytopenia. A-MPO, a-PR3, a-phosphatidil-serine tests were positive. Skin biopsy showed cutan vasculitis. After radioiodine therapy her symptoms resolved within three months.

A 55-year old woman was treated with PTU for six years. She complained arthralgia and a-MPO positivity was found. PTU treatment was stopped which resulted in the complete resolution of her symptoms.

A 53-year old woman received PTU for four years. After one year of treatment, a necrotising vasculitis was diagnosed with renal and pulmonary involvement. Screening for ANA and a-MPO were positive. She was treated six times with bolus cyclophosphamide and continuous oral prednisolone. The PTU therapy was discontinued recently.

The differential diagnosis between drug-induced and idiopathic vasculitis may be difficult in the individual patient, but failure to recognize the relationship with drug can lead to fatal organ damage. In two-thirds of the patients with PTU-induced autoimmune syndromes the stopping of the drug-therapy alone leads to rapid and complete resolution.

This work was supported by the Hungarian Research Found (OTKA) F037639/2002, F042912/2003.

P455

Difficult management of a thyrotoxic patient with abnormal liver function tests

Shabeeha Memon, Robin Polson & Hisham Maksoud
King's Mill Hospital, Sutton In Ashfield, United Kingdom.

Thyrotoxicosis is associated with abnormal liver function test through a poorly understood mechanism.

We report a case of a 67 years old lady presenting with retrosternal chest pain and weight loss. Examination was unremarkable other than marked spider naevi. The Liver function tests showed raised ALT, GGT and Alkaline Phosphatase. She had profoundly deranged thyroid function tests with raised T3 and T4 with highly suppressed TSH.

Hepatic ultrasound showed an irregular mass. A CT scan of Chest and Abdomen showed Liver malignancy (primary or secondary) with lung metastasis and retrocrural lymphadenopathy. A CT guided biopsy confirming Hepatocellular carcinoma.

She was referred to Oncology for further input and started on treatment with carbimazole.

This lady's liver mass could easily have been overlooked if weight loss was attributed solely to thyrotoxicosis, causing a delay in diagnosis.Treatment for this lady is far more complicated than it appears. She was admitted with neutropenic sepsis secondary to carbimazole even before chemotherapy was commenced, which complicated the management further.

She is not a candidate for Radio- iodine to avoid exposure to healthcare workers in the post radiation phase or surgery because of the progression of the tumour and thyrotoxic state.

She was treated with steroids and a limited course of Lugol's iodine until her white cell count recovered sufficiently to allow introduction of Propylthiouracil.

This case illustrates the importance of carbimazole-induced neutropenia and the need to be vigilant in the management of altered liver function tests with thyrotoxicosis.

P456

Dermatological manifestations of the neuroendocrine cancer: a four cases report of primitive and metastatic Merkerl cell tumor

Francesco Buda, Roberto Colle, Oreste Bergamo, Gordana Svenda & Concetta Buda
Dpt Int Med & Medical Oncology. City of Udine Hospital, Udine, Italy; Dpt Int Med & Medical Oncology. City of Udine Hospital, Udine, Italy; Dpt Int Med & Medical Oncology. City of Udine Hospital, Udine, Italy; Dpt Int Med & Medical Oncology. City of Udine Hospital, Udine, Italy; Dpt of Surgery, Univ of Messina, Messina, Italy; Dpt of Radiation Oncology,Univ of Messina, Messina, Italy.

Merkel cell cancer, is a very rare, malignant, neuroendocrine tumour. of the skin The cause is not known. Is and often aggressive malignancy with high tendency for local recurrence, lymph node involvement and distant metastasis and a poor prognosis and rapid progression. The Merkel cell is located in or near the basal layer of the epidermis and is closely associated with terminal axons. The aims in this study we report four cases of Merkel cell tumor of the skin(1 primary and 3 metastatic).The primary carcinoma occurred as multiple dermal nodules on the right arm showing a fast growth and spreading to regional lymph nodes.In the metastatic cases the primary tumor was often ulcerated and local regional metastatis were massive.The main diagnostic role of electron microscopic studies of the primary lesion and the importance of the immunohistochemistry are validated. Superficial lesions were easily detected by fine needle aspiration biopsy and histological examination of surgical excisions.The Surgical of primary tumor were followed by a high incidence of local recurrence and distal metastasis(3 /4 pts);median DSF was 10 months.A correct surgical treatment of primary lesions,independent of site, may influence the rate of local regional invasion.For this reason a close follow-up is advisable, including the seric control of NSE levels because of the good correlation of this enzyme to disease outcone.Since the role oh the complementary therapies has not been completelyestablished, adjuvant therapy may be reserved for high risk pts(young aged,with high L.I.,with lymphatic and /or haematic involvemen-t.As standardized protocols in Merkel cell tumour are lacking, AA. Suggest that the primary treatment consider a wide surgical excision of the primary lesion and regional lymph nodes followed bay local regional radiotherapy. Metastatic cases are treatated with chemotherapeutic regimens used for oat cell carcinoma of the lung because of the close morphobiological similarity exsting between these two tumors.

P457

Retrospective analysis of diagnostic and treatment outcomes of primary aldosteronism

Nikolette Szücs[1], Edit Gláz[1], Ibolya Varga[1], Miklós Tóth[1], Róbert Kiss[1], Attila Patócs[1], Ferenc Perner[2], János Horányi[3], Ferenc Molnár[4], László Fütö[4] & Károly Rácz[1]
[1]Semmelweis University, 2nd. Department of Medicine, Budapest, Hungary; [2]Semmelweis University, Department of Transplantation and Surgery, Budapest, Hungary; [3]Semmelweis University, 1st Department of Surgery, Budapest, Hungary; [4]Markhot Ferenc Hospital, Ist Department of Medicine, Eger, Hungary.

The authors retrospectively analyzed the efficacy of diagnostic procedures and the outcome of treatment by the analysis of data of 187 patients with primary aldosteronism (PA) examined between 1958 and 2004 at the 2nd Department of Medicine of Semmelweis University. Aldosterone-producing adenoma (APA) was detected in 135 patients, whereas idiopathic hyperaldosteronism (IHA) was found in 46 patients. Other subtypes of PA included 5 patients with unilateral primary adrenocortical hyperplasia and one patient with adrenocortical carcinoma. Molecular biological studies of the aldosterone-synthase/11 β-hydroxylase gene chimera were carried out in 30 patients but none of them showed the presence of the chimeric gene. When comparing the clinical parameters of patients with APA and IHA, no significant differences were found in the time period between the diagnosis of hypertension and the diagnosis of PA, in blood pressure, or in serum potassium values. Normokalemic PA was found in 7 cases. The ratio of plasma aldosterone concentration (ng/dl) to plasma renin activity (ng/ml/h) was above 20 in all patients with APA and in all but 5 cases with IHA. The postural test combined with furosemide administration differentiated APA patients from those with IHA with a sensitivity of 69% and a specificity of 92%. In cases of adrenocortical adenomas not clearly detectable by radiological imaging techniques and in cases with bilateral adrenocortical adenomas, selective adrenal vein sampling was performed ($n=55$). All but 4 patients with APA underwent adrenalectomy. After surgery serum potassium concentration returned to normal in all patients showing low serum potassium levels before surgery. Also, the moderate to severe preoperative hypertension disappeared or improved after surgery. The relatively low frequency of normokalemic PA and a less frequent occurrence of IHA in this cohort of patients suggests that a significant number of PA cases that are not accompanied with severe hypokalemia may remain undetected in Hungary.

P458

A case with hypercalcemia caused by hyperparathyroidism and multiple myeloma

Banu Avcioglu[1], Taner Bayraktaroglu[1], Ayse Kubat Uzum[1], Sevgi Kalayoglu Besisik[2], Ferihan Aral[1], Nese Colak[1], Fatma Harika Boztepe[1] & Faruk Alagöl[1]
[1]Istanbul University, Istanbul Faculty of Medicine, Endocrinology and Metabolism, Capa/Fatih, Istanbul, Turkey; [2]Istanbul University, Istanbul Faculty of Medicine, Haematology, Capa/Fatih, Istanbul, Turkey.

Aim
Hypercalcemia is particularly complicated with hyperparathroidism or malignancy such as myeloma. There were several cases report abouth primary hyperparathroidism coexistent with benign monoclonal gammopathy or multiple myeloma. We present clinical managament of a patient who have hypercalcemia caused by hyperparathyroidism and multiple myeloma.
Case
Fifthy-two years old a women, she was complaint with weakness by anemia due to ferrum deficiency. During the evaluation, hypercalcemia and monoclonal gammopathy were detected, and she was admitted to the hospital. Hyperparathyroidism was diagnosed by hypercalcemia (12.6 mg/dl), hypophosphatemia (2.5 mg/dl) and increased parathyroid hormone (149 pg/ml) values. Multiple myeloma was diagnosed by serum gamma-globulin component of 3.47 g/dl with a monoclonal gammopathy spike and peripheral plasmacytosis of 7%. Serum and urine immunoelectrophoresis revealed abnormal IgG and kappa arcs. Multiple myeloma was defined by kappa chain and IgG type plasma cell discrasia in bone marrow biopsy. Glucocorticoid suppression decreased serum calcium levels. Parathyroid sonography and scintigraphy showed an adenoma. She was referred previously to surgery before the management of myeloma.
Conclusion
The association between primary hyperparathyroidism and monoclonal gammopathy was discussed in terms of possible pathogenetic mechanisms by several cases report in the literature. Primary hyperparathyroidism should be suspected in patients with hypercalcemia and multiple myeloma. Most suitable management should be done for each clinical condition.

P459

Prostate specific antigen (PSA) in women with menstrual disturbances and mastopathy

Stanislaw Radowicki & Michal Kunicki
Departement of Gynaecological Endocrinology, Medical University, Warsaw, Poland.

The aim of the study was to assess free and total prostate-specific antigen-PSA in serum of women with menstrual ditubances and mastopathy.
Material and methods
We examined 176 patients who were admitted to the Departement of Gynaecological Endocrinology with benign breasts pathology. According to clinical examination and sonographical findings women were didvided into two groups:

- group I: 114 with fibrocystic breast disease. Sonographical findings revealed the presence of cysts <10 mm in diameter.
- group II: 62 women with fibrocystic breast disease, cysts >10 mm in diameter.

The control group – 46 healthy women aged 18–45 years with regular menses and no pathological finding in ultrasonography examination
The menstrual patterns were definied according to presented classification:

- Eumenorrhoea- cycle lengh 21 to 35 days., Polymenorrhoea- cycle <25 days
- Oligomenorrhoea- cycle >32 days, Amenorrhoea secundaria - absence of menstruation for >180 days.

One-way analysis of variance ANOVA was performed and Mann-Whitney test when apopriate. $P<.05$ was considered statistically significant.
The mean free and total PSA concentrations in relation to menstrual disturbances in women with mastopathy. Presented as $x \pm SD$; *=differ significantly ($P<0.05$)

Menstrual pattern	Free PSA concetration(ng/ml) Total PSA concentration		
	Group I x ± SD	Group II x ± SD	Control x ± SD
Eumenorrhoea	0.18 ± 0.46	0.26 ± 0.84	0.13 ± 0.58
	0.55 ± 1.48	0.90 ± 2.84	0.35 ± 0.88
Oligomenorrhoea	0.15 ± 0.42	Undetectable*	–
	0.48 ± 1.23	0.09 ± 0.18	
Polymenorrhoea	0.29 ± 0.80	0.31 ± 0.70	–
	0.95 ± 2.75	0.97 ± 2.14	
Am. Secundaria	1.07 ± 1.51	Undetectable*	–
	3.25 ± 4.60	0.02 ± 0.03	

Conclusions
1. The mean free and total-PSA concentrations did not differ significantly between healthy women and women with mastopathy and regular menstruation
2. Women with cysts < 10 mm (group I) and oligomenorrhoea or amenorrhoea secundaria had significantly higher free PSA concentrations than women with cysts > 10 mm

P460

Selected parameters of lipid metabolism in patients with Turner's syndrome

Beata Wikiera, Ewa Barg & Anna Noczynska
Medical University, Department of Endocrinology and Diabetology for Children and Adolescents, Wroclaw, Poland.

Women with Turner syndrome (TS) more frequently develop cardiovascular disease. Abnormal lipid metabolism is a well known risk factor for ischemic heart disease. Adiponectin as well as cytokines are useful tool in evaluation the fat tissue metabolism.
The aim of the study was to evaluate the relationship between adiponectin, TNF-alpha, IL-6 and lipids in patients with TS.
Patients and method
The study group consisted of 87 girls with TS without clinical signs of thyroid dysfunction or diabetes mellitus. The mean age was 14.05 ± 6.06 (2–25) years. X chromosome monosomy was found in 59%, mosaicism in 30.12%, structural aberration in the rest of the patients. Most of them (54%) received GH treatment, 30% finished treatment prior to the study, 16% didn't start it yet. Height, weight, BMI, BMISDS, adiponectin, TNF-alpha, IL-6, cholesterol, TG, HDL, LDL, Lp(a), insulin, HBA_1c, IGF_1, $IGFBP_3$ were determined.
Results
Thyroid hormones values were within normal ranges in all the patients. Mean concentration of IL-6 was 8.44 ± 14.07 pg/ml, TNF-alpha was 4.92 ± 3.59 pg/ml, adiponectin was 14783.02 ± 7558.25 mg/ml. There was correlation between IL6 and TNF-alpha ($r = 0.33$), but not other examined parameters. Adiponectin correlated inversely with BMISDS ($r = -0.38$) and HBA_1c ($r = -0.39$). Several correlation was found between: insulin and BMISDS ($r = 0.43$), insulin and TG ($r = 0.51$), insulin and IGF_1 ($r = 0.63$), insulin and $IGFBP_3$ ($r = 0.57$).
We compared the group of GH treated patients with girls who finished GH therapy or didn't start it yet. GH treated patients had lower level of IL6 (7.36 vs 9.16 pg/ml) and higher level of adiponectin (15587.27 vs 14241.69 ng/ml). The difference however was not statistically important.
Conclusion
GH therapy seems to reduce IL6 level and probably augment adiponectin concentration and thus can be protective for ischemic heart disease.

P461

Successive gestational hyperandrogenism with maternal virilization and female pseudohermaphroditism

Chi-Huang Chen, Jah-Yao Liu & Gwo-Jang Wu
Department of Obstetrics and Gynecology, Tri-Service General Hospital, National Defense Medical Center, Taipei, Taiwan.

Objective
Successive female pseudohermaphroditism born to gestational hyperandrogenism accompanied by maternal virilization is extremely rare in literature.
Patient(s)
A housewife, age 29, G2P1A1, revealed no hyperandrogenism before pregnancy. She gave her first child birth complicated by maternal virilization and female fetal pseudohermaphroditism due to hyperandrogenemia of bilateral 6-cm ovarian luteoma at age 27. Peak maternal serum testosterone level as high as 11539 ng/dl (normal: 20–86) was evident. Spontaneous regression of ovarian size and hyperandrogenemia during the puerperium revealed the natural course of pregnancy luteomas, not true neoplasms. She returned to regular menstruation without symptoms and signs of hyperandrogenemia the following two years except irreversible deepening voice in the aftermath of high androgen exposure. She conceived her second pregnancy at age 29. Elevation of maternal serum androgen level commenced as early as 5 weeks gestation, followed by rising androgen level that positively corresponds to acne formation and emerging facial hair by increasing gestational age. A 46 XX karyotype was confirmed after chorion villi sampling at 12 weeks gestation. Both parents made a fully informed decision to terminate the pregnancy until 14 2/7 weeks gestation. Maternal testosterone level reached 751 ng/dl while ovarian size is normal at termination.
Result(s)
The abortus revealed apparently clitoral hypertrophy. The patient returns to normal androgen level two weeks later and free from virilization afterward, leaving lowering of her voice.
Conclusion(s)
Placenta may be protective by virtue of its high capacity to convert androgens to estrogen. Conversion of testosterone to oestradiol was inadequate to protect from high maternal testosterone concentration and, undoubtedly, this fetus would have virilised if female in our observation (1). The risk for male fetus is unknown. Expectant management is the treatment option as there are no pharmacological options which are safe in pregnancy. Imprudent surgical intervention should be withheld in this regard.

P462

Tetraploid/diploid mosaicism: case report of a 35-year-old woman

Sophie Schuldiner[1], Aline Artières[1], Jean Chiesa[2], Anne marie Guedj[1], Nathalie Jourdan[1], Jean louis Richard[1] & Michel Rodier[1]
[1]Endocrinology Department, Caremeau Hospital, Nimes, France; [2]Genetic Department, Caremeau Hospital, Nimes, France.

A lot of chromomosomal abnormalities have been described of which some are very unusual. Mosaicism refers to a condition where chromosomal (abnormalities) altered structure or number of chromosomes are present in some but not all cells. Polyploidy is defined as a condition where cells contain more than two homologous sets of chromosome, due to fertilization abnormalities; tetraploidy rarely allows birth of a living child but accounts for 6% of spontaneaous abortion. We report the case of a 35-year-old woman suffering from severe obesity treated by bariatric surgery; she complained of dizziness attributed to a reactive hypoglycaemia. She had a complex medical history including idiopathic hyperprolactinemia and spaniomenorrhea treated by cabergoline, hypothyroidism treated with levothyroxine, arterial hypertension associated with hypokalemia, bilateral cataract, right carpal tunnel syndrome, patent ductus arteriosus requiring surgery at the age of 14, removal of nevi, papillary malformation and iris muscle dysfunction. Her weight was 106 kg, her height 151 cm. On examination, she presented with a shortened 4th metarcarpal bone, a moderately ogival palate, a short neck and multiple nevi throughout the body. Biologically, no evidence for reactive hypoglycaemia or hyperalmdosteronism was found. Karyotype was normal (46,XX). After stopping hypnotic treatment (antidepressants) and cabergoline, prolactin level was normal but there was a GH deficiency as evidenced by a low IGF-1 level and a GH peak < 2 mU/L after stimulation. Finally, a mosaicism was found on karyotyping a cutaneous biopsy showing both diploid and tetraploid (28%) cells (46,XX/92,XXXX).
Tetraploidy is caused by a mitotic failure during the early stage of zygotic development. Mosaicism is responsible for a great number of congenital abnormalities and a wide range of mental and growth retardation. Currently, fourteen cases have been reported in the literature: five patients deceased in the early life and the older living patient is 21 years of age.

P463

Genotype-phenotype correlation in Romanian patients with classical forms of 21-hydroxylase deficiency

Anca Zimmermann[1], Paula Grigorescu Sido[2], Camelia Al-Khzouz[2], Tiberiu Nistor[2], Matthias M Weber[1] & Egbert Schulze[3]
[1]University of Mainz, Mainz, Germany; [2]University of Cluj, Cluj, Romania; [3]Molekulargenetisches Labor Gemeinschaftspraxis Raue, Heidelberg, Germany.

Congenital adrenal hyperplasia (CAH) comprises autosomal recessive disorders mainly due to defects in the 21-hydroxylase (CYP21) gene. We aimed to perform a genotype-phenotype analysis in Romanian patients with classical 21-hydroxylase deficiency.
Patients and methods
We included 42 patients (13 males, 29 females, 19 with the salt wasting (SW) form, 29 with the simple virilizing (SV) form. Molecular analysis was performed by direct sequencing of PCR amplified products of the CYP21A2 gene.
Results
Age at diagnosis in SW patients was 23 ± 5 days in females, 30 ± 11 days in males. Female SV patients were diagnosed at 28.5 ± 43 months, males with SV were diagnosed at 8 ± 9.6 years. The most frequent mutation in Romanian patients with 21-hydroxylase deficiency was a splice site mutation in intron 2 (IVS2-13A/C>G) (43.2%), followed by deletions and large conversions and the I172N mutation in exon 4, accounting for 14.9% each, a triple mutation (P30L+ IVS2-13A/C>G+ deletion of 8 bp in exon 3) (13.5%), P30L (6.8%), different double mutations (5.4%) and R356W (1.4%). Genotypes were divided in 3 mutation groups (0, A, B), according to their predicted functional consequences and compared to clinical phenotype. Positive predictive values were 100%, 76.5% and 78.3% for group 0, A and B respectively. Overall genotype-phenotype correlation was 88.1%. In female patients we observed in genotype group 0 only severe virilization (Prader-IV), in group A there was a tendency to severe virilization (5 patients with Prader-IV, 3 with Prader-III and 2 with Prader-II), while in group B all Prader stages were encountered (2 patients with Prader-I, 4 with Prader-II and III, respectively and 6 with Prader-IV).
Conclusions
Genotype-phenotype correlation in our patients with 21-hydroxylase deficiency was high, with an overall value of 88.1%. Severe genotypes resulted in more pronounced clinical virilization, expressed as higher Prader stages.

P464

Cushing's syndrome in paediatric age – casuistic, evolution of investigation tests and treatment options in our institution throughout the last 20 years

Miguel Melo[1], Ana Fagulha[1], Isabel Paiva[1], Joana Guimaraes[1], Carla Baptista[1], Fernando Gomes[2], Francisco Belo[2] & Manuela Carvalheiro[1]
[1]Endocrinology Department, University Hospital of Coimbra, Coimbra, Portugal; [2]Nerosurgery Department, University Hospital of Coimbra, Coimbra, Portugal.

Cushing's syndrome is a rare disorder in children and adolescents. The diagnosis can be a challenge for the clinician, as its principal feature – obesity – is extremely common. We present three cases diagnosed in the last 20 years. The first one was a boy aged 17 that presented in 1984 with central obesity, acne, moon face with plethora, abdominal striae, easy bruising and skin atrophy. The investigations performed consisted in cortisol and ACTH plasma measurements (8/24 hours), low and high dose Dexamethasone Suppression Test (DST), and metyrapone test; the results were consistent with Cushing's disease. A head CT scan did not show evidence of any pituitary lesion. A trans-sphenoidal (TS) surgical exploration was performed with removal of a micronodular lesion; histology confirmed it was a corticotrophinoma. Since then, this patient has been in clinical and biochemical remission. The second case is a girl investigated in 1997 when she was 17 years old for secondary amenorrhea, obesity, hirsutism, acne and purple striae. She had cortisol and ACTH plasma measurements (8/24 hours), low and high dose DST and a CRH test that confirmed the hypercortisolism and were suggestive of a pituitary cause. A pituitary MRI scan showed a probable microadenoma. Before TS removal of the adenoma, she was treated with metyrapone. Six months after surgery she resumed regular menses. A third patient, aged 14, presented with slow growth pattern, obesity, hirsutism, striae and amenorrhea in 2002. The investigation was similar to the second case and a pituitary MRI showed an 8 mm adenoma. After TS surgery, she had biochemical remission. One year after, she had recurrence of the disease and a second surgery was performed. Since then, she has showed consistent remission, resumed regular menses and became pregnant without medical help. None of these patients has hypopituitarism now. These cases illustrate the importance of a timed diagnosis, as it may allow total remission of the disease with preservation of anterior pituitary function, a factor of major importance at this age. We analyze the evolution of investigations and therapeutic options available in our institution.

P465

Thyroidectomy as the last chance treatment for life threatening thyrotoxicosis: a case report

Margaret Gietka-Czernel, Helena Jastrzebska, Wojciech Zgliczynski, Agnieszka Dudek & Marek Szczepkowski
Medical Center of Postgraduate Education, Warsaw/Mazowieckie, Poland.

54-year old woman with Graves' disease was admitted to Endocrinology Department because of severe thyrotoxicosis and antithyroid drugs inolerance. The apathetic form of thyrotoxicosis was diagnosed; she lost 12 kg during 3 months and she had heavy muscle weakness. Previously she demonstrated allergic skin reactions (macular rushes) after both: Methimazole and Propylthiouracil. At admission her TSH was 0.001 mU/l, fT3 24 pg/ml, fT4 37 pmol/l. Lithium, propranolol and glucocorticoids were instituted but within few days she deteriorated and threatening thyroid storm was noted. She was given low doses of Methimazole, iopanoic acid, propranolol and glucocorticoids iv. Both clinical and biochemical performance improved during the next days but hepatitis probably due to Methimazole developed. Methimazole and iopanoic acid were stopped and after etablishing T24 RAIU 50%, 20 mCi 131-I was administered. Subsequently glucocorticoids, lithium and propranolol were continued. She became stable for several days and then deteriorated again. Her fT3 and fT4 were 9,4 pg/ml and 44 pmol/l respectively. She was transferred to Surgical Department and successful bilateral subtotal thyroidectomy was performed. Three days after surgery her fT3 and fT4 were within normal range. Substitution with L-thyroxine was started on the third week and no relapse of thyrotoxicosis has occured so far.
Conclusion
Thyroidectomy should be concidered as a method of treatment for severe life threatening cases of thyrotoxicosis.

P466

Insulinoma and gastrinoma in MEN 1: case report

Margaret Gietka-Czernel, Janusz Krassowski & Wojciech Zgliczynski
Medical Center of Postgraduate Education, Warsaw/Mazowieckie, Poland.

23-year old man was admitted to Endocrinology Department because of hypogonadism and pituitary tumor seen at CT. Diagnosis of prolactinoma was established based on high serum PRL level- 800 ng/ml and therapy with bromocriptine was instituted. On the next year a temporary loss of consciousness related to physical exertion occured. The neurological reasons were excluded and laboratory tests showed hypercalcemia, hypophosphatemia and elevated serum PTH levels. Prolactinoma and hyperparathyroidism made the diagnosis of MEN 1 so the insulinoma as the cause of consciousness loss was taken into account. During fasting test hypoglicemia 36 mg/dl and hyperinsulinemia 40 μU/ml was documented. Therapy with diazoxide was instituted and patient was transferred to Surgical Department. Insulinoma was not found no preoperatively nor during surgical exploration. Distal subtotal pancreatectomy was carried out but hyperinsulinemia persisted. Micoscopical analysis showed multiple pancreatic adenomas up to 0.5 cm in diameter.

On the next year subtotal parathyreoidectomy was established. 5 years later, abdominal pain and nusea occured. During gastro-duodenal endoscopy gastric hyperemia and wide duodenal ulcer was seen. Elevated levels of BAO- 15 mEq/h, MAO- 38 mEq/h and gastrin- 530 pg/ml were relevant to gastrinoma. The patient did not accepted further diagnostic procedures nor possible surgical treatment.

This case shows some different features of insulinoma associated with MEN 1 compared to sporadic insulinoma: 1/ insulinoma in MEN 1 is usually multifocal and surgery might be unsuccessful, 2/ GEP in MEN 1 can be multihormonal so strict clinical and biochemical surveillance is needed.

P467

Multiple endocrine dependent tumours in a dog patient without measurable endocrine consequences

Julianna Thuroczy[1], Aniko Tibold[1] & Lajos Balogh[2]
[1]SzIU, Faculty of Veterinary Science, Budapest, Hungary; [2]FJNCH, National 'FJC' Research Institute for Radiobiology and Radiohygiene, Budapest, Hungary.

Testicular neoplasmas are 5–15 percent of total tumours number in male dogs. Seminomas are the most common type of testicular tumours in dog.

The thyroid tumours are large, unilateral palpable masses in neck region in most of the cases. Although seventy percent of malignant thyroid neoplasmas are carcinomas, 5–20% of them are endocrinologically active which induce the clinical signs of hyperthyroidism.

Seven – twenty one percent of skin tumours are mastocytomas in dog but the incidence of them is higher in spayed female and intact male dogs, which should indicate the testosterone dependency.

Eight years old argentin dog was present at our clinic with clinical signs of alopecia, weight loss and ointment faeces. Plasma biochemical parameters were in reference ranges. The total thyroxin concentration was 30.11 nmol/l which is fit to euthyroid state. An altered density focus in right testis was visualized by the ultrasonographic examination. Neither testosterone nor estrogen serum concentrations were high. The Tc-pertechnetate uptake of left thyroid gland was increased in opposite the visualisation of right thyroid gland was decreased. The left thyroid gland, both testes and a 1 cm diameter nodule in skin were surgically removed.

Seminoma in both testes, follicular compact cell carcinoma and C-cell carcinoma in removed thyroid gland and Grade-II type mastocytoma in skin were histologically established.

The faeces got the normal consistency following the operation. The hair grows finished in sixth week after the operation. The thyroxin concentration after transient decrease reached the 35.48 nmol/l level in four month. Plasma TSH concentration was 0.272 ng/ml.

The combination of three different endocrine tumours with a suspected hormone dependent tumour suggests the relation of their development. I spite of hormone dependent tumours the plasma hormone levels were ambiguous and reached to diagnosis with use of complex diagnostic imaging techniques.

P468

Case report: Adrenal glands and stress: hypercortisolism in the course of urosepsis

Lucyna Papierska, Michal Rabijewski & Anna Kasperlik-Zaluska
Center for Postgraduate Medical Education, Endocrinological Department, Warszawa, Poland.

A 76-year obese women with diabetes mellitus t II, hypertension and urolithiasis was referred to our clinic for reason of high cortisol levels, which were detected in regional hospital, two days after cystoscopy and catheterization of right ureter. Cortisolemia was 35 μg% at 8.00 and 37 μg % at 22.00. Short dexamethasone test didn't cause cortisol suppression (cortisolemia after 1 mg DXM was 27 μg %). After two days of dexamethasone (4×2 mg) blood cortisol level was 17 μg %. Blood samples were taken during the antibiotic therapy.

In our clinic we performed a CT scans of kidneys and adrenal glands. It revealed little tumour of left adrenal gland (size 14 mm and low density). Then, Cushing syndrome of adrenal origin was suspected.

Eight hours after the examination, patient's temperature ran up to 39 °C and symptoms of urosepsis occurred. Cortisol level during this event (at the evening) was >50 μg %, DHEAS was low (348 ng/ml). Surprisingly, ACTH level was very high (323 pg/ml).

After ten days of the treatment with ciprofloxacin, when patient's general condition became good, endocrinological tests were repeated. Cortisol levels were normal, with maintained circadian rhythm (18.7–8.6 μg %), ACTH levels were 16 (8^{00}) and 5 (22^{00}) pg/ml. Dexamethasone caused proper suppression of serum cortisol (1,8 μg % after 1 mg), and MRI revealed little hypophysis, without adenoma. The tests were repeated after three months, results were also normal.

In conclusion

Observed disorders came out of normal physiological reaction of hypothalamo-pituitary-adrenal axis to stress – in described case to serious infection. Little adrenal adenoma might contribute to very brisk cortisol response to high, 'stressed' ACTH levels.

P469

Long-term experience and pharmacogenetic aspects of safety in 101 treatment-years with a long-acting formulation of testosterone undecanoate in substitution therapy of 66 hypogonadal men

Michael Zitzmann & Eberhard Nieschlag
Institute of Reproductive Medicine, Muenster, 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 new feasible modality is the intramuscular injection of the long-acting ester testosterone undecanoate (TU).

Design

Analysis of safety data accumulated during 101 treatment-years in 66 hypogonadal men receiving altogether 510 injections of 1000 mg TU in 10–14-week intervals. 35 men had primary, 27 secondary and 4 late-onset hypogonadism. A minimum of 4 injections was necessary for data entry, maximum duration of individual treatment was 9.5 years. Primary endpoints were PSA levels, prostate size, erythropoeisis, lipoprotein profiles and blood pressure. Putative modulators of safety parameters entering regression models were nadir total testosterone concentrations, age (range: 17–66 years), body mass index and androgen receptor CAG repeat length (range: 15 –29).

Results

The medication was well tolerated. PSA levels did not exceed 2.9 ng/ml. Overall, therapy-induced changes within the normal range of PSA, prostate size and erythropoeisis were more pronounced in men with higher nadir testosterone concentrations and shorter androgen receptor CAG repeats (independently and with high significance respectively). Factors leading to observations of adverse nature (assessments beyond normal limits) such as elevated hematocrit, increased blood pressure and unfavorable lipoprotein constellations were due to obesity and advanced age, but not testosterone levels or receptor properties. The absolute incidence of such events remained below 10% of all assessments respectively.

Conclusion

Intramuscular injections of testosterone undecanoate represent a feasible, safe and well-tolerated modality of androgen substitution in hypogonadal men. Testosterone treatment with this regimen is modulated by the androgen receptor CAG repeat polymorphism. Adverse observations are due to obesity and advanced age, but not testosterone levels *per se*.

P470

Thyrotoxic hypokalemic periodic paralysis in two Caucasian females

Cristina Preda, Letitia Leustean, Carmen Vulpoi, Cristina Cristea, Christina Ungureanu & Eusebie Zbranca
University of Medecine 'Gr.T.Popa', Iasi, Romania.

Hypokalemic periodic paralysis is an uncommon complication of thyrotoxicosis especially in Caucasian women. It is most frequent in east asian and Japanese males and is characterized by recurrent episodes of motor weekness of variable intensity associated with hyperthyroidism. It is usually associated with low plasma potassium levels and is often precipitated by physical activity. This condition is a self limiting disorder that is cured by the treatment of the underlying hyperthyroidism. We report two cases of acute onset weakness followed by paraplegia from periodic paralysis in two Caucasian female patients aged 69, 51 respectively. Both patients presented hypokalemia and thyroid function tests showed hyperthyroidism. Oral potassium and antithyroid drugs (thiocarbamides) resulted in disappearance of symptoms. Thyrotoxic hypokalemic periodic paralysis is often under-recognized. This cases shows that thyrotoxic hypokalemic periodic paralysis is not confined only to east-asian males but also to Caucasian females. The treatment with antithyroid drugs and oral potassium given as soon is possible is successful.

P471

Pseudophaeochrocytoma presenting with catatonia - a novel observation

Bahram Jafar-Mohammadi[1], Thomas Walsh[2], John Barry[1], James Ryan[1], Eleanor Mullan[3], Eugene Cassidy[3], Michael O'Connor[2] & Domhnall O'Halloran[1]
[1]Dept. of Endocrinology,Cork University Hospital, Cork, Ireland; [2]Dept.of Geriatric Medicine, Cork University Hospital, Cork, Ireland; [3]Dept. of Psychiatry, Cork University Hospital, Cork, Ireland.

A 74 year old lady was admitted with an agitated depression. She had been taking Lorazepam and Olanzapine throughout the preceding 6 months. Escitalopram had been introduced 2 months prior to admission and the dose was escalated 3 weeks prior to presentation. The dose of Olanzapine was doubled at the same time.

She was treated with sotalol for atrial fibrillation and she had documented labile hypertension (BP range 77/57–250/118). She had fluctuating levels of consciousness and developed catatonia on day 20 of her admission. 24 hour urinary cathecholamines were reported as:

- Noradrenaline 4100 nmol/24hrs (160–485)
- Adrenaline 854 nmol/24hrs (27–165)
- Dopamine 5486 nmol/24hrs(1300–3000)

The patient was referred to our endocrine service on day 21 of admission. Olanzapine and Escitalopram were stopped and she was commenced on phenoxybenzamine (via NG Tube). Within 24hours her level of consciousness had returned to normal. Her alpha-blockade therapy was escalated until a postural drop in BP was achieved.

A CT body (contrasted), MIBG scan and MRI brain were normal.

The patient has remained clinically well, with no features suggestive of phaeochromocytoma 8 months after presentation. These observations and the normalisation of her urinary cathecholamines and negative radiological investigations support a diagnosis of pseudophaeochromocytoma secondary to either Olanzapine or Escitalopram. Catecholamine levels have remained normal in this patient while off antipsychotic and SSRI therapy. This we believe is the first presentation of pseudopheochromocytoma with catatonia as a dominant feature.

This case illustrates the need for vigilance in making a diagnosis of phaeochromocytoma in patients who are on drugs which alter neurotransmitter metabolism.

Table 1 Urinary Volume and Cathecholamine excretion/24 hours

Day of admission	8	24	25	43	66	Range
Volume	1440	962	514	1745	2320	mls
Noradrenaline	4100	638	537	92	197	160–485
Adrenaline	854	176	101	–	–	1300–3000
Dopamine	5486	1100	1108	710	979	600–1300

P472

Relapse of hyperthyroidism in Graves' disease after long-term drug treatment

Emese Mezosi, Orsolya Nemes, Beata Bodis, Zsuzsanna Nagy, Beata Ruzsa, Karoly Rucz & Laszlo Bajnok
University of Pecs, School of Medicine, Pecs, Hungary.

The optimal treatment of hyperthyroidism in Graves' disease is still an unresolved question. Hyperthyroidism recurs in 50% of patients after discontinuation of antithyroid therapy. In this retrospective study, Graves' patients investigated in the endocrine unit of Pecs University between December 2004 and October 2006 were enrolled (68 women, 22 men, age 47 (15–79) years). Antithyroid drug therapy was applied for a minimum of one year and the treatment was withdrawn for at least 5 months. The duration of antithyroid therapy was much longer than usually recommended, on the average 3,4 years, the median follow up was 20 months. The relapse rate in the group of patients treated over two years (on the average 4,6 years) was even higher (59%) than in the group treated for 1–2 years (50%) ($P=0.008$). Predictors of the relapse were age <40 years at the onset of disease, enlarged thyroid gland, positive TSH-receptor antibody (TRAK) level, other autoimmune disease, endocrine orbitopathy and thiamazole allergy. The relapse rate was lower after block-replace treatment regimen (40% versus 64%, $P<0.001$). Recurrence of hyperthyroidism was more frequent in women (58%) than in men (45%, $P<0.001$). The nodularity of the thyroid gland and the negative TRAK level did not affect the recurrence of thyrotoxicosis. In conclusion, long-term (over two years) treatment of Graves' disease did not decrease the risk for relapse after discontinuation of drug therapy.

Clinical case reports – presented on Tuesday

P473

Bloch-sulzberger syndrome, hypothyroidism and a pituitary incidentaloma: a case report

Marko Stojanovic[1], Dragana Miljic[1], Mirjana Doknic[1], Marina Djurovic[1], Sandra Pekic[1], Milos Nikolic[2] & Vera Popovic[1]
[1]Institute of Endocrinology, Diabetes and Diseases of Metabolism, University Clinical Center of Serbia, Belgrade, Serbia; [2]Institute of Dermatology and Veneral Diseases, University Clinical Center of Serbia, Belgrade, Serbia.

A female patient, 34 years old, was referred to endocrinologist, for an incidentally discovered interstellar mass on MR, mild subclinical hypothyroidism and hyperprolactinemia, and irregular menstrual cycles. She was diagnosed with Bloch-Sulzberger syndrome (BSS) in neonatal age. Epilepsy, her most prominent component of BSS, was well controlled but only with triple anticonvulsant therapy (Valproate, Carbamazepine, Clonazepam). She was obese, clinically euthyroid, and exhibited dermal, ocular and dental signs of the late phase of BSS. Elevated serum lipids and insulin resistance were observed. Mild hypothyroidism, with negative anti-thyroid antibodies was confirmed, with a response in TRH test pointing to primary hypothyroidism accompanied by mild hyperprolactinemia, responsive to TRH. Normal basal gonadotropins with a slow response to LHRH test were observed. Slightly lower IGF-1 was accompanied by a low normal response of GH to GHRH-GHRP-6. The pituitary tumor apparently exhibited no hormonal activity and no mass effects were observed by profile craniography and computerized perimetry. It was thus decided that it currently demanded only surveillance. The mild thyroid, reproductive and metabolic disturbances were attributed to the known side effects of antiepileptics. Lacking the opportunity to exclude the antiepileptic drugs and thus revert their side effects, a decision was made to relieve the subclinical hypothyroidism by levothyroxine replacement. Two months after introducing the replacement therapy, a marked clinical and laboratory improvement was notable.

BSS is a rare, X linked syndrome caused by an inactivating mutation in the NEMO gene. Dermal manifestations are the most prominent, followed by neurological (including epilepsy), ocular, dental and other. It is also associated with a higher tumor incidence. There is a possibility that a pituitary tumor, as observed in our patient, can represent a component of BSS, which was never previously reported.

P474

Extreme obesity as an important obstacle in diagnosing a patient with MEN1

Przemyslaw Witek[1], Wojciech Zgliczynski[1], Piotr Zdunowski[1], Andrzej Cichocki[2] & Jaroslaw B. Cwikla[3]
[1]Department of Endocrinology Centre for Postgraduate Medical Education, Warsaw, Poland; [2]The Marie Sklodowska-Curie Institute of Oncology, Warsaw, Poland; [3]MSWiA Hospital, Warsaw, Poland.

Background
MEN1 is an autosomal dominant inherited syndrome. Primary hyperparathyroidism, tumors of the endocrine pancreas, and of the pituitary, are the characteristic features of the syndrome.
Objective
To present a case of MEN1 in a patient with extreme obesity, causing serious difficulties in diagnostic procedures leading to localization of pancreatic tumors.
Case presentation
22-year-old male with extreme obesity (BMI 59), hypogonadism, gynaecomastia, galactorrhoea and duodenal ulceration, came to our Department before a planned surgery for obesity. Hormonal tests showed elevated levels of PRL (2000 ug/l), low levels of LH/FSH (<0.1 U/l) and of testosterone (0.7 ng/ml) with heighten estradiol (55.6 pg/ml) and elevated levels of S-DHEA (12312 ng/ml). MR examination showed a pituitary macroadenoma, size $45\times32\times25$ mm. Treatment with bromocriptin lowered PRL to 45 ug/l. Further diagnostic confirmed hyperparathyroidism (PTH-115 pg/ml; Ca-10.41 mg/dl) and a left adrenal gland tumor. High levels of gastrin (1340 μU/ml; $N<115$) and of chromogranin A (833 U/l; $N<18$) led to suspicion of gastrinoma. Endosonography showed 4 hypoechogenic foci in the head of pancreas. Octreoscan confirmed a high expression of somatostatin receptors. It was impossible to perform computed tomography because of the extreme obesity. In spite of that subtotal splenopancreatectomy, left side adrenalectomy and subtotal gastrectomy were performed. Histopathological examination confirmed multifocal well differentiated

neuroendocrine carcinoma with a single metastasis to lymphatic node, and a benign adrenal tumor. Postoperative scintigraphy did not show abnormal uptake of radioisotope. The level of gastrin decreased to 113 μU/ml, and of CgA to 81 U/l. Patient is currently treated with IPP and bromocriptin. In case of relapse or liver metastasis radiotherapy will be considered, using radiolabeled somatostatin's analogs.

Summary

Localizing diagnostic and treatment procedures in cases of tumors of the endocrine pancreas as a part of MEN1 remain a significant challenge. In case of the above mentioned patient the decision of surgery was made on the basis of the result of octreoscan and endosonography because the extreme obesity made computed tomography impossible.

P475

Six months physiological DHEA substitution in female adrenal failure: impact on quality of life and sexual parameters.

Jens J Christiansen[1], Mimi Mehlsen[1], Anna Maria Geraldi[2], Claus Gravholt[1], Jens O Jørgensen[1] & Jens S Christiansen[1]
[1]Århus Universityhospital, Århus, Denmark; [2]Rigshospitalet, Copenhagen, Denmark.

Female adrenal failure involves impaired DHEA secretion and very low circulating androgens. To address the impact of a daily physiological substitution dose of capsule DHEA 50 mg on quality of life and sexual parameters, we performed a 6 month trial in a randomised, crossover and placebo controlled design. The trial was approved by the local ethical committee and conducted according to GCP guidelines. Ten patients were enrolled. Seven patients reported seborrhoic side effects in the DHEA treatment period. On this background two patients left the study.

Short Form 36 (SF36) and Female Sexual Function Index (FSFI) were obtained before and after each period. Delta values on physical function (pf), role-physical (rp), bodily pain (bp), general health (gh), vitality (vt), social functioning (sf), role-emotional (re), mental health (mh) were all positive in the DHEA treatment period but failed to reach statistical significance separately. Delta value on FSFI total score was not differently influenced by the treatments (delta placebo -2.1 ± 2.0, delta DHEA -3.2 ± 0.6; $P = 0.598$), neither were subheadings as desire, arousal, lubrication, orgasm, satisfaction and pain. A spousal questionnaire handling 15 questions recorded 15, 67, 7 (positive, neutral, negative observations) after placebo treatment and 32, 53, 5 after DHEA treatment. After both treatment periods, an interview was performed by a clinical psychologist. Topics as knowledge to DHEA and expectations to treatment effects were handled as well as side effects and clinical effects. In summary, this blinded study in a well-motivated group of patients recorded a high frequency of side effects due to DHEA treatment and no significant effects on quality of life or sexual parameters.

P476

Central hypothyrodism and dyslipidemia induced by bexarotene in patients with cutaneous T-cell lymphoma

Carlos Sánchez-Juan, Elena Roche Gamón, Xelo García Fabra, Victor Alegre de Miquel, Raquel Albalat Galera & Juan-Carlos Ferrer García
Universitary General Hospital, Valencia, Valencia, Spain.

Background and aims

Bexarotene is the first retinoid receptor X (RXR)-selective agonist approved for cutaneous T-cell lymphoma in patients resistant to at least one previus systemic treatment. However, it produces often two endocrine-metabolic alterations: central hypothyroidism and dyslipidemia. We assessed, in a group of patients with Mycosis Fungoide or Sezary syndrome treated with bexarotene, the endocrine-metabolic side effects.

Patients and methods

Descriptive and retrospective study of 13 patients (4 women) treated with bexarotene (300 mg/m^2) in the department of Dermatology of our Hospital between 2003 and June of 2006 by Mycosis fungoide or Sezary syndrome. We analyzed the clinical characteristics of the patients, the efficacy of the treatment and the endocrine-metabolic side effects relationated with the drug.

Results

Patients assessed were 59,53 years old (28–79). Median period of treatment was 11,3 months but 4 patients were continuing at the end of the period of the study. 3/13 patients (23,1%) achieved partial remission, 4/13 (30.8%) achieved complete remission, 4/13 (30.8%) were stable and 2/13 (15.5%) progressed. 3/13 cases (23.1%) were treated with oral bexarotene as monotherapy and 10/13 (76.9%) in combination with other active agents (included topic steroids).

The most frequent side effects were hypertriglyceridemia in 13/13 (100%), hypercholesterolemia in 12/13 (92.3%) and central hypothyroidism in 7/13 (53.8%). Thyroid hormone replacement therapy and additional treatment with statin or fenofibrate was used in these cases. In patients who discontinued bexarotene treatment, thyroid function and lipid levels returned to baseline values.

Conclusions

Bexarotene is an effective therapeutic option in patients with cutaneous T-cell lymphoma but usually it produces central hypothyroidism and dyslipidemia which require treatment with levothyroxine and lipid-lowering agents. These frequent alterations must be in mind when bexarotene treatment is prescribed.

P477

Thyroid dysplasia – 30 cases of lingual thyroids

Eusebie Zbranca, Cristina Preda, Voichita Mogos, Letitia Leustean, Carmen Vulpoi, Bogdan Galusca, Valeriu Rusu & Radu Negru
University of Medecine, Iasi, Romania.

Thyroid dysplasia (ectopy, hypoplasia or aplasia) is a common cause of congenital hypothyroidism. Lingual thyroid is a rare embryological aberration caused by failure of migration of the thyroid gland to its normal position in the neck.This retrospective study involved 30 patients with lingual thyroid diagnosed in our Department between 1970–2005. The diagnosis was based on physical examination, evaluation of the mental development (IQ) and following tests:TSH, fT4, ultrasound imaging of the neck and sublingual region and neck scintigraphy. Among the patients with congenital hypothyroidism the incidence of lingual thyroid was 29%. Females (83%)were affected more than males (17%). In our group the age at diagnosis was between 6 months and 35 years. The mental retardation (mild to moderate) was present in 85% of cases. The analysis of physical development reveal growth disturbances in 56% of cases. On the basis of this findings it may be stated that the early diagnosis and treatment are the most important for the normal development of children with lingual thyroid.

P478

Primary hyperparathyroidism during pregnancy – case report

Voichita Mogos, Simona Mogos, Mircea Onofriescu, Elena Cotea, Eugen Tarcoveanu, Niculina Florea & Eusebie Zbranca
University of Medicine, Iasi, Romania.

Until 2002 less than 200 cases of primary hyperparathyroidism identified during pregnancy were reported. We present a case of primary hyperparathyroidism discovered during pregnancy in a 28-year-old woman. The disease was suspected due to a hypercalcemia discovered during a routine blood assessment during pregnancy (calcium = 11 mg/dl, phosphorus = 2.4 mg/dl). The patient was investigated in the Clinic of Endocrinology and the diagnosis of primary hyperparathyroidism was made on biological investigations: calcium = 12.80–15.84 mg/dl, phosphorus = 1.06 mg/dl., alkaline phosphatase = 428 IU/l, urinary hydroxyproline = 118 mg/24 h. Ultrasound neck examination showed a solid formation of $33 \times 18 \times 20$ mm. under the lower pole of the right thyroid lobe outside of thyroid tissue. The gestational age was of 30 weeks. The patient was transferred to the 1st. Clinic of Obstetrics and Gynecology and treated with glucocorticoids on order to mature the lung surphactant of the fetus in case of premature labor induced by surgery. At 32 week of gestation the parathyroid adenoma was removed under local anesthesia and confirmed by pathological examination. After resection of the parathyroid adenoma patient's calcium dropped to 8.5 mg/dl. She gave birth to a healthy newborn at 38 weeks. The patient and her infant were seen after 1 year and both were normal biological parameters. We reported this case because very low incidence of such association and the successful management that prevented the birth of a newborn with severe hypocalcemia due to exposure to hypercalcemia during pregnancy

P479

Pituitary insufficiency after traumatic brain injury in southwest Hungary

Orsolya Nemes, Zsuzsanna Nagy, Beata Bodis, Laszlo Bajnok, Dora Szellar, Endre Czeiter, Andras Buki, Tamas Doczi & Emese Mezosi
University of Pecs, School of Medicine, Pecs, Hungary.

Traumatic brain injury (TBI) often results in long-term pituitary insufficiency. Regular endocrine screening of TBI patients is advised after the acute phase of the treatment period. We monitored pituitary functions in 32 TBI patients (28 men, 4 women). Endocrine tests were performed from 3 to 36 months after the brain injury. Thyroid functions, cortisol and ACTH levels, prolactin, sex hormone concentrations, GH/IGF1 axis and posterior pituitary function were evaluated. Additional stimulatory tests were done if data indicated pituitary hypofunction: insulin/arginine/glucagone/TRH tests. Mean age of the patients was 35.1 years (men: 35, women: 36). Endocrine abnormalities developed in 37.5% of the patients, 75% of these in one axis and 25% in two axes. Three patients had hyperprolactinemia. Normal endocrine functions were detected in 62.5% of TBI patients. GH deficiency was the most frequently found abnormality in TBI patients (9 cases-28.1%), central hypogonadism was diagnosed in 4 patients (12.5%), and central hypadrenia in 2 (6.25%). Central hypothyroidism and diabetes insipidus were not present in our studied patient group. In conclusion, approximately one third of monitored TBI patients had pituitary dysfunction during follow-up. The majority of these cases displayed single axis disturbance, with GH deficiency representing the leading abnormality. Systematic endocrine follow-up of TBI patients should be extended in Hungary.

P480

Bartter syndrome – a case of secondary hyperaldosteronism

Isabel Manita, Andreia Veloza, Catarina Coelho, Catarina Saraiva, Dolores Passos, Maria Cordeiro, Luísa Raimundo & Jorge Portugal
Hospital Garcia de Orta, Almada, Portugal.

Introduction
Bartter syndrome represents a set of closely related autosomal recessive renal tubular disorders characterised by hypokalemia, hypochloremia, metabolic alkalosis and hyperreninemia with normal blood pressure. The underlying abnormality results in excessive urinary losses of sodium, chloride and potassium. Bartter syndrome is classified into 3 main clinical variants: neonatal Bartter syndrome, classic syndrome and Gitelman syndrome.
Case report
We present a 19 year-old male caucasian, the only child of a consanguinuos marriage, referred for severe hypokalemia detected during investigation of anemia (spherocytosis). Data concerning pregnancy, delivery and early childhood is not available. There is a history of nocturnal enuresis that lasted until 12 years of age, and of persistent polyuria and polydipsia. Growth and pubertal development were normal. Symptoms such as parestesias, fatigue and spasms were absent.
Laboratorial tests revealed hypokalemia alkalosis, normomagnesiemia, hypercalciuria and hyperaldosteronism. Renal ultrasound did not show alterations. We are waiting for the opportunity to order genetic testing. Other causes of hypokaliemia were excluded such as surreptitious diuretic and laxative abuse, persistent vomiting and diarrhoea.
On the ground of clinical appearance and biochemical data, the Bartter syndrome in classic variant was diagnosed. Good therapeutic effect was achieved using spironolacton, indomethacin and potassium supplementation.
Conclusion
Bartter syndrome is a rare autosomal recessive disorder. Recent molecular diagnosis has revealed that Bartter syndrome results from mutation in 5 distinct genes that affect the function of ion channels of the distal nephron segments. The literature confirms a lack of correlation of genotype and phenotype in this disease. In this case the authors emphasize the unusual late and asymptomatic presentation.

P481

A Wellbeing patch induced Adrenal crisis

Rajagopalan Sriraman, Mary Armitage & Tristan Richardson
Royal Bournemouth Hospital, Bournemouth, BH7 7DW, United Kingdom.

A 29-year-old lady with known Addison's disease and hypothyroidism was admitted with a history of increasing lethargy and dizziness for 2 weeks. At the time of admission she was on (and compliant with) Hydrocortisone 20 mg twice daily, Fludrocortisone 100 mcg once daily and Thyroxine 150 mcg once daily. On the day of admission her BP was 128/92 mmHg with no postural drop. Her electrolytes were normal, however an early morning cortisol measured 28 mmol/l. She was treated with IV Hydrocortisone for 24 hrs following which she was changed to oral Hydrocortisone. She was discharged after 3 days on Hydrocortisone 10, 10, 5 mg and Fludrocortisone 100 mcg once daily.

Unfortunately she was readmitted 7 days later. Her symptoms included postural dizziness and pins and needles over her face. During this admission her blood pressure was 136/97 mmHg lying and 118/97 mmHg sitting. Her electrolytes were again normal. She was treated with IV Hydrocortisone for the first 24 hrs and Endocrinology review requested. On further questioning, it was noted that the only change in her medication within the last few weeks was use of 'Wellbeing Detox Patches'. She denied any previous Addisonian crisis and had been very well controlled previously on oral steroids. On stopping the patch, her steroid replacement has since been unproblematic
Discussion
Detox patches contain multiple natural ingredients (up to 15 different 'natural' products). They are sold on the pretext that they 'cleanse' the body of harmful by-products. Others are said to stimulate acupuncture points through action of wood/bamboo vinegar, far infrared (a form of safe radiated energy) or minus ion emissions (formed naturally).
Herbal medicines may contain several enzyme inducers that metabolise cortisol leading to hypocortisolaemia and crisis. Subjects on steroid replacement should be warned about the usage of over-the-counter medicines even those thought to be 'natural remedies'.

P482

Antidepressants and elevated catecholamines

Rajagopalan Sriraman, David Cavan & Tristan Richardson
Royal Bournemouth Hospital, Bournemouth, BH7 7DW, United Kingdom.

Urinary catecholamine assessment is one of the screening tests for phaeochromocytoma but false positives results can occur. The pretest probability for phaeochromocytoma is 0.5% (1 in 200 patients tested) in the presence of hypertension and suggestive symptoms. We present two cases of elevated urinary catecholamines in hypertensive subjects treated with serotonin and noradrenaline re-uptake inhibitors (SNRI).
Case1
A 27 year old male presented with palpitations, tremor, sweating, myalgia, nausea and fatigue. His past medical history included acute depression for which he took venlafaxine and then sertraline 50 mg/day. His BP fluctuated between 170/105 and 115/55 mmHg. General examination and investigations including thyroid function tests were normal. Three urinary catecholamine collections were mildly elevated (24 hr adrenaline 107, 105, 38 nmol/d (normal $<$100 nmol/d), dopamine 3796, 3584, 3048 nmol/d (normal $<$3000 nmol/d)). Further investigations excluded phaeochromacytoma.
Case 2
A 43 year old male with type 2 diabetes, anxiety and depression presented with palpitations, sweats and hypertension (BP 180/106). His other problems included lithium-induced thyroid abnormalities and sleep apnoea. In addition to bendrofluazide, felodipine, metformin, and lithium, he was taking venlafaxine 150 mg/day. His thyroid function was normal, but urinary catecholamines were mildly elevated (24 hr noradrenaline output 680, 806 nmol/d (normal $<$500 nmol/d), dopamine output 4811, 3821 nmol/d (normal $<$3000 nmol/d)). There was no further evidence of phaeochromocytoma radiologically.
Discussion
Medications may cause raised catecholamines and result in false positive tests for pheochromocytoma. Tricyclic antidepressants and phenoxybenzamine have been most commonly implicated, accounting for 40% of medication-associated false positive results. We present two cases where small rises in catcholamines have occurred in patients taking SNRIs, which could be consistent with their mode of action. Clinicians should be aware of this possible effect when assessing patients, particularly with a background of depression.

P483

Myasthenia gravis and autoimmune Addison's disease in a patient with thymoma

Mesut Seker, Hulya Iliksu Gozu, Berkant Sonmez, Fatih Yavuz, Taflan Salepci, Ekrem Orbay, Mehmet Sargin, Haluk Sargin, Mahmut Gumus, Ulku Turk Boru, Mustafa Yaylaci & Ali Yayla
Dr. Lutfi Kirdar Kartal Education Hospital, Istanbul, Turkey.

The association of thymoma with myasthenia gravis is well known, however association of these two syndromes (Thymoma + Myasthenia gravis) with Addison's disease is very rare. In here we report myasthenia gravis and autoimmune Addison's disease in a patient with thymoma.

A 32-year-old man was admitted to our hospital with symptoms of weakness, anorexia, nausea, vomiting, pigmentation of skin and mucous membranes for 2 years. He had undergone to the operation because of thymoma 17 months before to admission.

On physical examination, generalized pigmentation, especially in oral mucosa, and tongue, was observed. Except ptozis in the right eye, neurologic examination was normal. Unexplained pigmentation and other symptoms suggested possibility of diagnosis of adrenal insufficiency. He was diagnosed as Addison's disease on the basis of the findings of a high plasma ACTH level; >185 pmol/L (normal; <125 pmol/L), low plasma cortisol level; 1.85 ug/dl (normal; 5–25 ug/dl). ACTH stimulation test revealed that cortisol levels were not stimulated upon stimulation by ACTH (Basal ACTH level: 2.97 ug/dl, stimulated ACTH level: 2.84 ug/dl. Anticorticosurrenal antibodies was measured as 640 (N: <5). Thyroid stimulating hormone (TSH), free thyroxine (FT4) were normal. Anti TSH reseptor antibody was measured as 3.00 U/L (normal; 0.00–10.00 U/L).

He had complaint of ptozis in the right eye for 2 years. Skull radiographs and orbita MRI were normal. Although electromyography and edrophonium test were negative; myasthenia gravis was diagnosed on the basis of findings of a high titer of acetylcholin receptors levels (2.4 nmol/l; normal: 0.00–0.50 nmol/l). Prednisolon (7.5 mg/day) and prostigmine (180 mg/day) tablets have been started. Symptoms and signs were improving by this treatment.

In here we report another example of this rare syndrome in which myasthenia gravis, autoimmune Addison's disease and thymoma ocurred together.

P484

Severe hyperandrogenism during the entire course of pregnancy does not cause virilization of a female infant born

Rita Bertalan[1], Zita Halász[1], László Csabay[1], János Rigó Jr[1], Sándor Németh[2], Anna Blázovics[1], Judit Toke[1], Belema Boyle[1] & Károly Rácz[1]
[1]Semmelweis University, Budapest, Hungary; [2]G. Richter Ltd, Budapest, Hungary.

Objevtives
Maternal hyperandrogenism occurs rarely during pregnancy as the consequense of maternal ovarian or adrenal disorders, or placental aromatase deficiency.
Case
A 33-year-old pregnant women was referred because of high serum testosterone (240 ng/dl; normal, 20–60 ng/dl) measured at the 7th week of pregnancy. At presentation she had symptoms of moderate hyperandrogenism, which slightly increased until delivery. Abdominal and pelvic ultrasound exams showed no evidence for adrenal or ovarian masses. Serum hormone measurements indicated severe hyperandrogenism and marked increases of serum estradiol levels during the whole tenure of pregnancy. Serum hCG and SHBG levels were normal. The patient refused fetal karyotype exam. Fetal ultrasound indicated normal female external genitalia.

Mother's hormone levels during gestation	13th week	17th week	28th week	35th week	Postpartum 12 hours
Testosterone ng/dl	458	664	607	590	808
Estradiol pg/ml	3139	11073	28973	33733	609

At 39 weeks of pregnancy she delivered a girl with normal female external genitalia. Umbilical cord hormone levels were normal, except a modest increase of testosterone (94 ng/dl). At the age of six weeks the baby's androgen concentrations were normal and bone age was not accelerated. One week after delivery androgen levels of the mother decreased markedly, but they remained slightly above the upper limit of normal. Placental aromatase activity, measured by conversion of testosterone to estradiol in microsomal preparations was normal.
Conclusion
This case clearly shows that severe hyperandrogenism detected as early as 7 weeks of pregnancy and persisting until delivery presumably due to hyperreactio luteinalis does not necessarily cause virilization of a female fetus. The marked difference in maternal and umbilical blood testosterone levels, together with the largely increased maternal estradiol suggest that placental aromatase activity plays a key role in preventing fetal androgen excess.

P485

Regression of metastatic gastric carcinoid associated with atrophic gastritis and after octreotid treatment

Peter Gergics[1], Gabriella Dabasi[2], Eva Csoregh[1], Zsuzsa Jakab[1], Peter Nagy[3], Beatrix Sarman[1], Peter Pusztai[1], Mark Juhasz[1], Peter Reismann[1], Nikolette Szucs[1], Ibolya Varga[1], Miklos Toth[1], Karoly Racz[1] & Zsolt Tulassay[1]
[1]2nd Department of Medicine, Semmelweis University, Budapest, Hungary; [2]Isotope Laboratory, Department of Transplantation and Surgery, Semmelweis University, Budapest, Hungary; [3]1st Department of Pathology and Experimental Cancer Research, Semmelweis University, Budapest, Hungary.

A 57-year-old female patient was admitted for evaluation of multiple focal liver lesions diagnosed with abdominal ultrasound and CT. Her medical history included severe rheumatoid arthritis and pernicious anaemia treated with vitamin B12. Gastroscopic examination revealed numerous small polypoid lesions within the stomach, and histology of tissue samples obtained by biopsy showed carcinoid associated with atrophic gastritis. Although the patient had no symptoms of carcinoid syndrome, 24 hour urinary excretion of 5-hydroxyindole acetic acid (5-HIAA) was elevated and serum chromogranin A (CgA) was three times higher than the upper limit of the reference range. Octreoscan showed focal radionuclide accumulation corresponding to the stomach and the liver. Because of the severe rheumatoid arthritis surgical therapy was not considered. After 7 months of octreotide LAR treatment abdominal ultrasound and CT showed a complete remission of liver lesions and repeat octreoscan failed to show pathologic radionuclide accumulation. Repeat gastroscopy was also negative and biopsy revealed chronic atrophic gastritis and a scattered pattern of chromogranin-positive cell-nests. In accordance with regression of the carcinoid tumor, urinary 5-HIAA excretion and serum CgA levels returned to normal.

Although somatostatin analogues have been shown to induce regression of gastric carcinoid tumors associated with pernicious anemia-related hypergastrinemia, a complete regression of liver metastases after somatostatin-analogue treatment has rarely been documented. In addition, our case demonstrates not only the efficacy of octreotide for treatment of metastatic gastric carcinoid but also the importance of octreotide treatment in cases without carcinoid syndrome.

P486

Persistent fever after surgical removal of a craniopharyngioma: diagnosis pitfalls and therapeutic difficulties

Raluca Alexandra Trifanescu[1], Monica Livia Gheorghiu[1], Irina Ogrezean[2], Vasile Ciubotaru[2], Daniela Preotescu[3], Rudolf Fahlbusch[4] & Mihail Coculescu[1]
[1]Department of Endocrinology, 'Carol Davila' University of Medicine and Pharmacy, Bucharest, Romania; [2]'Bagdasar Arseni' Neurosurgery Hospital, Bucharest, Romania; [3]'Matei Bals' Institute of Infectious Diseases, Bucharest, Romania; [4]Endocrine Neurosurgery Department, International Neuroscience Institute, Hannover, Germany.

Background
Thermoregulatory disorders after neurosurgery of craniopharingyomas were seldom reported.
Aim
To present the difficulties of etiologic diagnosis and treatment of a persistent febrile syndrome in a patient with surgically removed craniopharyngioma.
Patient and methods
A 34 years old man with a giant craniopharyngioma situated in the basal-anterior part of the third ventricle is reported. Anterior pituitary hormones were measured by fluoroimmunoassay. MRI, CT, X-rays were used for imaging. Cultures from various biologic fluids were performed.

Results
The patient underwent two successive transfrontal neurosurgical interventions. Postsurgery, diabetes insipidus and panhypopituitarism occurred. Substitutive hormonal therapy was introduced. After the second operation, the patient presented fever (up to 39 °C), abdominal pains, hypodypsia with hypernatremia and hyperphagia. Suspected colitis was excluded by colonoscopy. Thereafter, the patient developed a left inferior pneumonia complicated with minimal pleuresia; the bronchial aspirate identified Klebsiella pneumoniae and the patient received antibiotics according to the antibiogram. The pneumonic and pleural opacities on X-rays and on CT scan resumed, but the fever persisted. No inflammation markers were noticed: normal C reactive protein (0.52 mg/dL) and fibrinogen (391 mg/dL) levels, negative procalcitonine. Repeated hemocultures and cerebrospinal fluid cultures were negative. The urocultures and the cultures from the ventriculo-subcutaneous shunts were also negative. The fever persisted despite intensive, wide spectrum antibiotherapy, combined tuberculostatic therapy or high doses of corticosteroids. Excluding the infection, we conclude that the fever had central origin.
Conclusion
Hypothalamic thermoregulatory dysfunction with fever should be considered in patients with surgically removed craniopharyngiomas of the third ventricle.

P487

Study of aldosterone secretion in patients with essential hypertension using a modified suppression test

Aggeliki Gouli, Ioannis Androulakis, Athina Markou, Despoina Ragkou, Konstantina Dimitriou, Alexandra Stathopoulou, Tilemachos Anagnostou, Georgia Kondyli & George Piaditis
General Hospital of Athens 'G. Gennimatas', Department of Endocrinology & Diabetes Center, Athens, Greece.

Background
The renin-angiotensin system is important for blood pressure control. Screening and diagnostic tests used so far to diagnose patients with aldosterone related hypertension does not take into consideration the stimulating ACTH effect on aldosterone secretion.
Objective
To assess the role of aldosterone in essential hypertension using a modified suppression test, performed under suppressed ACTH levels.
Subjects and methods
117 hypertensive patients with essential hypertension and 34 age and sex matched normotensive controls were studied. A modified fludrocortisone suppression test (FST) under suppressed ACTH levels was performed to all participants (fludrocortisone 0.4 mg daily in 4 divided doses for 4 days and overnight dexamethasone suppression with 1 mg on day 4). Basic biochemical parameters, ACTH, plasma aldosterone and plasma active renin were measured at 08.00 am on day 1 and 5. Median value of aldosterone to renin ratio (ARR) +2 standard deviations in the control group after the test was used to define normal cut-off.
Results
Basal aldosterone, renin, ARR, K^+, and urine 24-hour K^+ did not differ between the two groups. Post-test aldosterone and ARR were significantly higher in hypertensives compared to controls (47.79 ± 3.97 (mean $\pm$ S.E.M) vs 132.2 ± 11.18 pmol/L, $P < 0.0001$ and 23.4 ± 3.25 vs 55.54 ± 7.53 pmol/L/pg/ml, $P < 0.0001$). Baseline K^+ levels were inversely correlated to post-test aldosterone and ARR only in the hypertensive group ($r = -0.21$, $P < 0.05$ and $r = -0.24$, $P < 0.01$ respectively). A significant proportion of hypertensives (29.05%) failed to suppress aldosterone levels to normal range after the test.
Conclusions
A modified FST revealed that a high percentage (29.05%) of patients who were thought to have essential hypertension, have autonomy of aldosterone secretion. This observation could possibly explain the cause of the low renin levels of the 25% of patients with essential hypertension reported in literature.

P488

Abstract unavailable

P489

GH-secreting adenomas may disappear with long-acting somatostatin analoque (octreotide-LAR) treatment

Senay Arikan[1], Deniz Gokalp[1], Alpaslan Tuzcu[1], Mithat Bahceci[1] & Hatice Akay[2]
[1]Dicle University School of Medicine Department of Endocrinology, Diyarbakir, Turkey; [2]Dicle University School of Medicine Department of Radiology, Diyarbakir, Turkey.

Background and aim
Somatostatin analogues such as octreotide acetate were used in acromegalic patients as primary or secondary treatment. In this study we aimed to report completely disappearing of adenoma and clinical cure in 3 acromegalic cases that rejected surgical treatment.
Case1
E.A (62 years old, female) She reported enlargement of his hands, deepening of his voice and increases in shoe size. MRI revealed a macro adenoma which was spread to cavernous sinus (20×15 mm). She has been treated ocreotide-LAR 20 mg/per month for 24 month. Adenoma size gradually became small and completely disappeared after 24 months.
Case 2
S.S (46 years old, male) Magnetic resonance imaging (MRI) revealed a 12 mm pituitary macro adenoma. He has been treated with octreotide-LAR 20 mg/per month for 25 months. After Eight months octreotide LAR treatment adenoma disappeared. Octreotide-LAR treatment was continued because risk of enlargement of adenoma
Case3
B.U (44 years old, male) Magnetic resonance imaging (MRI) revealed a 9 mm pituitary adenoma. He had treated with short acting octreotide analogue for 6 month (octreotide 100 μ three times a day) then he has treated with octreotide-LAR 20–30 mg/month for 36 months. With this treatment pituitary adenoma of the patient completely disappeared in MRI of pituitary gland.

Age and sex matched serum IGF-1 levels decreased to normal range in case 1. IGF-1 levels of case 2 and case 3 decreased but not achieved to normal range. Growth hormone levels of the patients with the treatment achieved normal range in case 2. Growth hormone levels during oral glucose tolerance test decreased in case 1 and case 3 but not achieve normal range. Biochemical data were shown in the table.
Conclusions
1- Octreotide treatment decreased IGF-1and GH hormone levels in acromegalic patients.
2- Adenomas may completely disappeared with octreotide-LAR treatment
3- Octreotide-LAR treatment may be used in selected patients instead of surgical treatment.

P490

Adrenal rest tumours in 11-β hydroxylase deficiency

Tabinda Dugal[1] & Ken Laji[2]
[1]St Georges Hospital, London, United Kingdom; [2]St Richards Hospital, West Sussex, United Kingdom.

Adrenal rest tumours are well described in 21-hydroxylase deficiencies. However there are few reports in literature of rest tumours in 11-β hydroxylase (11- β OH) deficiency. We report a case with an established diagnosis of 11- β OH deficiency with non-compliance to steroid treatment and endocrine follow-up

He presented to Urology with haemospermia. He was found to have scrotal swellings. Ultrasound confirmed bilateral testicular tumours. CAT scan showed small para-aortic lymph nodes and one below the renal hilum. He was presumed to have bilateral testicular tumours with congenital adrenal hyperplasia (CAH). He had Oncology review with sperm banking for prospective orchidectomy.An endocrine referral for his CAH was sought.Scans were re-examined. The blood flow was found to be intralesional. An alternative diagnosis of adrenal rest was made. He had raised Androstenedione and 17-OH progesterone His blood pressure was also elevated. All these features are consistant with non-compliance of treatment with steroids. He was meant to be on Prednisolone 5 mg B.D. Compliance issues were discussed and the risks mainly infertility and complications of elevated blood pressure reiterated.

Adrenals and gonads both originate from the urogenital ridge and adrenal rest tissue can be found in the gonads. CAH has an incidence of 1:10,000 and 27–30% of them have adrenal rest (Vanzull etal 1992). 2/3 of these are salt losing. 18% not previously diagnosed. 83% are bilateral and palpable (up to 10 cm). With adequate replacement tumour shrinkage occurs in >30% (Stickelbroek etal). Compliance with treatment prevents occurrence (Srikanth MS etal). However adenomatous transformation can occur. Diagnosis is by imaging with ultrasound and MRI.

We suggest that sufficient replacement from the start should be ensured. Regular screening with ultrasound of the male CAH. Fertility issues should be discussed. Azoospermia patients may need screening for CAH.

P491

The possible role of genetics in severity of thyrotoxicosis

Nosheen Iqbal & George Thomson
Sherwood Forest Hospitals NHS Trust, Sutton in Ashfield, United Kingdom.

We report the cases of two identical twins with Graves' disease which proved very difficult to control and followed very similar stormy course. Twin A was a 20 years old female of 12 weeks gestation when she was referred to endocrine clinic with history of weight loss, palpitations and tremor. Her thyroid function tests revealed TSH <0.08 mU/L (0.03–4.30), FT4 82.5 pmol/L (12–22 pmol/L) and FT3 44.4 pmol/L (2.8–7.1) with positive thyroid receptor antibody. Twin B presented when she was aged 21 years with similar complaints. Her Thyroid Function Tests revealed TSH <0.08 mU/L (0.03–4.40), FT4 73.7 pmol/L (12–22), and FT3 38.4 pmol/L (2.8–7.1). On clinical examination they both had evidence of small goitre, tremor and tachycardia with significant thyroid eye disease. Due to the severity of their disease it was difficult to treat them medically as they did not respond to the maximal doses of antithyroid drugs. Radioablation was also not an option due to high risk of thyroid storm in view of incomplete response to high dose antithyroid drugs. Therefore after adequate pharmacological preparation (with Lugol's iodine and propylthiouracil) Twin A was referred for subtotal thyroidectomy and Twin B had intrapartum thyroidectomy at 24-weeks gestation recently. Biochemical euthyroid status was achieved in both the twins within 4-days post-operatively, and they are currently on thyroid replacement therapy. This is a rare presentation of identical twins presenting at around the same age with marked thyrotoxicosis and ophthalmic involvement in both siblings. Their disease course and severity was almost identical. This could be a serendipity, but raises the issue of the need for screening for thyroid disorders in siblings of those with known thyroid disease, particularly females and more so in identical twins. It also raises the interesting possibility that disease course and severity may have significant genetic determinants.

P492

The challenge of managing thionamide induced agranulocytosis in a patient with Graves' disease

Nosheen Iqbal, A Dissanayake & George Thomson
Sherwood Forest Hospital NHS Trust, Sutton in Ashfield, United Kingdom.

We report a 44 year old lady with a history of weight loss, anxiety and 'prominent eyes'. She was clinically and biochemically thyrotoxic (FT4: 158.1 pmol/L [12–22], FT3:56.5 pmol/L [2.8–7.], TSH: <0.08 mU/L [0.30–4.30]). She was commenced on carbimazole and propranolol. Failure to attend regular clinic appointments or comply with drug therapy over the next few years resulted in huge swings of her thyroid status from severe thyrotoxicosis to profound hypothyroidism (TSH: >100 mU/L). After several years lost to follow up she was admitted to hospital with severe neutropaenia (WCC: 2.9×10^9/L [$4–11\times10^9$/L], Neutrophil: 0.22×10^9/L [$2–7.5\times10^9$/l]) secondary to carbimazole, which was stopped. Treated with antibiotics, anti-fungals and G-CSF her cell count improved gradually. However she remained unwell and in persistent thyrotoxicosis (FT3: 13.3 pmol/L, TSH: <0.08 mU/L).

Due to issues around compliance she was kept hospitalised while on Lugol's iodine to render her euthyroid before more definitive treatment with subtotal thyroidectomy. Her blood results started improving and she was discharged home with elective thyroidectomy planned after a fortnight. Due to worsening of thyrotoxicosis again, she was re-admitted and her surgery was postponed. Her medical treatment continued but unfortunately she exhibited the phenomenon of 'iodine escape' and her thyroid function tests continued to deteriorate posing her at high risk of perioperative thyroid storm. After thorough consideration of all treatment options she was started on low dose Propylthiouracil and dexamethasone in addition to Lugol's iodine. Her thyroid function tests showed progressive improvement with a stable cell count rise until 5 days prior to surgery when she developed agranulocytosis. Her Propylthiouracil was therefore discontinued. She underwent subtotal thyroidectomy under antibiotic cover and made an uneventful recovery. Our case illustrated that although thyrotoxicosis is a common condition its treatment can remain a challenge. All treatment options of thyrotoxicosis has its own risks and benefits and therefore treatment should be tailored to patient specific considerations.

P493

Five-year treatment experience with metformin in polycystic ovary syndrome

Gyula Petrányi[1] & Maria Zaoura[2]
[1]Private practice, Limassol, Cyprus; [2]General Hospital, Nicosia, Cyprus.

In 2002, we introduced metformin as an alternative treatment option of the anti-androgenic contraceptive pill in polycystic ovary syndrome (PCOS). The analysis of our observations is presented here.

170 women (age 14–45 y) were diagnosed with PCOS according to the Rotterdam criteria. 88% had acne, 68% hirsutism, 46% irregular menstrual cycles, 39% BMI over 25 kg/m^2, 13.5% had apple-type obesity and 4.7% acanthosis nigricans. 104/170 patients were offered metformin 500 mg tablets three times daily who did not want to take the anti-androgenic contraceptive pill. Body mass index, waist-to-hip ratio, Global Acne Score, Ferriman-Gallwey score and the regularity of menstrual periods were registered every three months.

12 patients had transient vertigo, diarrhoea or abdominal discomfort at the beginning of the treatment; four patients discontinued metformin because of them. A 3 to 42 month follow-up period of 47 patients on metformin could be evaluated. Irregular menstrual cycles of 13/24 patients became regular within three months of treatment. Six women became pregnant during the 1st–17th months on metformin, two continued metformin throughout and delivered healthy babies. One of them who suffered from pre-eclampsia during all of her previous pregnancies remained symptom-free throughout this pregnancy. The Global Acne Score diminished from 20.0 ± 12.9 to 6.3 ± 7.1, and the Ferriman-Gallwey score from 16.9 ± 8.3 to 10.5 ± 6.8 in 15 patients during the first 12 months of treatment.

The direct comparison of these results cannot be made to those who opted for contraception because of the different indication of treatment, furthermore the metformin group contained more severe cases in many respects (obesity, acne and hirsutism). Despite this, metformin treatment resulted in favourable improvement of the symptoms in patients with PCOS and seems to be suitable for long-term use.

P494

Hand-Foot-Uterus syndrome in a patient with secondary amenorrhea: a rare case

Neslihan Kurtulmus[1], Kivanc Cefle[2], Sukru Ozturk[2], Sukru Palanduz[2], Solmaz Deniz[3] & Mahmut Remzi Savas[3]
[1]Vakif Gureba Educational Hospital, Department of Endocrinology, Istanbul, Turkey; [2]Istanbul University, Meducal Faculty of Istanbul University, Department of Genetic, Istanbul, Turkey; [3]Vakif Gureba Educational Hospital, Department of Radiology, Istanbul, Turkey.

Introduction

Hand-Foot-Uterus Syndrome (HFUS) is a rare genetic condition. It is characterized by abnormalities of the hand, foot, reproductive tract, and urinary tract. There are also wrist- and ankle-bone fusions, very small feet, short great toes, urinary-tract abnormalities, duplications of the reproductive tract in women, urethral openings on the underside of the penis in men, and curved penis. The genetic associations of HFUS is not fully understood. It seems that the most cases of HFUS is caused by a mutation in HOXA13, but other genes may be involved.

Case

We present a 27-years-old woman who had a history of secondary amenorrhea for several years. On physical examination, her secondary sexual chacteristics were normal, but she had strabismus and small feet and hands, as well as clinodactyly. We referred her to Genetic Department. X rays of the hands and feet, and imaging of reproductive tract were performed. On x-rays, clinodactyly, traphesium/scaphoid fusion and fusions of other bones in hands, and shortened thumbs were detected. On ultrasonography and MRI, There were bicornate uterus and bilateral ovarium hypoplasia of reproductive tract. Serum Luteinizing hormone and Follicule stimulating hormone were elaveted to 50.5 IU/L and 114 IU/L, respectively and estradiol level was low to 20 ng/ml. Other pituitary hormones and laboratory findings were within normal ranges. She had a normal karyotype(46XX). MRI of pituitary gland was normal. After above-mentioned physical and radiological examination, diagnosis of HFUS was obtained. In addition to these, she had a secondary amenorrhea along with HFUS.

Conclusion

It is very often observed the case of amonerrhea at endocrinologic clinics. The ethiological reasons are generally similar and caused by over or pituitary disorders. However as we present in our case that the amonerrhea could accompany to other syndrome. To our best knowledge that this is the first case of HFUS associated to amonerrhea.

P495

Neonatal ventricular septal defect and late diagnosis of Turner syndrome

Serafino Lio & Marino Cibin
Dpt Internal Medicine 3, Oderzo-TV, Italy.

The high morbidity and mortality rate of women with Turner syndrome (TS) is primarily a results of the cardiovascular complications and so it is necessary an accurate and precocious diagnosis of this disease. Congenital cardiac anomalies, whose causes remains unknown, are common in TS (21–40%), in particular among patients with 45 X; between these the ventricular septal defect (VSD) is very rare (in a recent review, 3/1092 cases) (Gravholt 2004) and so in neonatal with VSD may not suppose the presence of TS.

We describe a female with TS (45 X, dic.(Y,15)(q12;p11.2) and VSD. Pt is a 17-year-old Caucasian female who first presented to endocrine evaluation for no pubertal development. The patient, born at term of normal pregnancy, at 7-months-old is operated of VSD. Clinically is present short stature (<third percentile) and cubitus valgus. Endocrine function show an hypergonadotropic hypogonadism. The chromosomal analysis showed 45 X and the presence of dicentric chromosome (Y,15)(q 12;p11.2) and so the patient it has been submitted to prophylactic laparoscopic excision of the gonads for risk of gonadoblastoma. Moreover, a hormone replacement therapy has been begun with induction of puberty.

In summary, this is a patient with mosaic TS with VSD; it is important remember that the VSD is rare but possible in TS and so suggested in these patients for precociously treated each problem of this syndrome.

P496

Interleukin-6-producing pheochromocytoma presenting with fever of unknown origin

Ozer Taranoglu[1], Sema Yarman[1], Esma Altun[2], Taner Bayraktaroglu[1], Meral Mert[1] & Refik Tanakol[1]
[1]Istanbul University, Istanbul Faculty of Medicine, Department of Internal Medicine, Division of Endocrinology and Metabolism, Istanbul, Turkey; [2]Ministry of Health, Istanbul Research and Training Hospital, Department of Internal Medicine, Istanbul, Turkey.

Pheochromocytomas are tumors capable of producing catecholamines and a variety of biologically active resulting in unusual clinical manifestations. We report the case of 18-year-old female with pheochromocytoma exhibiting fever of unknown origin. She had experienced continuous fever (ranging between 37.1–41 °C) and chills for previous several weeks. Antipyretics had been ineffective in lowering the body temperature and she was referred to our hospital when an adrenal incidentaloma of 5.5 cm diameter was detected during evaluation for fever. At the time, specific and nonspecific blood and urine cultures yielded in no pathogenic agents with negative viral serological markers. On admission, physical and laboratory examinations revealed normotension, a fever of 38.7 °C, markedly elevated sedimentation rate and CRP level, anemia, thrombocytosis, anemia with high ferritin levels and elevated levels of urinary norepinephrine and normetanephrine. A diagnosis of pheochromocytoma was made and the fever resolved promptly after beginning treatment with adrenergic blockers. Serum interleukin-6 level was measured to be 12.5 (normal; <3.0) pg/ml before adrenergic blockade was started. Additional measurement of 9.9 pg/ml was obtained in the second month of the treatment. She was sent to operation where complete resection of the tumor was achieved. It is suggested that the elevation of interleukin-6 might play an important role in clinical and biochemical inflammatory response. To our knowledge, our paper represents a rare case of interleukin-6 secreting normotensive pheochromocytoma associated with clinical markers of inflammation. Pheochromocytoma should be considered in the differential diagnosis of unexplained fever even for normotensive patients.

P497

Neonatal severe hyperparathyroidism associated with a novel de novo heterozygous R551K inactivating mutation and a heterozygous A986S polymorphism of the calcium-sensing receptor gene

Judit Toke[1], Gábor Czirják[2], Attila Patócs[1], Balázs Enyedi[2], Péter Gergics[1], Violetta Csákváry[3], Péter Enyedi[2] & Miklós Tóth[1]
[1]Semmelweis University, 2nd Department of Medicine, Budapest, Hungary; [2]Semmelweis University, Department of Physiology, Budapest, Hungary; [3]Markusovszky Teaching Hospital of Vas County, Department of Pediatrics, Szombathely, Hungary.

Objectives
Neonatal severe hyperparathyroidism (NSHPT) is induced by inactivating mutations of human calcium-sensing receptor (CaSR). We report the case of a now 11 year-old boy with NSHPT. We characterize a novel inactivating mutation with the results of some functional analyses.
Case
In the neonatal age the patient presented the clinical syndrome of NSHPT. At the age of 6 years, persisting hypercalcemia without clinical symptoms was documented, and the patient remained completely symptom-free without parathyroid surgery until his present age of 11 years.
Methods
The entire coding region of the CaSR gene of the patient, and exons 6 and 7 from his family members were sequenced. Functional investigation was performed in HEK-293 cells, transiently transfected with wild type and mutant CaSR plasmid constructs.
Results
Sequence analysis revealed a novel de novo heterozygous mutation at codon 551 (AGG→AAG) predicting a change of arginine to lysine (R551K) and a known heterozygous polymorphism (A986S) on the same allele, which was inherited from the father. We demonstrated that the novel R551K mutation significantly reduced the calcium sensitivity of CaSR (EC50: from 3.38 ± 0.62 to 6.10 ± 0.83 mmol/l) which was not alleviated by the simultaneous presence of A986S polymorphism.
Conclusion
We present the fourth NSHPT case induced by a novel de novo heterozygous inactivating mutation (R551K) of the CaSR gene. The disease gradually reverted to a symptomless, benign condition resembling familial hypocalciuric hypercalcemia without any surgical intervention.

P498

A case of paraganglioma of *glomus caroticum* with lung metastasis

Ferhan Mantar[1], Meral Mert[2], Ayse Kubat Uzum[2], Ferihan Aral[2] & Nese Colak Ozbey[2]
[1]Okmeydani Training Hospital, Department of Internal Medicine, Endocrinology and Metabolism, Istanbul, Turkey; [2]Istanbul University, Istanbul Medical Faculty, Department of Internal Medicine, Division of Endocrinology and Metabolism, Istanbul, Turkey.

Introduction
We report a case of paraganglioma of glomus caroticum with lung metastases treated with 150 mCi 131I-MIBG.
Case
A 25-year-old woman was referred to our department for cachexia. She had low BMI (18) normotension, mild normochromic normocytic anemia with a mass under left mandibula. Radiologic imaging of neck revealed that $24 \times 36 \times 45$ mm diameter mass surrounding of carotis externa and interna at the level of bifurcation. She underwent surgical operation. Pathological examination revealed that the tumor was paraganglioma with index of Ki-67 2–3%. Further endocrine evaluation showed increased urinary normetanephrine (607 microgram/dl (normal: 88–444) and dopamine 405 microgram/dl (normal: 65–400). Radiological scan of thorax, abdomen and cervical region were performed for evaluation of metastases. Bilateral,small lung nodules were shown in thorax CT. 131I-MIBG scintigraphy was positive only on the right side of neck and octreotide scan was negative. Lung biopsy was performed for pathological confirmation of metastases. Pathological examination revealed that the lung tumors were paraganglioma with index of Ki-67 2–3%. 131I-MIBG therapy was performed with 150 mCi. Post-therapeutic MIBG scan was showing no uptake in the lung. No further elevation of urinary cathecolamine metabolites was observed during follow-up. Mass size and clinical findings were stable.
Conclusion
Paragangliomas are very rare tumours in the head and neck but should be considered in the differential diagnosis of neck masses. As these tumours can form part of a familial syndrome, long-term follow-up is necessary.

P499

Intracranial giant sarcoma in an acromegalic patient after radiotherapy

Ferhan Mantar[1], Meral Mert[2], Laika Karabulut[1], Yunus Aydin[3] & Murat Musluman[3]
[1]Okmeydani Training Hospital, Department of Internal Medicine, Endocrinology and Metabolism, Istanbul, Turkey; [2]Istanbul University, Istanbul Medical Faculty, Department of Internal Medicine, Division of Endocrinology and Metabolism, Istanbul, Turkey; [3]Sisli Etfal Training Hospital, Department of Neurosurgery, Istanbul, Turkey.

Introduction
Primary pituitary sarcoma causally unrelated to radiotherapy, but an increased risk of second brain tumors continues beyond 20 and 30 year after treatment.

We report an acromegalic patient with intrasellar giant adenoma invased suprasellar and nasal cavity during follow-up after radiotherapy.
Case
A 39-year-old woman had transsphenoidal and transcranial operation in 2002 and 2003 respectively. She had also conventional external beam radiotherapy after surgery. Thereafter, she treated with octreotide LAR 30 mg/month at the same time. Cabergoline had added one year later because of resistance of the therapy. She applied to outpatient clinic with severe headache and nevralgia on her face. Huge sellar mass invased suprasellar region and cavrnous sinus was found in sellar MRI. Sellar mass was bigger three times compared to previous MRI scan which was performed only three months ago. Growth hormone and IGF-I levels were markedly elevated. She underwent hypophyseal surgery immediately. Pathological examination and immunohistochemical stains revealed undifferentiated pleomorphic sarcoma. Though the surgery was performed, sellar mass regrew through the nasal cavity, clinical signs were progressively worsen and she died within two months.
Conclusion
In an acromegalic patient, persistently raised levels of growth hormone may associated with increased risk of second brain tumors in patients with pituitary adenoma treated with surgery and radiotherapy. An increased risk of second brain tumors usually appear more than 5 years after radiotherapy, in our case sarcomateous transformation was seen only two years later. It might be related either radiotherapy or high levels of GH or both.

P500

Short stature and neurofibromatosis type 1 – issues of diagnosis

Letitia Leustean, Cristina Hurjui, Cristina Preda, Carmen Vulpoi, Liliana Moisii & Eusebie Zbranca
University of Medecine and Pharmacy "Gr. T. Popa", Iasi, Rwanda.

Neurofibromatosis type 1 (NF1) is an inherited disorder characterized by formation of neurofibromas in the skin, brain and other parts of the body, in association with skin pigment changes. It is well known that this condition may be a risk factor for short stature with growth hormone deficiency (GHD) in children, due to suprasellar lesions. We present the case report of a 9-year-old boy admitted in our Service for short stature (-2DS). Physical examination revealed 'café-au-lait' spots, underarm and inguinal freckles (the same as his father and great father). No neurofibromas were found. The ophthalmologic exam was normal: no evidence of Lisch nodules or optic glioma. Psychological evaluation was also normal (IQ = 105). The serum GH levels were low (1.7 ng/dl), with no response to exercise test (1.3 ng/dl) and with inappropriate response to arginine provocative test (2.3 ng/dl). The serum levels of IGF-1 were low (25 ng/dl). Magnetic resonance imaging demonstrated no intrasellar mass lesion, but foci involving the cerebellum, globus pallidus and cerebral pedunculy. The final diagnosis was pituitary dwarfism and NF1, but with no connection between the two. The presence of GHD in short children with NF1 independent of organic, pituitary change is frequently quoted in the literature, the latest studies suggesting that NF1 could represent a novel etiology for GHD.

P501

Paraneoplastic Cushing's syndrome presenting as psychosis – case report

Cristina Spiroiu, Aurelian Emil Ranetti, Ana- Maria Mihai, Adina Mazilu & Nicolae Diaconu
Central Military Emergency Hospital, Bucharest, Romania.

We present the case of a 51 years old woman, nonsmoker, without relevant past medical history, who presented with acute psychotic state starting the third day of treatment with prednisone 30 mg indicated for allergy. She had also arterial hypertension and a significant and progressive loss of proximal muscle strength in her legs. The initial evaluation showed hyperglycemia, metabolic alkalosis and severe hypokalemia. Basal plasma cortisol was high (>90 μg/dl) and did not suppress after high dose of dexamethasone. Abdominal computed tomography revealed bilateral adrenal hyperplasia; thoracic computed tomography showed a lung mass, which proved to be a small cell lung carcinoma at fiberoptic bronchoscopy with brushing and cytology exam. The psychotic state resolved in a couple of days; despite intensive oral and intravenous potassium supplement, high doses of spironolactone and aminoglutethimide, the serum level of potassium increase but did not normalize. Combination chemotherapy did not improve the patient's condition. She died a month later of severe lung infection.

P502

Endocrine tumour registry – tools for endocrine epidemiology

Corin Badiu[1] & Sorin Sava[2]
[1]Institute of Endocrinology, Bucharest, Romania; [2]Infoworld, Bucharest, Romania.

Endocrine tumour registry is a web-based system which is divided in several categories of endocrine tumours: pituitary adenomas, thyroid cancer, parathyroid tumours, adrenal and other types.

The program is intended to give epidemiological data concerning the prevalence of each type, age and sex distribution, therapy and basic results of it. The centres involved are the medical universities and expertise centres in Romania, centres in which there are enough resources to diagnose, treat and monitories treatment of various endocrine tumours.

The data entered are personal patients ID's, tumour type, extension and complications, type of treatment and its results as tumour dimensions, and endocrine tumour markers. Thyroid cancer registry and pituitary tumour registry are subdivisions of the system.

From each centre, 2 persons dedicated to enter data in the system are designated by the system administrator, which will be located in the Institute of Endocrinology in Bucharest. The access to the site is web secured. The network started with 10 centres and will be developed afterwards using the already existent resources. An import software filter for this site was developed, which will allow dynamic recording of cases from an institutional database (in the last 5 years) towards the registry. This hardware and software infrastructure is the base of future epidemiological public health surveys in this thematic area.

P503

Tumour induced osteomalacia – a phosphaturic mesenchymal tumour

Polyxeni Bompoti, Grigoris Effraimidis, Kalliopi Bilariki, Alkmini Nikou & NC Thalassinos
Department of Endocrinology, Diabetes and Metabolism, Evangelismos General Hospital, Athens, Greece.

A case of a 29-year old woman presented with a 6-year history of bone pain located in the lower spine and gradually extended to the spinal skeleton and the lower extremities, worsening by activity. The progressive symptoms and the established weakness finally led to patient's complete disability. The investigation revealed low serum phosphorus and elevated 24 h urinary phosphate excretion, normal serum calcium and 24 h urine calcium excretion, normal to normal-high PTH and elevated serum alkaline phosphatase, particularly the bone isoenzyme. Calcidiol levels were normal and calcitriol values were low. Iliac bone biopsy showed osteomalacia. Renal phosphate wasting can occur in disorders of vitamin D metabolism, in the Fanconi syndrome or in primary phosphaturic syndromes, which can be inherited or acquired, either as idiopathic disorders or in association with mesenchymal tumors (tumor-induced osteomalacia TIO). TIO is more likely to be the diagnosis for this patient based on symptoms and the above findings (osteomalacia, acquired hypophosphatemia, renal phosphate wasting, inappropriately low plasma calcitriol concentration, negative family history). The major diagnostic challenge was the identification of the primary tumor. The scintigraphy using indium-111 labeled octreotide was negative. The total body CT scan showed a soft tissue mass, extensive osteolysis of the ala and the body of the left ilium and extension to the ipsilateral limbus of the acetabulum. FGF23, a potential phosphaturic hormone which has been implicated in TIO, was highly elevated in our patient (1625 RU/l normal values <100). She was treated with calcitriol 3 μg/day, phosphate 3 gr/day and calcium 1500 mg/day until the removal of the causative tumor, with substantial improvement. The surgical resection of the tumor took place at the Royal National Orthopeadic Hospital, Stanmore-Middlesex. The histology demonstrated a phosphaturic mesenchymal tumour without a high-grade component. The excision of the tumor led to reversal of the biochemical and the clinical abnormalities. Unfortunately, FGF23 levels were not measured postoperatively.

P504

Unusual onset of Graves' disease – case report

Cristina Cristea[1], Adriana Ciornohuz[2] & Eusebie Zbranca[1]
[1]University of Medecine "Gr.T.Popa", Iasi, Romania; [2]Medical Cennter "Praxis", Iasi, Romania.

Graves' thyrotoxicosis frequently occurs after delivery through immune rebound mechanism. A 34 years old patient, in postpartum period was referred to rheumatologist for gradually gait impairment. Examination showed only weakness of pelvic girdle muscles which required an extensive differential diagnosis including: neurological diseases and inflammatory/metabolic/toxic myopathies. Routine lab tests were unremarkable except low cholesterol (128 mg/dl) and slightly increase of total alkaline phosphatase (ALP). Immunological and inflammatory tests were negative and muscle enzymes were within normal range. Three month later the patient had significant weight loss, persistent muscular symptoms and bright-eyed stare. On examination performed by the endocrinologist Graves' disease was considered and confirmed by abnormal levels of TSH, FT4 and TRAb. The patient was treated with antithyroid drugs. After eight weeks muscular strength became nearly normal. FT4 was normal (14.4 pmol/l), but ALP level increased up to 3 times normal. Serum calcium and phosphorus were normal and so were the liver tests. Elevated ALP and osteocalcin levels were included in an accelerated bone turnover, which characterized hyperthyroidism.

Discussion

In women diagnosed with Graves' disease during the ages of 20 to 35 years, 66% have a postpartum onset. The diagnosis is often quite simple, but it can be challenging when extrathyroidal manifestations occur early in the course of disease.

P505

Growth hormone replacement therapy and metabolic parameters in adult-onset GH-deficiency: long-term effects.

Claudia Giavoli, Emanuele Ferrante, Silvia Bergamaschi, Ronchi Cristina L, Doanadio Francesca, Lania Andrea, Spada Anna & Beck-Peccoz Paolo
Department of Medical Sciences, University of Milan, Endocrine and Metabolic Unit, Fondazione Ospedale Maggiore Policlinico IRCCS, Milan, Italy.

Aim of this study was to evaluate the impact of rhGH treatment on glucose and lipid metabolism in in 26 patients (17M and 9F, age 47.0 ± 11.1 years) with adult onset GH deficiency. Metabolic parameters (fasting glucose and insulin, glycated haemoglobin, lipid profile, body composition, OGTT) and indices of insulin resistance (IR) and sensitivity (IS), i.e. homeostasis model assessment (HOMA-IR and derived ISI-HOMA), quantitative insulin check index (QUICKI), ISI-composite, insulinogenic index (IGI) and area under the curve (AUC) for glucose and insulin derived from OGTT, were evaluated at baseline, after 1 ($n=26$) and 3 years ($n=15$) of rhGH therapy (GH dose: 0.3 ± 0.2 mg/day). At baseline, all patients had low IGF-I levels, high BMI and percent of body fat. Two out of 26 patients had impaired glucose tolerance (IGT). After 1 year, IGF-I normalization, BF% reduction and lean mass increase occurred ($P<0.005$) and persisted after 3-years treatment. Fasting insulin, glycated haemoglobin, total cholesterol, triglycerides, HOMA-IR, QUICKI, ISI-HOMA, AUC for insulin, IGI and ISI-composite did not differ after 1 and 3 years from baseline, while glucose and LDL-cholesterol levels had a transient increase and reduction after 1 year, respectively. After 3 years HDL-cholesterol levels increased ($P<0.05$) and basal insulin secretion (HOMA-B%) decreased ($P<0.05$). AUC for glucose significantly increased after 1 and 3 years of treatment ($P<0.02$). One patients progressed to diabetes after 1 year, while 5 showed IGT after 3 years. In conclusion, rhGH therapy improves body composition and lipid profile, but causes a small transient increase in fasting glucose. Since deterioration of glucose tolerance, as indicated by increase in AUC for glucose and development of IGT, a strict monitoring of glucose metabolism during long-term GH replacement therapy should be performed.

P506

Conventional glucocorticoid replacement therapy in patients with Addison's disease: effects on metabolic and bone parameters

Marcella Balbo, Roberta Giordano, Lorenza Bonelli, Rita Berardelli, Andreea Picu, Andrea Benso, Angela Bertagna, Ezio Ghigo & Emanuela Arvat
Div Endocrinology And Metabolism, Dpt Internal Medicine, University Of Turin, Turin, Italy.

In primary adrenal insufficiency hydrocortisone or cortisone are commonly used at doses of 30–37.5 mg/day as replacement therapy, though recent studies showed that cortisol normal production is about 5.7 mg/m^2, equivalent to 20 mg/day of hydrocortisone, suggesting that supraphysiological doses are used. In 19 Addison's disease patients (8 M, 11 F, 23–71 yr) under conventional glucocorticoid replacement therapy (37.5 mg cortisone/day) with low DHEAS levels, BMI, fasting glucose and insulin, glucose response to OGTT, cholesterol, triglycerides (TG), homocysteine, calcium, phosphate, PTH, 25OH-vitaminD, bone formation and resorption markers as well as intima-media thickness (IMT) by eco-doppler ultrasonography, bone mineral density (BMD) by a DEXA and vertebral morphology by spinal radiograph were measured. Mean BMI was in the upper range of normal, though higher than 25.0 kg/m^2 in 8 patients; mean fasting glucose, insulin, HOMA as well as glucose response to OGTT were normal, though HOMA were high in 5 patients; mean lipid profile was in the normal range; none of the patients had low HDL levels, whereas LDL and TG were higher than normal in 3 patients. Homocysteine was normal, though high in 5 patients. IMT was below 0.9 mm in all patients. Decreased mean BMD was found (T score < -1.0), while osteoporosis (< -2.5) was present in 2 eugonadal men and 3 postmenopausal women, vertebral fractures were found in 1 osteopenic and 1 osteoporotic patient. Mean calcium, phosphate, PTH, 25OH-vitaminD and osteocalcin were in the normal range, whereas urinary cross-laps were higher than normal. In conclusion, our preliminary results suggest that conventional glucocorticoid replacement therapy, associated with low DHEAS levels do not have a significant impact on glico-lipidic metabolism in patients with primary hypocortisolism, even in presence of slight overweight. On the other hand, increased risk of bone loss and vertebral fractures is confirmed in these patients.

P507

Gastric electrical stimulation in patients with severe diabetes mellitus associated gastroparesis – a cost benefit analysis

Mark J Hannon[1], Obada Yousif[2], Sean Dineen[3], Christopher J Thompson[4], Domhnaill J O'Halloran[1] & Eamonn MM Quigley[1]
[1]Cork University Hospital, Cork, Ireland; [2]Wexford General Hospital, Wexford, Ireland; [3]University College Hospital Galway, Galway, Ireland; [4]Beaumont Hospital Dublin, Dublin, Ireland.

Introduction

The management of diabetic gastroparesis resistant to medical therapy is very difficult – the most severely affected patients often spend many days as hospital inpatients with intractable nausea and vomiting and consequent dehydration, leading to a marked reduction in quality of life. Recently, gastric pacing (also known as gastric electrical stimulation (GES)) has been tried in these patients as a means of correcting the physiological deficit. It has shown promise in some international trials although patient numbers are still quite small. It has seen use in four patients in Ireland. Here we outline our experiences with these patients.

Methods

The records of all four patients with gastric pacemakers inserted were reviewed. The number of days spent as an inpatient by each patient before and after pacemaker insertion was calculated. From these figures, a cost benefit analysis was performed to see if the commencement of GES led to a reduction in the costs incurred due to inpatient admission for gastroparesis. The costs were calculated using 2004 bed day costs for Cork University Hospital from the Irish Health Service Executive (costings department).

Results

The bed cost for the inpatient stays of all four patients in the twelve months preceding pacemaker insertion was €306,399. The corresponding extrapolated figure for the year following pacemaker insertion was €322,543. There was no HbA1c change following GES.

Conclusion

Severe diabetic gastroparesis leads to recurrent patient admissions and places a large cost burden on the Irish healthcare system. However, the cost benefits of GES are as yet unproven in Ireland. There is very little data available worldwide which convincingly shows a cost benefit with GES, although some studies have shown a subjective improvement in patients' symptoms. Therefore, more research is needed on this contentious area.

P508

Levels of serum and salivary cortisol during low dose ACTH test in young adult-onset diabetes mellitus Type 1 patients

Katerina Simunkova, Karel Vondra, Richard Hampl, Martin Hill, Hana Kvasnickova, Jana Vrbikova & Luboslav Starka
Institute of Endocrinology, Prague, Czech Republic.

Detailed information on adrenal function in autoimmune Type 1 diabetes with onset in adults is still lacking. This work aimed at gathering own data on adrenal response to low-dose (1 μg) ACTH in blood and saliva.

Twenty-three diabetics were investigated; age 44 ± 10 yrs (mean ± S.D.), age at diagnosis 28.5 ± 10 yr, disease duration 15 ± 8 yr, BMI 24.5 ± 2.7 kg/cm^2, HbA1c 7.2 ± 1.2%.

The control group had 16 healthy subjects; age 27 ± 6 yr, BMI 21.7 ± 2.3 kg/cm^2. Neither group showed any clinical signs of adrenal disorders.

The study was approved by the Ethical Committee.

Adrenal reserve was tested by low dose ACTH test. Fasting blood and saliva were collected between 8–9 a.m. Blood and salivary cortisol were determined at times 0, 20, 30, 40, 60 min, ACTH and adrenal autoantibodies at 0′ only.

Maximum stimulated value in serum above 500 nmol/l was reached in 15 out of 23 patients (65.2%), normal-responders, NR. This cut off value was not reached in 8 patients (34.8%), low-reponders, LR. The results were compared with the control group (C).

NR: Basal and stimulated serum cortisol levels did not differ significantly from those in controls, while salivary cortisol in this subgroup was significantly lower at 20th min and 30th min, $P < 0.05$.

LR: Both basal and stimulated serum cortisol, as well as salivary cortisol were significantly lower than C, $P < 0.001$ for all times.

LR did not differ from NR in either average insulin doses, or HbA1c or basal ACTH value. Adrenal cortex autoantibodies were negative in all subjects.

In conclusion, surprisingly, in 34.8% of young adults-onset with diabetes mellitus Type 1 without signs of adrenal autoimmunity, in 1 μg ACTH test serum cortisol levels corresponding to subclinical hypocorticalism were found. Investigation of salivary cortisol brought additional information, which should be further evaluated.

The study was supported by grant IGAMZCR No.NR/9154-3.

P509

Long-term pegvisomant treatment in acromegaly

Valentina Gasco, Alessandra Mainolfi, Damiano De Giorgio, Ezio Ghigo & Silvia Grottoli
University of Turin, Division of Endocrinology, Turin, Italy.

In acromegalic patients not suitable for first-line surgical treatment, pharmacotherapy is a valuable choice. Depot somatostatin analogs (SSA) represent efficacious and well-tolerated drugs; however, they normalize hormonal parameters in no more than 65–75%. Pegvisomant (PEGA), a GH receptor antagonist, has been shown to normalize IGF-I levels in more than 90% of patients. We report our experience in 13 acromegalic patients (7 M, 6 F; age: 50.2 ± 3.9 yrs; 7 macroadenomas, 3 microadenomas and 3 empty sella) treated for 3–44 months (mean 28.8 ± 3.7 month) with PEGA (5–25 mg/day, mean 15.8 ± 1.6 mg/day) alone (n. 8) or combined with SSA (octreotide 10–30 mg/month). Diabetes mellitus or IGT was present in 5 patients. IGF-I and IGFBP-3 levels, glucose metabolism, clinical picture, MRI and safety parameters were monitored. Basal IGF-I and BP-3 levels were 858.3 ± 90.4 μg/l and 6.2 ± 0.4 μg/ml, respectively. During PEGA IGF-I normalized (222.4 ± 20.6 μg/l, $P < 0.005$) in 12/13 patients within 12 months with a mean PEGA dose of 15.8 ± 1.6 mg/day. Also IGFBP-3 markedly decreased (3.8 ± 0.3 μg/ml, $P < 0.005$). Morning glucose levels decreased from 104.2 ± 6.3 mg/dl to 92.6 ± 6.2 mg/dl ($P < 0.05$) but HbA1c didn't change (5.7 ± 0.2% vs 5.9 ± 0.3%) even when only diabetic and IGT patients were considered (7.1 ± 0.9% vs 6.8 ± 0.4%). All patients improved clinical picture and acromegalic signs and symptoms. No change occurred at pituitary MRI imaging in any patient. One patient had slight and transient increase in transaminases. One female patient complained abdominal lipodistrophy in the injection site. Thus, PEGA normalizes IGF-I in almost all patients, improves the clinical picture and also glucose levels, in front of good safety profile.

P510

The assesment of life quality satisfaction in women with Turner's syndrome

Beata Wikiera, Elzbieta Szpich, Ewa Barg & Anna Noczynska
Medical University, Department of Endocrinology and Diabetology for Children and Adolescents, Wroclaw, Poland.

Quality life satisfaction is important for personal resources analysis and perspectives for coping with ilness.

The aim of the study was to present a psychological portrait of a woman with Turner's syndrome (TS) and assessing perspectives for increased well-being of such patients. The study concerns psychological aspects of TS women's own assessment of their health and illness.

The area of interest was:

- TS patients' own health assessment
- life quality satisfaction experienced by the above mentioned patients
- the level of Optimism Available in each patient as an important element of natural resources.

Patients and methods

26 women with TS aged 18–25 participated in the study. All the patients have experienced many years of treatment and coping with their illness.

The evaluation was based on medical files analysis, an individual patient – doctor and patient – psychologist conversations. The information was gathered in the form of structured interview containing questions concerning health – illness aspects, current life and family situation and life aims of the women analysed. SWLS – Quality of Life Assessment Test and LOT-R Life Orientation Test were used to assess the level of optimism.

Results

The backgrounds of the patients tested varied. In general, the assessment of their own health condition was positive. Establishing a family was placed as No 1. life aim. Life contentment was high. Average results on AWLS scale were 48%. High results on AWLS scale were 44%. As concerns Optimism Available, 52% of the patients described their optimism level of medium, 28% as high and 20% as low. Optimistic patients seem more effective in coping with stress, which means a potentially better adjustment to changing life situations.

Conclusions

The behaviour and suffering levels in patients with TS are closely related to their natural resources. Proper specialist care and general social support may greatly facilitate such patients' natural resources.

P511

Pheochromocytoma in pediatric age

Amaya Fernández-Argüelles García[1], Cristóbal Morales Portillo[1], Cristina Campos Martín[1], Rafael Espino[2] & Luis López-Canti[2]
[1]Universitary Hospital Virgen Macarena, Seville, Spain; [2]Universitary Hospital Ntra. Sra. de Valme, Seville, Spain.

Introduction

Pheocrhomocytomas are rare tumors, principally benigns, and with high risk of morbimortality because of secretion of big amountos of catecholamines. They are an unfrequently cause of arterial hypertension in pediatric age but physicians must remember it because they can be diagnosed, treated and cured in a proper way.

Objectives

To evalue the cases reports of pediatric pheochromocytoma found in our area, to analyze the differences in diagnosis, pronostics and treatment if we compare with adult age.

Material and method

Demographic, analitical, morphological and histological characteristics are analized in the three cases of pheochromocytomas found in our area in last fifteen years. A bilateral pheochromocytoma with asynchronous presentation is exposed.

Results

The average age was 12,5 years. The both children were male. Clinical presentation was arterial hypertension (66%), tonicoclonic seizures (33%), and atypical symptons as hypoglycemias, arterial hypotension, tremors and malnutrition (weight < p3). The catecholamines determination in 24 hours tinkles, abdomen TC, I123 gammagraphy were the way to diagnose these tumors. Before surgery a α and β block was required. Histological study confirmed the benignancy of three tumors.

Conclusions

-Atypical symptons in presentation, extraadrenal and bilateral tumors, are more frequent in children than in adult age.

-Malignant pediatric pheochromocytomes are very unusual.

-Physicians shoud practise a genetic study to these children, because of the high association with herediatary syndromes as Von Hippel-Lindau disease.

P512

Ectopic localization of the pituitary bright spot in a patient with idiopathic central diabetes inspidus

Nur Kebapci[1], Erhan Erdogan[2] & Baki Adapinar[2]
[1]Eskisehir Osmangazi University School of Medicine, Department of Endocrinology and Metabolism, ESKISEHIR, Turkey; [2]Eskisehir Osmangazi University, School of Medicine, Department of Radiology, ESKISEHIR, Turkey.

We report the MRI findings of an unusual case of posterior pituitary ectopia (PPE) in a young female patient with idiopathic diabetes inspidus (DI). She was 29 years- old and presented with polydipsia (7–8 lt/day), and polyuria (7–8 lt/day) that had been present for about 5 months. She had regular menstrual cycles. She didn't have any history of significant medical illness or any history of head trauma. An 8-hour fluid deprivation test followed by desmopressin (DDAVP, 0.03 μg/kg SC) was performed in which the results were consistent with pituitary DI. She had complete correction of her thirst after DDAVP treatment was started (10 μg, bid,) and her water intake was limited to 3 L/d and urinary output decreased to 2.6 L/d. We evaluated the patient with dynamic pituitary MRI to see whether she had any problems in the hypothalamo-pituitary axis. Her pituitary MRI showed a normal appearing adenohypophysis without any space occupying lesions, the infundibulum was in the midline and of normal thickness. The pituitary bright spot was not observed at its normal location within the sella, instead we observed two discrete foci of hyperintensity at the median eminence of hypotalamus. Insulin hypoglycemia test revealed increased cortisol (>20 μg/dl) and growth hormone (>20 ng/ml) responses. Chest radiographs were normal. Analyses of lymphocyte subgroups for Sarcoidosis were in normal range. C-ANCA was negative for Wegener's granulomatosis. Control MRI 6 months later revealed exactly the same findings as the initial MRI. This case is one of the few cases in the literature since it is a case of PPE with preserved anterior pituitary functions and without any space occupying lesion in the sella and traumatic or infiltrative lesion of the infundibulum.

P513

Diabetes insipidus due to pituitary metastasis of breast cancer

Ayse Kubat Uzum, Ilkay Kartal, Meral Mert & Ferihan Aral
Istanbul University, Istanbul Faculty of Medicine, Deparment of Internal Medicine, Division of Endocrinology and Metabolism, Istanbul, Turkey.

Introduction
We have reported a case of breast carcinoma complicated by diabetes insipidus due to pituitary metastasis.
Case
A 47 years old woman had been referred our clinic with the symptoms of polyuria, polydypsia, weight loss, and fatigue. She had a diagnosis of breast carcinoma for six years, underwent radically mastectomy, chemotherapy and radiotherapy, subsequently. Vertebral metastasis was detected and local radiotherapy was performed six months before admission. Symptoms of polyuria, polydypsia began in the first years of the disease and got worse over time. Her skin turgor was reduced and her mouth was completely dry. She had 11L urinary output and oral intake in a day. Laboratory findings on admission were as follows: serum sodium: 144 mmol/L (135–146 mmol/L), potassium: 4,9 mmol/L (3.5–5.1 mg/dl) and chloride: 100 mmol/L (95–107 mmol/L), serum creatinine: 0.4 mg/dL (0.7–1.4 mg/dl). Free T4: 17.7 pmol/L, TSH: 1.23 mIU/L, LH: 3,2, FSH: 1,9, estradiol: 23 mIU/L, Prolactin: 0,6 ng/ml, cortisol: 21.3 microg/dl. According to urinalysis, the density of the urine was 1000. Urinary and plasma osmolality were 101 and 324 mOsm/L, respectively. Her gonadotropine levels were not compatible with menoposis. On the day after admission, dDAVP 0,1 mg/day was administered orally. The urinary output decreased to 3 L/day and the oral intake was 4 L/day. Magnetic resonance imaging of sella revealed a huge mass filling sella turcica, arising from suprasellar cistern, surrounding cavernous sinus and compressing to optic chiasm and infindubulum. The mass was compatible with breast cancer metastasis to hypophysis, and radiotherapy was performed. Three months after irradiation, panhypopituitarism had developed. She is still alive under full replacement therapy.
Conclusion
Extension of breast cancer to the pituitary gland is a rare and late complication. Although life expectancy is limited in advanced breast cancer, hormonal insufficiency should be corrected to increase the life quality.

P514

Multicystic dysplastic kidney – a potential accelerant of complications in type I diabetes mellitus

A Hillick, B Callaghan, EH El Tayeb, LA O'Shea & AG Stack
Department of Reanl Medicine, LGH, Co. Donegal, Ireland.

A Multicystic dysplastic kidney (MCDK) is a congenital, renal, cystic transformation usually diagnosed perinatally with 1:1000-4,000 incidence[1]. The natural history of MCDK is disputed with involution[1], enlargement and development of hypertension[2], infection and malignant transformation reported in the literature. We describe the incidental detection of An occult MCDK was detected in a 25-year-old chef who presented with a 4 month history of diarrhoeal episodes and left flank discomfort. He had noted a sensation of fullness in the flank for a number of years but had not sought medical investigation. It had increased in size and discomfort with the onset of diarrhoeal episodes. He had a 13 year history of Type I Diabetes Mellitus Medications included Novorapid 8iu/10iu/8iu, glargine 22iu nocte and lisinopril 2 mg daily. Blood pressure was 160/103 mmHg, and bilateral pre-proliferative retinopathy with neovascularisation. Abdominal palpation revealed a large left flank mass, confirmed on CT Abdomen and a non-functioning left kidney on DMSA scan. Laboratory studies revealed striking polycythaemia (Hb 21 g/dL), elevated erythropoietin level 36 mIU/ml (normal range 6–25), HbA1c 12.2%, diabetic proteinuria (0.16 grams/24 hours) and glomerular hyperfiltration GFR 130 mls/min/1.73 m^2. Preoperatively laser treatment and repeated venesection was required to manage worsening diabetic retinopathy and secondary polycythaemia. Following nephrectomy, stabilisation of retinopathy, normalization of haemoglobin and an improvement in hypertension control was observed. This case strengthens the arguement[2] for removal of all MCDKs in childhood to prevent complications in adulthood.

1. Rabelo EAS et al. Conservative management of multicystic dysplastic kidney: clinical course and ultrasound outcome. *Jornal de Pediatria* 2005 **81** 400–404.
2. Webb NJA *et al.* Unilateral multicystic dysplastic kidney: the case for nephrectomy. *Archives of Diseases of Childhood* 1997 **76** 31–34.

P515

MEN-1 phenotype without detectible MEN-1 mutation

Ivana Bozic, Katarina Mirkovic, Djuro Macut, Bojana Popovic, Tatjana Isailovic, Milan Petakov, Sanja Ognjanovic & Svetozar Damjanovic
Institute for endocrinology, diabetes and metabolic diseases, Department for endocrine oncology, Belgrade, Serbia.

We describe a 52-year-old woman, with acromegaly, clival chondroid chordoma, meningioma and lung carcinoid. There was no family history of MEN-1. She was diagnosed as acromegaly in 2000. Radiological evaluation (MRI) revealed pituitary tumor, however, another infiltration of scull base was detected which invaded sphenoidal and ethmoidal sinuses, lamina cribroza and bilateral orbit walls. Pituitary tumor was completely removed and the reduction of extra-sellar mass was performed. Hystopathological and immunohistochemical analysis confirmed somatotroph pituitary adenoma and chondroid chordoma. After surgery, she almost normalized IGF-I levels (288 ng/mL) while GH remained unsuppressible during oGTT. In 2001 the second surgery was performed, para- and infra-sellar mass was reduced and pathohystology confirmed diagnosis of chondroid chordoma. In 2004 irradiation therapy gave no results regarding regression of scull base tumor, but IGF-I (113 ng/mL) and GH suppressibility normalized one year later. Atypycal bronchial carcinoid from the left lung was extirpated the same year and meningeoma arising from the falx cerebri was detected on MRI. Until now, the residual chordoma showed no further progression. On ^{111}Indium-labelled octreotid scintigraphy performed after lung operation, only meningeoma was detected. Even six years after the initial diagnosis there are no sings of primary hyperparathyroidism.

Possible mechanisms explaining MEN 1 phenotype with negative genetic result: the patient might have sporadic MEN 1 syndrome caused by double independent somatic events or to have germline mosaicism that has to be confirmed by genetic analyses of various tissues. Additionaly we have to exclude large deletion in MEN 1 gene.

P516

Composite medullary and papillary tumor with mixed lymph node metastases

Tatjana Isailovic, Milan Petakov, Djuro Macut, Bojana Popovic, Ivana Bozic, Sanja Ognjanovic, Jelena Nikolic, Jovana Vignjevic, Katarina Mirkovic & Svetozar Damjanovic
Institute of Endocrinology, diabetes and metabolic diseases, Belgrade, Serbia.

A 34 year-old female patient was admitted to the hospital because of a large nodule in the left thyroid lobe and elevated calcitonine level. A large encapsulated tumor was found and total thyroidectomy with left neck dissection was performed. Pathohistology revealed medullary and papillary carcinoma separate from each other in tumor tissue but mixed in regional lymph nodes. Papillary component was dominant in thyroid tissue but not in lymph nodes. Both calcitonine and thyroglobulin plasma levels were elevated after the surgery witch suggested distant metastases. I^{131} scintigrafy showed focal accumulation in the left side of the neck, thoracal vertebrae and diffuse accumulation in the ribs. DMSA and I^{131}MIBG scintigraphy revealed pathologic focuses in the left thyroid

lobe region. None of these focuses was confirmed by MRI. The octreoscan was negative. Genetic analysis of RET protooncogene was negative. The patient was treated with radiotherapy.
Conclusion
Synchronous occurrence of medullary and papillary carcinoma of the thyroid gland is very rare and a few cases were described in the literature. Concurrency of two distinct cell lines may suggest that they have a common stem cell origin or possible activation of a common tumorigenic pathway. However, synchronous coincidental genetic event cannot be excluded.

P517

Management of endocrine syndrome in a patient with plurihormonal neuroendocrine tumour

Bojana Popovic, Djuro Macut, Ivana Bozic, Tatjana Isailovic, Milan Petakov, Sanja Ognjanovic, Katarina Mirkovic, Jovana Vignjevic & Svetozar Damjanovic
Institute of endocrinology, diabetes and metabolic diseases, Belgrade, Serbia.

Neuroendocrine tumours (NETs) have a unique ability to produce and secrete a variety of biogen amines and peptide hormones. They arise from multipotential stem cells, which differentiate during tumorigenesis into specific cell lines. Some of these tumours are functional, producing characteristic clinical syndromes. We present a female patient incidentally discovered to have diffuse liver metastases of neuroendocrine tumour of unknown primary origin. She was free of symptoms at initial presentation, and pathohistological analysis after liver biopsy revealed the tumour to be well differentiated (Ki-67 – 4.5%) with somatostatin positive in more than 70% of tumour cells. Fasting glycemia was normal, but results of oral glucose tolerance test were in favor of diabetes. Thorough examination including the octreoscan did not reveal the site of primary tumour. Expression of somatostatin receptors was intensive in metastases. Three months later, she reported episodes of night sweats, tremulousness, tachycardia and amnesia. Hypoglycemia was recorded during first hour of fasting, with extremely high levels of insulin and C-peptide. Further imunohistological investigation of tumor bioptate revealed positivety for proinsulin in 30%, and insulin in less than 0.2% of tumour cells. After the initiation of diazoxide the glycemic control was improved but only after the initiation of combined therapy with short-acting somatostatin analogue she managed to have euglycemia during the whole day. In an attempt to control both endocrinological syndrome and tumour growth, the patient underwent hemoembolisation. Clinical syndromes caused by plurihormonal secretion make therapeutical treatment difficult, especially in cases of cosecretion of physiological antagonists.

P518

Brown tumor in hyperparathyroidism – clinical case

Zelija Velija-Asimi & B Helijc
University Clinical Centre of Sarajevo, Bosnia and Herzegovina.

A 66 years old woman on orthopedic clinic, was resection of tibias tumor in both legs because was suspicion of primary bone neoplasm or metastases (X-ray showed osteolytic lesion). Histology was: osteitis fibrosa cystica - Brown tumor. After resection the patient was referred to the endocrinologist because of persistently high calcemia (3.3 mmol/l). Blood tests showed normal CRP but elevated alkaline phosphatase of 173 U/l. Phosphate was low at 0.75 mmol/l (0.81–1.58). Parathyroid hormone (PTH) was elevated at 1450 pg/ml (10–65 pg/ml). Renal function was normal. CT scans of chest and abdomen was normal. But echosonography of parathyroid gland showed tumor structure size 4 cm of lower parathyroid gland in the right side. This tumor resected. The histological examination confirmed the parathyroid carcinoma. Post parathyroidectomy her calcium, phosphate, alkaline phosphates and PTH are in normal limits. When X-ray shows an osteolytic lesion, PTH and calcemia should be performed to exclude the primary hyperparathyroidism.

Neuroendocrine and pituitary behaviour – presented on Monday

P519

Investigation of early atherosclerotic changes in acromegalic patients

Ilkay Kartal, Huseyin Oflaz, Burak Pamukcu, Ferihan Aral, Nese Ozbey & Faruk Alagol
Istanbul University Istanbul Medical Faculty, Istanbul, Turkey.

Background
Functional and morphological changes of endothelium are risk factors for mortality attributed to atherosclerosis. Studies investigating early atherosclerotic alterations and the effect of the treatment of acromegaly on these alterations gave conflicting results.
Objective
Surrogate markers of early atheosclerotic changes, i.e. brachial artery flow-mediated dilation (FMD) and carotid artery intima-media-thickness (IMT) in active and inactive acromegalic patients are compared with control subjects matched to patients for age, sex, cardiovascular risk factors in order to find out the direct effects of GH/IGF-1 excess.
Methods
In 14 active acromegalics and their 14 matched controls, 14 inactive acromegalics and their 14 matched controls, carotid artery IMT and FMD of brachial artery were measured. Inactive acromegalics were in remission for at least 1 year.
Results
Active acromegalics had higher IMT than matched controls and inactive acromegalics (0.85 ± 0.20 mm, 0.64 ± 1.77 mm, 0.66 ± 0.20 mm respectively; $P<0.005$, $P<0.05$) and IMT of inactive acromegalics was not different from their matched controls (0.61 ± 0.12 mm). FMD was significantly lower in active acromegalics than in matched controls and inactive acromegalics (2.910 ± 2.00 mm, 6.5 ± 2.81 mm, 5.68 ± 2.9 mm respectively; $P<0.005$, $P<0.05$). FMD of inactive acromegalics was not significantly different from their matched controls (7.96 ± 3.12 mm). A significant inverse relationship was found between GH and FMD in active acromegalics ($r=-0.659$; $P=0.010$).
Conclusion
In active acromegalics, early atherosclerotic changes are not only attributed to the high prevalence of risk factors, but also to the abnormal GH secretion itself.

P520

Food preference, central serotoninergic activity, depression and insulin resistance in obese and lean healthy men: a pilot study

Ludmila Brunerova[1], Jana Potockova[1], Jiri Horacek[2] & Michal Andel[1]
[1]Diabetologic Centre, II. Department of Internal Medicine, Faculty Hospital Kralovske Vinohrady and 3rd Faculty of Medicine, Charles University, Prague, Czech Republic, [2]Psychiatric Centre Prague, 3rd Faculty of Medicine, Charles University, Prague, Czech Republic.

Objective
Higher anxiety/depression scores can be connected with food preference of carbohydrates, with increased insulin secretion and thus can lead to insulin resistance. Three hypotheses were postulated: 1. more depressive (still in the normal range) subjects do not differ in the central serotoninergic activity from those less depressive. 2. the former do not differ in food preference and 3. in insulin resistance from the latter.
Methods
healthy men, 30–55 years, 9 lean (44.5 ± 7.7 years, BMI 22.8 ± 1.8 kg/m^2) and 8 obese (45.3 ± 6.0 years, /ns/ BMI 30.5 ± 4.0 kg/m^2 /$P=0.0005$/) were involved in the study, which was approved by the local Ethical Comittee. The study protocol included filling in the SCL-90 questionnaire (for excluding psychopathology), self-assessing questionnaires for depression and anxiety (SAS, SDA), carbo-hydrate craving questionnaire (CCQ), 3-days diet records, 4-hours hyperinsuli-nemic euglycemic clamp on two insulin levels (1mU/kg/min and 10mU/kg/min) with calculating the metabolic clearance of glucose: MCR1, MCR2 and citaloprame challange test with 0.3 mg/kg citaloprame infusion followed by plasma sampling for prolactine at –30, −5,0,15,30,45,60,90,120,150 minutes and calculation of area under the curve for prolactine (AUC/PRL). ANOVA, Kruskall-Walis test and linear regression were used for statistical analyses.
Results
No correlations were found between AUC/PRL and SAS/SDA scores. Positive correlations were determined between SAS and SDA scores and % of carbohydrates in diet records ($r=0.74$; $P<0.01$, resp. $r=0.75$; $P<0.01$) and between depression/anxiety scores (SCL-90) and CCQ score (0.53; $P<0.01$, resp.0.54; $P<0.01$). We have not observed any relationships between central serotoninergic activity respectively SAS/SDA scores and MCR1/ MCR2.
Conclucions
We have proved that the subjects with higher depression/anxiety scores prefer more carbohydrates in the food. However, we have not observed any relationships between insulin resistance and depressive/anxiety scores or the central serotoninergic activity.
The study was supported by VZ MSM 0021620814.

P521

Somatic, body composition and psychological particularities in a group of untreated adult pituitary dwarves

Ioan Gotca, Dumitru Branisteanu, Letitia Leustean, Eusebie Zbranca & Voichita Mogos
University of Mediciine and Pharmacy "Gr.T.Popa", Iasi, Romania.

Aside its growth promoting effects, growth hormone (GH) displays other actions upon carbohydrate, lipid and protein metabolisms, and possibly also direct central nervous system effects. Fourteen adult pituitary dwarves (mean height of 132.3 ± 8 cm, mean age of 30.7 ± 9.6 y), 5 women and 9 men, never having received rGH therapy, were investigated. Body composition (BC) was assessed by bioelectrical impedance, and bone mineral density (BMD) was evaluated by quantitative ultrasound. Patients were submitted to psychological tests and examined by a psychologist and psychiatrist.

BC of GH-deficient adult dwarves was significantly modified: a reduced percentage of water (45.7 ± 13.6% compared to 69.4 ± 15% water in a BMI- and age-matched group with normal adult height) and an increased fat percentage (48.3 ± 12.9% compared to 25.2 ± 9.4% in normal-sized BMI-matched healthy controls, $P < 0.05$). BMD was decreased in the group of pituitary dwarves, with a mean T score of −1.45 ± 0.8 (in the range of osteopenia). When psychologically assessed, certain pituitary dwarves scored poorly at family and society adaptation (10 and 12 patients, respectively), whereas all but one patient had mild to profound self-esteem disturbances. Ten patients were resistant to refractory at any external help. Two dwarves had a high Beck depression score, three had suicidal thoughts and one had a suicidal attempt in her history. A strong correlation between the patients' IQ and their quality of life estimated with the Guilford-Zimmerman score was observed ($R^2 = 0.764$). Non-treated, childhood-onset GH deficiency leads therefore not only to dwarfism, but also to alterations in body composition and energy output. Modifications in their body image may have significant impact upon the adaptation of pituitary dwarves in the society, and on their quality of life. Their adaptation is dependent to a great extent of their mental capacity as well as of the degree of tolerance from the family and society.

P522

L-thyroid hormone enhancement of antidepressant treatment in major depressive episode

Paul Ivanov[1], Alexandra Raceala[2], Octavian Vasiliu[1] & Diana Paun[2]
[1]Emergency Military Hospital Dr. Carol Davila Department of Psychiatry, Bucharest, Romania; [2]Universitary Endocrinology Hospital Dr. C.I. Parhon, Bucharest, Romania.

Objective
To determine the impact of combined antidepressant drugs and LT4 enhancers in treatment of patients with Major Depressive Disorder.
Method
We conducted a randomized, placebo-controlled trial to determine whether LT4 supplementation had any augmentation effect on selective serotonin reuptake inhibitors (SSRIs). The study involved 70 patients with major depressive disorder; patients with hypothyroidism were excluded. Of the participants, 38 were assigned to receive LT4, and 32 received placebo. All of the patients received SSRI – paroxetine (50%), sertraline (28%) and fluoxetine (22%). A total of 66 patients completed the three month study. We made weekly psychological evaluations using clinical scale HAM-D (Hamilton Depression Scale). Thyroid data, consisting of values for thyroid-stimulating hormone TSH and LT4, measured by radioimmunoassay were collected before and after treatment.
Results
A decrease in HAM-D score was observed in both groups, with a medium improvement of 12.3 points and a significant difference in favour of LT4 group. In the LT4 group, 30 patients (83.3%) responded to treatment compared with 21 patients (70%) in the placebo group. The onset of antidepressant effect was earlier in the LT4 group with an average response in 2 weeks. Those in the group receiving LT4 supplements had lower levels of LT4 and TSH after the study when compared to baseline. Final TSH values correlated strongly with response to treatment as measured by change in HAM-D scores.
Conclusion
Supplements of levothyronine (LT4) enhance the antidepressant effects of SSRIs. LT4 is efficacious as an enhancer of antidepressant therapy. Low TSH values correlated with greater improvement in depressive symptoms.

P523

Evaluation of cognitive functions by using P300 auditory event related potentials (ERPs) in amateur kickboxers: a preliminary study

Fatih Tanriverdi[1], Hande Yapislar[2], Cem Suer[2], Ismail Kocyigit[1], Kursad Unluhizarci[1] & Fahrettin Kelestimur[1]
[1]Erciyes University Medical School Department of Endocrinology, Kayseri, Turkey; [2]Erciyes University Medical School Department of Physiology, Kayseri, Turkey.

Objective
Impaired cognitive function has been demonstrated in adults with GH deficiency (GHD) by using different neuropsychological tests. P300 ERP application is a well established neurophysiological approach in the assessment of cognitive function. Kickboxing is a novel cause of hypopituiatarism due to sports related traumatic brain injury (TBI) and isolated GHD is the most common problem (1).

The present preliminary study was therefore designed to investigate the effects of sports related head trauma induced GHD on cognitive function by using P300 ERPs.
Methods
The study comprised 15 amateur kickboxers (13 male, 2 female), with a mean age of 30.0 ± 5.9 yr. GHD was diagnosed in 6 kickboxers by using two stimulation tests (GHGH + GHRP-6 and glucagon). 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
The mean P300 latencies (at all electrode sites) of the kickboxers with GHD were prolonged when compared with those of GH normal kickboxers. However the difference did not reach to a significant level. There was a significant negative correlation between IGF-I levels and latencies at Fz electrode site (r: −0.530, P: 0.04).
Conclusions
P300 latency is related to stimulus evaluation time and prolonged P300 latencies suggest an impaired cognitive function in GH deficient kickboxers. The differences did not reach to a significant level due to a small sample size. This is an objective electrophysiological evidence for cognitive dysfunction in GHD and further data with high number of kickboxers are warranted.

P524

Changes in hypothalamo-pituitary-testicular axis sensitivity in aging male

Svetlana Vujovic, Milos Stojanovic, Miomira Ivovic, Branko Barac, Milina Tancic Gajic, Marija Barac, Ljiljana Marina & Milka Drezgic
Institute of Endocrinology, Belgrade, Serbia.

Involutive hypoandria (late onset hypogonadism) is characterized by decline in serum testosterone and increase of gonadotropins. Changes of hypothalamo-pituitary-testicular axis sensitivity are influenced by primary testicular changes and altered neuroendocrine regulation during aging. To evaluate age-related changes in gonadotroph and Leydig cell sensitivity two groups were formed: 1) 35 men, 51.8 ± 3.2 years old, BMI = 28.2 ± 3.1 kg/m^2; 2) 32 men, 63.2 ± 6.8 years old, BMI = 27.2 ± 3.1 kg/m^2. Blood samples for FSH, LH, prolactin, estradiol, testosterone, SHBG were taken at 8 am. LHRH test was then performed (100 microg LHRH i.v., FSH and LH were taken before, 20 and 60 min later). Next three days HCG test was done (Pregnyl amp. a 5000 i.j./day, testosterone, estradiol and SHBG were detected before and after test). Hormone analyses were done by RIA. Statistics: Spearman, Mann-Whitney test, area under the curve-AUC. Neither increase of LH (4.5 ± 3.1 vs. 5.8 ± 3.5 IU/L, $P > 0.05$) nor decrease of testosterone (19.7 ± 7.6 vs. 14.8 ± 6.9 nM/L, $P > 0.05$) reached significant difference. The maximal LH response in 20 minutes (17.6 ± 13.2 vs.27.0 ± 11.8 IU/L, $P = 0.03$) and LH AUC (962.5 ± 738.2 vs. 1428.5 ± 658 IU/L/min) were higher in older men. Higher sensitivity of Leydig cell testosterone response was observed in older group (19.2 to 33.1 vs. 14.2 to 31 nM/L, $P = 0.0021$). Negative correlation was found between testosterone and BMI ($P = 0.02$). Conclusion: Older men show significantly increased gonadotrophin release due to amplified secretory burst mass, diminished gonadal hormone negative feedback or primary alterations in hypothalamo-pituitary unit with aging. Leydig cell sensitivity is preserved during aging. Secondary testicular failure in aging male is due in part to decreased GnRH gene expression rather than to decreased pituitary responsiveness to LHRH.

P525

Glutamatergic neurons and synaptic contacts between glutamatergic axon terminals and chemically identified nerve cells in the rat hypothalamic suprachiasmatic nucleus

József Kiss[1], Zsolt Csaba[2], Ágnes Csáki[3] & Béla Halász[4]
[1]Hungarian Academy of Sciences and semmelweis University, Neuroendocrine Research Laboratory, Budapest, Hungary; [2]Department of Human Morphology and Developmental Biology, Semmelweis University, Budapest, Hungary; [3]Department of Human Morphology and Developmental Biology, Semmelweis University, Budapest, Hungary; [4]Hungarian Academy of Sciences and semmelweis University, Neuroendocrine Research Laboratory, Budapest, Hungary.

The hypothalamic suprachiasmatic nucleus (SCN) is the key-structure of the control of circadian rhythms. Several observations support the view that glutamate is the primary transmitter of the retinal projection to this cell group. The glutamatergic innervation of the nucleus is not limited to this projection, it is much more extended. The aim of our investigations was (1) to examine whether are glutamatergic neurons existing in the SCN and (2) to get information about the relationship between glutamatergic axon terminals and vasoactive intestinal polypeptide (VIP)-, GABA- and arginine-vasopressin (AVP)-containing neurons. Vesicular glutamate transporter type 2 (VGluT2) was used as marker of the glutamatergic elements. Single and double label immunocytochemistry was applied and the brain sections were examined by confocal laser scanning microscopy and under the electron microscope. We detected VGluT2 immunoreactive neurons in the SCN and observed VGluT2 axon terminals in synaptic contact with GABA, VIP, AVP and with VGluT2-positive perikarya or dendrites. The morphology of the contacts indicated asymmetric type synapses. Our observations provide the first neuromorphological evidence for the view that glutamatergic neurons exist in the SCN and further they demonstrate for the first time terminations of glutamatergic boutons on prominent cell groups of the SCN. The findings are in line with the view that the intranuclear organization of the circadian clock is extremely complex.

This work was supported by the National Scientific Research Fund (OTKA T-042516 to B.H., OTKA T-049455 to K.J.), the Ministry of Health (ETT 020/2006), National Research Program (NKFP 1A002-04 8/6/04) and the Hungarian Academy of Sciences.

P526

Laterality in the supraspinal innervation of the adrenal gland: a dual-virus tracer study

Ida Tóth[1], Ory Wiesel[1], Dorina Tóth[2], Zsolt Boldogkoi[3], Kamill Bálint[3] & Ida Gerendai[1]
[1]Neuroendocrine Research Laboratory, Hungarian Academy of Sciences and Semmelweis University, Department of Human Morphology and Developmental Biology, Budapest, Hungary; [2]Department of Physiology, Albert Szent-Györgyi Medical University, Szeged, Hungary; [3]Department of Biology, Albert Szent-Györgyi Medical University, Szeged, Hungary.

Recent studies using the viral transneuronal tracing technique allowed to reveal brain neurons synaptically connected with the adrenal gland, via specific infections within functionally related chains of neurons. The aim of the present study was to investigate whether cerebral neurons involved in the innervation of the adrenal gland exhibit asymmetry or not. In order to label simultaneously the supraspinal neurons connected with the right- and left-sided adrenal glands, dual-virus tracer was applied in young female rats. Two genetically almost identical, however, histochemically distinguishable, newly constructed Bartha strains of pseudorabies virus (PRV) were used, each expressing a unique marker antigen in infected host cells (green fluorescent protein: BDG-strain or ß-galactosidase, BDL-strain). The virus suspensions were injected into the left- or right-sided adrenal, then the spinal cord and brain were investigated by immunofluorescent staining of the marker genes. In the brain stem the nucleus of the solitari tract, dorsal vagal, ambigus, parapyramidal nuclei, caudal raphe nuclei and the ventrolateral areas were labeled, mainly by monolabeled neurons. In addition, neurons of the A5, lateral hypothalamus, paraventricular (PVN), periventricular, arcuate nuclei were infected. In PVN many neurons were double-labeled. Viral infection of the above cell groups projecting to the left adrenal was more prominent compared to that of the right gland. Data suggest predominance in supraspinal innervation of the left adrenal gland.

Supported by grants from the National Research Found (OTKA T-046624), the Ministry of Health (ETT 448/2006) and the Hungarian Academy of Sciences.

P527

Prolonged orocecal transit time and small intestinal bacterial overgrowth in acromegalic patients

Eugenia Resmini[1], Andrea Parodi[2], Vincenzo Savarino[2], Alfredo Greco[2], Alberto Rebora[1], Francesco Minuto[1] & Diego Ferone[1]
[1]University of Genoa, Department of Endocrinology, Genoa, Italy; [2]University of Genoa, Department of gastroenterology, Genoa, Italy.

Gastrointestinal abnormalities in acromegaly include dolichomegacolon and increased prevalence of colonic polyps. No data are available on the small intestine. The aims of this study were to investigate orocecal transit time (OCTT) and the presence of small intestinal bacterial overgrowth (SIBO) in acromegaly. 41 acromegalic patients and 30 controls entered the study. Acromegalics were classified according to whether they were on medical treatment with somatostatin analogs (SSA): "treated" and "untreated" and according to clinical control: "controlled", "uncontrolled" and "partially controlled". Acromegalics and controls were submitted to a 10 g lactulose hydrogen (H_2) breath test (LH-BT) in order to determine the OCTT and presence of SIBO.

There is an increased prevalence of SIBO in acromegalics comparing to controls ($P=0.000$). OCTT was significantly slower in acromegalics comparing to controls ($P=0.000$).

Nine treated and 9 untreated acromegalics were positive for SIBO, without a statistical significant difference. Six controlled, 9 partially controlled and 3 uncontrolled acromegalics were positive for SIBO, without a statistical significant difference.There was a significantly lower OCTT in treated compared with untreated patients ($P=0.02$) and between these two groups and controls ($P=0.00$). There was no statistically significant difference for OCTT between controlled and uncontrolled acromegalics.

These data demonstrate for the first time that SIBO occurs more frequently in acromegalics than in controls, and medical therapy with SSA does not influence the presence of SIBO. OCTT is significantly delayed in acromegalics both in treated and in untreated ones and this suggests that acromegaly determines *per se* impairment of intestinal motility. Clinical control does not influence the OCTT, suggesting that this may be an irreversible complication. The slower OCTT may represent a risk factor for the development of SIBO. These alterations might be related to the occurrence of an autonomic intestinal disorder, as we have previously demonstrated for cardiac autonomic activity in acromegaly.

P528

Adulthood follow-up of a large number of patients with congenital morphological alteration of anterior and/or posterior pituitary lobes

Danilo Ribichini[1], Cecilia Ragazzini[1], Stefano Zucchini[2], Piero Pirazzoli[2], Alessandro Cicognani[2], Valentina Vicennati[1], Uberto Pagotto[1] & Renato Pasquali[1]
[1]Unità Operativa di Endocrinologia, Bologna, Italy; [2]Clinica Pediatrica, Bologna, Italy.

Congenital morphological alteration of anterior and/or posterior pituitary lobes is a rare disease often associated with isolated or multiple hormonal deficiency. In this study we re-evaluated 24 adult patients, 20 males and 4 females, (23–46 years), in whom Nuclear Magnetic Resonance (NMR) showed congenital morphological alteration of the gland. Twenty out of 24 patients presented with an association of hypoplasic adenohypophysis and ectopic neurohypophysis, while 4 presented with hypoplasic adenohypophysis and undetectable neurohypophysis as by NMR. All patients are currently under hormonal replacement therapy. Fourteen out of 24 patients were panhypopituitaric (58.3%); 3 presented with multiple GH, LH-FSH, TSH deficit (12.5%); 2 with GH and combined LH-FSH deficit (8.3%); 2 with isolated GH deficit (8.3%); 1 with combined GH, ACTH, TSH deficit (4.2%); 1 with multiple GH, LH-FSH, AVP deficit (4.2%); and 1 with isolated AVP deficit (4.2%). Anthropometric parameters showed that 11 patients are overweight (BMI 25–29.3), and 4 obese (BMI 31.5–34.6). Biochemical evaluation showed that 9 patients were hypercolesterolemic (37.5%), 7 were hypertrygliceridemic (29.2%), and 4 patients presented with low levels of HDL-colesterol (16.7%); other metabolic or biochemical parameters were not significantly altered. Epilepsy has been recently observed in 2 of these patients presumably associated with a novel radiological findings of areas of subependymal heterotopia. In conclusion, the association of hypoplasic adenohypophysis and ectopic or undetectable neurohypophysis are congenital conditions frequently associated with single or multiple hormonal deficits. The etiopathogenesis of these conditions is still unknown, and our preliminary genetic observation seem to exclude that HESX1 or PROP1 mutations play a role in these congenital malformations (11 out of 24 patients were tested and no mutations were found for both genes). Moreover, periodic long-life radiological monitoring of the brain is necessary to detect areas of subependymal heterotopia in order to pharmacologically prevent epilepsy.

P529

Non-NMDA glutamate receptor antagonist injected into the mesencephalic dorsal raphe nucleus inhibits the suckling-induced prolactin release and administered into the lateral cerebral ventricle interferes with the diurnal fluctuations of plasma prolactin of male rats

Ibolya Bodnár, Zsuzsanna Bánky & Béla Halász
Hungarian Academy of Sciences and Semmelweis University, Neuroendocrine Research Laboratory, Budapest, Hungary.

We examined the functional significance of the glutamatergic innervation of the dorsal raphe nucleus (DR) in the mediation of the suckling stimulus inducing prolactin release. A non-NMDA (6-cyano-7-nitroquinoxaline-2,3-dione disodium, CNQX) or an NMDA glutamate receptor antagonist (dizocilpine hydrogen malate, MK-801) was injected into the DR of freely moving lactating rats at the end of 4 h separation. The litters were then returned and blood samples for prolactin were taken at different time points. In addition, we studied the effect of the non-NMDA receptor antagonist on the diurnal fluctuations of plasma prolactin and corticosterone. Adult male rats received by means of ALZET minipump CNQX (0.5 or 10 pM/µl/h) into the lateral cerebral ventricle for 72 hrs before and during blood sampling. CNQX, when injected into the DR in higher dose, inhibited the suckling-induced prolactin release. After MK-801 administration the prolactin response was significantly diminished. There were no diurnal fluctuations in plasma prolactin concentrations and only attenuated changes in corticosterone levels of rats treated with CNQX compared to controls getting physiological saline into the lateral ventricle. The findings suggest that (1) the glutamatergic innervation of the dorsal raphe nucleus is involved in the mediation of the neural signal of the suckling stimulus inducing prolactin release and (2) glutamatergic innervation of brain structures participating in the control of diurnal fluctuations of plasma prolactin and corticosterone concentration contributes to the maintenance of the circadian rhythm of these hormones.

This work was supported by the National Scientific Research Fund (OTKA T-042516 to B.H.), the Ministry of Health (ETT 020/2006 to B.H.) and the Hungarian Academy of Sciences.

P530

The expression of the neuroprotective factor seladin-1 is up-regulated by thyroid hormones in human neuronal precursor cells, but not in mature neurons

Susanna Benvenuti[1], Paola Luciani[1], Ilaria Cellai[1], Cristiana Deledda[1], Riccardo Saccardi[2], Serena Urbani[2], Gabriella B Vannelli[3], Fabio Francini[4], Roberta Squecco[4], Mario Serio[1], Aldo Pinchera[5] & Alessandro Peri[1]
[1]Endocrine Unit, Dept of Clinical Physiopathology, University of Forence, Florence, Italy; [2]Department of Haematology, Careggi Hospital, Florence, Italy; [3]Dept of Anatomy, Histology and Forensic Medicine, University of Florence, Florence, Italy; [4]Department of Physiological Sciences, University of Florence, Florence, Italy; [5]Department of Endocrinology and Metabolism, Section of Endocrinology, University of Florence, Florence, Italy.

Thyroid hormones (TH) play a fundamental role during brain development by modulating the expression of different genes involved in neuronal differentiation, proliferation, migration, myelinization, and synapse formation. *Seladin-1* (for SELective Alzheimer's Disease INdicator-1) is a recently identified anti-apoptotic gene, which has been found to be down-regulated in brain regions affected by Alzheimer's disease (AD). We hypothesized that seladin-1 might be a novel mediator of the effects of TH in the developing brain. Thus, in the present study we determined whether TH modulate the expression of seladin-1 in human neuronal precursors and/or in differentiated cells. Two different cell models were used: fetal human neuroepithelial cells (FNC) isolated previously from fetal olfactory epithelium; and human mesenchymal stem cells (hMSC), isolated from bone marrow, which have a demonstrated ability to differentiate into neurons. In our hands, hMSC were differentiated into neurons (hMSC-n), following previously established protocols. The neuronal phenotype was confirmed by the positivity for the specific markers nestin, glypican 4, necdin, neurofilament subunit L, neurofilament subunit M, neurite outgrowth-promoting protein, choline acetyltransferase, neuronal nuclei. Electrophysiological evaluation revealed the presence of inward Na and Ca currents typical of neuronal cells. In basal conditions, the amount of seladin-1 was significantly higher in undifferentiated cells than in mature neurons, as assessed by real-time RT-PCR. T3 and T4 (1 nM) significantly increased the amount of seladin-1 mRNA in both FNC (140% and 66% increase, respectively) and hMSC (61% and 16% increase, respectively), but not in hMSC-n. The amount of the protein, evaluated by Western blotting, changed accordingly. This is the first demonstration that TH stimulate the expression of seladin-1 in human neuronal precursors, but not in terminally differentiated neurons. These results suggest that this neuroprotective factor may play a prevalent role during brain development, together with other well-known TH-dependent factors.

P531

Long-term evaluation of hypotalamic-pituitary-adrenal (HPA) axis in acromegalic patients during somatostatin analogs therapy and after successful surgery

Cristina L Ronchi, Erica Rizzo, Emanuele Ferrante, Claudia Giavoli, Andrea G Lania, Paolo Beck-Peccoz & Anna Spada
Unit of Endocrinology, Dep. Medical Sciences, Fondazione IRCCS Ospedale Maggiore Policlinico, Mangiagalli e Regina Elena, Milano, Italy.

Long-term effects of trans-naso-sphenoidal surgery (TNS) and long-acting somatostatin analogs (SSTa) on hypotalamic-pituitary-adrenal (HPA) function have been poorly investigated. Aim of the study was to evaluate over time the integrity of HPA axis in acromegalic patients with baseline preserved adrenal function and treated with one or both available treatments. We selected 23 patients (15F & 8M, age ($\pm$S.D.) = 46.8 $\pm$ 13.7 yrs) with normal (n = 19) or subnormal HPA axis not requiring replacement therapy (n = 4). In particular, 15 patients well responsive to chronic SSTa therapy (11 previously operated and 4 de novo) were investigated before and during treatment (median = 63 months), while 8 patients cured by TNS were studied 2–3 months after surgery and during follow-up (median = 100 months). HPA function was studied by morning circulating cortisol and ACTH levels, urinary free cortisol (UFC) and cortisol response to low-doses short Synacthen test (LDSST, 1 mcg). The cut-off for a normal function was a cortisol peak $>$ 500 nmol/liter while a peak between 450 and 500 indicated a partial hypoadrenalism. All patients were studied for serum GH and IGF-I, basal thyroid and gonadal function and MRI. Basal cortisol, ACTH and UFC levels did not significantly change over time and remained in the normal range. Considering the cortisol peak after LDSST, 3 patients with subnormal function at baseline developed overt hypoadrenalism (peak $<$ 450 nmol/liter), 7 with normal adrenal function developed partial (n = 4) or overt hypoadrenalism (n = 3), while HPA function remained unchanged in 13. No significant correlations between HPA axis deterioration and GH/IGF-I levels, type of treatment, SSTa formulation, occurrence of other pituitary deficiencies, presence of secondary empty sella, changes in tumor or residual volume were observed. In conclusion, the HPA axis integrity must be carefully monitored over the time in all acromegalic patients, independently from the type of treatment, and not limited to patients undergoing radiotherapy.

P532

Growth hormone deficiency and recombinant hGH (rhGH) replacement in children with idiopathic isolated GH deficiency: effects on the hypothalamus-pituitary-adrenal axis

Silvia Bergamaschi[1], Claudia Giavoli[1], Emanuele Ferrante[1], Roberto Rusconi[2], Andrea G Lania[1], Anna Spada[1] & Paolo Beck-Peccoz[1]
[1]Endocrine-metabolic Unit, Department of Medical Sciences, University of Milan, Fondazione Ospedale Maggiore Policlinico, Mangiagalli e Regina Elena IRCCS, Milan, Italy; [2]Pediatric Unit, University of Milan, Fondazione Ospedale Maggiore Policlinico, Mangiagalli e Regina Elena IRCCS, Milan, Italy.

Cortisol and cortisone are interconverted by type 1 and type 2 11ßhydroxysteroid dehydrogenase (11ßHSD) isoenzymes. The type 1 isoenzyme is a widely expressed reductase that converts cortisone to cortisol regulating glucocorticoid tissue exposure. Its activity is inhibited by GH and IGF-I, being increased in GH deficiency (GHD) and decreased in acromegaly. In our experience rhGH therapy unmasked a central hypoadrenal state in adults with organic GHD, likely by normalizing 11ßHSD1 activity and reducing cortisone to cortisol conversion.

Aim of this study was to evaluate the hypothalamus-pituitary-adrenal (HPA) axis in 9 children (5M and 4F, mean age 12.0 $\pm$ 1.1 (S.E.) yrs, mean height SDS -2.1 ± 0.4) with idiopathic isolated GHD. Measurements were performed at baseline and on rhGH therapy (mean duration: 12 $\pm$ 3 months, mean dose: 0.03 $\pm$ 0.01 mg/kg bw/day). HPA function was assessed by basal serum cortisol levels and after 1 mcg ACTH test (n = 4 patients) or insulin tolerance test (ITT, n = 5 patients). Central hypoadrenalism was excluded for both tests by the presence of either a peak of cortisol $>$ 500 nmol/L or a cortisol absolute delta $>$ 200 nmol/L. Serum IGF-I levels normalized on rhGH. Mean basal serum cortisol levels on rhGH, though showing a slight decrease, did not significantly differ from those recorded at baseline (215 $\pm$ 28 *vs* 256 $\pm$ 52 nmol/L, respectively, P = NS). The serum cortisol peak either after 1

mcg ACTH and after ITT was the same on rhGH therapy and at baseline (515±126 *vs* 574±131 nmol/L, respectively, P=NS). Plasma ACTH levels did not vary significantly. In conclusion, according to the diagnostic criteria, no child became central hypoadrenal on rhGH, contrary to what observed in adult patients with organic GHD and multiple pituitary deficits. This finding further supports the view that only in patients with organic multiple pituitary hormone deficiency GH deficiency may mask the presence of a hidden central hypoadrenalism.

P533

Effects of glucocorticoid replacement on bone mass in women after long-term remission of Cushing's syndrome

Maria-Jose Barahona, Nuria Sucunza, Pilar Lluch, Jose-Manuel Fedrnandez-Real, Wifredo Ricart, Teresa Puig, Ana M Wäger, Caroline van Elzelingen, Jordi Farrerons & Susan M Webb
Endocrinology, Hospital Sant Pau, UAB, Barcelona, Spain; Internal Medicine, Hospital Sant Pau, UAB, Barcelona, Spain; Epidemiology, Hospital Sant Pau, UAB, Barcelona, Spain; Endocrinology, Hospital J Trueta, Girona, Spain.

High dose and long-term glucocorticoid (GC) therapy reduce bone mass and negatively affect the metabolic profile. Patients in remission after successful treatment of Cushing's syndrome (CS) often present hypoadrenalism and require long-term GC replacement.
Objective
To evaluate the effect of GC therapy on bone and metabolic parameters in women after long-term remission of CS. Materials and methods: Thirty-two women (mean age: 50±14 years) with cured CS were enrolled. Mean time of cure was 11±6 years. Twenty-three patients had pituitary and 9 adrenal tumours. Bone mineral density (BMD) and body composition was measured by dual-energy x-ray absorptiometry scanning (DEXA). Anthropometric and laboratory parameters were measured (lipid profile, adiponectin, glucose, insulin, serum calcium, alkaline phosphate, fibrinogen, IGF-I and free T4). Duration of GC treatment, GC dose, and duration of hypercortisolism (including duration of CS symptoms pre-diagnosis and from diagnosis until cure) were calculated. Results were compared with those of 25 age-matched control women. Results: Duration of GC treatment, GC dose and duration of hypercortisolism were negatively correlated with bone mineral content (BMC) and BMD, and positively with fibrinogen. After multiple linear regression analysis, duration of GC treatment (P=0.003) and current age (P=0.019) were significantly related to BMC; only duration of GC treatment was related to BMD (P=0.002); whereas duration of hypercortisolism was significantly related to fibrinogen (P=0.004) and insulin (P=0.015). Daily GC dose was related to adiponectin (P=0.012). Patients treated longest with GC therapy (>24 months) had less BMC (P=0.002) and BMD (P=0.001) than those treated for <24 months and controls.
Conclusions
'Replacement' therapy with GC in women in remission after successful therapy for CS who are adrenal insufficient, is correlated with a reduction in bone mass and adiponectin. Thus, GC should be prescribed in the lowest dose and shortest time possible.
Supported by FIS 05/0448and CIBER 06 07/044.

P534

Factors influencing the rhythmic secretion of melatonin in human

Roxana Rosca[1], Olga Ianas[2], Dana Manda[2], Suzana Vladoiu[2], Carmina Busu[2], Dana Dinu[2], Carmen Chioasca[3] & Camelia Procopiuc[2]
[1]"Carol Davila" University of Medicine and Farmacy, Bucharest, Romania; [2]"C.I. Parhon" Institute of Endocrinology, Bucharest, Romania; [3]Neuropsychiatry Hospital, Buzau, Romania.

Objective
The present study examined the influence of light and dark, seasonal differences, electromagnetic fields, adrenergic and GABA-ergic control, age on melatonin secretion and also which factors affect rhythmicity of melatonin secretion, both in health and disease.

Data were combined from a series of studies conducted between 1997 and 2006. Hormones were temporally assayed by immunometric assays.

The results showed that melatonin is secreted in a circadian pattern with high levels during the night and low ones in the day time and constant from day to day in the same subject when the individual behavior and environment remain relatively unchanged but there is very large variability in amplitude among subjects. In a structured environment there are changes in melatonin production in seasonal photoperiod. In short photoperiod seasons the melatonin circadian profile amplitude is the highest while in long photoperiod seasons it is diminished. Occupational exposure to extremely low frequency magnetic fields altered profiles of melatonin secretion in electric power station workers.

Inhibition of the beta-adrenergic receptor by beta-blockers accounts for approximately 80% of the nightly increase in melatonin production. Benzodiazepine receptors have been found to modulate melatonin production.

The amplitude of circadian rhythm of melatonin decreases with age. Changes in melatonin secretion in puberty development and menstrual cycle and also in disorders of hypothalamic-pituitary-gonadal axis suggest that melatonin by its circadian secretion regulates the temporal organization of HPG axis. Results related to epileptic disorders in children showed disturbances of melatonin circadian rhythm.
Conclusion
The results showed that melatonin is a chronobiotic hormone regulated by light with the ability to modulate various bodily functions using hormones and restore the balance when disorders of circadian regulation occur.
Acknowledgements
The study was done under research project CEEX 100/2006.

P535

Neurotropic profiles of androgens - mechanisms and targets

Franziska Goetz[1], Vladimir K Patchev[2], Wolfgang Rohde[1] & Alexandre V Patchev[1]
[1]Institute of Experimental Endocrinology, Charité - University Medicine Berlin, Berlin, Germany; [2]Schering AG/Jenapharm, Jena, Germany.

Identification of pure neural androgenic effects is difficult due to 1) regional distribution of androgen receptors (AR) in the CNS; 2) cross-talk between molecular pathways of steroid hormone signalling, and 3) chemical nature and biotransformation of androgens in the CNS. Testosterone is transformed in the CNS by 5α-reductase and aromatase to the pure AR-agonist dihydrotestosterone (DHT) and the estrogen receptor-agonist estradiol, respectively. Decreased sexual activity is a symptom of hypogonadism, whereas anxiety and poor control of pituitary-adrenal responsiveness to stress are hallmarks of affective disorders (e.g. major depression). Age-related androgen deficiency has been associated with affective disorders, and androgens have been sporadically used as treatment. Three androgens with different pharmacological profiles were investigated in rats to elucidate whether 1) biotransformation to estrogens and 2) pronounced anabolic properties differentially contribute to behavioural and neuroendocrine actions. We used the aromatizable and 5α-reducible testosterone and the non-aromatizable dihydrotestosterone as well as the synthetic steroid anadrol (oxymetholone), a 5α-reduced androgen with pronounced anabolic properties. By chronic administration in castrated rats only testosterone was able to fully restore mounting activity to the level seen in intact rats, the non-aromatizable AR-agonist DHT showed merely a trend towards induction of sexual behaviour, while anadrol failed to induce male sexual activity. Anadrol displayed significant anxiolytic effects, whereas testosterone was effective only at higher doses; DHT failed to produce anxiolysis. Stress-induced cortico-sterone secretion was suppressed in all treatment groups, but most pronounced under testosterone. The results of this comparative examination of pure AR-agonists (DHT), aromatizable androgens (testosterone) and androgenic-anabolic steroids (anadrol) indicate differential neurotropic profiles and, consecutively, applicability to defined neurological symptoms (e.g. sexual dysfunction, anxiety or inadequate responsiveness to emotional stress).

P536

Age-related changes of circadian rhythmicity: relationship with melatonin.

Dana Manda, Olga Ianas, Suzana Vladoiu, Carmina Busu & Roxana Rosca
"C.I. Parhon" Institute of Endocrinology, Bucharest, Romania.

The suprachiasmatic nucleus (SCN) is the 'master clock' of the mammalian brain. It coordinates the peripheral clock in body, including the pineal clock that receives SCN input via a multisynaptic noradrenergic pathway. Melatonin is exclusively involved in signaling the 'time of day', 'time of year' to all tissues and is thus considered to be the chronological pacemaker or 'zeitgeber'.
Objective
To determine the chronology of age-related changes in melatonin secretion and relationship with gonadotropin and cortisol levels.
Subjects and methods
Data were combined from a series of studies conducted between 1997 and 2006. A total of 60 healty subjects, aged 3 to 70 years, without sleep complaints or histories of endocrine psychiatric disorders were enrolled. Twenty-four hour

profiles of urine aMT6 s, cortisol and gonadotropins were assayed by cosinor analysis.
Results
The circadian patterns of melatonin secretion exhibited a significant decline around pubescence; in younger adults there was no significant change or sex-differences. Correlations between melatonin secretion and gonadotropins showed a positive correlation at the onset of puberty and negative one in both premenopausal women (at ovulation) and men (<60 y). In menopausal women there was a very large variability in chronobiological parameters associated with an increase in gonadotropin excretion, LH and FSH. An age-related decline in melatonin was found after 55–60 years of age. Whereas circadian rhythms persisted, they were associated with earlier timing acrophases and blunted amplitudes. Cortisol secretion exhibited significant circadian rhythm but with a surprisingly long time log; the acrophase occurred across the 24 h.
Conclusion
Aging influences both the amplitude and phase of circadian rhythmicity and melatonin could be an index of circadian rhythm function.
Acknowledgements
This study was done under research project CEEX nr. 100/2006

P537

Idiopathic isolated GH deficiency (IGHD) and combined pituitary hormone deficiency (CPHD) in Italy: genetic screening and clinical correlates

Ginevra Corneli[1], Daniela Vivenza[1], Simona Mellone[2], Luigi Tiradani[2], Yari Carlomagno[2], Michela Godi[2], Simonetta Bellone[1], Gianluca Aimaretti[3], Mara Giordano[2], Patricia Momigliano-Richiardi[2] & Gianni Bona[1]
[1]Unit of Paediatrics, Department of Medical Sciences, University of Piemonte Orientale "Amedeo Avogadro", Novara, Italy; [2]Laboratory of Human Genetics, Department of Medical Sciences, University of Piemonte Orientale "Amedeo Avogadro", Novara, Italy; [3]Endocrinology, Department of Clinical and Experimental Medicine, University of Piemonte Orientale "Amedeo Avogadro", Novara, Italy.

Mutations in genes encoding pituitary-specific factors have been identified in patients with idiopathic isolated GH deficiency (IGHD) or combined pituitary hormone deficiency (CPHD), with or without neuro-morphological abnormalities. We screened 205 IGHD (M/F:131/74; 183 sporadic and 22 belonging to 12 families) for mutations in *GH1*, *GHRH-R, HESX1* and 129 CPHD, (M/F:75/54; 118 sporadic and 13 belonging to 9 families) for mutations in *PIT1*, *PROP1*, *LHX4* and *HESX1*. We considered as familial cases both patients with family history of the disease and those with consanguineous parents. All the CPHD patients had GH deficiency. All IGHD were diagnosed during childhood. Among CPHD patients 82 were diagnosed in childhood, 14 during adolescence and 33 in adulthood. Neuroradiological abnormalities at MRI scan were found in 26.8% of IGHD and 65.1% of CPHD. Mutations were detected in the *GH1* gene in two IGHD familial cases (a homozygous tandem duplication within exon 2 and a heterozygous IVSdel+56–77) and in two CPHD familial cases, one in *PIT1* (IVS2+3insA heterozygote) and one in *PROP1* (R73C/R73H compound heterozygote). Among sporadic cases likely causal mutations were identified in one IGHD in *HESX1* (IVS2+3G→A heterozygote) and in three CPHD, of which two in *PROP1* (296delGA and 150delA, both in homozygosis) and one in *HESX1* (Q6H heterozygote). No mutations were found in the *LHX4* gene. Thus, we found mutations in 4 out of 21 families (19%) and 4 out of 301 sporadic cases (1,3%). In four further sporadic cases sequence variations were detected (one V10G in *GHRH-R* and three V129I in *HESX1*) but there is still no evidence of their pathogenic role. In conclusion, most causal mutations in the genes analysed in this study were found in familial cases. Thus, the inclusion criteria for the genetic analysis, at least for sporadic patients, should be better clarified, prior to offering genetic testing.

P538

Increased intraovarian levels of noradrenaline and NGF precede the follicular changes in the rat ovary at the end of reproductive period

Alfonso H Paredes, Eric Acuña, Romina Fornes & Hernan E Lara
University of Chile, Santiago, Metropolitan, Chile.

Reports in the literature have demonstrated an increased number of nerve fibers and the presence of a follicle development similar to polycystic ovary during perimenopausal ovary in women. Since differentiation, proliferation and growth of nerves depends of nerve growth factor (NGF), changes in the content of nerves fiber could be preceded by increases in NGF and p75 neurotrophin receptor (p75NTR). Our purpose was to evaluate the changes in noradrenaline (NA) at the celiac ganglion at the ovary and plasma levels through the establishment of the anovulatory condition associated with age. We also measured NGF and p75NTR mRNA, in relation to the changes in ovarian morphology. We used Spraque-Dawley rat between at 6 and 16 month old. The NA was determined by HPLC, NGF proteins by ELISA and NGF and p75NTR mRNA by real time PCR. The results show that plasma NA content decreased gradually with age, while in the ovary NA content increased at ageing. NA content in celiac ganglion only decreased after 12 month old,. The local changes of NA are accompanied by an intraovarian increase of NGF mRNA. Nevertheless, the content of p75NTR not changed. The ovarian morphological analysis shows after rat 12 month old present an increased number of type III, luteinized as cystic follicles. In conclusion, the increase of cystic and type III follicles in the rat aging, after 12 month old and at the end of reproductive function are preceded by a local increase of intraovaric neurotrophin and nerves sympathetic activity.

P539

Incorporation and release of ^{3}H-norepinephrine by granulosa cells: Novel functionality for endocrine cells

Monika Greiner & Hernán E Lara
University of Chile, Santiago, Metropolitan Area, Chile.

Ovarian function in mammals is regulated by gonadotropins and by sympathetic nervous system. Norepinephrine (NE) is one of the major neurotransmitters present in the fibres innervating the gonad and regulates follicular development and ovarian steroids release. Surgical section of the sympathetic fibres partially decreased the release of NE as compared with non-denervated rats. The remnant release capability supposes the existence of an intraovarian compartment able to incorporate and release NE independent of the sympathetic innervation. To study one of these compartments, we used fresh isolated rat granulosa cells and observe that they incorporate and release ^{3}HNE in response to a depolarizing stimulus. These cells are immunoreactive for the dopamine transporter (DAT), and cocaine, a selective inhibitor of DAT, blocks the norepinephrine incorporation. In contrast to granulosa cells, luteal cells presented a weak immunoreactivity to DAT and a diminished capability for incorporate and release norepinephrine. This data provide information for a role of granulosa cells in the control of intraovarian norepinephrine homeostasis and possibly to the ovarian function.
Supported by: Fondecyt 1050765, PG 63/2004

P540

Treatment of Cushing's disease by transsphenoidal pituitary micro-surgery: prognosis factors and long-term follow-up

Svetlana Arapova, Evgeniy Marova, Galina Kolesnikova, Andrey Grigorev & Anastasia Lapshina
National Research Center for Endocrinology Russian Academy of Medical Sciences, Moscow, Russia.

In our study we included retrospective analyses of 101 patients (mean age 35 years, 85 women and 16 men) with Cushing's disease (CD), who underwent transsphenoidal surgery (TSS) treatment. CD is based on clinical suspicion, hormonal research of cortisol (F), ACTH, 24-hous urine F, results of dexamethasone suppression tests low (1 mg) dose (LDDST) and high (8 mg) dose (HDDST) and MR-imaging (MRI). Before the operation all patients have high F, ACTH, negative LDDST and positive HDDST, abnormal responses to tests desmopressin (DDAVP), insulin and pituitary adenomas on MRI (76% - microadenoma and 24% - macroadenjma). Post-operative pituitary and adrenal functions were assessed after 5–10 days (serum F - post F), then every year. 74% of patients had adrenal deficiency after TSS. The results of serum post F, circadian rhythm F, ACTH, LDDST, desmopressin, and insulin tests were the criteria to define cure or remission. 82% of patients had clinical and biochemical remission over 6 month, 84% over 12 month after TSS.

75% of the patients have prolonged remission during long-term follow-up (in average 8,6 years).

Recurrent (R) in 12.4% of patients initially deemed to be remission, at a mean of 69 months. After 12 months the patients with R had post F >50 nmol/l, evaluation ACTH and F after DDAVP, but normal test LDDST.
Conclusion
Results of the study confirm the facts that the predictive value for long-term remission CD are: postoperative 09.00 h serum cortisol values $<$ 50 nmol/l; normal 24-hous urine F; normal circadian rhythm F, ACTH; normal LDDST, negative test with (DDAVP), normal response F and ACTH to insulin test over 12 month after TSS.

P541

Ketoconazole before transsphenoidal surgery in Cushing's disease patiens as a good alternative to glucocorticoids perioperatory treatment

E Venegas[1], A Soto[1], M Vazquez[1], R Guerrero[1], A Pumar[1], JM Montero[2], N Garcia[1], MA Mangas[1], A Leon[3] & A Leal-Cerro[1]
[1]Departament of Endocrinology and Nutrition, Hospital Universitario Virgen del Rocío, Sevilla, Spain; [2]Departament of Neurosurgery, Hospital Universitario Virgen del Rocío, Sevilla, Spain; [3]Departament of Laboratory, Hospital Universitario Virgen del Rocío, Sevilla, Spain.

Cushing's disease is a debilitating endocrinopathy characterized by excessive cortisol levels in the blood which may be produced from tumours of the pituitary gland. The only way to achieve long term cure of Cushing's disease is by Transphenoidal removal of the adenoma. ketoconazole, inhibit steroid (cortisol) production in the adrenal glands.
The use of glucocorticids treatment before and after hypophisectomy is a classic management in the perioperative Cushing disease patients.
Aim
To assess if ketoconazole treatment previous to pituitary surgery could free the plasma cortisol postsurgical determination from any interference from steroid substitute treatment without clinical risks for patients. To evaluate in how many patients we can avoid systematic substitutive treatment.
Method
We have treated 38 Cushing's disease patients with ketoconazole (400–800 mg/d) during 3–6 weeks before the pituitary surgery and we have evaluated the plasmatic cortisol levels immediately after the surgery. Neither intraoperatory nor immediately postoperatory glucocorticoids were administrated until hypocortisolims were diagnosticated.
Results
In 9 of 38 patients (23.68%) substitutive treatment was not necessary. 26 of 38 patients needs glucocorticoids treatment: 11 in the 3–7 days after the surgery, (2 of them with symptomatic hypocortisolims), and 13 about 30 days after the surgery. In 12 cases (31.58%) the substitutive treatment was iniciated because of laboratory hypocortisolims and in 14 cases (36.8%) the treatment was started because of clinical suspicious of hypocortisolims.
Conclusions
The treatment with Ketoconazole before pituitary surgery can allow us the measure of plasmatic cortisol postoperatory without the interference of de substitutive treatment in a security way, and in some patients we can avoid systematic substitutive treatment.

P542

Analysis of three different tests in the diagnosis of growth hormone deficit (GHD) in patients with severe cerebral trauma

A Soto[1], E Venegas[1], A Caro[1], R Gurrero[1], MD Rincon[2], S Garcia[2], A Pumar[1], A Leon[3], JM Flores[2], F Murillo[2] & A Leal-Cerro[1]
[1]Department of Endocrinology, HH.UU Virgen del Rocío, Sevilla, Spain; [2]Department of Critical Care, HH.UU Virgen del Rocío, Sevilla, Spain; [3]Department of Laboratory, HH.UU Virgen del Rocío, Sevilla, Spain.

Introduction
Patients with severe cerebral Trauma are a risk population for developing hipopituytarism. Diagnosis of GHD need to study pituitary gland reserve with a stimulation test. Insulin Tolerance test (ITT) is the best standard test, although has important contraindication in these patients and its realization is not always a possible. We evaluate the diagnostic capacity of two alternative test (Glucagon Test an GHRH-GHRP6 test) and compared them with ITT.
Material and methods
In 52 adult patients with severe cerebral trauma (Glasgow < 8) occurred at least 12 month before study, we perform three consecutive test, with a minimum period of 72 hours among each other, in the following order: (1) Glucagon test. Glucagon 1 mg s.c with extractions at 90, 120, 150, 180, 210 and 240 minutes to determinate GH and glucose. (2) GHRH-GHRP-6 test. GHRH 100 mg and GHRP-6 100 mg, bolus i.v, and extractions in 0, 30, 60, 90 and 120 minutes to determinate GH. (3) ITT. Regular insulin 0.05–0.15 UI/Kg, bolus i.v and extractions in 0, 30, 60 minutes to determinate GH and Glucose, to get glucose level <40 mg/dl. In 10 patients ITT could not be done because of contraindications. GH values used for diagnosis of GHD are those published in de literature for each of these test. (Glucagon test, <3 mg/dl, GHRH-GHRP-6 <15 mg/dl and ITT < 3 mg/ml).
Results
15/47 (31.91%) patients were diagnosticated of GHD with ITT, 8/58 (13.57%) patients were diagnosticated of GHD with GHRH-GHRP-6 test and 23/46 (41.07%) patients were diagnosticated of GHD with Glucagon test. Glucagon test sensitivity and specificity was 73.3%, Positive Predictive Value was 57.8% and Negative Predictive Value 84.61%, when we compared with ITT. GHRH-GHRP-6 test sensitivity was 40% and specificity was 100%, Positive Predictive Value was 100% and Negative Predictive Value 82.92%, when we compared with ITT.
Conclusions
Variability among three test is important. Glucagon test is not a good test since it has neither good sensitivity nor specificity. GHRP-GHRP-6 test is very specific, and may be valuable as confirmation test.

P543

Meningiomas in patients diagnosed with acromegaly: the report of two cases

Malgorzata Trofimiuk[1], Dariusz Adamek[2], Ryszard Czepko[3], Grzegorz Sokolowski[1], Agata Baldys-Waligorska[1] & Bohdan Huszno[1]
[1]Chair and Department of Endocrinology, Collegium Medicum, Jagiellonian University, Krakow, Poland; [2]Neuropathology Department, Collegium Medicum, Jagiellonian University, Krakow, Poland; [3]Neurosurgery Department, Collegium Medicum, Jagiellonian University, Krakow, Poland.

Introduction
Only several cases of co-existing meningiomas and pituitary tumours secreting growth hormone (GH) have been described so far in patients not treated previously with irradiation.
Aim
The aim of the study was to describe two cases of co-occurrence of acromegaly and meningioma and to discuss their relationship.
Case reports
Case 1. 52-year old female complained of visual disturbances. She was diagnosed with pituitary microadenoma secreting GH, and subsequently underwent successful transsphenoidal surgery. MRI performed after surgery revealed the presence of the second tumour invading right optic nerve canal. She was re-operated, meningioma was confirmed on histopathological examination. After the surgery her visual field has improved.
Case 2. 26-year old female was admitted to the hospital due to rapidly progressing apathy and extremities paralysis. Head CT showed the giant tumour of parasellar region invading neighbouring central nervous system structures. She was operated by transcranial approach). The histopathological assessment showed fibrous meningioma. After second transsphenoidal surgery, the GH-secreting pituitary tumour was confirmed. Although the operation did not removed the whole tumour, the patient improved substantially. One year later she was re-operated because of high levels of GH not controlled by somatostatin analogue injections. Unfortunately, the surgery did not normalised GH levels. The patient declined irradiation.
Conclusions
Co-existence of meningiomas and acromegaly may result from pro-proliferative action of high levels of GH and/or IGF-1 on central nervous system tumours expressing growth hormone and insulin-like growth factors receptors, although most of the clinical observations argue against the close relationship between increased IGF-1 levels and development of meningiomas in humans.

P544

Topoisomerase II alpha expression in pituitary tumours – preliminary results

Malgorzata Trofimiuk[1], Dariusz Adamek[2], Ryszard Czepko[3], Grzegorz Sokolowski[1] & Bohdan Huszno[1]
[1]Chair and Department of Endocrinology, Collegium Medicum Jagiellonian University, Krakow, Poland; [2]Neuropathology Department Collegium Medicum Jagiellonian University, Krakow, Poland; [3]Neurosurgery Department Collegium Medicum Jagiellonian University, Krakow, Poland.

Introduction
Topoisomerase II alpha is regarded as the important marker of cellular proliferation. Pituitary tumours are usually benign, but some of them are characterized by rapid growth, high recurrence rate and local invasiveness. Proliferation marker Ki-67 most commonly used in pituitary tumours is not entirely reliable as the indicator of the aggressive growth of the lesion. Topoisomerase II alpha expression assessment may be a valuable tool for identification such pituitary neoplasms.
Aims
The aim of the study was to assess topoisomerase II alpha expression in pituitary tumours as a factor influencing tumour behaviour.
Material and methods
The study included 24 subjects (15 males and 9 females aged 24–79 years, mean age 53 years) who had underwent surgery due to pituitary tumour. The tissue

samples were stained immunohistochemically for ACTH, FSH, LH, GH, PRL, TSH and topoisomerase II alpha. Topoisomerase index (IT) was assessed as a number of positive-stained nuclei per 100 tumour cells.
Results
The IT in studied subjects varied from 0 to 93 (median value – 0.8; males – 0.2; females – 0.8). The highest IT value was observed in the case of pituitary germinoma. Among the patients diagnosed with pituitary adenoma, the highest expression of topoisomerase was noted in GH positive- (IT value of 1.35) and ACTH positive tumours (IT of 0.8). The lowest IT values were noted in adenomas co-expressing LH/FSH and PRL/GH (IT of 0.3 and 0.1, respectively). Only in 8% of all studied tumours no expression of topoisomerase was found. The IT in larger tumours invading neighbouring structures was higher but the difference did not reach the statistical significance.
Conclusion
Topoisomerase II alpha seems to be useful marker for assessment of proliferation activity of pituitary tumours, particularly in case of rapidly growing tumours such as germinal neoplasms or metastases. We have presented preliminary results.

P545

Is there an endocrine explanation for persistent neuropsychological disabilities long after traumatic brain injury (TBI)?

Edwige Yollin[1], Odile Kozlowski[2], Benoît Soudan[3], Michèle D'Herbomez[4], Pierre Fontaine[1], Marc Rousseaux[4] & Christine Cortet-Rudelli[1]
[1]Department of Endocrinology and Diabetology, Clinique Marc Linquette, Centre Hospitalier Régional Universitaire, Lille, Nord, France; [2]Department of Reeducation, Hôpital Swynghedaw, Centre Hospitalier Régional Universitaire, Lille, Nord, France; [3]Laboratory of biochemistry, Centre Hospitalier Régional Universitaire, Lille, Nord, France; [4]Laboratory of nuclear medicine, Centre Hospitalier Régional Universitaire, Lille, Nord, France.

The aims of this study were to determine the prevalence of pituitary dysfunction in patients keeping neuropsychological disabilities long after TBI (at least 1 year), to research predictive factors and to evaluate consequences of endocrine abnormalities on metabolism and quality of life in these patients.
We studied 50 patients (42 men, mean age 36, range 20–59 years, mean BMI 25, range 17–42 kg/m^2) who had survived severe ($n=38$), moderate ($n=2$) or mild TBI ($n=10$) at a mean of 59 months (range 13–247) post event. 52% had moderate, 32% had severe disability (GOS score: 2 or 3 respectively), 30% had anosognosia.
No patient showed posterior pituitary dysfunction, hyperprolactinemia or gonadotropin deficiency. Six patients (12%) showed TSH deficiency. Ten patients (20%) had partial ACTH deficiency (diagnosed by ITT or metyrapone test). Severe GH deficiency was diagnosed in 44.5% (glucagon stimulation test confirmed by ITT or arginine + GHRH test) and was isolated in 40% of cases. GHD patients had significantly higher BMI, triglycerid, fasting and postprandial insulin plasma levels than no-GHD patients, but mean QoL-AGHDA or NHP questionnaires scores were not significantly different in the 2 groups even among non-anosognosic patients. Totally 46% of the patients showed at least one anterior pituitary deficiency requiring a substitutive treatment (multiple and isolated hormone deficiency in 24% and 22% respectively). Hypopituitarism was not related to GCS score, initial CT scan lesions, GOS score, self-sufficiency (EBIS scale score) or resumption of work.
The high risk for anterior pituitary deficiency in patients with persistent neuropsychological disabilities long after TBI justify a pituitary exploration in all of them, with reference tests, even long after the TBI. Evaluation of quality of life must be adjusted to TBI patients, with specialised neuropsychological testing and multidisciplinary collaboration.

P546

ACTH and cortisol responses to ghrelin and DDAVP in patients with Cushing's disease (CD)

Dragana Miljic, Mirjana Doknic, Sandra Pekic, Marina Djurovic, Zorana Penezic, Svetozar Damjanovic, Djuro Macut, Mirjana Sumarac-Dumanovic, Bojana Popovic, Mohammad Ghatei & Vera Popovic
Institute of Endocrinology, Belgrade, United States; Imperial College, London, United Kingdom.

Aim of this study was to investigate head to head ACTH and cortisol responses to DDAVP and ghrelin in patients with CD. Study was approved by the local ethics committee and informed consent obtained. Nine patients with CD were submitted to ghrelin 1 μg/kg and DDAVP 10 μg bolus iv administration on two separate occasions. Blood was sampled at 0.15, 30, 45, 60, 90 min. for ACTH (ELISA) and cortisol (RIA, Cis, Bio International, France) measurements.
Ghrelin induced significant ACTH (65.3 ± 54.7 vs 188.6 ± 128.8 pg/ml; $P<0.05$) and cortisol responses (642.5 ± 357.2 vs 856.0 ± 447.4 nmol/l; $P=0.05$) in our patients. After DDAVP there was also a significant increase in ACTH (53.5 ± 49.3 vs 227.6 ± 359.2 pg/ml; $P=0.05$) and cortisol levels (444.5 ± 249.2 vs 658.8 ± 369.6 nmol/l; $P<0.05$). When compared peak ACTH and cortisol values after both tests were not statistically different. Integrated ACTH (pg/ml.min) (ghrelin:11 677.7 ± 7 253.6 vs DDAVP: 12 470.4 ± 16 911.2) and cortisol (nmol/l.min) secretion (ghrelin: 81 810.6 ± 49 437.6 vs DDAVP: 64 677.0 ± 38 399.9) were not significantly different after two tests.
Although limited by size our study shows that ghrelin compared to DDAVP induces similar cortisol and ACTH responses. The mechanisms of ghrelin action on ACTH and cortisol secretion in patients with CD merit further investigation.

P547

Effect of treatment with somatostatin analogue on glucose homeostasis in patients with acromegaly

Nikos Gavalas, Andrea Sabo, Lida Papastathopoulou, Efi Botoula & Marinella Tzanela
Evangelismos Hospital, Athens, Greece.

Long acting somatostatin analogues (SMS) are extensively used as second and even first line treatment of acromegaly. Except of the inhibition of GH secretion, somatostatin is a potent inhibitor of insulin secretion from the pancreatic b- cells. As defects of glucose homeostasis are very common in acromegaly, we decided to examine the effect of the control of GH hypersecretion with SMS on glucose metabolism.
We study 44 acromegalic patients divided in 3 groups. Patients of group I ($n=18$) were evaluated at the time of diagnosis and before any therapeutic intervention, while patients of groups II and III were evaluated after control of their disease (indicated by normal IGF-I values for age and sex and GH levels <1 μg/L during OGTT, Consensus 2000) either by transphenoidal surgery, alone or followed by pituitary irradiation, (group II, $n=16$) or by somatostatin analogue administration (group III, $n=10$).
Insulin levels were significantly lower in groups II and III compared to group I (7.5 ± 0.6 and 5.2 ± 0.8 vs 15.7 ± 2.7 μIU/ml, $P<0.05$) with a parallel drop of insulin resistance (as estimated by HOMA-IR) from 4.9 ± 0.9 in group I to 1.8 ± 0.2 and 1.4 ± 0.2 ($P<0.05$) in groups II and III respectively. Insulin secretion (as estimated by HOMA-β) was statistically lower in group III than in group I and II (42.4 ± 6.97 vs 117.2 ± 18.8 and 85.6 ± 7.49 respectively, $P<0.05$). These alterations led to lower mean glucose levels in group II compared group I (99 ± 4.9 vs 120.9 ± 8.3 mg/dl, $P<0.05$) but not in group III (108.4 ± 3.1 mg/dl). The incidence of Diabetes Mellitus dropped from 50% in group I to 12% in group II and 10% in group III, while that of Impaired Glucose Tolerance from 33% in group I to 18.7% in group II but to 30% in group III.
In conclusion, despite of treatment modality, successful control of acromegaly reduces the incidence of Diabetes Mellitus. However, control of GH hypersecretion with SMS treatment seems to be less effective to fully reverse the impaired glucose tolerance, probably due to inhibition of insulin secretion by SMS.

P548

Effects of combined treatment with cabergoline and somatostatin analogues (SAA) on GH and IGF-I levels and tumor volume in patients with acromegaly not fully responsive to SAA

Renata S Auriemma, Mariano Galdiero, Maria Cristina De Martino, Monica De Leo, Gaetano Lombardi, Annamaria Colao & Rosario Pivonello
Department of Molecular and Clinical Endocrinology and Oncology, "Federico II" University, Naples, Italy.

Dopamine agonists have been used as first or adjunctive therapy for acromegaly for many years, but relatively few studies have assessed the efficacy of a newer agonist, cabergoline (CAB) alone or in combination with somatostatin analogues (SSA). The aim of this study was to evaluate the efficacy of combined treatment with SSA *plus* CAB in patients with acromegaly and resistance to SSA, defined as lack of normalization of IGF-I levels after long-term (>1 year) and high dose (30 mg/month) treatment with SSA. Twelve patients (8 men and 4 women, age 32–70 years) with active acromegaly after unsuccessful surgery entered the study: 10 patients had been treated with octreotide LAR and 2 with lanreotide; 7 had a pituitary

macroadenoma, 2 a microadenoma and 3 an empty sella. None of the patients had hyperprolactinemia. CAB was added at the initial dose of 1 mg/week for 1 month, then increased to 3.5 mg/week. After long-term SSA treatment, no significant difference in GH ($P=0.56$) and IGF-I ($P=0.08$) levels was found, whereas tumor volume was significantly reduced ($P=0.014$) as compared to baseline. After 6-month treatment with SAA *plus* CAB, both GH ($P=0.004$) and IGF-I ($P=0.005$) levels as well as tumor volume ($P=0.014$) were significantly decreased compared to baseline. Moreover, GH ($P=0.02$) and IGF-I ($P=0.002$) levels, as well as tumor volume ($P=0.014$), measured after SAA *plus* CAB treatment were also significantly lower than those measured after SSA treatment alone. The addition of CAB to SSA induced a percent GH, IGF-I and tumor volume decrease of $46\pm41\%$, $24\pm23\%$ and $17\pm37\%$ respectively. After six months of combined treatment, six patients (50%) showed a normalization of GH and IGF-I levels. In conclusion, combined treatment with SAA *plus* CAB can be effective in inducing IGF-I normalization in acromegalic patients resistant to SSA and deserves an important role as alternative treatment in the therapeutic algorithm of acromegaly.

P549

Macroprolactin: the clinically and diagnostically importance

Larisa K Dzeranova, Ekaterina N Giniatullina, Nikolai P Goncharov, Anna D Dobracheva & Galina S Kolesnikova
Research Centre for Endocrinology, Moscow, Russia.

Recently, the phenomenon of macroprolactinaemia has manifested itself into a great interest for physicians. This problem forces both physicians and patient to waste sizeable resources, and can lead to iatrogenic and unjustified emotional stress and further material losses. At the same time the problem of differential diagnostics of pseudoprolactinomas and true prolactinomas remains challenging. The purpose of the present study was to determine the clinic-analytical repercussion of the presence of maPRL in female patients with hyperprolactinaemia.

The patients and methods

321 patients with hyperprolactinaemia (PRL level was more than 700 mU/l) were examined (36 male and 285 female). The age mediana was 29 ± 3 years. The quantitative estimation of biologically active monomeric PRL was conducted. A polyethylene glycol (PEG) precipitation test (Delfia System) was used to detect the presence of maPRL in all consecutive samples with prolactin levels >700 mU\L). A recovery $<60\%$ was taking as indicating of maPRL.

The results

maPRL was found in 57 (18%) of 321 patients with total PRL >700 mU\l; all other 264 patients (82%) had maPRL below 60%. Mediana of PRL level in the group with macroprolactinaemia was – 1167 mU/l (700–1635); the mediana of maPRL-997 mU/l (700–1295). The most frequent reason for the initial PRL request was menstrual disturbance (36.8% patients). As for clinical presentations, Galactorrhea was noted in 19.2% cases; the headaches -in 38.5% patients, the increasing of the mass of the body in 24.5% of cases. The microadenomas were revealed in 38.7% events, and macroadenomas – in 4.5% cases.

The conclusion

Macroprolactinaemia is a frequent condition. The estimation of PRL fractions is an important problem and nessesary for diagnostic mistakes elimination, to avoid the unnecessary diagnostic procedures, to the needless medical treatment or surgery prevention. The determination of maPRL in routine studies is recommended.

P550

OASIS: observational study on the international clinical practice for the treatment of recently diagnosed acromegalic patients

Lesly Portocarrero[1], Güngör Akçay[2], Hakim Bouterfa[3], Davide Carvalho[4], Yona Greenman[5], Feng Gu[6], Mirtha A Guitelman[7], Sungwoon Kim[8], Christine Kriesche[9], Moises Mercado[10], Chiung-Chi Shen[11], Antonio Silva[3], Cesar Varela[12], Henri Wallaschofski[13] & Susan Webb[14]
[1]National Institute of Neurology and Neurosurgery, Department of Endocrinology, Mexico City, Mexico; [2]Ataturk University Hospital, Erzurum, Turkey; [3]Novartis Pharma AG, Basel, Switzerland; [4]Hospital de São João, Porto, Portugal; [5]Tel Aviv Medical Center, Tel Aviv, Israel; [6]Peking Union Medical College Hospital, Beijing, China; [7]Department of Endocrinology, Hospital Carlos G. Durand, Buenos Aires, Argentina; [8]Kyung Hee University, Seoul, Korea, South; [9]Chemnitz, Germany; [10]Endocrinology Service, Hospital de Especialidades, Centro Medico Nacional Siglo XXI, IMSS, Mexico City, Mexico; [11]Taichung, Taiwan; [12]Hospital Ramón y Cajal, Madrid, Spain; [13]University of Greifswald, Greifswald, Germany; [14]Hospital Sant Pau, Barcelona, Spain.

Background

There is significant interest in how the use of different treatment regimens (e.g. surgery, medical therapy) impacts the clinical course of Acromegaly. This study has been designed to better understand clinical decision making in the context of various treatment options.

Methods

OASIS is an international, observational study in recently diagnosed acromegalic patients. Ethical committee approval was obtained where applicable. Observations include biochemical control, acromegaly symptoms, tumor volume, safety and tolerability. Patient data are collected under normal practice conditions over a 12 months period.

Results

As of November 2006, 380 patients are enrolled from 103 centres in 21 countries. Baseline characteristics of the first 133 patients with available data are reported here. About half of the patients are female (56%), the majority (82%) are Caucasian, and the mean age is 48 years. Most patients (70%) have a diagnosis of macroadenoma. At baseline, 61% of patients had a planed treatment with Sandostatin LAR alone or combined with surgery. 39% of patients received other treatment options (e.g. surgery alone, radiotherapy or non- Sandostatin LAR medical therapy). The most common starting dose for Sandostatin LAR was 20 mg (74% of the patients treated with Sandostatin LAR). At baseline median levels of GH were 8.8 ng/mL in 58 patients treated with Sandostatin LAR (alone or in combination) and 12.8 ng/mL in 46 patients treated with other therapies. IGF-1 levels were 626 ng/mL in 56 patients with Sandostatin LAR and 713 ng/mL for patients with other therapies. At first quarter follow-up data were available for 35 patients with GH and for 27 patients with IGF-1 levels. The median values of GH showed a 40% decrease in the Sandostatin LAR group and 70% decrease in patients with other therapies. Similarly, IGF-I decreased by 22% and 40%, respectively.

Conclusions

These first data show a large proportion of patients treated with Sandostatin LAR as first treatment option. Observation of the treatment practice over the complete course of the study will provide a more complete picture of the treatment choice for these patients.

P551

Comparison of basal ghrelin and leptin serum levels and after an oral glucose tolerance test in active and inactive acromegalic patients

Josefine Roemmler, Bärbel Otto, Birgit Steffin, Martin Bidlingmaier & Jochen Schopohl
Medizinische Klinik – Innenstadt, Munich, Germany.

Leptin and ghrelin are correlated to acute and chronic nutritional status. Elevated BMI and fat mass as well as food intake increase leptin levels whereas ghrelin levels are reduced. Ghrelin stimulates growth hormone (GH) secretion. The influence of GH on ghrelin is unclear. Since GH reduces fat mass and is dependent on nutritional status we performed this prospective cross sectional study in order to investigate any interaction between GH, ghrelin and leptin levels in active and inactive acromegalic patients (pat).

We measured glucose, insulin, ghrelin, leptin and GH concentration during a 3 h oral glucose tolerance test (OGTT) and IGF-I in 36 acromegalic patientsients (19f/17m, median age 53.3y (20-75)). 29/36 patients underwent surgery. At time point of evaluation none of the patients had received radiotherapy or any medication for acromegaly. Concentration of GH and IGF-I were determined by a single laboratory using the same immunoassay (Nichols Advantage, San Clemente, CA). Active disease was defined as IGF-I above the upper limit of age- and sex-adjusted normal.

11 patients had active acromegaly, 25 were inactive. BMI was not significantly different between active and inactive patients. Baseline ghrelin levels were significantly reduced in active compared to inactive patients ($P<0.01$), baseline leptin levels were only slightly reduced (p=n.s.). Basal leptin was positively correlated to baseline blood glucose in active patients ($P<0.01$) and to BMI in inactive patients ($P<0.05$). During OGTT ghrelin and leptin significantly decreased (active: $P<0.01$; inactive: $P<0.001$). The ghrelin decline was significantly higher in inactive patients ($P<0.05$).

In active acromegalic patients the ghrelin regulation by nutritional status and food intake is reduced which could be due to a negative feedback of GH and IGF-I on ghrelin secretion. The tendency of lower leptin levels in active acromegalic pat might be caused by lipolytic effect of elevated GH levels.

P552

Determinants of the acromegalic cardiomyopathy: a prospective, controlled study in 205 patients

Annamaria Colao[1], Rosario Pivonello[1], Renata S. Auriemma[1], Mariano Galdiero[1], Letizia Spinelli[2], Maurizio Galderisi[3] & Gaetano Lombardi[1]
[1]Department of Molecular and Clinical Endocrinology and Oncology, section of Endocrinology, University "Federico II" of Naples, Naples, Italy; [2]Department of Internal Medicine I, Section of Cardiology; University "Federico II" of Naples, Naples, Italy; [3]Department of Experimental Medicine, Section of Cardiology, II University of Naples, Italy, Naples, Italy.

The most frequent cause of death in acromegaly is cardiomyopathy. To evaluate determinants of the acromegalic cardiomyopathy we performed an analytical, observational, open, prospective, controlled study in 205 patients with active acromegaly (108 women and 97 men) and 205 non-acromegalic subjects sex- and age-matched with the patients. We determined the prevalence of Left Ventricular (LV) hypertrophy (LVH), diastolic and systolic dysfunction, by echocardiography- measured LV mass index (LVMi) early-to-late mitral flow velocity (E/A) and LV ejection fraction (LVEF). The role of age, estimated disease duration, BMI, GH and IGF-I levels, systolic and diastolic blood pressure, lipid profile and glucose tolerance was investigated. Compared to sex- and age-matched controls, the patients had lower BMI, E/A, LVEF, HDL-cholesterol levels and higher LVMi, total and LDL-cholesterol, triglycerides, glucose and insulin levels, HOMA-R and HOMA-β. The relative risk to develop mild [Odds ratio (OR) = 2.2 (1.3–3.8) $P = 0.002$] or severe hypertension [OR = 3.2 (1.7–6.0), $P < 0.0001$], arrhythmias [OR = 3.7 (1.1–5.6), $P = 0.017$], impaired glucose tolerance [OR = 2.6 (1.5–4.6), $P = 0.0002$], diabetes [OR = 2.1 (1.2–3.8), $P = 0.006$], LVH [OR = 11.5 (7.1–19.0), $P < 0.0001$], diastolic [OR = 5.4 (3.2–9.2), $P < 0.0001$], and systolic dysfunction [OR = 6.3 (3.1–13.8), $P < 0.0001$], was higher in acromegaly. Disease duration and systolic blood pressure level was the most important predictor of LVH ($t = 2.4$, $P = 0.02$) and systolic dysfunction ($t = -2.8$, $P = 0.006$) while diastolic dysfunction was predicted by patient's age ($t = -3.3$, $P = 0.001$). The patients were divided into three groups based on disease duration: short ($\leq$60 months), intermediate (60–144 months; 75 percentile) and long ($>$144 months). Patients with long estimated disease duration had a relative risk to present LVH 9.9 times, diastolic dysfunction 4.8 times and all cardiac complications 3 times higher than patients with shorter estimated disease duration. In conclusion the prevalence of different features of cardiomyopathy is 5.4–11.5 times higher in the acromegalic than in the non-acromegalic population. The major determinant of cardiomyopathy is disease duration.

P553

Growing incidence of idiopathic isolated secondary adrenal insufficiency.

Anna Kasperlik-Zaluska[1], Barbara Czarnocka[1], Lucyna Papierska[1], Sophie Bensing[2], Anna Hulting[2] & Patricia Crock[3]
[1]Centre for Postgraduate Medical Education, Warsaw, Poland; [2]Karolinska Institutet, Stockholm, Sweden; [3]University of Newcastle, Newcastle, Australia.

Objective
The origin of idiopathic isolated secondary adrenal insufficiency (IISAI) is uncertain, however autoimmunity seems to be the most probable cause. Within last eight years the initial number of about 100 such cases, increased in our registry by 250%. We searched for features of autoimmune diseases in our group of patients to prove autoimmune etiology in a majority of these patients.
Materials and methods
The material consisted of 260 patients with IISAI (female/male ratio 10.8, age 17–78 years). The diagnosis was based on clinical characteristics and hormonal (especially cortisol and ACTH) examinations, including $^{1\text{-}24}$ACTH stimulating test. Methods: clinical examination, hormonal investigations (TSH, LH, FSH, PRL, fT_4), immunological studies (routine antithyroid autoantibodies + pituitary autoantibodies by an immunoblotting assay with human pituitary cytosol as autoantigen, in 65 patients), imaging methods (MRI of the pituitary – in a part of patients).
Results
Autoimmune disorders were diagnosed in 181 patients (70%), the most frequently thyroid diseases (especially hypothyroidism), vitiligo and premature ovarian failure. The thyroid autoantibodies were detected in 65% of the patients, while pituitary autoantibodies in 34% of the patients under study (immunoreactivity to a 49-kDa and to a novel 36-kDa pituitary autoantigen). Partially empty sella was the most frequent finding in MRI.
Conclusions
1/ The incidence of the diagnosed idiopathic isolated secondary adrenal insufficiency is growing in last years, probably mainly due to a better detectability of disease. 2/ Association of autoimmune disorders with IISAI in 70% of the patients suggests autoimmune origin of pituitary disease, confirmed by the presence of pituitary autoantibodies in 34% of the patients under study.

P554

The role of fibrinogen and CRP in cardiovascular risk in patients with acromegaly

Marcin Kaluzny & Marek Bolanowski
Dept. of Endocrinology, Diabetology and Isotope Therapy, Medical University, Wroclaw, Poland.

Patients with acromegaly have 2–3 fold increased mortality from cardiovascular diseases. It is associated with elevated growth hormone (GH) levels. Alterations of acute phase proteins, observed in patients with acromegaly, could lead to increased cardiovascular mortality. There are limited data on influences of GH excess on acute phase reactants.
The aim of the study was to evaluate selected acute phase proteins levels: fibrinogen and C-reactive protein (CRP) in patients with acromegaly.
Seventy-seven patients were divided into two groups: active acromegaly (AA, $n = 56$) and controlled acromegaly (CA, $n = 21$) according to minimal GH level during an oral glucose tolerance test and IGF-1 levels. Twenty sex matched healthy subjects were controls. The following parameters were measured: fibrinogen, CRP, fasting glucose, insulin, total cholesterol, LDL and HDL cholesterol, triglycerides and BMI.
Comparison of all groups using Mann-Whitney U testing revealed statistically significant: higher LDL cholesterol and insulin levels and lower CRP levels and BMI values in AA than CA groups ($P < 0.04$, 0.02, 0.01 and 0.03, respectively); higher fibrinogen, triglycerides, glucose levels and BMI values in AA group than controls ($P < 0.000001$, 0.002, 0.01 and 0.001, respectively); higher CRP, fibrinogen, triglycerides levels and BMI values in CA group than controls ($P <$ 0.01, 0.002, 0.04 and 0.001, respectively).
Fibrinogen levels in all patients with acromegaly were significantly higher than in healthy subjects irrespective of disease status. CRP levels were significantly and paradoxically lower in patients with active acromegaly than in patients with well controlled disease and did not explain increased cardiovascular mortality in acromegaly. The role of CRP levels as a cardiovascular risk factor in the mortality of patients with uncontrolled acromegaly ought to be better explained in future studies.

P555

Evaluation of insulin sensitivity with euglycemic hyperinsulinemic clamp technique in non-obese patients with microprolactinoma

Alpaslan Tuzcu, Serkan Yalaki, Senay Arikan, Deniz Gokalp & Mithat Bahceci
Dicle University Department of Endocrinology, Diyarbakir, Turkmenistan.

Background and aim
Hyperprolactinemia may associate with insulin resistance. This fact has been determined by many studies with methods which show insulin sensitivity. In this study we aimed to search insulin resistance with golden standard euglycemic hyperinsulinemic clamp technique on hyperprolactinemic patients.
Subjects and methods
This study was performed in Endocrinology Department of Dicle University. Sixteen patients with microprolactinoma (mean age: 32.06 ± 11.60 year and BMI: 24.43 ± 3.23 kg/m^2) 12 healthy subjects (mean age: 31.25 ± 9.40 year and BMI:24.33 ± 3.42 kg/m^2) were included to the study. Fasting glucose, insulin levels and lipid parameters were measured in both groups. HOMA-B and HOMA-IR values of groups were calculated. Euglycemic hyperinsulinemic clamp technique was performed to the both group and M value of the groups was defined. Mann-Whitney U and Chi-Square tests were used in statistically analysis.
Result
Age, BMI, total cholesterol, triglycerides, LDL-cholesterol, HDL-Cholesterol and fasting glucose levels of the groups were not show statistically difference. Basal insulin level of hyperprolactinemic patients were higher than control group (6.85 ± 4.68; 3.66 ± 0.88 pU/ml respectively; $P < 0.05$). Mean HOMA-IR and HOMA-B values of patients were higher than control group (1.49 ± 1.30, 0.78 ± 0.27 respectively; $P < 0.05$) (136.28 ± 72.53, 64.77 ± 23.31 respectively, $P < 0.05$). Insulin resistance was determined on 5 patients by euglycemic

hyperinsulinemic clamp technique (M<4). M values of the patients were statistically lower than control group (5.64±2.36, 7.05±1.62 kg/mg/m^2 respectively, $P<0.05$).
Conclusions
1- Hyperprolactinemia is associated with an insulin-resistant state
2- Insulin resistance of hyperprolactinemic patients is not associated with obesity.

P556

Abstract unavailable

P557

Comparative analysis of reactivity of macroprolactin in first and second-generation prolactin assays

Erzsébet Rigó[1], Erzsébet Toldy[2], Zoltán Löcsei[1], Erzsébet Toldy[4], István Szabolcs[3] & Gábor L. Kovács[5]
[1]1st.Department of Medicine Markusovszky Teaching Hospital of County Vas, Szombathely, Hungary; [2]Central Laboratory Markusovszky Teaching Hospital of County Vas, Szombathely, Hungary; [3]Department of Endocrinology National Medical Centre, Budapest, Hungary; [4]Department of Practial Diagnostics of Institute of Diagnostics and Management University of Pécs, Szombathely, Hungary; [5]Institute of Laboratory Medicine, University of Pécs, Pécs, Hungary.

Issue
Macroprolactin (MPRL) is a high molecular mass complex of prolactin with minimal bioactivity in vivo that may be the cause of elevated serum prolactin (PRL) as determined by immunoassay. Unrecognised macroprolactinemia can lead to misdiagnosis and mistreatment. The frequency of MPRL is highly dependent on the affinity of the antibody used in the assay.
The aim of this study
Was to compare the frequency and quantity of MPRL measured by a first and second-generation prolactin assay.
Methods
109 sera sent for PRL estimation were analysed: PRL was measured both in the native sera and after PEG-precipitation by a first and second-generation electrochemiluminscence immunoassay (ECLMA1 and ECLMA2, Elecsys 2010, Roche).
Results
The mean PRL concentration was lower if measured by ECLMA2 (961±687 versus 1419±1079 IU/l, $P<0.001$). The rate of elevated PRL was 59% by ECLMA1 and 51% by ECLMA2 respectively. The mean recovery following PEG-precipitation was not different (89±23 and 89±15), but the rate of macroprolactinemia defined (as less than 40% recovery) was 10-times more often by ECLMA1 ($N=19$) than ECLMA2 ($N=2$). Normalisation of elevated PRL levels after PEG precipitation occurred in 10% ($N=11$) and 12% ($N=12$), respectively of the sera with not difference according to the method used and typically in cases with slight hyperprolactinemia.
Conclusion
The affinity of the second-generation ECLMA2 assay to MPRL seems to be less than that of the first generation assay. Approximately 8% less cases of macroprolactinemia are to be expected by the novel assay even is the normal "cut off" level of PRL is decreased from 700 to 500 IU/l. The dramatic decrease of cases with less than 40% recovery raises the proposition that instead of %-recovery the normalisation of the PRL concentration following PEG precipitation should be used to define cases with macroprolactiemia.

P558

Cabergoline treatment in Cushing's disease: effect on hypertension, glucose intolerance and dyslipidemia.

Rosario Pivonello[1], Maria Cristina De Martino[1], Antongiulio Faggiano[1], Monica De Leo[1], Gaetano Lombardi[1], Leo J Hofland[2], Steven WJ Lamberts[2] & Annamaria Colao[1]
[1]Department of Molecular and Clinical Endocrinology and Oncology, "Federico II" University, Naples, Italy; [2]Department of Internal Medicine, Erasmus Medical Center, Rotterdam, Netherlands.

Cabergoline has been recently demonstrated to normalize cortisol secretion in more than one third of patients with Cushing's disease (CD). The aim of this study was to evaluate short-term (3-months) and long-term (12-24 months) effects of cabergoline treatment on the main systemic complications of CD, including hypertension, glucose intolerance and dyslipidemia. Twenty patients with CD unsuccessfully treated by neurosurgery entered the study. Cabergoline was admistered at the initial dose of 1 mg/week and a maximal dose of 7 mg/week. At 3-months follow-up, 15 (75%) patients were responsive whereas 5 (25%) were resistant to cabergoline treatment. Systolic and diastolic blood pressure, serum glucose and insulin levels, HOMA index, and serum cholesterol levels significantly decreased in parallel with the normalization of cortisol secretion. A significant improvement of blood pressure and a slight improvement in glucose tolerance and cholesterol levels was found both in responsive and resistant patients. Cabergoline treatment was continued in the 15 responsive patients, although treatment escape was observed in 5 patients, so that the long-term study was performed in 10 patients, who was followed-up for 12-24 months. During long-term treatment, urinary cortisol levels remained within the normal range. Serum glucose and insulin levels, HOMA index and serum cholesterol levels further decreased. At the last follow-up, the prevalence of hypertension decreased from 50% to 0%, glucose intolerance from 62.5% to 30%, and dyslipidemia from 33.3% to 0%. In conclusion, the results of the current study confirmed that cabergoline treatment is effective in controlling cortisol secretion for at least 1-2 years in more than one third of patients with CD, and demonstrated that it is able to improve hypertension, glucose intolerance and dyslipidemia in patients responsive and, partially, also in patients resistant to the treatment. Therefore, cabergoline is confirmed to be a useful treatment option in patients with CD unsuccessfully treated by neurosurgery.

P559

Development and validation of a questionnaire to evaluate health-related quality of life in patients with Cushing's syndrome

Susan M Webb[1], Xavier Badia[2], Maria- Jose Barahona[1], Annamaria Colao[3], Christian J Strasburger[4], Antoine Tabarin[5], MO van Aken[6], Rosario Pivonello[3], Gunther Stalla[7], O Chabre[8], Steven WJ Lamberts[6] & Joan E Glusman[9]
[1]Hospital S Pau, UAB, Barcelona, Spain; [2]IMS, Barcelona, Spain; [3]University Federico II, Naples, Italy; [4]Charité Campus Mitte, Berlin, Germany; [5]Hôpital Haut Lévêque-CHU, Bordeaux, France; [6]Erasmus Medical Center, Rotterdam, Netherlands; [7]Max-Planck-Institut für Psychiatrie, Munich, Germany; [8]CHU Hopital Nord, Grenoble, France; [9]Novartis, New Jersey, United States.

Chronic exposure to hypercortisolism has a significant impact on patient's health and Health-Related Quality of Life (HRQoL), as demonstrated with generic questionnaires. Objective: Develop and validate a disease-generated questionnaire to evaluate HRQoL in patients with Cushing's syndrome-CS- (Cushing QoL). Methods: After a literature review, interviews with expert endocrinologists and 10 patients identified HRQoL domains and clinical aspects of the disease; an analysis of the content allowed a qualitative reduction of items and design of version-1 (V-1) of the questionnaire, which was administered to 5 Spanish patients to detect and correct comprehension problems (cognitive debriefing); this allowed the obtaining of the V-2 version, the items of which were scored by 10 endocrinologists in terms of importance and frequency to select the most relevant ones and design the V-3 questionnaire, which was translated into 16 languages. This questionnaire was presented to 125 patients in an observational, international, multi-center, cross-sectional study, including 14 investigators from Spain, France, Germany, The Netherlands and Italy; the generic SF-36 questionnaire and a question on self-perceived general health status, as well as clinical and hormonal data were also collected. Results: 107 were pituitary-dependent and 18 adrenal- dependent CS; 83% were females, median age 45 yrs; 34% were currently hypercortisolemic and 38% adrenal insufficient. CushingQoL was feasible (94% of patients fully responded to the questionnaire in 4 minutes), reliable (Crohnbach's alpha =0.87) and valid (factorial analysis demonstrated unidimensionality and Rasch analysis lead to a final version with 12 items). A significant ($P<0.001$) correlation was observed between CushingQoL score and patients self-perceived general health status and dimensions of SF-36 (Pearson correlation coefficient >0.597). Patients with hypercortisolemia (56±22 vs 48±20, P=0.043) and increased UFC (56±19 vs 46±23, P=0.009) scored worse than those without.
Conclusion
Conclusion: CushingQoL is useful to evaluate HRQoL in patients with CS and correlates with clinical parameters.

P560

Differential expression of genes related to aggressiveness in non-functioning pituitary adenomas

Maria Asuncion Martinez-Brocca[1], Carmen Saez[2], Alfonso Soto[1], Carolina Castilla[2] & Miguel Angel Japon[2]
[1]Department of Endocrinology. Hospitales Universitarios Virgen del Rocio, Seville, Spain; [2]Department of Pathology. Hospitales Universitarios Virgen del Rocio, Seville, Spain.

Prediction of the biological behavior in non-functioning pituitary adenomas (NFPA) according to morphological criteria is highly inaccurate. Reliable prognostic molecular markers could be useful in providing guidance in NFPA post-surgical follow-up.
Aim
To identify differentially expressed genes between aggressive and non-aggressive NFPAs and to assess their prognostic value.
Methods
Samples analyzed were selected from a series of 60 NFPAs consecutively resected in our institution between 1998 and 2005 and kept frozen at $-80\,^{\circ}C$. Criteria for aggressive NFPA were invasion of surrounding structures or central nervous system at diagnosis (Hardy III/IV), recurrence and/or regrowth of post-surgical remnants. cDNA from pooled aggressive and non-aggressive NFPAs samples were labelled and hybridized on cDNA arrays (Superarray Bioscience), containing 192 genes related to invasiveness and angiogenesis, and normalized expression for each gene was calculated. Overexpression of selected genes was individually assessed by RT-PCR and its association to clinical parameters of agressiveness was analyzed.
Results
61.6% adenomas were classified as aggressive, and 38.4% as non-aggressive NFPAs. The expression of a subset of genes was 1.5 to 3.9 fold higher in aggressive NFPAs; among them, growth factors and their receptors (KGF, HGF, PDGFa, TGFb1, TGFb3, FGFR2, FGFR3), chemokines (CXCL1, CXCL4), metalloproteases (Meth1, MMP9) and other proteins related to cellular adhesion and migration, such as osteopontin and cadherin-5, were identified. By RT-PCR, cadherin-5 was found to be expressed in 100% of aggressive-NFPAs but only in 8.7% of non-aggressive NFPAs. Moreover, a trend toward a higher expression of osteopontin in NFPAs invading cavernous sinus was found. Differences in CXCL4 expression were not individually detected.
Conclusions
cDNA arrays are useful to identify differentially expressed genes in NFPAs with discordant clinical behavior. Cadherin-5 and osteopontin are potential markers of aggressiveness in NFPAs, a fact that might be related to a pro-angiogenic and pro-invasive state.

P561

Effects of CST-8, a synthetic cortistatin analogue, in humans

Andrea Benso[1], Flavia Prodam[1], Elena Gramaglia[1], Fabrizio Riganti[1], Barbara Lucatello[1], Aart Jan van der Lely[2], Romano Deghenghi[1], Giampiero Muccioli[3], Ezio Ghigo[1] & Fabio Broglio[1]
[1]Div Endocrinology and Metabolic Diseases, Dpt Internal Medicine, University of Turin, Turin, Italy; [2]Div Endocrinology, Dpt Internal Medicine, Erasmus University Rotterdam, Rotterdam, Netherlands; [3]Dpt Anatomy, Pharmacology and Forensic Medicine, University of Turin, Turin, Italy.

Cortistatin (CST), a neuropeptide with high structural homology with somatostatin (SS), binds all SS receptor (SS-R) subtypes but, unlike SS, also shows high binding affinity to ghrelin (GRLN) receptor (GRLN-R). In humans CST exerts the same endocrine activities of SS, suggesting that the activation of the SS-R might mask the potential interaction with the GRLN system.
CST-8, a synthetic CST-analogue devoid of any binding affinity to SS-R but capable to bind the GRLN-R, has been reported able to exert antagonistic actions on GRLN actions either *in vitro* or *in vivo* in animals. We studied the effects of CST-8 (2.0 μg/kg iv as a bolus or 2.0 μg/kg/h iv as infusion) on both spontaneous and GRLN- or hexarelin (HEX) (1.0 μg/kg iv as bolus)-stimulated GH, PRL, ACTH and cortisol secretion in 6 normal volunteers. The effect of CST-8 iv infusion at 4.0 μg/kg/h on the GH response to GRLN was also studied in 3 subjects. The study was approved by an independent Ethical Committee.
During saline, spontaneous ACTH and cortisol decrease was observed while no change occurred in GH and PRL levels. GRLN and HEX increased ($P<0.05$) GH, PRL, ACTH and cortisol levels. CST-8 administered either as bolus or as continuous infusion did not modify both spontaneous and GRLN- or HEX-stimulated GH, PRL, ACTH and cortisol secretion. The GH response to GRLN was unchanged even under exposure to the highest CST-8 dose.
In conclusion, CST-8 seems devoid of any modulatory action on either spontaneous or GRLN-stimulated somatotroph, lactotroph and corticotroph secretion. Thus, CST-8 seems an inactive peptide in humans, at least in term of modulation of pituitary hormone secretion.

P562

Prevalence of anterior pituitary dysfunction in patients following traumatic brain injury (TBI) in a German multi-centre screening program

Christian Berg[1], Alexandra Oeffner[4], Petra Maria Schumm-Draeger[4], Georg Brabant[2], B Gerbert[3], Matthias Weber[5], Klaus Mann[1] & Burkhard Herrmann[6]
[1]University of Duisburg-Essen, Clinic of Endocrinology, Essen, Germany; [2]University of Hannover, Hannover, Germany; [3]University of Dresden, Dresden, Germany; [4]University of Munich, Klinikum Bogenhausen, Munich, Germany; [5]University of Mainz, Mainz, Germany; [6]Institute of Cardio-Diabetes, Technology-Centre, Bochum, Germany.

Recent data suggest that hypopituitarism is a common complication of TBI. Prevalence differs between 10–40% and is based on different diagnostic tests and criteria. Hence, under field conditions TBI-mediated hypopituitarism may be less frequent than previously thought. We determined the prevalence of anterior pituitary dysfunction in a multi-centre screening program across five German endocrine centres in patients rehabilitating from TBI (GCS <13).
Patients & methods
246 patients (43 ± 14 yrs; 133 males, 12 ± 8 months after TBI) underwent baseline endocrine testing with central assessment of TSH, free T4, prolactin, LH, FSH, testosterone (m), estradiol (f), cortisol and IGF-I. If IGF-I was <-1 SDS GHRH + arginine or insulin tolerance test was performed. GHD was defined according to BMI-dependent cut-off values for GH response to GHRH + arginine of <4.2, <8.0 and <11.5 ng/ml in obese, overweight and lean subjects, respectively, and <3 microg/L in ITT. Hypocortisolism was defined when basal cortisol was <200 nmol/l and confirmed by ITT.
Results
In TBI patients some degree of impaired pituitary function was shown in 21% ($n=52/246$). Total, multiple and isolated deficits were present in 1%, 2% and 18% respectively. 19% ($n=46$) had an IGF-I of <-1 SDS. In 4% ($n=9$) GHD was confirmed. IGF-I did not correlate with BMI, gender or time after injury, but with age ($P=0.03$). 9% ($n=23$) had hypogonadism (total testosterone <9.5 nmol/L /low estradiol and low gonadotropins). Total testosterone levels did not correlate with BMI or age. 10.7% ($n=35$) had mild hyperprolactinemia. 4% ($n=11$) had hypocortisolism and 1% ($n=3$) had confirmed ACTH-deficiency. 12% ($n=29$) had TSH-deficiency.
Conclusion
In summary, in this large series carried out on an unselected group of TBI survivors we could not confirm a high prevalence of anterior pituitary dysfunction. Only every fifth patient with low IGF-I had confirmed GHD according to strict criteria and based on BMI-dependent cut-off values for GHRH + arginine testing. Hence IGF-I is a poor predictor for GHD in TBI.

Neuroendocrine and pituitary behaviour – presented on Tuesday

P563

Distribution of type 1 cannabinoid receptor (CB1) immunoreactive axons in the mouse hypothalamus

Gábor Wittmann, Levente Deli, Imre Kalló, Erik Hrabovszky, Masahiko Watanabe, Zsolt Liposits & Csaba Fekete
Department of Endocrine Neurobiology, Institute of Experimental Medicine, Hungarian Academy of Sciences, Budapest, Hungary; Department of Anatomy, Hokkaido University School of Medicine, Sapporo, Japan; Department of Neuroscience, Faculty of Information Technology, Pázmány Péter Catholic University, Budapest, Hungary; Tupper Research Institute and Department of Medicine, Division of Endocrinology, Diabetes, Metabolism and Molecular Medicine, New England Medical Center, Boston, MA, United States.

Type 1 cannabinoid receptor (CB1) is the principal receptor for endocannabinoids in the brain which mainly occurs in preterminal/terminal axons and mediates

retrograde neuronal signaling mechanisms. A large body of physiological and electrophysiological evidence indicates the critical role of CB1 in the regulation of hypothalamic functions. Conversely, the distribution of CB1-containing axons in the hypothalamus is essentially unknown. Therefore, we have analyzed the distribution and the ultrastructural characteristics of the CB1-immunoreactive (IR) axons in the mouse hypothalamus using an antiserum against the C-terminal 31 amino acids of the mouse CB1. We found that CB1-IR axons innervated densely the majority of hypothalamic nuclei, except for the suprachiasmatic and lateral mamillary nuclei where only scattered CB1-IR fibers occurred. CB1-IR innervation of the arcuate, ventromedial, dorsomedial and paraventricular nuclei and the external zone of the median eminence corroborated the important role of CB1 in the regulation of energy homeostasis and neuroendocrine functions. Ultrastructural studies to characterize the phenotype of CB1-IR fibers established that most CB1-immunoreactivity appeared in the preterminal and terminal portions of axons. The CB1-IR boutons formed axo-spinous, axo-dendritic and axo-somatic synapses. Analysis of labeled synapses in the paraventricular, supraoptic and arcuate nuclei detected approximately equal numbers of symmetric and asymmetric specializations.

In conclusion, the study revealed the dense and differential CB1-IR innervation of most hypothalamic nuclei and the median eminence of the mouse brain. At ultrastructural level, CB1-IR axons established communication with hypothalamic neurons via symmetric and asymmetric synapses indicating the occurrence of retrograde signaling by endocannabinoids in hypothalamic neuronal networks.

P564

Immunohistochemistry of pure growth hormone-containing and mixed growth hormone/prolactin-containing pituitary adenomas

Evgeniya Marova, Petr Yushkov, Natalja Molitvoslovova & Elena Luljeva
National Endocrinology Research Centre, Moscow, Russia.

Transsphenoidal surgery is the most efficient primary treatment for acromegaly. However, some patients do not meet remission criteria after operation. Mixed growth hormone (GH)/prolactin (PRL)-secreting pituitary adenomas are known to predict poor surgical outcome. The aim of our study was to evaluate immunohistochemical markers in pure GH-and mixed GH/PRL-containing tumors and to investigate their prognostic value. In our study we included 39 acromegalic patients, who underwent transsphenoidal surgery as primary treatment. We used immunohistochemical staining of removed adenomas for PRL to evaluate hormonal content of adenomas' cells; for proliferation marker (Ki-67), angiogenesis index (CD31) and marker for malignancy potential (galectin-3) to assess the biological tumor behavior. In addition to immunostaining of removed pituitary adenomas we evaluated clinical, hormonal and radiological data based on magnetic resonance imaging (MRI). Immunohistochemistry showed mixed GH/PRL-containing adenomas in 9 patients (23%), whereas pure GH-secreting adenomas in 30 cases (77%). Ki-67 was present in all mixed adenomas, but not in pure GH-secreting tumors. Galectin-3 was positive in 2 GH/PRL-cosecreting tumors (22%) and 9 pure GH adenomas (30%). CD31 was found in 3 mixed tumors (33%) and 13 pure GH adenomas (43%). In patients with GH/PRL co-secreting tumors MRI-predictors of unsuccessful surgical outcome were present: large size ($P=0.0007$, under Mann-Whitney's test) and intracavernous extension of adenomas ($P=0.0262$, under two-tailed Fisher's exact test). In addition, there were no cases of remission in patients with mixed GH/PRL-containing tumors. In conclusion, evaluation of immunohistochemical predictors of removed adenomas in combination with immunostaining for PRL in acromegalic patients gives the additional information which can determine surgical outcome and postoperative adjunctive therapy for such patients.

P565

The effects of pasireotide (SOM230) on glucose metabolism and growth hormone (GH) nadir during oral glucose tolerance test (OGTT) in 12 patients with acromegaly from a Phase II study

Stephan Petersenn[1], Joan Glusman[2], Nicole Unger[1] & Klaus Mann[1]
[1]University of Essen, Essen, Germany; [2]Novartis Pharmaceuticals Corporation, East Hanover, United States.

Introduction
Pasireotide (SOM230) is a novel multi-ligand somatostatin analogue with high binding affinity for four of the five somatostatin receptor subtypes ($sst_{1,2,3}$ and sst_5). A randomized study of 59 patients showed that pasireotide effectively controls GH and IGF-I levels in patients with acromegaly and reduces pituitary tumor size. The impact of pasireotide on GH levels during glucose suppression and glucose metabolism in 12 patients enrolled in the study is reported.
Methods
Patients in this study had GH levels >5 μg/L, elevated IGF-I and lack of suppression of GH to <1 μg/L post-OGTT. After treatment with octreotide 100 μg sc tid for 28 days, patients received pasireotide 200, 400 and 600 μg sc bid in random order for 28 days each. Glucose and GH levels were measured during OGTT in 12 patients prior to treatment, after octreotide treatment and after each pasireotide treatment phase.
Results
During glucose suppression, 4 of the 12 patients had a similar GH nadir ($<10\%$ difference) after pasireotide (-71.0%) or octreotide (-72.3%) treatment, and 8 patients had a stronger GH suppression with pasireotide (-75.1%) than with octreotide (-22.8%). Under fasting conditions prior to therapy, 7 patients had normal glucose tolerance (NGT), 2 patients had impaired glucose tolerance (IGT), and 3 patients had diabetes mellitus (DM). At the last assessment during treatment with pasireotide, 9 patients remained in the same category, 1 patient improved, and 2 patients had increased glucose levels. Similar results were seen for glucose metabolism 120 minutes post-OGTT.
Conclusions
Pasireotide suppressed GH levels during OGTT to a similar extent (4/12 patients) or greater extent (8/12 patients) than octreotide, indicating that it may be effective in patients with octreotide-resistant acromegaly. Furthermore, using stringent criteria, the majority of patients did not demonstrate relevant changes in glucose metabolism by the end of the pasireotide treatment period.

P566

Cerebrospinal fluid (CSF)/serum albumin ratio shows no alteration of the blood-brain barrier in patients with pituitary adenomas and high CSF levels of pituitary hormones

Monica Livia Gheorghiu[1], Mariana Purice[2], Catrinel Rusu[3], Simona Galoiu[1], Ruxandra Tanasescu[1], Dan Niculescu[1] & Mihai Coculescu[1]
[1]'C. Davila' University of Medicine & Pharmacy, Bucharest, Romania; [2]'C.I.Parhon' Institute of Endocrinology, Bucharest, Romania; [3]'N. Kretzulescu' Medical Center of the Internal Affairs Ministry, Bucharest, Romania.

Some patients with pituitary adenomas show high CSF levels and/or high CSF/serum ratio for peptidic pituitary hormones (PR), potentially due to a blood-brain barrier (BBB) damage. We evaluated albumin.CSF/serum ratio (AR) in patients with pituitary adenomas and elevated PR, as an accurate index for a BBB damage.
Patients and methods
We evaluated 10 controls (21–79 years, 6M /4 F) before undergoing abdominal or peripheral surgery and 52 patients with pituitary adenomas (PA) (17–79 years, 25 M/27F, 16 before and 36 after pituitary surgery), with the approval of the local Ethical Committee. Anterior pituitary hormones and albumin were measured in simultaneously sampled serum and CSF by rapid fluoroimmunoassay and nephelometry, respectively. AR >0.007 was considered abnormal.
Results
In PA, median albumin in serum (4625 ± 1134 mg/dl) and CSF (24.7 ± 37.7 mg/dl) was not statistically different from controls ($3710+710$ mg/dl and 20.2 ± 8.2 mg/dl, respectively). In 1/7 (14%) controls and 9/52 (17%) PA, AR was >0.007 (NS).
PR >1 for at least one pituitary hormone was found in significantly more patients with tumors in contact with BBB (suprasellar extension + neuroophtalmic syndrome or intracavernous sinus invasion), either before pituitary surgery ($10/21=47\%$) or after surgery ($9/16=56\%$), compared with only 1/15 (6%) in PA without contact with BBB before surgery ($P=0.001$). Albumin CSF, serum and R were not statistically different between contact and non-contact tumors or in patients with PR >1 compared to those with PR <1.
Conclusion
CSF/serum albumin evaluation shows that there is no alteration of the CSF flow rate in patients with pituitary adenomas and increased CSF/serum ratio for the anterior pituitary hormones, compared to controls. It is tempting to believe that the increased hormonal level in CSF is due to the tumor secretion.

P567

Medial cerebral artery occlusion 25 years after cranial radiation therapy in acromegaly

Pietro Maffei[1], Chiara Martini[1], Martina Barban[1], Marta Carli[1], Renzo Manara[2], Massimo Scanarini[3], Renzo Mazzarotto[4], Giorgio Meneghetti[5], Carla Menegazzo[1], Roberto Mioni[1], Eugenio De Carlo[1], Roberto Vettor[1] & Nicola Sicolo[1]
[1]Department of Medical and Surgical Sciences, Padua, Italy; [2]Department of Neuroradiology, Padua, Italy; [3]Department of Neurosurgery, Padua, Italy; [4]Department of Radiotherapy, Padua, Italy; [5]Department of Neurology, Padua, Italy.

Introduction
Epidemiological studies indicate that patients with acromegaly have increased mortality rates for cardiovascular and cerebrovascular disease (CVD). Potentials risk factors for increased CVD include hypopituitarism and cranial radiation therapy (CRT).
Case Report
A 43-year-old acromegalic woman was admitted because of worsening headache, left lower facial numbness and left arm weakness. Two weeks later she developed a central palsy of the left 7th cranial nerve. Past medical history was remarkable for gigantism-acromegaly which was diagnosed at age 17. At that time a pituitary macroadenoma was partially removed by transsphenoidal surgery and few months later she underwent conventional radiation therapy (two-fields, 50 Gy, 25 sessions). The patient's current medications were estroprogestin (since age 18), L-thyroxin (since age 39) and cortisone (since age 42). GH deficiency was also diagnosed (IGF-I 70 ug/L). Finally, a prothrombin mutation (G20210A) was discovered in family members including the patient.
Results
On examination there were a left lower facial palsy and bilateral cutaneous brownish of temporal regions. Laboratory investigations were uneventful for homocysteine, glucose and lipid metabolism. Thyroid and adrenal glands were regularly replaced. Intensive general (EKG; BP values; echocardiogram; 24-h Holter EKG; chest X-ray; abdominal US) and neurological study (carotid US; transcranial US; EEG; CT; MRI; MR-angiography; CT-angiography) disclosed several ischemic lesions of right parietal lobe, lenticular nucleus, anterior limb of internal capsule and occlusion of right medial cerebral artery near its origin. The diameter of right carotid artery was also reduced. Post-radiotherapy brain damage was visible by MRI. Atrial septal defect was excluded. The patient was treated with aspirin plus low dose heparin s.c. and neurological disturbances relieved completely.
Discussion
Life-long follow-up of acromegalic patients receiving CRT is essential so that early diagnosis of radiation-induced vascular damage can be made. In this particular context, treatment and monitoring of cerebrovascular thrombosis remain almost empiric.

P568

The hypothalamic-pituitary-adrenal axis in premenopausal females with psoriatic arthritis

Miroslav Vlcek[1], Jozef Rovensky[2], Adela Penesova[1], Zofia Radikova[1], Helena Raffayova[2] & Richard Imrich[1]
[1]Institute of Experimental Endocrinology, Slovak Academy of Sciences, Bratislava, Slovakia; [2]National Institute of Rheumatic Diseases, Piestany, Slovakia.

Objective
Changes of hypothalamic-pituitary-adrenal (HPA) axis activity, in particular decreased production of adrenal androgens, have been observed in patients with rheumatic diseases. However, data on adrenal steroid status in patients with psoriatic arthritis (PsA) are scarce. The present study was aimed at evaluation of HPA status in context of chronic inflammation in glucocorticoid-naïve premenopausal females with PsA.
Subjects and methods
Concentrations of ACTH, cortisol, androstenedione (ASD), 17OH-progesterone (17OHP), dehydroepiandrosterone (DHEA), and DHEA-sulphate (DHEAS) were analyzed before and during insulin-induced hypoglycaemia in 16 female premenopausal patients (age 40.1 ± 1.4 y, BMI 23.5 ± 1.1 kg/m^2) with PsA and in 11 age and BMI matched healthy women. Basal levels of IL-1alpha, IL-1beta, IL-6, TNF alpha and CRP were measured in all studied subjects as well. The study was approved by local ethical committee.
Results
The disease activity of PsA patients was low. No significant differences in levels of IL-1alpha, IL-1beta, TNF alpha or CRP were found between patients and controls. IL-6 levels were higher in PsA compared to controls ($P=0.045$). Basal levels and response to stimulation of ACTH and cortisol did not differ between the study groups. PsA patients had lower basal levels of ASD (2.79 ± 0.24 nmol/l vs. 4.89 ± 0.87 nmol/l; $P=0.013$) and DHEAS (2.42 ± 0.32 μmol/l vs. 3.79 ± 0.63 μmol/l; $P=0.044$) and levels of DHEA tended to be lower (13.2 ± 1.9 nmol/l vs. 20.4 ± 3.5 nmol/l; $P=0.065$). During stimulation PsA patients had significantly lower response of 17OHP and ASD when compared to controls ($P=0.046$, $P=0.004$ respectively). We did not find any significant correlation between basal levels of steroid hormones and cytokines.
Conclusions
The results suggest a shift in production of adrenal steroids from adrenal androgens towards production of cortisol in patients with PsA. Whether or not the observed changes in production of adrenal androgens are secondary due to ongoing inflammatory process remains to be elucidated.

P569

Cortisol and dexamethasone exert different negative feedback action in humans

Roberta Giordano, Rita Berardelli, Andreea Picu, Marcella Balbo, Lorenza Bonelli, Anna Barberis, Ezio Ghigo & Emanuela Arvat
Division of Endocrinology and Metabolism, Department of Internal Medicine, University of Turin, Turin, Italy.

HPA response to glucocorticoids (GCs) feedback is usually tested by dexamethasone (DEX), a synthetic GC; it poorly crosses BBB and preferentially activates pituitaric glucocorticoid receptor (GR), with a binding potency to GR 7 fold higher and an anti-inflammatory potency about 35 fold higher than cortisol. Cortisol, which easily penetrates into CNS, could better evaluate the GC feedback by acting also at supra-pituitary level. We studied the effects of 150 min infusion of hydrocortisone (HC: 15, 30 or 60 μg/kg/h) or DEX (0.4, 0.8, 1.6 or 2.1, 4.2, 8.5 μg/kg/h, covering either HC:DEX 1:35 or 1:7) on ACTH and cortisol levels in 9 normal subjects who underwent also a testing session with placebo. The study had been approved by an independent Ethical Committee. During placebo, ACTH and cortisol levels showed progressive decrease ($P<0.05$). The different doses of HC induced dose-dependent cortisol increases ($P<0.05$) coupled with dose-dependent ACTH decreases ($P<0.05$). 0.4, 0.8 and 1.6 μg/kg/h DEX doses did not modify cortisol levels; 0.8 and 1.6 but not 0.4 μg/kg/h DEX doses induced a dose-dependent ACTH decrease ($P<0.05$). Conversely, 2.1, 4.2 and 8.5 μg/kg/h DEX doses inhibited cortisol levels in dose-dependent manner ($P<0.05$) and induced more marked ACTH decrease ($P<0.05$). In conclusion, based on the potency of binding to GR, similar doses of hydrocortisone and dexamethasone are needed to reduce ACTH levels. Conversely, taking into account the anti-inflammatory potency, doses of dexamethasone higher than hydrocortisone are needed to inhibit ACTH secretion. These latter findings are likely to reflect different sites where natural and synthetic GCs exert their feedback action, i.e. mainly the CNS for hydrocortisone and the pituitary for dexamethasone. It is suggested that the HPA sensitivity to the feedback action of GCs in various pathophysiological conditions would better be evaluated by using natural GCs.

P570

The role of vasopressin in the hypothalamo-pituitary-adrenal axis regulation during the perinatal period: paradoxical corticosterone elevation without an ACTH rise

Dóra Zelena, István Barna, Zsuzsa Mergl, Ágnes Domokos, Katalin Csabai & Gábor B Makara
Hungarian Academy of Sciences, Institute of Experimental Medicine, Budapest, Hungary.

Early life events have special importance in the development and may affect the lifetime vulnerability to diseases. For correct interpretation of the long-term consequences it is crucial to understand the immediate effects. The role of vasopressin in hypothalamo-pituitary-adrenal axis regulation as well as in stress-related affective disorders is important therefore we addressed the question if the lack of this hormone will modify the perinatal stress reactivity.
Vasopressin producing (di/+) and deficient (di/di) Brattleboro rat pups were used. The separation of the 9-day-old pups from their mother for 24 h resulted in a remarkable corticosterone elevation in both genotypes without an ACTH increase in di/di rats. As the time-course of ACTH and corticosterone can be different we examined the 1-4-12-24 h separation period, too, with similar result (no ACTH elevation at any time point in di/di rats parallel with a remarkable corticosterone increase). Altered sensitivity of the adrenal gland might also explain the findings,

so we examined adrenal secretion *in vivo* with exogenous ACTH administration, but failed to find a significant difference between the genotypes. Tenth postnatal day is in the middle of the stress hyporesponsive period so we examined earlier (4–5 day old) and later (20 day old) postnatal phases too. After 24 h separation the ACTH levels did not change in di/di, but increased in di/+ pups with the highest rise at 10 days old, although corticosterone was significantly higher in both genotype at each time-point.

We can conclude that the role of vasopressin is an important factor in ACTH-secretion regulation during the postnatal period. However in the absence of ACTH other secretagogues may become important in the regulation of the adrenal gland secretion. The marked corticosterone elevation in the absence of ACTH rise is possibly not due to the different time-course of the two hormones or an altered sensitivity of the gland and it is present during the whole postnatal period up to 20 day.

P571

Riluzole treatment does not affect growth hormone (GH) secretion in amyotrophic lateral sclerosis

Lisa Linda Morselli[1], Paolo Bongioanni[2], Maura Genovesi[1], Rosaria Licitra[2], Bruno Rossi[2], Luigi Murri[2], Maurizio Gasperi[1] & Enio Martino[1]
[1]Department of Endocrinology, Pisa, Italy; [2]Department of Neurosciences, Pisa, Italy.

Amyotrophic lateral sclerosis (ALS), the most common motor neurone disorder in human adults, presents is characterized by selective and progressive degeneration of upper and lower motor neurones in central nervous system. GH secretion, evaluated by GHRH + arginine test, has been recently reported to be impaired in about 70% of untreated ALS patients. The currently available drug for ALS treatment is riluzole, a compound acting through inhibition of glutamate relase, post-synaptic receptor activation and voltage sensitive channel inhibition.

The aim of the present study was to evaluate whether riluzole administration can interfere with GH secretion and the diagnosis of adult GH deficiency. Ten patients (6 M, 4 F, mean age 59 ± 11 years) were studied. GHRH + arginine test was performed before and 1–3 months after starting riluzole treatment (100 mg/die). Blood samples for GH were collected at baseline and 30 and 60 minutes. Two patients showed severe (peak GH < 9 ng/ml), 5 patients mild (9 < peak GH < 16 ng/ml) GH deficiency and 2 patients had a normal GH response (peak GH > 16 ng/ml). Mean peak GH levels were similar before and during riluzole treatment (13.4 ± 10 vs 14.2 ± 10.1 ng/ml; P = NS). No significant correlation was observed between peak GH concentrations and age, BMI, disease duration, severity or clinical form. In conclusion, the present data confirm, in a new series of ALS patients, that GH secretion is impaired in these patients and indicate that riluzole treatment does not interfere with GH secretion. Therefore adult GH deficiency can also be diagnosed during riluzole therapy.

P572

Impairment of GH secretion by ghrelin stimulation test in primary hyperparathyroidism (PHP)

Elisabetta Cecconi[1], Clara Giovannetti[1], Luca Manetti[1], Valentina Raffaelli[1], Massimo Procopio[2], Ezio Ghigo[2], Maurizio Gasperi[1] & Enio Martino[1]
[1]Department of Endocrinology, Pisa, Italy; [2]Division of Endocrinology, Turin, Italy.

Pituitary GH secretion is regulated by the interplay of at least two hypothalamic hormones, GH-releasing hormone (GHRH) and somatostatin, through their interaction with specific cell surface receptors on the anterior pituitary somatotrophs. A third type of receptor, the growth hormone secretagogue receptor, called GHS receptor type 1a (GHSR1a), was identified in the pituitary and the hypothalamus. Ghrelin is an acylated peptide produced predominantly by stomach and a natural ligand of the GHS-R1a. In HEK-293 cells expressing the GHS-R1a, ghrelin induces a biphasic cytosolic calcium elevation. We recently reported that untreated PHP patients have an impaired GH secretion, as demonstrated by a blunted GH response to maximal stimulation with GHRH + Arginine test. The aim of the present study was to evaluate effects on GH secretion induced by ghrelin in PHP. Eleven patients (2 male/9 female, age range 41–67 yrs, mean 54 yrs, BMI 26.6 ± 3.4) with PHP were studied. The control group consisted of 35 normal age- and sex-matched subjects (12 male/23 female, age range 23–78 yrs, mean 59 yrs, BMI 26.3 ± 3.1). Patients and controls were submitted on two separate days to ghrelin administration (1 μg/Kg iv) and to GHRH + arginine test.

Serum GH secretion was reduced (GH response to GHRH + arg test: 9.54 ± 3.1 μg/liter) in 7 patients (64%) and normal (38.57 ± 10.5 μg/liter) in the remaining 4 (36%); in the control group no GHD was found (peak GH 38.0 ± 3.5 μg/liter, P < 0.001).

The mean peak GH response to ghrelin in PHP was significantly lower than in normals (17.99 ± 8.3 vs. 84.0 ± 36 μg/L, P < 0.001) in accordance to the values obtained by GHRH + arginine test.

In conclusion, this study confirms the impaired GH secretion to GHRH + Arg stimulation in PHP patients and represents the first demonstration that ghrelin administration unveils GH deficiency in PHP.

P573

The influence of cabergoline treatment on seminal fluid

Ekaterina N Giniatullina, Larisa K Dzeranova, Ludmila Y Rozhinskaya & Anna D Dobracheva
Research Center for Endocrinology, Moscow, Russia.

This study evaluated the effects of the long treatment with cabergoline on seminal fluid parameters and sexual and gonadal function in hyperprolactinemic males. Eleven males with macroprolactinoma were treated with cabergoline at a dose of 1.5–2.5 mg a week for 6 months. All the patients suffered from libido impairment, reduced sexual potency, six had infertility. In three patients provocative bilateral galactorrhea was found.

Seminal fluid analysis, functional seminal tests, prolactin and testosterone concentrations and cerebrum magnetic resonance imaging were assessed before and after 6 months of cabergoline treatment. Baseline prolactin was 11530.7 ± 222.6 mU/l. Baseline testosterone was 6.25 ± 0.2 nmol/l. Before treatment, all patients had a low sperm count with oligoasthenospermia, reduced motility and rapid progression with an abnormal morphology and decreased viability, and a low number of erections.

After 6 months, serum PRL level was significantly reduced 682 ± 16.6 mU/l (P < 0.005). Testosterone level significantly increased to 19.8 ± 0.04 nmol/L (P < 0.002). After 6 months, a significant increase of sperm volume, number, total motility, rapid progression and normal morphology was recorded in patients treated with cabergoline. An increase in the number of erections during the first 3 months of treatment was noted. The number of erections was normalized after 6 months of treatment in all patients. Positive dynamics of the tumors volume was noted at 9 patients (81.2%) - adenoma has reduced. No dynamics observed in 2 men (18.8%). The bilateral galactorrhea in all three patients was not found.

The treatment with cabergoline normalized prolactin and testosterone levels, improving gonadal and sexual function and fertility in hyperprolactinaemic males and can be successfully used as primary therapy in men with large macroprolactinomas.

P574

Fractionated stereotactic conformal radiotherapy for skull base benign tumours: an endocrinological follow-up

Giuseppe Minniti, Daphne Traish, Sue Ashley, Adam Gonsalves & Michael Brada
Neuro-oncology Unit1, The Institute of Cancer Research and the Royal Marsden NHS Foundation Trust, Downs Road, Sutton, Surrey SM2 5PT, UK, and Fulham Road, London SW3 6JJ, United Kingdom.

Background
Stereotactic radiotherapy techniques have been recently employed in the control of skull base tumours, such as pituitary edenomas, craniopharyngiomas and meningiomas.
Purpose
To assess the long-term endocrinological effect of fractionated stereotactic conformal radiotherapy (SCRT) in patients with residual and recurrent sellar and parasellar tumours treated at Royal Marsden Hospital.
Patients and methods
245 patients (median age 50 years) with residual or recurrent pituitary adenomas (n = 98), meningiomas (n = 108) and craniopharyngiomas (n = 39) were treated between 1995 and 2004 at The Royal marsden Hospital. 102 patients had partial or complete hypopituitarism before SCRT (69, 29 and 5 patients with pituitary adenomas, craniopharyngiomas and meningiomas), including 44 with a complete and 58 with a partial hypopituitarism. Patients were treated supine and immobilized in a Gill-Thomas-Cosman relocatable frame. High-resolution planning CT scan was fused with magnetic resonance imaging (MRI) scan.

The treatment was delivered by 4–6 noncoplanar conformal fixed fields using a 6-MV linear accelerator to a dose of 45–55 Gy in 25–33 fractions.
Results
At a median follow-up of 38 months (range 3–120) the 5 year actuarial progression free is 98.9%, 93%, 92% and overall survival is 98%, 97% and 100% for adenomas, meningiomas and craniopharyngiomas. The treatment was well tolerated with minimal acute and long-term toxicity. Hypopituitarism was the most common long-term effect and 26%, 42% and 6% of patients with a pituitary adenoma, a craniopharyngioma and a meningioma worsened pituitary function. Hypopituitarism was more common in patients with pre-SCRT pituitary hormone abnormalities.
Conclusion
SCRT is an effective treatment for patients with benign skull base tumours and is associated with low toxicity. Tumour control was equivalent to that seen following conventional radiotherapy and radiosurgery. Longer follow-up is needed to assess a potential reduction in long-term morbidity. Hypopituitarism develops in a significant number of patients requiring a regular follow-up in these patients.

P575

The GH releasing activity of ghrelin is insensitive to the negative growth hormone (GH) autofeedback in humans

Fabrizio Riganti, Flavia Prodam, Elena Gramaglia, Andrea Benso, Barbara Lucatello, Ezio Ghigo & Fabio Broglio
Division of Endocrinology and Metabolism, University of Turin, Turin, Italy.

Growth hormone (GH) secretion is regulated by a complex interplay between GH-releasing hormone (GHRH), somatostatin and several other central and peripheral modulatory signals. Ghrelin has been hypothesized as physiological amplifier of GH pulsatility and acts via mechanisms, at least partially, independent of GHRH and somatostatin. The GH response to GHRH is strongly inhibited by previous administration of recombinant human GH (rhGH), likely as a consequence of a somatostatin-mediated negative GH auto-feedback. The effect of exogenous rhGH on the GH-releasing effect of ghrelin has never been tested so far. In 5 normal young volunteers we studied the acute GH response to ghrelin (2.0 mcg/kg iv at 0 min) during saline or rhGH infusion (4.0 μg/Kg/h i.v. from −180 min to +60 min). Mean GH levels during saline infusion were: 0.7 ± 0.4 mcg/l. The rhGH administration increased mean GH levels to: 22.1 ± 2.3 mcg/l ($P<0.01$). During saline, ghrelin administration induced clear cut increase of GH secretion (Δpeak: 55.0 ± 6.7 mc/l; ΔAUC: 2096.4 ± 193.2 mcg/l/h; $P<0.01$ vs baseline). During rhGH infusion, ghrelin elicited the same potent GH-releasing effect (Δpeak: 92.2 ± 53.4 mcg/l; ΔAUC: 2298.3 ± 684.4 mcg/l/h; $P<0.01$). In conclusion, these results show that the acute rhGH administration does not modify the GH-releasing action of ghrelin. As GH auto-feedback is known to act by a concomitant reduction in the activity of GHRH-secreting neurons and increase of somatostatinergic tone, these data further indicate that the impact of the ghrelin system on somatotroph function is remarkably independent of either GHRH or somatostatin.

P576

Impaired GH secretion in women with HIV-related lipodystrophy

Vincenzo Rochira[1], Lucia Zirilli[1], Bruno Madeo[1], Giovanni Caffagni[1], Alessia Sanguanini[1], Antonio RM Granata[1], Maria Cristina De Santis[2], Gabriella Orlando[3], Nicola Squillace[3], Giovanni Guaraldi[3] & Cesare Carani[1]
[1]Integrated Department of Medicine, Endocrinology, Metabolism and Geriatrics, University of Modena and Reggio Emilia, Modena, Italy; [2]Laboratory of Endocrinology, Policlinico of Modena, Modena, Italy; [3]Department of Medicine and Medical Specialties, Clinics of Infective Diseases, University of Modena and Reggio Emilia, Modena, Italy.

Introduction
Patients with human immunodeficiency virus-1 (HIV-1) infection develop a lipodystrophic syndrome characterised by accumulation of central fat both in visceral and in subcutaneous compartment. In recent studies approximately 20% of male patients with HIV-related lipodystrophy presented an inadequate peak of GH secretion in response to GHRH-arginine testing, which is strongly inversely related to visceral adipose tissue-(VAT).
Aim of the study
To investigate GH secretion in female patients with HIV-related lipodystrophy according to their body composition.
Subjects and methods
We included 35 HIV-infected female patients (mean age 44.6 ± 7.6 S.D.) with lipodystrophy according to the Marrakech scale. We investigated their GH response to standardised GHRH-arginine testing in order to compare it with BMI, VAT and subcutaneous adipose tissue (SAT) evaluated by CT scan. On the basis of current clinical guidelines we considered a severely impaired GH secretion (IGHS) when the GH peak after GHRH-arginine testing was ≤5 μg/L; a mildly IGHS when it was >5 μg/L but <9 μg/L and a normal GH secretion with a peak ≥9 μg/L, according to the degree of obesity together with preliminary data obtained in male HIV-related lipodystrophy.
Results
The 37.5% of our patients had IGHS (12.5% a severe IGHS, 25% the mild form). The average GH peak in the three group and the compared data among them are shown in the table:

IGHS	GH peak	IGF-1	IGFBP3	BMI	VAT cm^2	SAT cm^2	VAT/SAT
Severe	3.2+	112.8+	1682.5±	27.1±	102.3±	154±	0.66±
	1.6	23.5	606.1	6.6	66.7	46	0.32
Mild	6.5+	157+	2149.3±	25.9±	119.8±	307.2±	0.43±
	0.9	67.9	650.2	3.1	70.4	132.8	0.22
Normal	21.5+	183.9+	2144.4±	26.1±	106.7±	215.8±	0.57±
	8.1	84.6	639	2.4	45.1	93.6	0.29

Conclusion
The pituitary GH secretion may be impaired in HIV-positive women. The percentage of subjects with IGHS seems to be higher in HIV-positive women than in men. IGF-1 results lower in IGHS subjects. Furthermore, body composition does not change according to GH-peak status.

P577

Midnight salivary cortisol vs. urinary free cortisol for the diagnosis of Cushing's syndrome

Maria Yaneva, Georgi Kirilov & Sabina Zacharieva
Clinical Center of Endocrinology and Gerontology, Medical University, Sofia, Sofia, Bulgaria.

Introduction
Midnight salivary cortisol measurement (MSC) has been recently introduced as a diagnostic test for hypercortisolism. The aim of our study was to compare the diagnostic value of two methods of screening for Cushnig's syndrome (CS): MSC and 24-h urinary free cortisol (UFC), wildely accepted as a 'gold standard' for this diagnosis.
Patients and methods
Three groups were studied: 30 patients with CS (mean age ± S.D., 39.9 ± 12.8 y, f/m 25/5, BMI 29.5 ± 7.2 kg/m^2), 34 with metabolic syndrome (MS) (41.1 ± 13.6 y, f/m 24/10, 36.5 ± 4.8 kg/m^2) and 40 healthy normal weight controls (37.2 ± 9.3 y, f/m 24/16, 23.4 ± 2.8 kg/m^2). Saliva was sampled at midnight (Salivette, Sarstedt®). Urine was collected over 24 hours at the same day. An electrochemiluminescence immunoassay was used to measure salivary cortisol. UFC was assessed by a radioimmunoassay.
Results
Mean MSC in healthy volunteers, patients with MS and CS was 8.3 ± 3.6, 8.1 ± 4.5 and 33.1 ± 21.7 nmol/l, respectively. Mean UFC was 129.1 ± 72.7, 124.25 ± 106.1 and 773.7 ± 761.7 nmol/d. No significant difference was found between MSC and UFC in healthy controls and MS ($P>0.05$). By contrast, MSC and UFC were significantly higher in patients with CS ($P<0.0001$) as compared to both other groups. The cut-off point of 14.2 nmol/l for MSC yielded a sensitivity of 93.3% and a specificity of 94.2%. The cut-off point of 222 nmol/d for UFC showed a sensitivity of 100% and a specificity of 90%. Analysis of the areas under the curve (AUC) showed no significant difference between MSC and UFC ($P<0.05$, AUCMSC = 0.984 ± 0.01 (0.965–1.000); AUCUFC = 0.975 ± 0.01 (0.948–1.000) (mean ± S.E.M. (confidentail interval of 95%)).
Conclusion
MSC and UFC determination have comparable diagnostic value. They both have reliably high sensitivity and specificity. We recommend the use of MSC as a first-line screening test for CS because of its convenience, escpecially in the ambulatory practice.

P578

Behavioural and biological effects of des-Gln14-ghrelin

Roberta Dochnal, Mónika Mácsai & Gyula Szabó
University of Szeged, Department of Pathophysiology, Szeged, Hungary.

Ghrelin, ligand for the growth hormone secretagogue receptor (GHS-R), was isolated from the stomach. Immunoreactive neurons were observed in the hypothalamic nuclei and the ependymal layer of the third ventricle. Lower amounts are produced in the small intestine, pancreas, liver, kidney, placenta, and pituitary. Receptors have widespread distribution in the body, mainly concentrated in the hypothalamus-pituitary unit.

Ghrelin, a 28-amino acid peptide, has an *n*-octanoyl modification at its third serine residue. This modification is necessary for biologic activity. A second endogenous ghrelin form was discovered which is derived from an alternative splicing of the ghrelin gene. This 27 amino acid peptide is called des-*Gln*14-ghrelin, and has an *n*-octanoyl modification at its third serine residue, identical to ghrelin, except for deletion of one glutamine.

Considerable amount of data has accumulated regarding biological effects of ghrelin 28 but des-*Gln*14-ghrelin was less studied. No experiment investigating behavioral effects of des-*Gln*14-ghrelin has been carried out in mice. Therefore in the present study we aimed to elucidate how des-*Gln*14-ghrelin influences locomotion, anxiety, body temperature, and pain threshold in CFLP mice. The peptide was injected intracerebroventicularly (icv.) and we performed open-field, plus-maze, and tail flick tests.

Our experiments showed that des-*Gln*14-ghrelin increased locomotion and exploratory behavior. The most effective dose was 2 μg/μl, which induced a significant increase in both the vertical and horizontal locomotor activity in the open field test. The increased locomotion was confirmed by the plus maze test also, where the number of entries was increased. In addition, the peptide in higher doses (4 μg/μl) seems to induce anxiolytic effect. Lower doses did not change the anxiety level. Analgesia and body temperature seems to be influenced by des-*Gln*14-ghrelin, but our results were not statistically significant.

This work was supported by ETT (050/2003), NKFP (1/027/2001), OTKA (T043095).

P579

Diagnostic accuracy of bilateral inferior petrosal sinus sampling performed following a combined stimulation with CRH and desmopressin

Stylianos Tsagarakis[1], Dimitra Vassiliadi[1], Ioannis Kaskarelis[2], Ioannis Komninos[1], Emmanouil Souvatzoglou[1] & Nicolaos Thalassinos[1]
[1]Evangelismos Hospital, Department of Endocriology, Diabetes and Metabolism, Athens, Greece; [2]Evangelismos Hospital, Department of Radiology, Athens, Greece.

Although bilateral inferior petrosal sinus sampling (BIPSS) is the most accurate procedure for the differential diagnosis of ACTH-dependent Cushing's syndrome, a false-negative rate of 4–15% has been reported. An even lower sensitivity has been shown in patients with equivocal responses to CRH and/or high-dose dexamethasone suppression test (HDST). In the present study we investigated whether the administration of CRH plus desmopressin (DDAVP) during BIPPS, which is considered to be a more potent stimulus, improves the sensitivity without compromising the specificity of the procedure.

The results in 55 patients, 48 with confirmed Cushing's disease (CD) (36 women, 12 men, mean age 42.4 ± 12.5 years) and 7 with confirmed occult ectopic ACTH syndrome (oEAS) (1 woman, 6 men, mean age 44 ± 20.4 years) that underwent BIPSS using a combined stimulus with CRH plus DDAVP were retrospectively analysed. The sensitivity for a basal IPS/P gradient >2 was 60.4%, with 100% specificity and a diagnostic accuracy of only 65.5%. After stimulation with DDAVP and CRH, 47/48 patients with CD had an IPS/P gradient $>$ 2 but, none of the patients with oEAS, resulting in a sensitivity of 97.9%. The specificity was 100%, diagnostic accuracy 98.18% and the positive and the negative predictive values were 100% and 87.5%, respectively. A subgroup of 19 patients (17 with CD and 2 with oEAS) had contradictory responses to routine tests with CRH and/or HDST; sensitivity, specificity and accuracy of BIPSS in this subgroup were 100%.

In conclusion, the application of a combined stimulation with CRH plus DDAVP may be the preferred stimulus during BIPSS, since it seems to substantially decrease the false negative rate resulting in higher sensitivity but with no loss of specificity.

P580

The empty sella syndrome – particularities of the clinical features depending on etiology

Cristina Ghervan, Cristina Olarescu, Daniel Leucuta & Ileana Duncea
Iuliu Hatieganu University of Medecine and Pharmacy, Cluj-Napoca, Cluj, Romania.

The empty sella syndrome (ESS) is caused by the herniation of the suprarahnoidian space into the sella turcica, generating the compression of the pituitary gland and, in most cases, a remodelling of the sella. The purpose of this study was to evaluate the etiology, the degree of hormonal deficit and the occurrence rate of the signs and symptoms accompanying ESS.

Material and methods

We performed a descriptive, retrospective study by analysing the medical records of the patients admitted to the Endocrinology Clinic, between 1995 and 2005. We identified 49 patients with ESS (39 women and 10 men) with ages between 18 and 68 years, with a mean age of 49.81 ± 10.14. The following parameters were examined: ESS etiology, clinical symptoms, hormonal values, neurological and ophthalmologic evaluation. The following statistical tests were used: Fisher's exact test, the Hi^2 test, the paired t test (student) and the Mann-Whitney U test.

Results

Regarding etiology: 38 patients (77.6%) had primary ESS (pEES) and 11 patients (22.4%) had secondary ESS (sEES). Total hormonal deficit was identified in 3 patients, all with pESS. Gonadal insufficiency was identified in 12 patients (11/1), central hypothyroidism in one patient with sEES and functional hyperprolactinemia in 6 patients (5/1). Diabetes Insipidus was found in 3 patients (2/1). Headaches were present in 43 patients (33/10), psychological disturbances in 20 patients (15/5), visual disturbances in 18 patients (10/8), obesity was present in 29 patients (21/8), and arterial hypertension in 27 patients (21/6).

Conclusions

Primary ESS was more frequent than the secondary form, and was more often accompanied by different degrees of pituitary insufficiency. Headaches, psychological disturbances, hypertension and obesity had high occurrence rates in both categories, while visual disturbances and gonadal insufficiency were more frequent in the patients with secondary ESS. Diabetes insipidus can be (rarely) present in both forms.

P581

Effects of successful transsphenoidal surgery on cardiovascular function in elderly acromegalics

Giuseppe Minniti[1], Carlo Moroni[2], Vincenzo Esposito[1], Rosario Cassone[2], Gianpaolo Cantore[1] & Marie Lise Jaffrain-Rea[3]
[1]Neurological Sciences, IRCCS NEUROMED, Pozzilli (IS), Italy; [2]Internal Medicine, University of Rome "La Sapienza", Rome, Italy; [3]Department of Experimental Medicine, University of L'Aquila, L'Aquila, Italy.

Background

Transsphenoidal surgery (TSS) is able to determine the biochemical remission of acromegaly in 45%–80% of the patients, thereby inducing an improvement of cardiovascular function and glucose metabolism. Only 3–5% of acromegalics are diagnosed over 65-years-old, so few data are available about post-operative cardiovascular and metabolic changes in this group.

Patients and Methods

Fifteen acromegalic patients $\geq$65 years-old who underwent successful TSS were studied. Doppler-echocardiography and 24-hour ambulatory blood pressure monitoring (ABPM) studies were performed immediately before and 6 months after TSS. Calculation of insulin sensitivity was derived from OGTT.

Results

Both left ventricular mass (LVM) and LVM index decreased significantly after surgery ($P=0.0021$ and $P=0.0015$, respectively). Nine out of 13 patients who fulfilled echocardiographic criteria for left ventricular hypertrophy (LVH) before surgery normalized LVMi, whereas LVH persisted in 3 hypertensive patients. Significant post-operative improvement of diastolic function was also observed. 24-h systolic BP (123.5 ± 12.2 *vs* 131.1 ± 15.6 mmHg, $P=0.003$) and diurnal diastolic BP (76.9 ± 7.8 *vs* 81.6 ± 6.3 mmHg, $P=0.04$) decreased after surgery. Three out of the 9 patients who were pre-operatively defined as hypertensive according to ABPM had normal post-operative diurnal BP values. Glucose metabolism improved after surgery, with a significant decrease of fasting ($P<0.05$) and post-load ($P<0.01$) glucose and insulin levels. This was associated with an improvement on insulin sensitivity ($P<0.003$).

Conclusions

Successful TSS is able to induce a significant improvement of cardiac mass and function even in elderly acromegalics, and this is associated with a slight decrease in BP values and improvement of glucose metabolism abnormalities. Long-term studies are necessary to evaluate the effect of biochemical cure on cardiovascular morbidity and mortality in such patients.

P582

Clinicopathologic correlation in cases with macronodular hyperplasia

Ayse Kubat Uzum[1], Meral Mert[1], Yesim Erbil[2], Nese Colak Ozbey[1] & Aral Ferihan[1]
[1]Istanbul University, Istanbul Faculty of Medicine, Department of Internal Medicine, Division of Endocrinology and Metabolism, Istanbul, Turkey; [2]Istanbul University, Istanbul Faculty of Medicine, Department of General Surgery, Istanbul, Turkey.

Introduction
We designed a retrospective study to investigate the clinical presentation, laboratory and pathological findings of 14 patients with ACTH-independent macronodular adrenal hyperplasia.
Materials and methods
Diagnose of Cushing' s syndrome was confirmed by biochemical tests, adrenal hyperplasia was confirmed by pathological examination in all patients.
Results
No supression was observed in overnight, low and high dose dexamethasone supression tests. Thirteen (92.9%) were females. Mean age was 39.71±9.18. ACTH concentrations were 23.20±9.70 (12–40 pg/ml). Two patients (14.3%) were diagnosed incidentally, whereas 12 patients had clinical findings. Two patients had diabetes mellitus (14.3%), eight (57.1%) had hypertension. Patients were found to have dorsocervical fat pad (n:9; 64.3%), central obesity (n:9; 64.3%), striae (*n*:6;42.9%), plethora (*n*:7;50%), amenorrhea (*n*:4;28.6%), acantosis (*n*:4;28.6%), hirsutismus (*n*:2;14.3%) and myopathy (*n*:1;7.1%). One had vertebral fracture during follow-up. One of 14 patients was diagnosed as subclinical cushing syndrome and underwent bilateral adrenalectomy. Seven patients underwent unilateral and seven patients underwent bilateral adrenalectomy. Hypocortisolemia developed in six patients after unilateral adrenalectomy and continued for 12.50±9.29 months. Radiotherapy for hypophysis was performed for four patients (*n*:1;before unilateral adrenalectomy, *n*:3;after adrenalectomy). Nelson syndrome developed in two patients against radiotherapy in 9th and 10th years. Eight (57.14%) of 14 patients had macronodular, five (35.71%) had micronodular, and one (7.14%) had primary pigmentated nodular adrenocortical nodules (PPNAD). Compact and clear cells were the most frequent cells in pathologic examination.
Conclusion
We have concluded that patients who underwent adrenalectomy had ACTH-dependent adrenal hyperplasia at first, by long and continuous stimulation of ACTH, bilateral nodular hyperplasia had developed in adrenal glands. Through years, nodules may became autonomous and partially lose ACTH dependence and secreted cortisol continously. Therefore, treatment should be chosen as unilateral or bilateral adrenalectomy.

P583

The endocrine and behavioural actions of neuromedine S

Miklós Jászberényi[1], Zsolt Bagosi[1], Gyula Szabó[1] & Gyula Telegdy[2]
[1]University of Szeged, Department of Pathophysiology, Szeged, Hungary; [2]Hungarian Academy of Sciences, Neurohumoral Research Group Szeged, Szeged, Hungary.

Since earlier publications revealed a prominent and versatile impact of the neuromedin peptide family on several neuroendocrine processes, in the present experiments we focused on the effects of a recently discovered member of neuromedines, neuromedine S on such phenomena as open-field behaviour and hypothalamic-pituitary-adrenal (HPA) activation. The peptide was administered intracerebroventricularly to freely moving rats and 30 minutes later the aforementioned neuronedocrine parameters were investigated. We also investigated the putative effect of neuromedine S on dopamine and GABA release from rat striatal slices in a superfusion system. Our results disclosed that neromedine S has a profound and dose-dependent action on the HPA system, evoking a threefold increase in plasma corticosterone level in a dose of 1 μg. It also activated grooming in a dose of 0.25 μg. The latter action displayed a bell-shaped dose-response curve. However, the neuropeptide does not influenced neither such open field paradigms as square crossing, rearing and defecation nor has an impact on the release of GABA and dopamine. Our results reinforce the hypothesis that, indeed, neuromedines are important regulators of neuroendocrine processes and shed light on the possible functions of the newly described neuromedine S in the central nervous system. It appears, that centrally administered neuromedine S can stimulate such CRF dependent processes as corticosterone release and grooming. However, further experiments are needed to clarify the exact mediation of these processes.

P584

Acute and long-term pituitary insufficiency in traumatic brain injury: a prospective cohort study

Marianne Klose[1], Michael Kosteljanetz[2], Lars Poulsgaard[2], Jannick Brennum[4], Anders Juul[3] & Ulla Feldt-Rasmussen[1]
[1]Dept. of Endocrinology, Rigshospitalet, Copenhagen, Denmark; [2]Dept. of Neurosurgery, Rigshospitalet, Copenhagen, Denmark; [3]Dept. of Growth and Reproduction, Rigshospitalet, Copenhagen, Denmark; [4]Dept. of Neurosurgery, Glostrup County Hospital, Copenhagen, Denmark.

Objective
To estimate the occurrence of hypopituitarism 12 months following traumatic brain injury (TBI), describe the time course, evaluate the predictive value of early hormonal changes and trauma related parameters, as well as out-come.
Methods Forty-six patients with TBI (mild (GCS:13–15) $n=22$; moderate (GCS:9–13) $n=9$; severe (GCS <9) $n=15$) were included. Patients were tested early post-injury (baseline hormone levels + Synacthen-test), and re-tested at 3 and 12 months post-injury (baseline + post-stimulatory hormone levels performing an insulin tolerance test or if contraindicated an arginineGHRH-test).
Results
In the early post-traumatic phase, pituitary hormone alterations were observed in 34/46 (74%) of TBI patients, primarily affecting the gonadal (31/46) and thyroidal (15/46) axes. These changes were most prevalent in severe TBI. At three months, 6/46 patients failed anterior pituitary testing. Twelve months post-injury, one patient had recovered, whereas one developed GH-deficiency in addition to existing ACTH-deficiency. No patients being sufficient at 3 months developed insufficiency during the 9 months follow-up. All insufficient patients had GH-deficiency (5/46 (11%)), followed by ACTH- (3/46), TSH- (1/46), LH/FSH- (1/46) and ADH-deficiency (1/46). The risk of long-term hypopituitarism was positively related to trauma severity ($P=0.04$; 4=severe TBI; 1=moderate TBI), but unrelated to early hormonal alterations when adjusted for trauma severity ($P>0.1$). Insufficient patients had lower self-evaluated health status ($P=0.05$), and a higher increase in BMI ($P=0.01$) and total cholesterol ($P=0.04$) as opposed to sufficient patients.
Conclusion
Head trauma patients had a high frequency of non-specific early hormonal alterations being non-predictive of long-term posttraumatic hypopituitarism. The prevalence of long-term posttraumatic hypopituitarism is clinically relevant in patients with severe TBI, and these patients should be referred to neuroendocrine evaluation in the stable posttraumatic phase. Clinicians should moreover become aware of potential hypoadrenalism in the initial posttraumatic period, as insufficiencies are most certainly present in some patients already from the eliciting trauma.

P585

Genetic analysis of PROP1 gene in patients with childhood-onset combined pituitary hormone deficiency (CPHD)

Zita Halász[1], Judit Toke[2], Attila Patócs[2], Rita Bertalan[2], Zsófia Tömböl[2], Ágnes Sallai[1], Éva Hosszú[1], Ágota Muzsnai[3], László Kovács[4], János Sólyom[1], György Fekete[1] & Károly Rácz[2]
[1]Semmelweis University, 2nd Department of Pediatrics, Budapest, Hungary; [2]Semmelweis University, 2nd Department of Medicine, Budapest, Hungary; [3]Buda Children's Hospital, Department of Endocrinology, Budapest, Hungary; [4]National Medical Center, Department of Medicine, Division of Endocrinology, Budapest, Hungary.

Introduction
Combined pituitary hormone deficiency (CPHD) may be associated with mutations of genes coding for pituitary transcription factors, of which the PROP1 and Pit1, gene mutations have been most extensively studied. However, there are controversial data about the prevalence of these gene mutations in non-acquired childhood-onset CPHD patients.
Objectives
To examine the prevalence and spectrum of PROP1 and Pit1 gene mutations in CPHD patients a multicenter study was performed.
Patients and methods
Patients were selected on the basis of evidence of childhood-onset growth hormone deficiency combined with at least one other pituitary hormone defect. Twenty-nine sporadic and 6 familial cases (2 affected siblings from 3 families) were examined. Genomic DNA was extracted from peripheral blood leukocytes. Mutational analysis of the coding exons of the PROP1 gene was carried out in all patients. In 14 patients in whom disease-causing mutation of the PROP1 gene was absent, mutational analysis of exon 6 of the Pit1 gene was also performed.
Results
Genetic testing indicated disease-causing mutations of the PROP1 gene in 15 patients (homozygous mutations in exon 2: 296-302delGA in 4 patients, 150delA

in 4 patients, C217T in one patient; homozygous mutations in exon3: F117I in one patient; and compound heterozygous mutations: 150delA/296-302delGA in 3 patients, 150delA/F117I in one patient, R99X/296-302delGA in one patient). No novel PROP1 gene mutation was detected. Mutational analysis of exon 6 of the Pit1 gene did not reveal disease-causing mutation.
Conclusion
With our selection criteria for genetic testing, disease-causing PROP1 gene mutations can be detected in a high proportion of childhood-onset, non-acquired CPHD in the Hungarian population.

P586

The role of G-protein- and β-arrestin dependent signaling mechanisms in the tonic regulation of prolactin secretion

Márk Oláh[1], Zsuzsanna Szepesi[2], Ibolya Bodnár[1], György M. Nagy[1] & Zsolt Tidrenczel[1]
[1]Hungarian Academy of Sciences-Semmelweis University Neuroendocrine Research Group, Budapest, Hungary; [2]Hungarian Academy of Sciences-Eötvös Loránd University of Sciences Neurobiology Research Group, Budapest, Hungary.

It is well known that hypophyseotrophic dopamine (DA) exhibits a tonic inhibitory effect on pituitary lactotrops in vivo. We have previously observed that prolactin (PRL) cells obtained from lactating rats become partially resistant to DA following a brief suckling period compared to non-suckled control female rats. This, so-called "desensitization" (and a parallel appearance of "tolerance") to DA is mediated through by a selective change of protein phosphatase 2A (PP2A) in the pituitary lactotrops. Besides the known G_i-protein-cAMP-PKA pathway, stimulation of D_2-receptor (D_2-R) leads to the activation of the p44/42 extracellular-regulated kinase (ERK1/2) in the pituitary gland. Moreover, an additional signal-transduction pathway has recently been described in case of the striatal D_2-R that is a G-protein independent and β-arrestin dependent mechanism. In this signaling β-arrestin is coupled with PP2A that dephosphorilates, therefore inactivates protein kinase B (Akt). We have investigated the changes in phosphorilation of ERK1/2 and Akt following physiological (suckling) and/or pharmacological (inhibitor of DA biosynthesis and/or D_2-R antagonist) manipulations of the hypophyseotrophic DA system using western-blot technique. Suckling stimulus compared to 4 h separation of lactating rats resulted in higher phosphorilation level of ERK1/2 in the AL as well as in male rats treated with DA biosynthesis inhibitor α-methyl-p-thyrosine (αMPT, 250 mg/kg b.w. ip.). Phospho-ERK1/2 content of the NIL was also higher after αMPT treatment in male rats. Suckling had no effect on Akt phosphorilation, but systematic administration of D_2-R blocker, haloperidol (2.5 mg/kg b.w. ip.) as well as αMPT significantly increased the level of phospho-Akt (Thr308) in both the AL and the NIL in male rats. These observations may help to explain the differences in the regulatory mechanism between male and female rats as well as the development of DA "tolerance" and "dependence" on the tonic regulation of lactotrops in lactating animals.

This work was supported by the National Scientific Research Fund (OTKA T-04337) and the Ministry of Health (ETT 177/2006) to GMN.

P587

Secondary hypothalamic amenorrhea as the initial manifestation of HIV infection

Garyfallia Tzifa, George Kalikakis, Ioannis Zaganas, Martha Spiliotis, Diamantis Kofteridis, Vasiliki Daraki, Valentina Petkova, Paraskevi Tzoutzouraki & Stathis Papavasileiou
University Hospital Of Crete, Iraklion, Crete, Greece.

Hypothalamic hormonal deficiency and anterior pituitary hormonal deficiency is a rare occurrence in patients presenting with HIV infection. We describe a patient with HIV infection who presented with secondary amenorrhea as the initial manifestation.
Case report
A 34-year-old woman with previously regular menses presented with secondary amenorrhea by 9 months. The patient had mild gait instability for 7 months; anorexia and weight loss (10 kg) for the last 4 months was also reported. Pregnancy test was negative. Gonadotropins were at the lower normal limits (FSH: 2.69 m U/ml. LH: 2.18. m U/ml) with low oestrogen values (E_2: 37. 4 pg/ml). Pelvic ultrasound confirmed the lack of oestrogen activity (endometrium 4 mm thick). A GnRH stimulation test showed an adequate response, pointing to the hypothalamic cause for the amenorrhea. The patient underwent a brain MRI that revealed an empty sella turcica, with accompanying multifocal leukoencephalopathy of unknown aetiology.
Due to the MRI findings and development of chorea serological and immunological tests were performed. Serological tests were positive for HIV1, HIV2 and CMV virus. The absolute number of CD4 was 39. The patient was diagnosed with CMV encephalopathy due to HIV infection (Stage C3) and was managed with combined antiviral therapy. The patient showed dramatic improvement in her symptoms. The CD4 number increased (225) and the viral load became undetectable. The hypothalamus – pituitary – gonad axis as well as the menstrual cycle was fully restored.
Conclusion
CMV encephalopathy, secondary to HIV infection may present with hypothalamic amenorrhea as the initial manifestation. Systemic and neurological symptoms and signs follow this setting. Combined antiretroviral and anti-CMV therapy can result in dramatic improvement and restoration of menses.

P588

Hypopituitary patients have an increased prevalence of cardiovascular risk factors

Beata Matuszek[1], Mariusz Kowalczyk[1], Tomasz Zapolski[2] & Andrzej Nowakowski[1]
[1]Department of Endocrinology, Medical University, Lublin, Poland; [2]Department of Cardiology, Medical University, Lublin, Poland.

Introduction
Hypopituitary patients receiving conventional hormone replacement, 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 an 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 the metabolic syndrome markers and cardiovascular risk factors.
Material and methods
The study included 18 patients (13-M and 5-F) within the age range from 21 to 59 years ($x=39$) with multihormonal hypopituitarism which lasted from 1 to 24 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 the metabolic syndrome were evaluated: body mass index (BMI), waist, arterial pressure, insulin resistance ratios, HOMA–IR and QUICKI, lipidogram, fibrinogen, homocysteine, adiponectin and echocardiography. The control group consisted of 12 healthy individuals.
Results
Hypopituitary patients had an obesity value ($P=0.0063$), independently of sex and age, with a higher circumference of waist ($P<0.0001$). Mixed hyperlipidemia was found in 88% of the studied patients, a higher low-density lipoprotein cholesterol ($P=0.001$), and triglyceridemia ($P=0.003$). Serum homocysteine was significantly higher ($P=0.02$) and adiponectin concentration was significantly lower in patients than in controls ($P<0.005$). Furthermore, the patients had a significantly increased left atrium size ($P=0.05$), but no difference was observed for other cardiac measure.
Conclusions
In patients with multihormonal hypopituitarism, it is necessary to apply simultaneous replacement treatment with regard to all hormonal deficiencies, including GH. Close monitoring for premature development of the metabolic syndrome risk factors is important, especially in the young.

P589

The evaluation of ghrelin concentration in patients treated for acromegaly and of ghrelin expression in pituitary somatotropinomas

Magdalena Jaskula[1], Ryszard Wasko[1], Wodzimierz Liebert[2], Elzbieta Poreba[3], Robert Plewa[4], Anna Gozdzicka-Jozefiak[3] & Jerzy Sowinski[1]
[1]Department of Endocrinology, Metabolism and Internal Diseases, University of Medical Sciences, Poznan, Poland; [2]Department of Neurosurgery, University of Medical Sciences, Poznan, Poland; [3]Department of Molecular Virology; Adam Mickiewicz University, Poznan, Poland; [4]Department of Experimental Biology, Adam Mickiewicz University, Poznan, Poland.

Ghrelin has been considered one of the factors that might contribute to the development of pituitary somatotropinoma.
The aim of our study was to assess whether serum concentrations of ghrelin differ in patients with acromegaly treated with surgery or/and long-acting octreotide (LAO) and also to evaluate the presence of ghrelin mRNA in tissues of somatotroph adenomas. The appoval of Ethical Committee to perform the study was obtained.
Materials
Serum ghrelin was measured with the use of radioimmunoassay RIA (Phoenix Pharmaceuticals) in 42 acromegalic patients and in 18 healthy control subjects. Acromegalic patients were divided into groups according to the treatment that had been administered: 1) surgery /−/, LAO/+/ 2) surgery /+/, LAO /+/ 3) surgery /−/, LAO /−/ 4) surgery /+/, LAO /−/. Human pituitary somatotroph adenoma tissues were obtained at transsphenoidal surgery from 3 acromegalic patients with macroadenomas and studied for ghrelin mRNA expression. Before surgery patients received long acting octreotide at doses 20 mg, 30 mg, 30 mg at 30 days intervals. The reverse transcription and real-time PCR were performed according to *Korbonits et al.* method.
Results
The difference between mean ghrelin level in the healthy subjects and acromegalic patients was not statistically significant ($P=0.08$), neither between patients who had and who had not undergone surgery ($P=0.1$). Patients treated with somatostatin analogue (Sandostatin LAR) had serum ghrelin levels significantly lower than patients who had undergone surgery and than healthy subjects (p=0.001). Ghrelin mRNA was not detected in any examined tissues.
Conclusions
Ghrelin concentrations were significantly lower in acromegalic patients who had been receiving long acting somatostatin analogue treatment; the absence of ghrelin mRNA might be due to the treatment with somatostatin analogue administered preoperatively, which could have suppressed the ghrelin gene transcription.

P590

Ghrelin, inhibits AMPK (AMP-dependent protein kinase), a regulator of cell proliferation and metabolism

Chrysanthia Leontiou, Blerina Kola, Paolo Dalino, Nabila Salahuddin, Giulia Franchi, Ashley Grossman & Marta Korbonits
Barts and the London Medical School, Queen Mary University of London, London, United Kingdom.

Background Ghrelin stimulates cell proliferation in a number of tissues including pituitary. AMPK, a heterotrimer kinase enzyme, is an important sensor and regulator of cellular energy balance. We have shown that ghrelin can change AMPK activity in various tissues and this mechanism could play a role in its metabolic effects. AMPK has recently been established to strongly inhibit cell proliferation and tumorigenesis. We therefore hypothesised that ghrelin stimulates cell proliferation *via* inhibition of AMPK activity in the pituitary.
Methods
The GH3 cell line was treated with ghrelin 10^{-6}, 10^{-7} and 10^{-9} M and cells were harvested in lysis buffer at 30 min, 60 min, 90 min, 2 h, 3 h, 6 h and 24 h. The effect of ghrelin on AMPK activity was studied with a kinase assay using γ^{32}P-ATP and with immunoblotting using phosphorylation-specific antibodies for alpha-AMPK.
Results
AMPK activity was significantly decreased in the ghrelin-treated cells compared to the media treated controls at 60 and 90 minutes for the 10^{-6} and 10^{-7} M, but also at the 6 h for the 10^{-9} M. The peak effect was at 60 minutes (control 21.0$\pm$ 0.7 pmol ATP/min/mg protein vs ghrelin 10^{-7} M 4.7$\pm$0.4 pmol ATP/min/mg protein; $P<0.01$). Immunoblotting for pAMPK showed a reduction in pAMPK content at 60 min after 10^{-6}M ghrelin treatment (88% of control).
Conclusion
We propose that in pituitary cells the proliferative effects of ghrelin involve the inhibition of AMPK which could lead to upregulation of the Akt and/or mTOR-S6kinase pathways and downregulation of the p53–p21 pathway, leading to increased protein synthesis and cell cycle progression.

P591

Self-concept in patients with PCOS

Ioan Gotca[1], Cristina Cristea[2], Letitia Leustean[2], Draghici Ioana[2] & Eusebie Zbranca[2]
[1]"Socola" Hospital, Iasi, Romania; [2]Unversity of Medecine " Gr.T.Popa", Iasi, Romania.

Polycystic ovary syndrome (PCOS) is a major source of psychological morbidity and can negatively affect quality of life. The aim of the study was to identify characteristics of self-concept in female patients diagnosed with PCOS ($n=22$, mean age 26+/− 11 years) and comparative analysis with a control group ($n=22$).
Methods
Psychology questionnaire, selection tests based on geometrical figures/ words and also graph logical analysis were applied to all patients.
Results
PCOS was accompanied by a significantly depreciation of self-concept (68%). Global and symptomatic depression was more severe in persons without important masculinity. Manifest masculinity was significantly associated with reduction of global and symptomatic anxiety and hostility (70%). Superior adaptability was seen for subjects with a lower masculinity. For patients diagnosed with PCOS the domains of interest and behaviour indicated right brain laterality. Graph logical analysis revealed for all patients a masculine / mixture script trend. Protection tendency evaluated by geometrical figure tests were more important for these patients. In summary: hormonal changes modify self-concept, psychological pattern and behaviour of patients with PCOS.

P592

Evaluation of hypotalamic-pituitary-gonadal (HPG) axis and GH-IGF-I axis in adult patients with celiac disease

Emanuele Ferrante[1], Claudia Giavoli[1], Silvia Bergamaschi[1], Luca Elli[2], Annamaria De Bellis[3], Cristina L Ronchi[1], Andrea Lania[1], Maria Teresa Bardella[2], Antonio Bellastella[3], Paolo Beck-Peccoz[1] & Anna Spada[1]
[1]Department of Medical Sciences, University of Milan, Endocrine and Metabolic Unit, Fondazione IRCCS Ospedale Maggiore Policlinico, Mangiagalli e Regina Elena, Milan, Italy; [2]Department of Medical Sciences, University of Milan, Fondazione IRCCS Ospedale Maggiore Policlinico, Mangiagalli e Regina Elena, Milan, Italy; [3]Departement of Clinical and Experimental Medicine and Surgery, Chair of Endocrinology, Second University of Naples, Naples, Italy.

Celiac disease is a chronic inflammatory autoimmune disorder often associated with other endocrine autoimmune diseases, such as type I diabetes mellitus, Addison's disease and Hashimoto's thyroiditis. In these patients, LH, FSH and GH secretion has been poorly investigated. Aim of this study is to evaluate anterior pituitary function, and in particular hypotalamic-pituitary-gonadal (HPG) axis and GH-IGF-I axis, in adult patients with treated celiac disease. For this purpouse, 22 celiac patients (15 M, 7 F, mean age: 34 years, range: 19–74 years) were studied by GHRH+arginine test. In male patients (mean age: 30 years, range: 19–47 years), GnRH test was also performed. All patients were evaluated for serum IGF-I, testosterone (M), basal thyroid and adrenal function and antithyroid antibodies. In 20 out of 22 patients, antipituitary antibodies (APA) were also evaluated.
No alterations in basal TSH, FT4, FT3, ACTH, cortisol, LH, FSH and testosterone levels were detected. Three patients (2 F, 1M) resulted positive for antithyroid antibodies. A normal response to GnRH test was detected in all cases. Four out of 22 patients (18.8%) showed an impaired GH secretion after GHRH+arginine test; in particular, four male patients (4/15, 26.7%) showed a GH deficiency (GHD)(1 patient with complete GHD and 3 with partial GHD), while in none female patients an impaired GH response was recorded. IGF-I levels where low in the patient with complete GHD. All patients, including these with complete or partial GHD, resulted negative for APA. No correlation between GHD deficiency and onset of disease was found.
In conclusion, adult celiac patients show an impaired GH secretion in a significant proportion of cases, this alteration seeming to be predominant in males and independent from disease onset. Given the absence of APA, the cause of this pituitary dysfunction is still unclear and requires further elucidations.

P593

The effects of salsolinol on the peripheral sympathetic activity of hypophysectomized, adrenalectomized and medullectomized rats

Dániel Székács, Ibolya Bodnár, György M Nagy & Márton IK Fekete
Semmelweis University and Hungarian Academy of Sciences, Neuroendocrine Research Laboratory, Budapest, Hungary.

Salsolinol (1-methyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline), is a recently identified endogenous prolactin (PRL) releasing factor. Salsolinol (SALS) seems to be a selective and potent stimulator of PRL secretion both *in vivo* and *in vitro*.

1-Methyl dihydroisoquinoline (1MeDIQ) is an antagonist of salsolinol induced prolactin release and causes increase in plasma norepinephrine (NE) level. SALS decreased the peripheral tissue dopamine (DA) level dose dependently, consequently increased the NE/DA ratio, indicating reduced release of newly formed NE from sympathetic terminals. These effects can be antagonized by 1MeDIQ pretreatment. The aim of our study was to investigate the effect of medullectomy (MEDX), adrenalectomy (ADX) and hypophysectomy (HYPOX) on the interaction of SALS and 1MeDIQ on the catecholamine concentration of the selected sympathetically innervated peripheral tissues (spleen, atrium, etc). We used HPLC-EC method for measurement of NE and DA concentrations. In ADX as well as in MEDX rats, SALS was able to reduce DA level and increase the NE/DA ratio that could be prevented by 1MeDIQ pretreatment. Therefore the presence of adrenal gland is not required for the reduction of peripheral sympathetic activity induced by SALS. Investigating the possible role of pituitary hormones on the peripheral sympathetic system, the effect of SALS has been tested in HYPOX rats. We have found that the effect of SALS on peripheral sympathetic terminals is not affected by HYPOX, conseqently pituitary hormones do not play any role in the catecholamine depleting activity of SALS. The possible physiological significance of these observations need further clarifications.

This work was supported by the National Scientific Research Fund (OTKA T-04337) and the Ministry of Health (ETT 177/2006) to GMN.

P594

Cardiovascular risk and hypopituitarism: evaluation of the global cardiovascular absolute risk, using the individual score of the Progetto CUORE of the Istituto Superiore della Sanità

Carolina Di Somma, Maria Cristina Savanelli, Francesca Rota, Gaetano Lombardi & Annamaria Colao
Department of Molecular and Clinical Endocrinology and Oncology Federico II University, Naples, Italy.

Adults with hypopituitarism are known to have reduced life expectancy with a 2-fold higher risk of death for cardiovascular disease compared with controls. In Italy, to identify individuals at high risk for cardiovascular disease, the function of the Progetto CUORE has been identified and the global cardiovascular risk score has been built using data from different Italian cohorts. To assess the global cardiovascular risk score in adult hypopituitaric patients: 108 hypopituitaric GHD patients (m:45, f: 47; 35–69 yrs), 62 hypopituitaric non GHD patients (m:21, f: 41; 35–69 yrs) and 108 matched controls were studied. At study entry, all subjects were tested with GHRH + ARG and serum IGF-1, total cholesterol, HDL-cholesterol; systolic blood pressure (SBP), smoking habit, diabetes and hypertension treatment were assessed in all subjects. The score was calculated using a test on the website www.cuore.iss.it. At baseline, the global cardiovascular risk score, total cholesterol and SBP were higher ($P<0.001$), while HDL cholesterol ($P<0.0001$) GH peak and IGF-I levels were lower in patients than in controls ($P<0.001$). In particular, the global cardiovascular risk score and total-cholesterol ($P<0.05$) were higher, while GH peak and IGF-I levels ($P<0.001$) were lower in GHD patients than in non GHD patients. No significant difference was found in age, SBP, HDL-cholesterol between two patient groups. An inverse correlation was found between the risk score and GH peak and serum IGF-1 both in patients and in controls. In conclusion, a significant impairment of the global cardiovascular risk score was found in hypopituitaric patients who were replaced for the other pituitary hormones except for GH, indicating a high risk for the development of major coronary or cerebrovascular events in the next ten years. However, whether GH replacement can reduce this risk remains to be established.

P595

Traumatic brain injury (TBI) and lipid profile abnormalities: study 12 months after the brain injury

Carolina Di Somma[1], Francesca Rota[1], Silvia Rovere[2], Maria Rosaria Ambrosio[3], Alessandra Fusco[4], Ginevra Corneli[2], Antonio Bianchi[4], Laura De Marinis[4], Ettore Ciro degli Uberti[3], Gaetano Lombardi[1], Ezio Ghigo[2], Annamaria Colao[1] & Gianluca Aimaretti[4]
[1]Department of Molecular and Clinical Endocrinology and Oncology University of Naples Federico II, Naples, Italy; [2]Division of Endocrinology and Metabolism, Department of Internal Medicine, University of Turin, Turin, Italy; [3]Department of Biomedical Sciences and Advanced Therapies Section of Endocrinology, University of Ferrara, Ferrara, Italy;\ [4]Division of Endocrinology Catholic University Rome, Rome, Jamaica.

Aim of this study was to evaluate lipid profile and the severity of GHD, in a large group of TBI patients with or without GH deficiency. We assayed lipid profile (Total-, HDL- Cholesterol, Triglycerides) in 62 TBI subjects 12 months after TBI (41 M, 21 F, 13–81 yrs, BMI: 24.6 ± 0.6 kg/m^2), and in 62 sex-, age- and BMI-matched controls. Based on the GH peak after GHRH + ARG test, patients were stratified as: 1) severe GHD (GH peak $\leq$ 9 μg/l; $n=13$; 20.9%); 2) partial GHD (GH peak in between 9.1–16.5 μg/l; $n=6$; 9.7%); 3) non-GHD (GH peak >16.5 μg/l; $n=43$; 69.3%). IGF-I levels were lower ($P<0.001$) in patients with severe GHD (88.7 ± 11.1 μg/L) than in those with partial GHD, non-GHD and in controls (148.1 ± 33.9, 219.2 ± 10.7, and 251.8 ± 10.8 μg/L, respectively). HDL-cholesterol were lower ($P<0.01$) in patients with severe GHD (44.1 ± 2.7 mg/dL) than in those non-GHD and in controls (54.4 ± 1.3 and 59.3 ± 1.1, respectively), while, no significant differences was found in partial GHD. In patients with severe GHD, total- and HDL-cholesterol ratio (4.9 ± 0.4, $P<0.01$) were higher than in those with partial GHD (4.4 ± 0.2,), non-GHD (3.9 ± 0.2), and controls (2.9 ± 0.1). In addition, partial GHD patients had total- and HDL-cholesterol ratio (4.4 ± 0.2, $P<0.01$) higher than those non-GHD (3.9 ± 0.2), and controls (2.9 ± 0.1). Triglycerides levels were not different among severe GHD, partial GHD and non GHD TBI patients and controls. In all subjects, a significant correlation was found between the GH peak and age ($r=-0.41$; $P<0.01$), BMI ($r=-0.33$; $P<0.05$), IGF-I ($r=0.36$; $P<0.01$), total cholesterol ($r=-0.37$; $P<0.05$), HDL cholesterol ($r=0.36$; $P<0.05$), total- and HDL- cholesterol ratio ($r=-0.47$; $P<0.01$). IGF-I was correlated with age ($r=-0.54$, $P<0.001$), total cholesterol ($r=-0.46$; $P<0.01$), HDL cholesterol ($r=0.39$; $P<0.05$), total- and HDL- cholesterol ratio ($r=-0.51$; $P<0.01$). In conclusion, impairment of lipid profile was evident in TBI patients with severe GHD.

P596

Abstract unavailable

P597

Does concealment of bad news stimulate the HPA or the SAS axis?

Gesthimani Mintziori[1], Efharis Panagopoulou[1], Dorothea Kapoukranidou[2] & Alexios Benos[1]
[1]Department of Hygiene, Medical School, Aristotle University of Thessaloniki, Thessaloniki, Greece; [2]Department of Physiology, Medical School, Aristotle University of Thessaloniki, Thessaloniki, Greece.

According to recent research in the field of Psychoneuroendocrinology each stressor appears to have its own neurochemical signature. The present study examined whether keeping a secret stimulates the HPA or the SAM axis as well as cortisol involvement in lying.

Methods

Sixty seven (N = 67) healthy young male medical students participated in the study. Students were randomly assigned in 3 groups. All students were informed that they were about to have a 5 min consultation with a 26 year-old woman with non-operable brain tumour. They were also given information about prognosis, treatment and side effects. Group A (disclosure group) was instructed to reveal the information about the diagnosis, prognosis, and treatment. Group B (concealment group) was instructed not to reveal the truth concerning the diagnosis, and prognosis, while students in group C (control group) were instructed to conduct a structured interview concerning dietary habits. Mood, cardiovascular reactivity and salivary cortisol was assessed at baseline (T1), 30 minutes later (T2), and immediately after the task (T3). In addition heart rate was assessed during the consultation using a digital signal extraction pulse oximeter.

Results

Compared to the control group, there was a significant increase in anxiety and negative affect in both experimental groups from T1 to T2 that significantly decreased from T2 to T3 to baseline levels only in the concealment group. In the concealment group there was also a significant decrease of heart rate throughout the consultation ($F=5.304$, $P=0.011$). The salivary cortisol significantly changed in all three groups throughout the process ($F=5.557$, $P=0.007$).

Conclusions

Results show that performance anxiety is involved in cortisol secretion. However concealment/ secrecy only results in SAM activation. Further research is needed to ascertain the endocrine proceedings taking place and eventually design a strategic plan on training for handling bad news in medical settings.

P598

Non-dopaminergic neurons expressing individual enzymes of dopamine synthesis in the arcuate nucleus: development and functional significance

Michael Ugrumov
Institute of Developmental Biology RAS, Moscow, Russia.

Although non-dopaminergic neurons expressing individual enzymes of dopamine (DA) synthesis are widely distributed in the brain, their functional significance remains uncertain. This study was aimed to evaluate the development and functional significance of the neurons expressing one of the enzymes of DA synthesis, tyrosine hydroxylase (TH) or aromatic L-amino acid decarboxylase (AADC), in the arcuate nucleus of rats *in vivo* and *in vitro* by using immunocytochemistry, *in situ* hybridization, image analysis, confocal microscopy, high performance liquid chromatography with electrochemical detection and the radioimmunoassay. According to our data:

- The number of so-called monoenzymatic TH-expressing or AADC-expressing neurons highly exceeded that of DA-ergic neurons expressing both enzymes in fetuses and neonates, whereas there was a reverse in adult animals;
- Monoenzymatic TH-neurons and AADC-neurons synthesize DA in cooperation: synthesis of L-DOPA from L-tyrosine in TH-neurons is followed by its release and uptake by the neighbouring AADC-neurons, where L-DOPA is further converted to DA;
- The 6-hydroxydopamine(neurotoxin)-induced degeneration of DA-ergic neurons in the arcuate nucleus and the development of hyperprolactinemia were accompanied by the increase of the number of monoenzymatic neurons and cooperative synthesis of DA that is considered as a compensatory reaction.

Thus, non-dopaminergic neurons expressing individual complementary enzymes of the DA synthetic pathway produce this neurotransmitter in cooperation that is a compensatory reaction to the failure of DA-ergic neurons.

P599

Impact of somatostatin analogs on the heart in acromegaly: a meta-analysis

Patrick Maison[1], Anne- Isabelle Tropeano[1], Isabelle Macquin-Mavier[1], Andrea Giustina[2] & Philippe Chanson[3]
[1]Assistance Publique-Hôpitaux de Paris, Hôpital Henri Mondor, Créteil, France; [2]University of Brescia, Brescia, Italy; [3]Assistance Publique-Hôpitaux de Paris, Hôpital de Bicêtre, Le Kremlin-Bicêtre, France.

Context
Acromegaly can be complicated by cardiomyopathy. Treatment with somatostatin analogs has been shown to improve some cardiac parameters, but most published clinical trials involved few patients and were not randomized or controlled. In addition, their results are rather variable.
Objective
To conduct a meta-analysis aimed at obtaining a more accurate picture of the effect of somatostatin analogs on the heart in patients with acromegaly.
Design
We systematically reviewed all studies of somatostatin analogs in acromegaly. Eighteen studies were identified in three databases. We conducted a combined analysis of the effects of somatostatin analogs by using the overall effect size to evaluate significance and by computing the weighted mean differences with and without treatment to assess the effect size.
Results
Somatostatin analog treatment was associated with significant reductions in the heart rate (−5.8 [2.1] beats/min), the left ventricular mass index (−22.3 [6.7] g/m^2), inter-ventricular septum thickness (−0.3 [0.2] mm), left ventricular posterior wall thickness (−0.8 [0.4] mm) and the ratio of the E-wave and A-wave peak velocities of the mitral flow profile (0.2 [0.1]). It was also associated with improved exercise tolerance (+1.6 [0.4] min]. Trends towards beneficial effects were noted for the left ventricular end-diastolic dimension (−1.5 [2.2] mm) and the left ventricular ejection fraction (3.3 [1.7] %). Overall effect sizes were not significant for blood pressure, left ventricular end-systolic dimension or fractional shortening. Bigger improvements were observed in studies with larger falls in IGF-I and/or GH levels, and in studies of younger patients.
Conclusion
This meta-analysis confirms that somatostatin analog therapy aimed at achieving stringent control of serum GH/IGF-I concentrations in patients with acromegaly is associated with significant positive effects on morphological and functional hemodynamic parameters.

P600

Thyrotropinoma response to somatostatin receptor ligand (SRL) – key feature in preoperative treatment

Wojciech Zgliczynski[1], Piotr Zdunowski[1], Malgorzata Gietka-Czernel[1] & Grzegorz Zielinski[2]
[1]Medical Center Postgraduate Education, Warsaw, Poland, [2]Army's Medical Institute, Warsaw, Poland.

Background
TSH-secreting tumors appears as extremely rare cause of hyperthyroidism. Major clinical feature is preserved TSH level in subjects with apparent thyrotoxicosis. Misdiagnosis of primary thyroid hyper function led to mistreatment with anti-thyroid agents. This worsens disease course and outcome. Neurosurgery success rate is limited by large tumor size and extrasellular expansion. Somatostatin plays key role in regulation of TSH secretion. Tumors in most cases expresses receptors for somatostatin therefore SRL are potent option in TSH-oma treatment.
Aim
Of the study was to determine SRL efficacy in patients before neurosurgical treatment of TSH-oma. Secondary aim was to verify long-period outcome of SRL in cases of neurosurgery failure.
Material
Comprise of 9 patients with secondary thyrotoxicosis, 6 women and 3 men, aged 35 to 69 yrs (mean 49) presenting with pituitary macroadenoma (18 to 45 mm). Before diagnosis was established, 5 out of 9 received antithyroid medication, and in 1 case strumectomia was performed.
Intervention
Somatostatin analogue octreotide long-acting repeatable (LAR) administration 3 months prior to the surgery.
Results
Initially, all patients had abnormal fT4 and alfa-SU levels (mean 38.8 pmol/l SD 11.6 and 6.1 ng/ml SD 6.4, respectively) as well as lack of TSH increase after TRH stimuli (mean rise 15% from basal value, SD 52). 3 months of SRL treatment led to marked TSH and alfa-SU levels decrease (to 1.2 mU/l SD 1.1 and 0.8 ng/ml SD 0.6, resp.), normalization of thyroid hormones (fT4 mean 15.7 pmol/l SD 5.0) and clinical improvement. Patients in euthyroid state were referred to neurosurgery unit. Tanssphenoideal adenomectomy was successful in 8 out of 9, and in this group TRH stimuli performed 3 months after surgery provokes significant TSH response (mean rise 210% SD 310). In one case after unsuccessful surgery euthyroid state is achieved during SRL treatment for 2 years, without noticeable adverse events.
Conclusions
Somatostatin analogue treatment is efficient in TSH-secreting tumors in inhibition of TSH secretion, thyroid hormone normalization, visual field improvement, thyroid volume decrease and neurosurgery success rate. Post-surgery TSH increase during TRH test indicates restored pituitary-thyroid axis. In cases of surgery failure prolonged SRL may be efficient option.

P601

Glucose resistance in acromegaly is reversible during somatostatin analogues treatment

Piotr Zdunowski & Wojciech Zgliczynski
Medical Center of Postgraduate Education, Warsaw, Poland.

Background
Insulin resistance leading to glucose intolerance and even diabetes mellitus is common in acromegaly and is partially caused by pathological high concentrations of growth hormone (GH) and somatomedin C (IGF-1). On the other hand, somatostatin analogues, common treatment option, can cause inhibition of insulin secretion and glucose tolerance disturbances.
Aim
Of the study was to determine impact of prolonged somatostatin analogues administration on insulin resistance in acromegaly.
Material
27 acromegalics 16 women and 11 men, aged 23 to 65, mean 43, previously untreated and with excluded diabetes mellitus was enrolled into this study.
Intervention
Primary octreotide LAR treatment for 6 months prior to neurosurgery.
Methods
Prolonged (0–180') oral glucose tolerance test (OGTT) with glucose, GH and insulin assessment was performed initially, 2 weeks after first octreotide injection and after 6 months of treatment. Insulin resistance was calculated as fasting glucose to fasting insulin ratio (FG/FI), sum of insulin levels during OGTT (sI).

Also, HOMA and Quicki indexes was calculated. Control group consists of healthy volunteers from department database. Disease activity was calculated with clinical symptoms score, GH and IGF-1 levels.
Results
Initially, 21 out of 27 (77%) patients was insulin-resistant (FG/FI <6), HOMA index was significantly higher than in controls (3.2 S.D. 1.4 v. 1.6 S.D. 0.8 $P<0.001$). After 6 months of treatment insulin-resistance presented 16 (59%), insulin levels drop significantly in fasting state and during OGTT (sI 659 S.D. 160 v. 430 S.D. 180 $P<0.05$ initially v. 6 months therapy) whereas glucose levels did not differ significantly ($P<0.01$). HOMA index fall close to controls (2.1 S.D. 0.7), and Quicki was slightly higher than initially (mean 0.329 v. 0.369 respectively), but difference did not reach statistical significance ($P=0.12$).
Conclusions
Somatostatin analogue therapy could improve insulin-sensitivity and did not worsen glucose metabolism in patients with acromegaly.

P602

Endocrine and neuro-ophtalmologic correlates of primary empty sella

Antonio Bianchi[1], Bruno Gregori[2], Eugenia Sacco[1], Antonella Giampietro[3], Domenico Milardi[3], Alessandra Fusco[1], Neri Accornero[2], Alfredo Pontecorvi[1] & Laura De Marinis[1]
[1]Department of Endocrinology, Catholic University School of Medicine, Rome, Italy; [2]Department of Neurological Sciences, University of Rome 'La Sapienza', Rome, Italy; [3]International Institute 'Paolo VI', Catholic University School of Medicine, Rome, Italy.

Background
Primary empty sella (ES) can be asymptomatic or associated with endocrine and neurological alterations, such as visual defects. Studies in a large number of patients is still lacking.
Objective
To study visual deficit in ES and its relationship with hormonal status.
Material and Methods
We recorded visual evoked potentials (VEP) by white/black, red/black, blue/black patterns. Isoluminance between red and blue checks allowed to compare potentials. We measured P1 latencies and computed a chromatic (blue-red/blue+red) contrast index (CC). Chromatic visual field perimetry was performed with a dedicated computerised system which provides quantitative chromatic maps for each eyes.
We included 64 eyes of 32 normal volunteers (age: 44 ± 14.8) and 10 eyes of 10 ES patients with no systemic disease and increased intracranial pressure (age 50 ± 16.1). On basis of clinical and laboratory data, patients were divided in two groups: with (group A) and without (group B) endocrine abnormalities.
Results
VEP and Visual field perimetry studies showed a significant alterations of both P1 latencies and visual field indices in ES patients as compared to controls. In group A visual alterations appeared more pronounced as compared to group B.
Discussion
Chromatic studies can selectively analyse parallel visual pathways which differ in their physiology and susceptibility to visual pathologies. Data suggest a different disorder of visual systems in ES patient with and without endocrine abnormalities. Studies in a large number of ES patients can provide insights in the pathophysiology of syndrome and more accurate indications for treatment.

P603

Growth hormone deficiency in patients with acromegaly after 'successful' transsphenoidal surgery

Milan Petakov[1], Tatjana Isailovic[1], Marina Djurovic[1], Djuro Macut[1], Miloje Joksimovic[2], Bojana Popovic[1], Ivana Bozic[1] & Svetozar Damjanovic[1]
[1]Institute of Endocrinology, Clinical Center of Serbia, Belgrade, Serbia; [2]Clinic for Neurosurgery, Belgrade, Serbia.

The diagnosis of adult growth hormone deficiency (GHD) in patients with pituitary disease relies predominantly on provocative tests of GH secretion. The incidence of GHD in treated acromegalic patients has not been fully documented. Therefore, the aim of the present study was to elucidate GH response to insulin-induced hypoglycaemia (ITT, 0.15 IU/kg i.v.) in a cohort of 10 patients with acromegly considered cured solely by transsphenoidal surgery (6 females and 4 males, mean age 51 ± 2.6 years), and 6 healthy age-matched controls (3 females and 3 males). All patients cured for acromegaly (biochemical criteria for remission-'cure" were the normalization of IGF-I level and GH suppression to less then 1 μg/l during the OGTT) had normal residual pituitary function i.e. had no signs of pituitary ACTH and TSH deficiency. The mean ($\pm$S.E.M) peak GH response to ITT in cured acromegalics was significantly lower in comparison with healthy subjects (8.19 ± 2.05 vs. 17.45 ± 3.1 μg/l; $P<0.05$). In five 'cured' acromegalic patients (50%) we confirmed the presence of severe growth hormone deficiency (peak GH during ITT less then 3 μg/l). In conclusion, it has been increasingly recognized that some patients previously concerned cured after surgery for acromegaly, in fact have the GH deficiency. It is necessary to check GH secretory capacity in every cured patient previously operated for acromegaly even if no other pituitary hormone deficit exist. Possibly, some of so-called cured patients with acromegaly should be treated with GH substitution, concerning the possible premature morbidity and mortality due to GH deficiency.

P604

Excess mortality in women with pituitary disease: results of a meta-analysis

Eigil Husted Nielsen, Jörgen Lindholm & Peter Laurberg
Dept. of Endocrinology, Aalborg Hospital, Aarhus University Hospital, Aalborg, Denmark.

Background
Several studies of rather heterogeneous groups of patients have shown an increased mortality in patients with pituitary diseases. In patients without hypersecretion of growth hormone or ACTH the increased mortality has mostly been attributed to pituitary insufficiency. Some studies have suggested sex-specific differences in standard mortality rates (SMR) whereas others have shown increased cardiovascular and/or cerebrovascular mortality. A recent study of patients who had undergone surgery for non-functioning pituitary adenoma showed a normal SMR in men, whereas SMR was significantly increased in women. We explored this sex related difference by a meta-analysis.
Material and methods
We performed an internet-based meta-analysis using major medical science databases of MedLine, Embase and Web of Science to identify publications on mortality in patients with pituitary disease. Both Thesaurus-term and free-text searches were applied. Articles were required to provide exact information on standard mortality rates in both men and women separately, 95% confidence interval (CI) and a well-defined normal reference population. Studies including patients with Cushing's disease or acromegaly were excluded as were studies with a majority of patients carrying a diagnosis of craniopharyngioma. Sex-specific overall SMR values for men and women in the meta-analysis were calculated as weighted averages of SMR from individual studies, using the inverse variance method. An additional analysis of association between first year of inclusion of new patients and SMR values in each study was also performed.
Results
Six studies fulfilled our criteria for inclusion in the meta-analysis. The weighted overall SMR for men was calculated to 2.06 (CI: 1.94–2.20), whereas weighted SMR for women was 2.80 (2.59–3.02). Mortality rates were thus significantly higher than in the reference population in both men and women, and SMR in women was significantly higher than in men. Analysis of association between first year of inclusion of new patients and SMR showed a statistically significant negative correlation in men reaching a normal value in the most recent study. In women SMR was always higher and did not normalize in recent studies.
Conclusion
Our meta-analysis showed that SMR is increased in both men and women with pituitary disease, with a significantly higher SMR in women than in men. SMR seems to be reaching normal levels in male patients treated in recent decades, whereas SMR is still clearly elevated in women. The reason for this is unknown, but most likely the high mortality in women reflects suboptimal diagnosis and/or suboptimal therapy of pituitary insufficiency.

P605

Familial acromegaly – the role of the AIP gene

M Gueorguiev[1], F Lolli[1], JP Chapple[1], R Quinton[2], A Ribeiro-de-Oliveira[3], MR Gadelha[4], V Popovic[5], JP Monson[1], JAH Wass[6], LA Frohman[7], AB Grossman[1] & M Korbonits[1]
[1]Barts and the London Medical School, London, United Kingdom; [2]Royal Victoria Infirmary and University of Newcastle-upon-Tyne, Newcastle-upon-Tyne, United Kingdom; [3]Federal University of Minas Gerais, Belo Horizonte, MG, Brazil; [4]Clementino Fraga Filho University Hospital, Federal University of Rio de Janeiro, Brazil; [5]University Clinical Centre, Belgrade, Serbia; [6]Churchill Hospital, Oxford, United Kingdom; [7]University of Illinois, Chicago, United States.

Pituitary adenomas are present in ~25% of autopsy samples, and recent studies have also suggested that clinically important pituitary adenomas are some 5 times more common than previously recognised. Acromegaly is almost always due to a sporadic growth-hormone secreting pituitary adenoma, but familial acromegaly has been reported occasionally. Linkage and loss of heterozygosity studies have shown that it is caused by a tumour suppressor gene located at 11q13; very recently 3 families have been reported with a very low penetrance mutation in the gene coding for the aryl hydrocarbon receptor (AhR) interactive protein (AIP), a molecular chaperone, which has been linked to the induction of hepatic detoxifying gene products in response to environmental toxins such as dioxin. However, an additional function appears to be regulation of the cell cycle, suppressing cyclin E and increasing expression of p27, which we have previously shown to be involved in pituitary tumorigenesis.

We studied 19 families with familial pituitary adenoma and identified mutations in the AIP gene in a 4/19, which were either stop codons or mutations disrupting the protein-binding segments of the protein. The penetrance of the disease at the time of the study was 64%, suggesting a much higher level of penetrance than previously reported; in some families there was 100% penetrance. A selected group of young-onset sporadic acromegalic patients, including 3 with gigantism, showed no germline mutations. We found AIP protein expression in normal pituitary and in sporadic pituitary adenomas, while mRNA expression of AIP and its putative partner AhR showed up-regulation, suggesting a compensatory mechanism. Somatic mutations of somatotroph tumours were not seen.

In summary, AIP mutation has been identified in one in four of familial acromegaly kindreds and shows a relatively high penetrance; while mutations of this gene are not involved in the pathogenesis of sporadic somatotroph adenomas, more subtle defects are currently under investigation.

P606

Validation of different insulin sensitivity indices in GH deficiency children using roc curve analysis

R Baldelli[1], S Bellone[2], G Corneli[2], A Giampietro[3], V Cimino[3], A Pontecorvi[3], L De Marinis[3] & G Bona[2]
[1]Regina Elena Cancer Institute, Rome, Italy; [2]University of Piemonte Orientale, Rome, Italy; [3]Catholic University of Sacred Hearth, Rome, Italy.

Insulin sensitivity in GHD patients tends to decrease with age and variations in body composition. Several indices of insulin sensitivity have been considered and among these HOMA, ISI and QUICKI are based on mathematical calculations taking into account glucose and/or insulin levels either in basal conditions or after OGTT. Aim of present study was to validate the different indices in a population of pre-pubertal GHD children ($n=66$) by ROC curve analysis. All patients underwent OGTT with evaluation of glucose and insulin. To validate the different indices the ROC curve analysis has been used with the aim to provide the cut-off limit, sensitivity and specificity for each index. The lowest limit of normality was defined as the value that provided the best pair of highest sensitivity/specificity for HOMA, ISI and QUICKI. Evaluating data derived from ROC curve analysis we have found that ISI index was the most robust index of insulin sensitivity. Using a cut-off of 0.6, HOMA shows a sensitivity of 29% and a specificity of 83.7%; using a cut-off of 0.4, QUICKI shows a sensitivity of 32.3% and a specificity of 88.4%; using a cut-off of 9.2, ISI shows a sensitivity of 43.5% and a specificity of 100%. Applying the cut-off point for ISI, among the patients we found that 42% of GHD children were insulin resistant. This kind of diagnosis was difficult before, because the specific cut-off limits of ISI had not been calculated. Data from the current study demonstrate that ISI was more potent respect to HOMA and QUICKI and represent a convenient test for the diagnosis of insulin resistance.

P607

The transition phase in GHD patients and metabolic alterations: life span variations of insulin sensitivity?

R Baldelli[1], S Bellone[3], G Gorneli[2], J Bellone[3], A Petri[3], S Vannelli[4], C De Sanctis[4], L Benso[4], G Bona[3] & G Aimaretti[2]
[1]Regina Elena Cancer Institute, Rome, Italy; [2]University of Turin, Turin, Italy; [3]Univeristy of Piemonte Orientale, Rome, Italy; [4]Regina Margherita Pediatric Hospital, Rome, Italy.

GH has well documented insulin antagonistic effects. By inference, GHD may be expected to result in increased insulin sensitivity. Young GHD children have a tendency to both fasting and readly provoked hypoglycaemia probably resulting from impaired hormonale counter-regolation. Increased insulin sensitivity could also contribute to their hypoglycaemia; however, this has not been directly demonstrated. Interestingly, susceptibility to hypoglycaemia in GHD children diminishes with increasing age and, paradoxically, GH deficient adults demonstrate insulin resistance even prior to GH replacement therapy. The mechanism underlying this apparent age-related deterioration in insulin sensitivity in GHD subjects is unknown (changes in body composition or metabolic responses to GH, or interaction with pubertal increases in sex steroids e.g.). The transition period is defined as the period between end of linear growth and attainment of full adult somatic development. It can be defined as late teenage years, 'Post-adolescence', 'Young adulthood' with a duration of ~ 3–10 years and the ESPE consensus of december 2003 underline the transition period defined as ending around 25 years. In order to examinate the life span insulin sensitivity index a group of GHD patients have been selected ($n=81$); in particular **group A** ($n=10$) (<25 yrs), **group B** ($n=4$) (26–30 yrs), **group C** ($n=11$) (31–40 yrs), **group D** ($n=14$) (41–50 yrs), **group E** ($n=30$) (51–60 yrs) and **group F** ($n=12$) (>60 yrs). The insulin sensitivity was evaluated using HOMA index (basal insulin levels x blood glucose/22) reflecting, in particular, the 'value' of insulin resistance. Our preliminary results indicated that insulin sensitivity decresed significantly in the group of patients after the transition phase (group B) respect to the other period of life ($P<0.05$, vs A, C, E). We are not aware of any other works evaluating insulin sensitivity in a large group of GHD patients. In our patients reduced insulin sensitivity in the period after transition age could support the hypothesis to treat this patients also in this period of life due to possible high incidence of insulin resistance after the transition period. There is some debate as to whether a reduced insulin sensitivity is only a transiet phenomenon or a persistent one. This data is reflecting somatic immaturity of patients who suffer for two components: developmental existing since childhood (in childhood onset GHD) and metabolic acquired in the transition period so this data support the hypothesis that patients must be treated also in transition phase due to the possible incidence of metabolic alteration in the following period of life.

P608

Growth hormone deficiency in succesfully treated acromegalic patients is not protective from cardiac complications

R Baldelli[1], C Di Somma[3], M Ambrosio[4], A Bianchi[5], R Pivonello[3], V Gasco[2], P Maffei[6], M Appetecchia[1], N Sicolo[6], E degli Uberti[4], L De Marinis[5], S Grottoli[2], A Colao[3] & E Ghigo[2]
[1]Regina Elena Cancer Institute, Rome, Italy; [2]University of Turin, Turin, Italy; [3]Federico II Univeristy of Naples, Naples, Italy; [4]University of Ferrara, Ferrara, Italy; [5]Catholic University of Rome, Rome, Italy; [6]University of Padua, Padua, Italy.

GH hypersection results in biventricular concentric hypertrophy and a progressive contractile impairment whereas cardiac hypotrophy and impaired diastolic filling and left ventricular function have been reported in GH deficiency (GHD). No information on cardiac performances and structure are available about those acromegalic patients in whom successful treatment made their GH and IGF-I secretion similar to those in GHD patients. In order to study the functional and structural cardiac consequences of optimal treatment for acromegaly, we enrolled 12 active acromegalic patients (group A), 14 post-surgical cured acromegalic patients with selective secondary GHD (group B), 11 cured acromegalic paties under treatment with SS analogs (group C), 21 GHD (group D) and 18 controls (group E). GHD diagnosis was based on GHRH+arginine test. In all the subjects LVMi, EF and E/A was studied by M-B mode echo-Doppler. IGF-I levels were higher in group A respect to groups B, C, D ($P<0.0005$, $P<0.005$, $P<0.0005$, respectively) whereas it was lower in group B than group C ($P<0.005$) but similar to group D. LVMi in group A was higher than in group E ($P<0.0005$) in which it was similar to group D. LVMi in group B were similar than in group A, whereas in group C it was lower than in groups A and in B ($P<0.0005$, $P<0.05$, respectively), still persisting higher than in group D and in group E ($P<0.05$, $P<0.0005$, respectively). EF in group A was similar to group E in whom it was higher

than in group D ($P<0.05$). EF in group B was similar as in group A, while in group C it resulted higher than in group D and E ($P< 0.0005$, $P<0.005$) but still similar to group A. E/A in group A was lower than in group E ($P<0.005$) in which it was higher than in group D ($P< 0.0005$). In group B, E/A was lower than in group A ($P<0.05$) but similar to in group D. In group C, E/A were similar to in group A, but still lower than in group E ($P<0.05$) and similar to group D. In conclusion these data suggest that GH deficiency induced by successful treatment of acromegaly does not per se counteract cardiac abnormalities induced by acromegalic cardiomyopthy. Despite similar GH and IGF-I levels, treatment with SS analogues appears more effective in reducing cardiac mass and to improve diastolic function suggesting a potential GH-independent direct role of SS at the cardiac level.

Reproduction – presented on Tuesday

P609

Implications for molecular mechanisms of glycoprotein hormone receptors using a new sequence-structure-function analysis resource

Gunnar Kleinau[1], Mara Brehm[2], Urs Wiedemann[1], Dirk Labudde[1], Ulf Leser[3] & Gerd Krause[1]
[1]Leibniz-Institut für Molekulare Pharmakologie, Berlin, Germany; [2]FH-Gießen, Gießen, Germany; [3]Institute for Computer Science, Humboldt-Univerität, Berlin, Germany.

Comparison between wild type and mutated glycoprotein hormone receptors (GPHRs) TSHR, FSHR and LHCGR is established to identify determinants involved in molecular activation mechanism. The basic aims of current work are the discrimination of receptor phenotypes according the differences between activity states they represent and hit-assignment of classified phenotypes to 3D-structural positions to reveal functional-structural hotspots and interrelations between determinants that are responsible for corresponding activity states. Since it is hard to survey the vast amount of pathogenic and site-directed mutations at GPHRs and to improve an almost isolated consideration of individual point mutations, we present a system for systematic and diversified sequence-structure-function analysis (SSFA) (http://www.fmp-berlin.de/ssfa). In order to combine all mutagenesis data into one set, we converted the functional data into unified scaled values. This at least enables their comparison in a rough classification manner. In this study we describe the compiled data set and a wide spectrum of functions for user driven searches and classification of receptor functionalities such as cell surface expression, maximum of hormone binding capability, and basal as well as hormone induced Gαs/Gαq mediated cAMP/IP accumulation. Complementary to known databases our data set and bioinformatics tools allow to link functional-, biochemical- specificities with spatial features to reveal concealed structure-function relationships by a semi-quantitative analysis. A comprehensive discrimination of specificities of *pathogenic mutations* and *in vitro* mutant phenotypes and their relation to signalling mechanisms of GPHRs demonstrates the utility of SSFA. Moreover, new interrelations of determinants important for selective G-protein mediated activation of GPHRs are resumed.

P610

A comparison between the efficacy and safety of pegvisomant to that of octreotide LAR in patients with acromegaly

Philip Harris[1], Gwyn D'Souza[1], Anthony Good[1], Gary Layton[1], Annamaria Colao[2], Biller Beverly[3], Anne Klibanski[3] & Ezio Ghigo[4]
[1]Clinical research and Development, Pfizer Ltd, Sandwich, United Kingdom; [2]Department of Endocrinology, Federico II University of Naples, Naples, Italy; [3]Neuroendocrine Unit, Massachusetts General Hospital, Boston, United States; [4]Division of Endocrinology and Metabolism, University of Turin, Turin, Italy.

Two medical therapies are now available for the treatment of acromegaly. Pegvisomant is a growth hormone (GH) receptor antagonist. Somatostatin analogues, in contrast, act by inhibiting the release of GH from the pituitary. The primary objective of this study was to compare the efficacy of pegvisomant (P) to that of octreotide LAR (LAR) in terms of IGF-1 normalisation. The secondary objective was to compare safety and tolerability between the two treatments.

The study was a 52 week, multi-centre, open label, parallel group, randomised trial in acromegalic subjects who were either *de novo*, or post-surgical, with IGF-1 levels $\geq 1.3\times$ upper limit of normal (ULN). Subjects were randomised to either P or LAR, using stratification with respect to baseline severity (mild (IGF-1 ≥ 1.3 ULN; severe (IGF-1 $\geq 2\times$ ULN), The dose of P was started at 10 mg sc and titrated at 8 week intervals to normalise IGF-1 up to a maximum of 40 mg. The dose of octreotide was 50 μg sc three times daily, switching at 4 weeks to 20 mg LAR im monthly. The dose was titrated to normalise IGF-1 at 16 week intervals up to a maximum of 30 or 40 mg monthly, according to local practice. During the study, the Nichols IGF-1 radioimmunoassay (RIA) became unavailable and analysis was switched to the Immulite chemiluminescent assay. The difference in number of subjects who achieved IGF-1 normalisation (responders (R)) between the two treatment groups was analysed by Fisher's Exact test, while changes from baseline in efficacy parameters were analysed by ANCOVA. The R rate was higher in the P group compared to LAR, but the difference was not statistically significant. In P, R rates using the Immulite and RIA assays respectively were 51%, 83%, compared to 34% and 67% in LAR. The number of subjects with treatment-related adverse events was 21 in P and 29 in LAR. Four subjects in both groups had abnormal ($\geq$ 3x ULN $\leq 10\times$) hepatic transaminases. There was a higher incidence of biliary tract abnormalities with LAR.Treatment with P was at least as efficacious as LAR. It is hypothesised that the lower than expected R rates and non-significant difference in IGF-1 normalisation between the 2 treatment groups are due to a change in assay methodology and non-optimal dose titration with P.

P611

Androtest: a structured interview for the screening of hypogonadism in patients with sexual dysfunction

Giovanni Corona[1], Luisa Petrone[1], Edoardo Mannucci[2], Alessandra D Fisher[1], Valerio Chairini[3], Giancarlo Balercia[4], Gianni Forti[1] & Mario Maggi[1]
[1]Andrology Unit, University of Florence, Florence, Italy; [2]Geriatric Unit, Diabetes Section, University of Florence, Florence, Italy; [3]Endocrinology Unit, Maggiore-Bellaria Hospital, Bologna, Italy; [4]Endocrinology Unit, Polytechnic University of Marche, Ancona, Italy.

Objectives
Detecting hypogonadism is crucial in patients with sexual dysfunctions because hypogonadism can have a causal role for them and testosterone (T) substitution represents a milestone for the therapy. At present, three different inventories have been developed for screening of hypogonadism in aging male. All these instruments demonstrated a good sensitivity but low specificity. No inventories are available for the screening of hypogonadism in patients with sexual dysfunction. We wished to set up a brief structured interview providing scores useful for detecting hypogonadism defined as low total T ($<$10.4 nmol/L, 300 ng/dL) in a symptomatic population (sexual dysfunction).
Methods
A minimum set of items was identified within a larger structured interview through iterative ROC curve analysis, with assessment of sensitivity and specificity for hypogonadism in a sample of 215 patients. Sensitivity and specificity were verified in a further sample of 664 patients. Correlation of test scores with PSA, testis volume, and others clinical and psychological parameters, was assessed for concurrent validity.
Results
In the validation sample, the final 12-item version of the interview (ANDROTEST) had a sensitivity and specificity of 68% and 65% with an accuracy of $0.700+0.03$ ($P<0.0001$), in detecting low total testosterone ($<$ 10.4 nmol/l) and of 71% and 65% with an accuracy of 0.716 ± 0.03 ($P<0.0001$), in the screening for low free testosterone ($<$37 pmol/l). Furthermore, patients with pathological test (i.e score $>$ 8) showed higher prevalence of hypogonadism related signs, such as lower testis volume and higher depressive symptoms. Finally, when younger patients only ($<$54 years, which represents the median age of the sample), were considered, Log10 [PSA] levels were significantly lower in those with ANDROTEST score $>$8.
Conclusion
ANDROTEST is a quick, and easy-to-administer interview that provides scores for the screening of male hypogonadism in patients with sexual dysfunction.

P612

Assessment of the relational factor in male patients consulting for sexual dysfunction: the concept of couple sexual dysfunction

Giovanni Corona[1], Luisa Petrone[1], Edoardo Mannucci[2], Angela Magini[1], Francesco Lotti[1], Valdo Ricca[3], Valerio Chiarini[4], Gianni Forti[1] & Mario Maggi[1]
[1]Andrology Unit, University of Florence, Florence, Italy; [2]Geriatric Unit, Diabetes Section, University of Florence, Florence, Italy; [3]Psychiatry Unit, University of Florence, Florence, Italy; [4]Endocrinology Unit, University of Florence, Florence, Italy.

Objectives
To date it is not clear to which extent a clinical, or even a subclinical, sexual dysfunction in the female partner might associate with erectile dysfunction (ED) in the male partner. The present study is aimed at the assessment of clinical features of ED associated with relational disturbances.
Methods
In a consecutive series of 1140 male subjects reporting a stable couple relationship we evaluated the impact of relational factors, as assessed by SIEDY Scale 2 (exploring, as reported by the patient, menopausal symptoms, partner's medical illness interfering with sexual activity and reduced partner desire and climax). SIEDY is an easy to administer instrument for the first screening of ED patient, providing scores for the relational component besides those to quantity the organic and intrapsychic components. Several hormonal, biochemical and instrumental parameters were also studied, along with psychopathology scores (Middlesex Hospital Questionnaire modified MHQ).
Results
We found that SIEDY Scale 2 is significantly and independently from other factors (as the organic ones) associated with ED, delayed ejaculation, hypoactive sexual desire and decreased number of intercourses. In particular, the chance of being affected by severe ED increased by 10 [1–10] % for each increment of SIEDY Scale 2 score ($P<0.05$). SIEDY Scale 2 scores are associated with an advanced age of the partner and a long couple relationship (>10 years), independently from patient's age. In addition, an increased relational factor significantly ($P<0.0001$) correlates with increased extra-marital affairs ($r=0.111$), conflicts in the couple ($r=0.279$), alcohol abuse ($r=0.155$) and presence of depressive symptoms ($r=0.182$), as assessed by MHQ questionnaire.
Conclusion
Our result should encourage the andrologist to consider the context in which the sexual symptom develops, analysing the relationship and partner's behaviour and diseases. Resolving, or at least ameliorating, the relational background and the sexual framework might help in treating male sexual dysfunction.

P613

Effect of hormone replacement therapy apart from growth hormone on the endothelial functions in patients with Sheehan's syndrome

Mithat Bahceci[1], Semir Pasa[1], Hatice O Akay[2], Alpaslan Tuzcu[1], Senay Arikan[1] & Deniz Gokalp[1]
[1]Dicle Univ. School of medicine Department of endocrinology, Diyarbakir, Turkey, [2]Dicle Univ. School of medicine Department of radiology, Diyarbakir, Turkey.

Aim
To examine the endothelial functions of patients with Sheehan syndrome (SS) and to eveluate the effects of hormone replacement treatment except growth hormone on endothelial functions.
Subjects and methods
Twenty-four patients with Sheehan syndrome (PSS) aged 40.83±6.43 yr and 25 healthy control women aged 41.13±6.51 yr (C) were included. Endothelial functions were evaluated with high resolution ultrasonography (flow mediated dilatation:FMD) and serum nitric oxide (NO) levels before and after the treatment [15 months with prednisolon (5–7.5 mg/d), L-thyroxin (100–200 μg/d), and conjugated estradiol (0.625 mg/d)- medroxyprogesteron acetate (5 mg/d) patients <40 years].
Results
1-Before treatment
Baseline (16.87±4.04 μol/L and 11.8±2.14 μol/L) and stimulated NO levels were higher (18.79±4.4 and 14.92±2.44); whereas, baseline arterial diameter (3.74±0.68 mm, 4.62±0.42 mm, $P=0{,}0001$), FMD stimulated NO increment ratio (13.16±5.57% and 26.38±8.89%, $P=0{,}0001$) and arterial dilation ratio (13.42±6.57% and 18.93±5.64, $P=0{,}003$) of PSS were lower than C group.
2- After treatment
Elevation of baseline (17.58±4.3 vs 11.8±2.14) and stimulated NO levels of PSS (21.12±4.85 vs 11.92±2.44, $P=0.0001$) insisted on. On the contrary FMD stimulated arterial dilation ratio of PSS increased to the similar level of C group with treatment. FMD stimulated NO levels (18.79±4.4 vs 21.12±4.85), NO increment ratios (13.16±5.57% and 22.83±8.57%) and FMD stimulated arterial dilation ratio increased with treatment significantly (13.42±6.57% vs 21.73±10.13%) ($P=0{,}0001$).
Conclusions
1- Although patients with Sheehan syndrome had high NO levels, they had small FMD stimulated NO increments and arterial dilation ratio. 2-Increased but little effective NO may responsible for this result. 3- HRT apart from GH may restore endothelial functions in patients with Sheehan's syndrome.

P614

Family history of diabetes mellitus determines insulin sensitivity and beta cell dysfunction in polycystic ovary syndrome

Jana Vrbikova, Katerina Dvorakova, Tereza Grimmichova, Martin Hill, Sona Stanicka & Karel Vondra
Institute of Endocrinology, Pragie 1, Czech Republic.

Aim
To examine the secretion of insulin and glucagon in PCOS in the context of insulin sensitivity.
Patients and methods
13 healthy women (BMI 21.8(2.2) kg/m^2), 21 PCOS without family history of DM2 (FH-); BMI 24.3 (4.4) kg/m^2 and 16 PCOS with the 1st degree relative affected by DM2 (FH+); BMI 26.7 (4.2) kg/m^2. Euglyceamic hyperinsulinaemic clamp (1mIU kg^{-1}.min^{-1}; with the determination of insulin sensitivity index (ISI)) and arginine secretion test to measure insulin (AIR) and glucagon (AGR) secretion after arginine bolus at fasting glycaemia (AIRf and AGRf) and at hyperglycaemia (AGRf and AGRg). Kruskal-Wallis ANOVA followed by Kruskal-Wallis multiple comparisons and Spearman correlations adjusted to a constant BMI were used for data evaluation.
Results
PCOS had higher basal insulin ($P=0.004$) and higher HOMA-R than C ($P=0.002$). Higher basal glucagon ($P=0.005$) and higher glucagon secretion at hyperglycemia (AGRg; $P=0.05$) in PCOS than in C was seen. PCOS FH+ had higher insulin secretion at fasting glycaemia ($P=0.05$) with no difference at hyperglycemia. Insulin sensitivity index (ISI, ISI $_{LBM}$) was lower in PCOS FH+ ($P=0.002$) than in C or PCOS FH-.Concerning beta cell function, disposition indices calculated from ISI and slope I or from AIRg were lower in PCOS FH+ than in PCOS FH- or C ($P=0.05$ for both). Basal glucagon correlated significantly with lean body mass ($r=-0.322$, $P=0.03$), basal insulin ($r=0.308$; $P=0.05$) and AGRg ($r=0.31$; $P=0.04$), with T ($r=0.479$; $P=0.001$), DHEAS ($r=0.335$; $P=0.028$) and with SHBG ($r=-0.356$; $P=0.015$). AGRg correlated with T ($r=0.32$; $P=0.03$), DHEAS ($r=0.40$; $P=0.008$), DHEA ($r=0.36$; $P=0.02$) and with SHBG ($r=-0.28$; $P=0.06$).
Conclusions
Higher basal glucagon levels are present in PCOS irrespective of obesity and family history of DM 2. Insulin resistance and beta cell secretory dysfunction are detectable only in PCOS with the family history of DM 2.
Supported by grant IGA MHCR 8759-3.

P615

Retinol-binding protein-4 in polycystic ovary synrome - relationship with obesity and androgen levels

Jana Vrbikova, Martin Hill, Bela Bendlova, Tereza Grimmichova, Katerina Dvorakova & Karel Vondra
Institute of Endocrinology, Prague, Czech Republic.

Aim and background
Retinol binding protein 4 (RBP 4) is an adipocyte-secreted molecule causing insulin resistance in transgenic animals. RBP was increased in subjects with impaired glucose tolerance and diabetes type 2. The levels of RBP-4 in PCOS were not investigated till now.
Subjects and methods
16 lean PCOS (BMI 21.4 (1.75) kg/m^2, age 24.1 (4.1) years), 25 obese PCOS (BMI 30.3 (4.8) kg/m^2, age 26.3 (5.0) years) and 13 healthy women (BMI 21.5 (1.6) kg/m^2, age 29.4 (7.0) years) were evaluated using euglyceamic hyperinsulinaemic clamp (1 mIU kg^{-1}.min^{-1}) with the determination of insulin sensitivity index (ISI; mmol.kg^{-1}.min^{-1} per mIU l^{-1}). In basal sample, RBP-4 levels (mg/l) were determined using ELISA (ImmundiagnostikAG, Bensheim, Germany). Results are given as mean (SD). ANOVA and multiple backward stepwise regression was used for data analysis. NCSS 2002 statistical software was used for calculations.
Results
Insulin sensitivity index was lower only in O-PCOS (33.2(22)) comparing L-PCOS (70.1 (22)) or C (77.4 (22.7)); ($P=0.0003$). RBP-4 levels were not different between L-PCOS (27.6 (6.8)), C (33.7 (8.2)) or O-PCOS (32.6 (9.9)).
To explain RBP-4 levels in PCOS women, a regression model consisting of ISI, BMI and 17-OHP was suggested. Only ISI ($P=0.04$) and 17 OHP ($P=0.03$) influenced significantly and independently RBP-4 levels; explaining 21.9% of the total variability in the dependent variable. When ISI was taken as dependent variable, and testosterone, RBP-4 and BMI as independent variables, final model contained only BMI ($P=0.0001$) and explained 33.7% of the variability in insulin sensitivity.
In conclusion, RBP-4 levels in PCOS are influenced negatively and independently by both androgen levels and insulin sensitivity. Hence, the RBP-4 levels in PCOS

could not be taken as a marker for the description of insulin sensitivity.
Supported by grants of IGA MH CR 8759-3 and GACR 301/04/1085.

P616

Protein metabolism in a model of premature ovarian failure, Turner syndrome, and the impact of hormone replacement therapy

Claus H Gravholt, Anne-Lene Riis & Jens Sandahl Christiansen
Medical Department M, Århus Sygehus NBG, Århus University Hospital, Århus, Denmark.

Background
Several studies have documented an altered body composition in Turner syndrome (TS), a model of premature ovarian failure. Body fat is increased and muscle mass is decreased. The ovarian failure necessitates substitution with female hormone replacement therapy (HRT) for a number of years, and HRT induces favourable changes in body composition with a decrease in body fat and an increase in fat free mass. It is unknown how HRT affects protein metabolism.
Aim
To study protein metabolism in TS in detail, and evaluate the distinct impact of HRT action.
Design
Randomized crossover study with active treatment (HRT in TS and P-pill in controls) or no treatment for 2 month each.
Material
We studied women with Turner syndrome ($n=8$, age 29.7 ± 5.6 (mean$\pm$S.D.) years), verified by karyotype, and age-matched controls ($n=8$, age 27.3 ± 4.9 years).
Methods
All subjects underwent a 3-h study in the postabsorptive state. After regional catheterization, protein dynamics of the whole body and of the forearm muscles were measured by amino acid tracer dilution technique using [^{15}N]phenylalanine and [$^{2}H_4$]tyrosine. Substrate metabolism was examined by indirect calorimetry.
Results
Estradiol increased and FSH decreased during active treatment in TS. Energy expenditure was comparable among TS and controls, and did not change during active treatment. Whole body phenylalanine and tyrosine fluxes were similar in the untreated situations, and did not change during active treatment. Amino acid degradation (TS vs C: 4.0 ± 0.9 vs 4.8 ± 0.8 μmol*kg^{-1}*h^{-1}, $P=0.1$) and protein synthesis (36.8 ± 5.2 vs 35.2 ± 3.0 μmol*kg^{-1}*h^{-1}, $P=0.5$) was similar in the untreated situations and did not change during treatment. Muscle protein breakdown was similar among groups, and was not affected by treatment. Muscle protein synthesis rate and forearm blood flow did not differ among groups or due to treatment.
Conclusions
Protein metabolism in TS is comparable to controls, and is not affected by a short course of HRT.

P617

Differences in the onset of puberty in selected inbred mouse strains

Otto Pinter, Zoltan Beda, Zsolt Csaba & Ida Gerendai
Neuroendocrine Research Laboratory, Hungarian Academy of Sciences and Semmelweis University, Budapest, Hungary.

Puberty is the final stage of maturation of the hypothalamo-pituitary-gonadal axis and is characterized by changes in circulating gonadotropins and increased levels of sex steroids. There are both genetic and external factors (e.g. nutrition, stress) which can alter the onset of puberty. The aim of this study was to determine the onset of puberty in genetically homogeneous inbred mouse strains. Five strains were used: 129X1/SvJ, DBA/2J, C57BL/6J, CBA/CaJ and A/J. Various pubertal markers were determined: vaginal opening (VO), first vaginal cornification, onset of cyclicity in females, and balanopreputial separation (BPS) in males. There were significant differences between strains in the onset of puberty. The earliest VO was detected in A/J (day 20,8), then CBA/CaJ (day 24,45), DBA/2J (day 25,78), C57BL/6J (day 26,45) and the latest was in 129X1/SvJ (day 29,38). The earliest day for the first vaginal cornification was in CBA/CaJ (day 30,4), followed by C57BL/6J (day 33,18), A/J (day 34,3), 129X1/SvJ (day 36,28) and the latest was in DBA/2J (day 38,33). The earliest onset of cyclicity was detected in CBA/CaJ (day 40,3), then A/J (day40,4), 129X1/SvJ (day 47,19), C57BL/6J (day 48,67) and the latest was in DBA/2J (day 51,11). There was no correlation between the weight and the age at either VO, cornification or the onset of cyclicity among strains. The occurrence of BPS was later in males then the first sign of puberty (i.e. VO) in females. The earliest BPS was in CBA/CaJ (day 27,75), followed by 129X1/SvJ (day 29,37), C57BL/6J (day 29,71), A/J (day 30,8) and the latest was in DBA/2J (day 34). There was no correlation between the weight and the age at BPS among strains. Data indicate significant differences in pubertal parameters of inbred mouse strains.

This work was supported by grant from the EU Commission (PIONEER FOOD-CT-2005-513991).

P618

The investigation of hypothalamo-pituitary-gonadal axis in patients with epilepsy

Fusun Erdogan[1], Sevgi Arik Yuksel[1], Ali Ozdemir Ersoy[1], Fatih Tanriverdi[2], Kursad Ünlühizarci[2] & Fahrettin Kelestimur[1]
[1]Erciyes University Medical School Department of Neurology, Kayseri, Turkey; [2]Erciyes University Medical School Department of Endocrinology, Kayseri, Turkey.

Polycystic ovary syndrome (PCOS) is a common endocrine disorder affecting approximately 4–8% of premenopausal women. The main clinical features are clinical and/or biochemical hyperandrogenemia, oligomenorrhea and polycystic ovarian changes. It has gained a great attention during the last two decades with the realization that this syndrome affects more than the reproductive system. Epilepsy is a common neurologic disorder affecting women during the reproductive years. Seizures and some antiepileptic drugs (AEDs) can compromise reproductive health. During the last years, several reports in the literature suggest a relationship between PCOS and epilepsy. The pathophysiology of the increased prevalence of PCOS and/or hyperandrogenism is not well established in patients with epilepsy and hypothalamus-pituitary-gonadal axis is not investigated in detail.

Forty-eight women with epilepsy were recruited in order to investigate the prevalence of PCOS, glucose intolerance and ovarian functions by using a GnRH analogue buserelin. All the patients were on valproic acid or carbamazepin treatment. Fasting blood chemistry, basal hormone levels (including FSH, LH, estradiol, DHEAS, testosterone, androstenedione SHBG, 17-OHP), OGTT, buserelin test were performed and ultrasonography of the ovaries obtained. Twenty age and BMI matched healthy women served as a control group.

Serum free testosterone and SHBG levels were significantly ($P<0.05$) higher in patients than in the control group. Three patients (7.5%) had glucose intolerance. Glucose and insulin responses to OGTT (either peak or area under the curve: AUC) were significantly ($P<0.05$) higher in the patients than in the control subjects. Patients with epilepsy had sigificantly ($P<0.05$) higher peak and AUC 17-OHP responses to buserelin test. Overall 15 (31.2%) patients had PCOS.

Our results suggest that women with epilepsy treatment have a high prevalance of PCOS, increased insulin resistance and ovarian dysfunction.

P619

Varicocele sclerotherapy improves serum inhibin B levels and seminal parameters

Matteo Baldi[1], Cataldo Di Bisceglie[1], Fabio Lanfranco[1], Angela Bertagna[1], Milena Tagliabue[1], Carlo Gazzera[2], Giovanni Gandini[2] & Chiara Manieri[1]
[1]Div. Endocrinology and Metabolic Diseases, Dep. Internal Medicine, University of Turin, Torino, Italy; [2]Dep. Radiodiagnostics IV, Dep. Medical-Surgical Disciplines, University of Turin, Torino, Italy.

Varicocele is a state of varicosity and tortuosity of the pampiniform plexus around the testis caused by retrograde blood flow. The association between varicocele and male subfertility has been questioned, thus the usefulness of treating varicocele in order to improve fertility is still a matter of debate. Inhibin B levels reflect the functional state of the seminiferous epithelium, and have been found to be a sensitive index of spermatogenesis. Serum inhibin B levels have been reported to increase after surgical varicocelectomy along with the improvement of sperm concentration. The aim of this study was to evaluate variations of seminal parameters and inhibin B concentrations in a group of 38 males affected by varicocele and treated by percutaneous retrograde sclerotherapy. Serum inhibin B, FSH, testosterone levels and seminal parameters were performed before and 6 months after sclerotherapy. Twenty age-matched patients with left varicocele who did not undergo any treatment were studied as controls. A significant increase of inhibin B levels and a significant decrease of FSH levels were observed 6 months after treatment (mean$\pm$S.E.M., 125.8 ± 15.7 vs 106.4 ± 12.7 pg/ml, $P<0.01$; 4.5 ± 0.6 vs 5.6 ± 1.0 mIU/ml $P<0.05$); no significant

change of testosterone levels was observed. After treatment semen analysis showed a significant improvement of sperm concentration (66.3 ± 10.4 vs 39.0 ± 6.6 million/ml, $P < 0.05$) and progressive motility (52.2 ± 3.7 vs 40.2 ± 4.1%, $P < 0.01$); no significant change of sperm normal morphology was observed. In the control group no significant variations of hormonal and seminal parameters were observed 6 months after the basal examination. In conclusion, percutaneous retrograde sclerotherapy in varicocele improves inhibin B levels and seminal parameters, confirming its positive effect on spermatogenesis and Sertoli cell function.

P620

LH receptor gene expression and splicing variants in marmoset (*Callithrix jacchus*) testis and adrenal gland at puberty

Caroline Michel, Jörg Gromoll, Ramesh Chandolia & Manuela Simoni
Institute of Reproductive Medicine, Muenster, Germany.

Background
The LHR is a crucial mediator for normal sexual development and fertility. In the marmoset monkey (*Callithrix jacchus*), LHR type II, lacking exon 10, is the native receptor type. In addition to the testis, the LHR is expressed in the adrenal gland where its function remains unknown.
Aim
To characterise marmoset LHR expression at different stages of puberty in the testis and adrenal gland and examine different splice variants in the testis.
Material and methods
We analysed 25 male marmosets of five age groups ($n = 5$/group): 21.5 ± 0.1, 43.3 ± 0.7, 52.8 ± 0.3, 70.1 ± 0.4 and 116.8 ± 20 weeks (mean ± S.E.M.). Total RNA was isolated from testes and adrenal glands, reverse-transcribed and used for real-time PCR. Splice variants were detected using primers directed to exon 2 and 11. PCR products were analysed by densitometric analysis, cloned into pGEM-T-Easy-vector and sequenced.
Results
The expression levels of the full transcript were lowest at the beginning of puberty and increased progressively both in the testis and in the adrenal gland. The full-length transcript expression values in the testis (2.244 ± 0.9 AU) were 4.2-fold higher compared to the adrenal gland (0.537 ± 0.5 AU). We detected eleven LHR splicing variants in the testes. Seven of these showed exon skipping, lacking one to seven exons, and four were alternatively spliced. As expected, exon 10 was absent in all variants. While each variant is expressed 0.7-fold, the overall amount of all splice variants is much more abundant (6.1 ± 0.5) than the wild type. Two thirds of all isoforms lack four or less exons and densitometric analysis recognized no pubertal-associated variance. Alternative splicing was much less evident in the adrenal glands.
Conclusion
LHR expression increased progressively in both tissues while the splicing patterns itself does not change during puberty, and different splice variants exist in the testis.

P621

Protamine 1 and Protamine 2 sequence variants in teratozoospermia

Sonja Römer, Frank Tüttelmann, Jörg Gromoll, Eberhard Nieschlag & Manuela Simoni
Institute of Reproductive Medicine of the University, Münster, Germany.

Background
During spermatogenesis protamines replace histones in sperm head. Haploinsufficiency of the protamine (*PRM*) 1 or *PRM2* gene causes infertility in mice. A mutation in *PRM1* was associated with increased abnormal sperm morphology in infertile men[1]. We assessed the frequency of mutations and SNPs in the *PRM1* and *PRM2* gene in infertile patiens with normal sperm concentration and reduced morphology, a phenotype similar to that of the *Prm1* deficient knockout mice.
Material and Methods
Using the institutional database (Androbase©) we identified 29 infertile men with normal sperm concentration and severe idiopathic teratozoospermia (<7% normal forms). *PRM1* and *PRM2* were sequenced in the patients and in 29 controls with normal spermatogenesis.
Results
Two single SNPs were identified in the *PRM1* gene. One (A230C) was known (rs737008) as a synomynous polymorphism in exon 2 with a heterozygosity of 0.5, and occured with similar frequencies in teratozoospermic men (heterozygous $n = 11$; homozygous minor $n = 4$) and controls (heterozygous $n = 13$; homozygous minor $n = 3$). We identified a novel synonymous SNP in exon 1 (G54A) in two patients and one control. The G197T mutation in *PRM1* previously reported[1] was not found. A meta-analysis of our and the literature data showed that the mutation G197T is not associated with teratozoospermia. Four SNPs were found in intron 1 of the *PRM2* gene. C298G and C373A are listed in the NCBI database (rs1646022; rs2070923). The remaining two (C366T; C406T) were rare heterozygous SNPs, evenly distributed with a frequency of 3,4% in both groups. The prevalence of all SNPs was similar in infertile men and controls. No SNP was found in the exons.
Conclusion
Mutations of *PRM1* and *PRM2* are rare in teratozoospermic men with normal sperm count. Common polymorphisms of the *PRM* genes are not associated with idiopathic teratozoospermia.
(1) Iguchi, Yang, Lamb, Hecht (2006) *J. Med. Genet*. **43**, 382–384.

P622

Ghrelin effects on spontaneous and stimulated LH secretion in human males

Lorenza Bonelli, Matteo Baldi, Fabio Broglio, Angela Bertagna, Cataldo Di Bisceglie, Milena Tagliabue, Chiara Manieri, Ezio Ghigo & Fabio Lanfranco
Division of Endocrinology and Metabolism, Department of Internal Medicine, University of Turin, Italy, Torino, Italy.

Acylated ghrelin (AG) participates in the modulation of the hypothalamic-pituitary-gonadal axis functions, with a predominantly inhibitory effect upon the reproductive system in animals. Animal studies have shown that ghrelin suppresses LH secretion *in vivo*, decreases LH responsiveness to GnRH *in vitro* and partially delays the timing of puberty in males. Aim of this study was to evaluate the effects of AG infusion on spontaneous and stimulated gonadotropin secretion in male subjects. In 6 eugonadal males (age mean ± S.E.M.:28.7 ± 1.1 yrs; BMI:22.4 ± 2.1 kg/m^2) we evaluated LH and FSH levels every 15 min during: a) i.v. isotonic saline infusion (SAL) from 0 to +480 min; b) i.v. SAL from 0 to +240 min followed by AG (1.0 μg/kg as a bolus at +240 min, and AG infusion 2 μg/kg/h in 500 ml isotonic saline from +240 to +480 min); c) GnRH test (100 μg i.v. as a bolus at +120 min) during saline or AG infusion from 0 to +240 min. No significant changes in FSH pulsatile secretion were recorded in test sessions a) and b). Under SAL infusion, significant LH pulses were recorded in all subjects. AG infusion significantly decreased LH pulse number and frequency, pulse height (MSPH: 0.04 ± 0.02 mU/ml; −84% vs. SAL) and pulse mass (MSPM: 0.65 ± 0.46 mU/ml; −89% vs. SAL). LH and FSH responses during saline (LH peak 18.2 ± 3.9 mU/ml, FSH peak 12.7 ± 2.6 mU/ml) were similar to those recorded during AG (LH peak 21.6 ± 4.4 mU/ml, FSH peak 11.2 ± 2.9 mU/ml). These findings demonstrate that AG inhibits pulsatile LH secretion but not LH responsiveness to GnRH in males. Therefore ghrelin, at least the acylated form, exerts an inhibitory effect on the gonadal axis in men through a hypothalamic mechanism.

P623

Differential effects of two-week treatment with atorvastatin or elocalcitol, two RhoA/ROK signalling modulators, on erectile function and sildenafil responsiveness in spontaneously hypertensive rats

Annamaria Morelli[1], Xin-Hua Zhang[1], Sandra Filippi[2], Linda Vignozzi[1], Sara Mungai[1] & Luciano Adorini[3]
[1]University of Florence, Dept. of Clinical Physiopathology, Andrology Unit, Florence, Italy, [2]University of Florence, Interdepartmental Laboratory of Functional and Cellular Pharmacology of Reproduction, Departments of Pharmacology and Clinical Physiopathology, Florence, Italy; [3]Bioxell, Milan, Italy.

Increased RhoA/Rho-kinase (ROK) signalling is known to impair erectile function. Spontaneously hypertensive rats (SHR) over-express penile RhoA and show an impaired erectile response. We tested treatments known to inhibit RhoA activation, on erectile function and sildenafil responsiveness in SHR. SHR have been treated for two weeks with atorvastatin (5 and 30 mg/Kg/day), or with elocalcitol (30 μg/Kg/day), a vitamin D receptor (VDR) agonist. The normotensive Wistar Kyoto (WKY) rats have been used as controls. At the selected doses, neither atorvastatin affected cholesterol, nor elocalcitol affected calcaemia in both SHR and WKY. In WKY, sildenafil (25 mg/Kg by oral gavage) greatly increased erectile function, evaluated as intracavernous pressure/mean arterial pressure (ICP/MAP) ratio after electrical stimulation (ES) of the cavernous nerve. In SHR, both basal and sildenafil-stimulated ICP/MAP ratio were depressed. Atorvastatin did not affect basal ICP/MAP at any concentration tested. However, it dose-dependently increased

sildenafil effect on ES-induced erection, significantly potentiated by 30 mg/Kg dosing. At this dose, atorvastatin normalized the over-expression of RhoA mRNA (real time RT-PCR) observed in SHR, without affecting other genes such as ROK1, ROK2, PDE5, nNOS, eNOS. Conversely, elocalcitol, at a dose known to ameliorate bladder overactivity by inhibiting RhoA activation, failed to restore ICP/MAP ratio, sildenafil responsiveness and RhoA expression in SHR. Finally, SHR rats expressed high levels of VDR mRNA in the bladder (almost 5-fold increase over WKY), but not in corpora cavernosa (CC). In conclusion, our data confirm that an increased RhoA signalling impairs erectile function and sildenafil responsiveness in SHR. Atorvastatin, at a dose unable to affect lipids, ameliorates sildenafil effectiveness and down-regulates RhoA expression. Conversely, elocalcitol was ineffective in restoring erectile function in SHR, either alone or with sildenafil. The differential quantitative VDR expression in bladder and CC suggests a plausible mechanism for the tissue-specific effect of elocalcitol on RhoA/ROK contractile pathway.

P624

Testosterone regulates RhoA/Rho-kinase signalling in two distinct animal models of chemical diabetes

Linda Vignozzi[1], Annamaria Morelli[1], Sandra Filippi[2], Stefano Ambrosini[4], Rosa Mancina[1], Michaela Luconi[3], Sara Mungai[1], Gabriella Barbara Vannelli[4], Xin-Hua Zhang[1], Gianni Forti[3] & Mario Maggi[1]
[1]University of Florence, Dept. of Clinical Physiopathology, Andrology Unit, Florence, Italy; [2]University of Florence, Interdep. Lab of Functional and Cellular Pharmacology of Reproduction, Depts. of Pharmacology and Clinical Physiopathology, Florence, Italy; [3]University of Florence, Dept. of Clinical Physiopathology, Endocrinology Unit, Florence, Italy; [4]University of Florence,Department of Anatomy, Histology and Forensic Medicine, Florence, Italy.

The contractile RhoA/Rho-kinase (Rock) signalling pathway is up-regulated in penile tissue in animal models of experimental diabetes and has been proposed to contribute to diabetes-related erectile dysfunction (ED). In previous studies we demonstrated that testosterone (T) restores diabetes-induced ED by influencing the NO/cGMP/PDE5 pathway.
Aim
To investigate the effect of T on the RhoA/Rock signalling in course of diabetes.
Methods
We used two distinct animal models of chemical diabetes (alloxan-induced in the rabbit and streptozotocin-induced in the rat) with or not T supplementation.
Results
In both models, hypogonadism was observed, characterized by reduced T plasma level and androgen-dependent accessory glands atrophy. Diabetic animals showed a significant increase in responsiveness to increasing concentrations of Y-27632, a highly selective Rock inhibitor, as evaluated either by '*in vitro*' contractility study (diabetic-rabbit) and '*in vivo*' as erectile response elicited by intracavernous injections (diabetic-rats). T-substitution (30 mg/kg, weekly) completely reverted hypogonadism and diabetes-induced penile hypersensitivity to Y-27632. To test whether this effect was due to a T-dependent regulation of RhoA/Rock gene expression, we measured RhoA/Rock mRNA. Both isoforms of Rock (Rock1/-Rock2) were analyzed by real time RT-PCR in rat penile samples. We found that Rock1 mRNA was significantly increased ($P < 0.05$) in penile tissues from diabetic animals and restored to the control values by T, as also confirmed by semiquantitative RT-PCR in rabbit. Conversely, RhoA and Rock2 mRNA expression was not influenced neither by diabetic condition and by T administration. Accordingly, Rock1 protein expression, as evaluated by western blot and immunohistochemistry analysis, resulted increased in penile samples from diabetic animals and normalized by T.
Conclusions
Our data further support the hypothesis that the activation of RhoA/Rock signalling contributes to diabetes-related erectile dysfunction. Moreover, treating hypogonadism in course of diabetes, may restore erectile function also by normalizing RhoA/ Rock pathway over-activity.

P625

Effect of sildenafil administration on penile hypoxia induced by cavernous neurotomy in the rat

Linda Vignozzi[1], Annamaria Morelli[1], Sandra Filippi[3], Gabriella Barbara Vannelli[4], Gianni Forti[2] & Mario Maggi[1]
[1]University of Florence, Department of Clinical Physiopathology, Andrology Unit, Florence, Italy; [2]University of Florence, Department of Clinical Physiopathology, Endocrinology Unit, Florence, Italy; [3]University of Florence,Interdepartmental 2Laboratory of Functional and Cellular Pharmacology of Reproduction, Departments of Pharmacology and Clinical Physiopathology, Florence, Italy; [4]University of Florence, Department of Anatomy Histology and Forensic Medicine, Florence, Italy.

Objectives
Radical prostatectomy is an effective therapy for men with clinically localized prostate cancer. A significant number of men develop erectile dysfunction after radical prostatectomy (PPED) due to intraoperatory cavernous nerve injury causing hypoxia and fibrosis of corpus cavernosus. We established an experimental model of bilateral cavernous neurotomy (BCN) in the rat in order to investigate whether sildenafil treatment in PPED patients could prevent penile tissue damage.
Design and methods
One, 5 and 10 days after neurotomy, animals were treated or not with a single dose of sildenafil (25 mg/kg orally) one hour before sacrifice. To analyze penile oxygenation, rats of each experimental group received (one hour before sacrifice) an intraperitoneal injection of the bio-reductive drug pimonidazole hydrochloride (hypoxyprobeTM-1, 60 mg/Kg), which has been recognized as a standard marker for *in vivo* imaging and quantification of hypoxia.
Results
With immunohistochemistry for hypoxiprobeTM, we found that BCN induced massive hypoxia at all times investigated in corpora cavernosa sections from the experimental rats, as revealed by computer-assisted quantitative image analysis. This tissue hypo-oxygenation was significantly reduced in sections from sildenafil treated rats at 1 and 5 days after neurotomy, while at 10 days this reduction was less evident and not significant. In addition, functional studies indicated that hypoxic corpora cavernosa tissues were hypersensitive to the relaxant effect of the endothelin receptor type B (ETB) agonist IRL-1620, due to the previously described hypoxia-induced overexpression of ETB receptors. Accordingly, ETB mRNA expression (real time RT-PCR) was significantly increased in corpora cavernosa from BCN rats, and was restored to control levels by sildenafil administration at all times investigated.
Conclusions
Our results indicate that sildenafil treatment can positively influence penile tissue oxygenation after cavernous nerve injury, with its effect being more evident the earlier it is administered.

P626

Androgenicity, androgen receptor polymorphism and pharmacogenetics

Domenico Canale, Cecilia Moschini, Carolina Caglieresi, Agnese Moscatelli, Silvia Gavioli, Enrico Macchia & Enio Martino
University, Pisa, Italy.

Exon 1 of the androgen receptor (AR) gene contains a variable number of CAG triplets, (CAGr)n 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 the transcriptional activity induced by the AR and therefore modulates target organs responsiveness to androgens.

The (CAGr)n is inversely associated with the transcriptional activity of target genes. The (CAGr)n has been analysed in a variety of cross-sectional studies, investigating its influence on clinical conditions and parameters affected by T action, such as bone density, spermatogenesis, mood variations, cognitive functions and hair development in both men and women. Zitzmann *et al* have correlated the prostate growth induced by T replacement therapy in hypogonadal men with (CAGr)n, demonstrating an impressive modulating effect by the CAGr polymorphism. A role of the (CAGr)n has been also demonstrated in determining the androgenicity of an individual: hypoandrogenized patients compared to a control group have an increased (CAGr)n (24.0 *vs* 21.5) with a significant shift toward higher numbers.

We will two patients affected by the same disease, that is congenital selective hypogonadotropic hypogonadism, treated with similar doses of androgens. Androgenization, though, was completely different, as the pictures will show: one had a 'female' hair pattern, no beard, no hair in the chest and lower abdomen, pubic hair 3, depressed mood, the other one was well androgenized, with 'extraordinary male' hair pattern, good muscular development, married with children. The first one had a (CAGr)n equal to 30 the second one 15.

Our data further support a pharmacogenetic approach which stresses the evaluation of AR polymorphism to be performed before initiating a long term androgen replacement treatment to provide satisfactory androgen effect at target organs.

P627

Characterization and functional role of an androgen-dependent PDE5 activity in bladder

Sandra Filippi[1], Annamaria Morelli[2], Peter Sandner[6], Benedetta Fibbi[2], Rosa Mancina[2], Mirca Marini[4], Mauro Gacci[5], Linda Vignozzi[2], Gabriella Barbara Vannelli[4], Marco Carini[5], Gianni Forti[3] & Mario Maggi[1]
[1]University of Florence, Interdepartmental Laboratory of Functional and Cellular Pharmacology of Reproduction, Departments of Pharmacology and Clinical Physiopathology, Florence, Italy; [2]University of Florence, Department of Clinical Physiopathology, Andrology Unit, Florence, Italy; [3]University of Florence,Department of Clinical Physiopathology, Endocrinology Unit, Florence, Italy; [4]University of Florence, Department of Anatomy, Histology and Forensic Medicine, Florence, Italy; [5]University of Florence,Department of Urology, Florence, Italy; [6]Bayer Healthcare, Wuppertal, Germany.

Benign prostate hyperplasia (BPH) is the most common disease in the aging male, often comorbid with erectile dysfunction (ED). PDE5 inhibitors (PDE5i, sildenafil, tadalafil and vardenafil) decrease lower urinary tract symptoms (LUTS) in patients with ED and BPH. We studied PDE5 expression and activity in the human bladder and PDE5i effects both *in vitro* (human and rat) and *in vivo* (rat). PDE5 is highly expressed in rat and human bladder and immunolocalized in vascular endothelium and muscle fibers. Sildenafil, tadalafil and vardenafil blocked 70% of the total cGMP catabolizing activity, with vardenafil being the most potent ($IC_{50}=0.3$ nM). In human bladder cells and in rat strips, a PDE-resistant cGMP analogue, SP-8-Br-PET-cGMPS, induced, respectively, a consistent anti-proliferative and relaxant effect. In contrast, the nitric oxide (NO) donor sodium nitroprusside (SNP) was almost ineffective. However, blocking PDE5 with vardenafil increased SNP anti-proliferative and relaxant activity up to the level observed with SP-8-Br-PET-cGMPS. We also found that castration decreased, and T supplementation restored, PDE5 gene expression in rat bladder. Accordingly, bladder strips from castrated rats were more sensitive to SNP- induced relaxation than strips from control or T-replaced rats, while in the presence of vardenafil, all groups showed the same SNP sensitivity. To discover whether vardenafil affects bladder activity *in vivo*, the rat bladder outlet obstruction (BOO) model was used. Chronic treatment with 10 mg/kg/d vardenafil significantly reduced non-voiding contractions (47%, $P<0.05$ vs. placebo) up to tamsulosin level (51%). Overall, these results demonstrate that PDE5 regulates bladder smooth muscle tone, strongly limiting the NO/cGMP signalling, and that vardenafil, by blocking PDE5, may be a possible therapeutic option for bladder dysfunction, by ameliorating irritative LUTS.

P628

Testosterone levels correlate positively with HDL cholesterol levels in men with Type 2 diabetes

Roger D Stanworth[1], Dheeraj Kapoor[1], Kevin S Channer[2] & T Hugh Jones[1]
[1]Academic Unit of Endocrinology, University of Sheffield, Sheffield, United Kingdom; [2]Faculty of Health and Wellbeing, Sheffield Hallam University, Sheffield, United Kingdom.

Low testosterone levels are a common finding in men with coronary artery disease and Type 2 diabetes and predict the future development of the metabolic syndrome and Type 2 diabetes in healthy men. Testosterone replacement therapy has been shown to improve insulin sensitivity and glycaemic control in men with diabetes and improves numerous other cardiovascular risk factors. Interest in testosterone as a potential treatment for cardiovascular disease continues to grow. Low HDL cholesterol (HDL-C) levels are now recognised as an independent cardiovascular risk factor and comprise part of the metabolic syndrome. The effect of testosterone treatment on HDL-C in clinical trials has been inconsistent. Testosterone may be acting through differing processes with opposite effects on HDL.

We present data on the link between testosterone levels and blood lipid levels in a sample of 293 men with Type 2 diabetes. Lipids were assessed by standard methods. Total testosterone (TT) and SHBG levels were assessed by ELISA. Bioavailable testosterone (BioT) was measured by ammonium precipitation. Calculated bioavailable (cBioT) and free testosterone (cFT) were also derived using recognised formulae.

Regression analysis revealed that HDL-C levels were positively associated with TT (regression coefficient $r=0.253$, $P<0.001$), BioT ($r=0.172$, $P=0.003$), cBioT ($r=0.219$, $P=<0.001$), cFT ($r=0.139$, $P=0.18$) and SHBG ($r=0.169$, $P=0.004$). Total cholesterol levels were not significantly associated with testosterone levels but there was a trend towards a negative association of testosterone with total cholesterol ($P=0.051$).

Thus, in our group of men with Type 2 diabetes, testosterone is positively associated with HDL-C suggesting that the dominant effect of testosterone in this group may be to increase HDL. Further clinical trials of testosterone replacement therapy in men with type 2 diabetes are warranted.

P629

Mutations of GnRH receptor and GPR54 in a cohort of patients with idiopathic hypogonadotropic hypogonadism

Nicolas Richard[1], Céline Leprince[1], Céline Gonfroy[1], Jacques Young[2], Didier Dewailly[3], Michel Pugeat[4] & Marie-Laure Kottler[1]
[1]CHU Caen, Caen, France; [2]CHU Kremlin Bicêtre, Kremlin bicêtre, France; [3]CHU Lille, Lille, France; [4]Gpmt Hospitalier est, Lyon, France.

Objective
To determine the frequency of mutations of the gonadotropin-releasing hormone receptor (GnRHR) and of the G protein-coupled receptor 54 (GPR54) genes in normosmic idiopathic hypogonadotropic hypogonadism patients (IHH).
Methods
In a retrospective study we analysed the *GnRHR* and the *GPR54* genes of 327 IHH patients including 105 females (36.5%) and 183 males (63.5%). Among the index cases (288 siblings) 267 were sporadic form (92.7%) and the others were included in 21 families (7.3%) with at least two affected siblings. Only 170 patients were tested for *GPR54* mutations. All females were diagnosed with primary amenorrhoea and 30.4% males presented with cryptorchidism. After informed consent, genomic DNA was amplified by PCR to obtain partially overlapping amplicons encompassing the exon-intron boundaries of the GnRHR and GPR54 genes and analyzed by DNA sequencing.
Results
Familial cases: ten of 21 (47.6%) IHH patients tested had mutations in either the *GnRHR* or the *GPR54* gene. Among the eight (38.1%) individuals bearing *GNRHR* mutations, 5 (23.8%) were homozygous or compound heterozygous and 3 (14.3%) were simple heterozygous. Among the 11 remaining patients, mutations of *GPR54* were found in two patients (18.2%): one (9.1%) at the homozygous state and the other one at the heterozygous state. *GnRHR* and *GPR54* mutations account for 7.5% and 2.5% respectively of sporadic cases.

The phenotype is depending on the nature of the genetic defect. GnRH administration fails to stimulate gonadotropin secretion when the biological activity of the mutated GnRHR is abolished (R139H, A129D, R139C) while a response is observed when the defect is partial (Q106R, R262Q).
Conclusion
The prevalence of GnRHR mutations is about three fold higher than that of GPR54. No genetic defect was identified in half of familial cases suggesting that additional genes play an important role in normal puberty.

P630

Assessment of non-enzymatic antioxidant profile of women on contraceptives

Oluyemi Akinloye[1], Oyabiyi S Adewale[2] & Ayo O Arowojolu[3]
[1]Department of Chemical Pathology, College of Health Sciences, Ladoke Akintola University, Osogbo, Oyo State, Nigeria; [2]Department of Chemical Pathology, University College Hospital, Ibadan, Oyo State, Nigeria; [3]Department of Obstetrics and Gynaecology, University College Hospital, Ibadan, Oyo State, Nigeria.

Objective
The use of contraceptives has been reported to interfere with absorption of micronutrients many of which serve as dietary antioxidant. The Changes in antioxidant levels may play significant role in the risk and pathophysiology of diseases associated with the use of contraceptives. This study was designed to assess the comparative antioxidant status of women on oral contraceptive pills (OCP), Injectables (INJ), Intrauterine Contraceptive Devices (IUCD) and Norplant (NP).
Methods
Participants were recruited (with informed consent) from the family planning clinics of the University College and Adeoyo Maternity Hospitals, Ibadan, Nigeria. A total of 118 apparently healthy (no alcohol and cigarette smoking), non-pregnant women consisting of 31 subjects on IUCD, 10 on OCP, 10 on INJ and 9 on NP. Likewise, 58 aged matched women not on contraceptives were recruited from same community as controls. Anthropometric indices and non-enzymatic antioxidant were evaluated using conventional methods. The study was approved by Ethical Committees of the Oyo State Government of Nigeria.
Results
Subjects on OCP had significantly lower vitamin C (50%), vitamin E (25%), albumin (20%), uric acid (31%) and selenium (69%) ($P<0.05$) when compared to

the controls. Significantly higher systolic BP and lower BMI were also observed in these subjects ($P<0.05$). The extend of lipid peroxidation (LPO) as evaluated by the level of malondialdehyde in the serum was significantly higher in subjects on OCP (62%) and IUCD (21%) ($P<0.05$) when compared to the controls.
Conclusions
These results indicate that while INJ and NP have no significant influence on antioxidant profile, IUCD remains the most acceptable in this community. Also, OCP has a tendency to depress the antioxidant status of its users. A routine monitoring of the antioxidant status of women on contraceptives especially OCP and IUCD and possible supplementation with dietary antioxidant may be warranted, particularly in developing countries.

P631

Hypothalamic-pituitary-gonadal axis responses of the male rats short and long time static magnetic fields (50 Hz) exposure

Akram Ahangarpour, Hadi Fathi Moghaddam, Mohammad Javad Tahmasebi Birgani, Hajie Shahbazian & Mohammad Badavi
Department of Physiology, School of Medicine, Jundishapour Medical Sciences, University of Ahwaz, Ahwaz, Iran.

Different studies have been done in the field of effect of magnetic field exposure on the biological organs. The aim of this study was to investigate the effects of static magnetic field (SMF) exposure on the secretion of hypothalamic-pituitary-gonadal axis in the male rats during short and long time exposed to SMFs (50 Hz).
Methods
Experiments have been done in four protocols. Each protocol included four groups (Wistar male rats, same range of age and weight) and each group contains 12 rats. After one-week adaptation they placed in exposure to SMF (0, 6, 12 and 24 mT) for 40 or 120 minutes daily for 17 or 34 days. All of protocols were started from 9:00 a.m. After experiments animals were anaesthetized, their blood has collected in separated tubes. Their serums were removed and kept frozen under -20 °C until use. Hormones were measured using gamma counter equipment with IRMA and RIA methods. The results were analyzed by ANOVA statistical method, followed by Tukey posthoc test ($P<0.05$).
Results
Subchronic exposures (40 min/day for 17 days) to SMFs have no effect on serum testosterone, LH and FSH levels. In contrast, SMFs (2 h/day for 17 days) induces a decrease of serum testosterone sham, vs. 6, 12 and 24 mT respectively (6.97 ± 1, vs. 4.66 ± 1.5; 2.6 ± 0.59 and 2.8 ± 0.64 ng/mL, $P<0.05$) and FSH levels (3.918 ± 1, vs. 2.1 ± 0.8; 0.765 ± 0.037 and 0.715 ± 0.01 mIU/mL, $P<0.05$).
Our results from third and forth protocols of experiments (40 min/day for 34 days) to 6 mT, SMF induces a increase of serum testosterone 6 mT *vs* sham, 12, and 24 mT respectively (7.53 ± 2, vs. 1.84 ± 0.6; 1.78 ± 0.3 and 1.63 ± 0.3 ng/mL, $P<0.05$) and 6 mT, SMF (2 h/day for 34 days) induces a increase of serum testosterone 6 mT *vs* 12 mT respectively (10.99 ± 3, vs. 2.6 ± 1; ng/mL, $P<0.05$).
Conclusions
Our results suggest that SMFs probably causes dysfunction in gonadal axis at the hypothalamic-pituitary level in male rats in different protocols. Subchronic exposure to short duration SMFs failed to alter hormonal levels in rat. In contrast, chronic exposure at low intensities increases testosterone.
Keywords: Magnetic fields; Rat; Testosterone; LH and FSH
All procedures carried out according to current and local National guidelines.

P632

Adrenal rest tissue in gonads in 70 French patients with classical congenital adrenal hyperplasia (21 hydroxylase deficiency)

Pierre Peggy[1], Despert Francois[1], Tranquart Francois[1], Chabrolle Christine[1], Kerlan Veronique[2], Sonnet Emmanuel[2], Metz Chantal[2], Baron Sabine[3], De Kerdanet Marc[6], Coutant Regis[4], Emy Philippe[5], Monceau Francoise[5], Bekka Said[1] & Lecomte Pierre[1]
[1]University, Tours, France; [2]University, Brest, France; [3]University, Nantes, France; [4]University, Angers, France; [5]Hospital, Orleans, France; [6]University, Rennes, France; [7]Hospital, Chartres, France.

Congenital adrenal hyperplasia (CAH) due to 21 hydroxylase deficiency is one of the most frequent endocrine genetic diseases. Adrenal rests have been described and can decrease fertility in men[1]. In a retrospective multi-center study we wanted 1.to evaluate the frequency of adrenal rests in classical forms of CAH (21-OH deficiency) by systematic ultrasonography (US); 2.to try to find the cause of this abnormality looking for a relationship between genotype and phenotype or with their therapeutic equilibrium. All patients with classical form of CAH and who have had an US were studied. In 24 women, none had adrenal rest in their ovaries, in accord with a very few cases published in the literature[2]. On the contrary, adrenal rests were detected in 30,4% of 46 men aged 1 to 38 years. We observed an increased frequency in testicular adrenal rest with age: none in the group less than 10 years, 15% in the group 10–17 years and 66,7% above 18 years. The role of an insufficiency in treatment in the development of adrenal rests has been evaluated. The therapeutic equilibrium is judged upon bone age and growth chart evolution in infants and upon 17 OHP or urinary pregnanetriol in adults. The appropriate equilibrium seems more often observed in patients without testicular adrenal rests: among patients, 55% without testicular adrenal rests were good treated during infancy compared to 14,3% with testicular adrenal rests, 44,4% compared to none during adolescence and 25% compared to 9% during adulthood. No relationship could be figure out between genotype and phenotype but the number of cases was probably too small in this cohort. Among 6 patients with adrenal rests and wishing fatherhood, an azoospermia was observed in 3; 2 had a very low sperm count and only 1 patient was able to procreate without any difficulty (2 children). In conclusion, ultrasonography of the ovaries is not usually necessary. On the opposite, testicular ultrasonography must be done during infancy, puberty and every five years during adulthood. This should reinforce better control of the disease by a more intensive treatment to try to reduce the number and volume of adrenal rests and improve fertility.

P633

Three months exercise training improves cardiopulmonary functional capacity in polycystic ovary syndrome

Francesco Orio[1], Francesco Giallauria[2], Stefano Palomba[3], Teresa Cascella[4], Laura Vuolo[4], Domenico Tafuri[5], Francesco Manguso[6], Gaetano Lombardi[4], Annamaria Colao[4] & Carlo Vigorito[2]
[1]Endocrinology, Faculty of Motor Science, "Parthenope" University of Naples and Department of Molecular & Clinical Endocrinology and Oncology, "Federico II" University of Naples, Naples, Italy; [2]Department of Clinical Medicine, Cardiovascular and Immunological Sciences, Cardiac Rehabilitation Unit, Institute of Internal Medicine and Metabolic Disease, "Federico II" University of Naples, Naples, Italy; [3]Department of Obstetrics and Gynecology, University "Magna Graecia" of Catanzaro, Catanzaro, Italy; [4]Department of Molecular & Clinical Endocrinology and Oncology, "Federico II" University of Naples, Naples, Italy; [5]Teaching and Methods of Sportive Activity, "Parthenope" University of Naples, Naples, Italy; [6]Gastroenterology Unit, "Cardarelli" Hospital, Naples, Naples, Italy.

Introduction and Aim
Polycystic Ovary Syndrome (PCOS) is an endocrine disease closely related to several risk factors for cardiovascular disease. Previous study demonstrated an impaired cardiopulmonary functional capacity in PCOS women. The present study was performed to evaluate the effects of 3-months exercise training (ET) programme on cardiopulmonary functional capacity in young women with PCOS.
Patients and Methods
The study was conducted according to the guidelines of the Declaration of Helsinki, and the institutional ethical committee approved the study protocol. The purpose of the protocol was explained to each subject, and written informed consent was obtained from each patient before beginning the study.

Ninety young PCOS women were randomly subdivided into two groups each composed of 45 subjects: PCOS-T (trained) group (age $=21.7\pm2.3$ years, BMI $=29.3\pm2.9$) underwent 3-months ET whereas PCOS-UnT (untrained) group (age $=21.9\pm1.9$ years, BMI $=29.3\pm3.1$) did not. At baseline and after 3 months, all patients were studied for their hormonal and metabolic profile, and underwent cardiopulmonary exercise test.
Results
After 3-month ET, PCOS-T showed a significant improvement in peak oxygen consumption ($+35.4\%$, $P<0.001$) and in maximal workload ($+37.2\%$, $P<0.001$). In PCOS-T we also observed a significant reduction of BMI (-4.5%, $P<0.001$) and C-reactive protein (-10%, $P<0.001$), and a significant ($P<0.001$) improvement of insulin sensitivity indexes. After 3 months, no changes were observed in PCOS-UnT.
Conclusions
Three-months ET improves cardiopulmonary functional capacity and insulin sensitivity in young PCOS women.

P634

Abnormal heart rate recovery after maximal cardiopulmonary exercise stress testing in polycystic ovary syndrome

Francesco Giallauria[1], Stefano Palomba[2], Teresa Cascella[3], Alessandra Vitelli[1], Luigi Maresca[1], Alessandra Grieco[3], Domenico Tafuri[4], Annamaria Colao[3], Gaetano Lombardi[3], Carlo Vigorito[1] & Francesco Orio[5]

[1]Department of Clinical Medicine, Cardiovascular and Immunological Sciences, Cardiac Rehabilitation Unit, Institute of Internal Medicine and Metabolic Disease, "Federico II" University of Naples, Naples, Italy; [2]Department of Obstetrics and Gynecology, University "Magna Graecia" of Catanzaro, Catanzaro, Italy; [3]Department of Molecular & Clinical Endocrinology and Oncology, "Federico II" University of Naples, Naples, Italy; [4]Teaching and Methods of Sportive Activity, "Parthenope" University of Naples, Naples, Italy; [5]Endocrinology, Faculty of Motor Science, "Parthenope" University of Naples and Department of Molecular & Clinical Endocrinology and Oncology, "Federico II" University of Naples, Naples, Italy.

Introduction and aim
Polycystic ovary syndrome (PCOS) is associated with an adverse metabolic and cardiovascular risk (CVR) profile, including: diabetes, insulin resistance, dyslipidemia and hypertension. Heart rate recovery (HRR) is a measure of autonomic dysfunction and an abnormal HRR is also associated with increased mortality. To date, no evaluation of autonomic function in young PCOS women has been performed, therefore the aim of the present study was to evaluate the HRR in PCOS.
Patients and methods
The study was approved by the local Ethical Committee. Forty-eight PCOS patients matched with 48 healthy women mean age (21.7 ± 2.2 vs. 21.9 ± 1.8, yrs$\pm$SD, respectively) and body mass index (29.5 ± 3.1 vs. 29.7 ± 3.6, kg/m^2 $\pm$ SD, respectively). Hormonal and metabolic pattern, cardiopulmonary functional capacity, as expressed by: maximal oxygen consumption (VO_{2max}) and oxygen consumption at anaerobic threshold (VO_{2AT}), and autonomic function, as expressed by HRR, were evaluated.
Results
In PCOS women we observed a significant ($P<0.001$) abnormal HRR (12.7 ± 2.1 vs. 20.8 ± 3.1 beats/min), and a significant impairment of: VO_{2max} (17.9 ± 2.3 vs. 29.0 ± 3.9, ml/Kg/min) and VO_{2AT} (13.1 ± 2.6 vs. 24.1 ± 3.1, ml/Kg/min) compared to healthy women. In PCOS patients, abnormal HRR was inversely correlated to BMI ($r=-0.700$, $P<0.0001$), HOMA ($r=-0.680$, $P<0.0001$) and AUC_{INS} ($r=-0.640$, $P<0.0001$).
Conclusions
Our data are the first to demonstrate an abnormal HRR after maximal cardiopulmonary exercise stress testing in young overweight PCOS patients, adding HRR as a further potential marker of increased CVR in PCOS.

P635

Age at menarche in relation to adult obesity

Taner Bayraktaroglu, Ayse Sertkaya Cikim, Adil Dogan Azezli & Yusuf Orhan
Istanbul Faculty of Medicine, Endocrinology and Metabolism, Endocrinology and Metabolism, Capa, Fatih/Istanbul, Turkey.

Introduction
Age at menarche reflects various health aspects including the timing of sexual maturation, growth and nutritional status, and enviromental conditions. This study carried out assesment obesity risk and the relationship between obesity, metabolic parameters and age at menarche in a series of Turkish women.
Materials and methods
In a survey of obesity outpatient clinic, 4212 women who have mean age 38.5 ± 12.1 years and ages at menarche between 9 – 18 years enrolled tertiles to the study: Group I, age at menarche 9 – 11 years ($n=476$, 11.3%); group II, 12 – 14 years ($n=2319$, 55.1%); group III, > 14 years ($n=1417$, 33.6%). According to ages at menarche, body size variables (height, weight, BMI, waist circumferences) and metabolic parameters were determined and compared between groups.
Results
There were 270 (6.4%) subjects with BMI < 25 kg/m^2, 800 (19.0%) with overweight (BMI 25 – 30 kg/m^2) and 3142 (74.6%) with obesity (BMI > 30 kg/m^2). Mean adult height was shorter (157.7 ± 6.3 yr, 158.4 ± 6.2 yr and 158.9 ± 6.4 yr, respectively) and BMI values (35.6 ± 7.4 kg/m^2, 34.8 ± 7.2 kg/m^2 and 34.6 ± 7.3 kg/m^2, respectively) were greater in group I with the lowest age at menarche than others ($P<0.05$). However, blood pressures, fasting glucose, insulin, total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides levels, waist to hip ratio and HOMA values were not different between groups.
Conclusion
Few studies have examined inverse association of age at menarche with adult BMI and the tendency of BMI to track between childhood and adult life. Age at menarche may simply be a marker for the pace of sexual maturation, leads to differences in adiposity that track into adult life. Our data suggest that children with earlier ages at menarche should be nearest follow-up to prevent the adulthood obesity.

P636

The multi-PDZ domain protein MUPP1: a scaffolding protein controlling the acrosome reaction in mammalian spermatozoa

Frauke Ackermann[1], Dorke Meyer[1], Diana Heydecke[1], Beate Wilhelm[2], Thomas Gudermann[1] & Ingrid Boekhoff[1]
[1]Department of Pharmacology, Philipps University, Marburg, Germany; [2]Department of Anatomy and Cell Biology, Philipps University, Marburg, Germany.

Upon adhesion to the zona pellucida, mammalian sperm undergo regulated exocytosis of the acrosome. Despite the difference in size, some parallels can be drawn concerning the signal transduction processes controlling the sperm acrosome reaction and synaptic vesicle exocytosis. Since components of signal transduction pathways are often organized in multiprotein signalling complexes, attempts were made to identify scaffolding proteins expressed in the acrosomal region of mammalian spermatozoa. Using RT-PCR approaches and immunohistochemical experiments, the Multi-PDZ domain Protein MUPP1, which comprises 13 potential protein interaction modules, was identified in mouse testis. Immunocytochemical experiments combined with immunogold electron microscopy revealed that MUPP1 is exclusively detectable within the acrosomal region of different mammalian spermatozoa and that the MUPP1 protein is most prominent at the outer acrosomal membrane. To assess the possible function of MUPP1, the acrosome reaction was monitored using the photosensitive calcium chelator NP-EGTA-AM and an inhibitory anti-MUPP1 antibody. This functional assay revealed that antibody treatment significantly reduces acrosome reaction compared to control conditions. These results together with the observation that MUPP1 co-migrates in detergent-insoluble lipid rafts along with proteins involved in acrosomal exocytosis, like syntaxin-2, indicates that MUPP1 in different mammalian species may assemble similar, if not identical signaling molecules controlling acrosomal exocytosis.

P637

Expression of the G-protein α-subunit gustducin in mammalian spermatozoa

Dorke Meyer[1], Johanna Fehr[1], Heike Borth[1], Patricia Widmayer[2], Beate Wilhelm[3], Thomas Gudermann[1] & Ingrid Boekhoff[1]
[1]Department of Pharmacology, Philipps University, Marburg, Germany; [2]Department of Physiology, University of Hohenheim, Stuttgart-Hohenheim, Germany; [3]Department of Anatomy and Cell Biology, Philipps University, Marburg, Germany.

The G protein subunit α-gustducin is generally accepted as a marker for chemosensitive cells. Since chemosensation is especially important for the navigation of sperm towards the egg, attempts were made to explore whether α-gustducin might also be expressed in spermatozoa. RT-PCR experiments revealed that a gustducin PCR specific RNA fragment with the predicted size could be amplified from total mouse and rat testis. To identify the testicular cell type in which α-gustducin is expressed, immunohistochemical experiments were performed with an anti-gustducin-specific antibody. The most intense immunoreactivity was visible in differentiating spermatids localized in the lumen of the seminiferous tubules whereas no staining was detectable in spermatogonia. To verify whether α-gustducin is still expressed in mature spermatozoa, mouse and rat sperm were subjected to immunocytochemistry as well as electron microscopy. A strong staining of the innerdense fibres was obtained within the flagellum. Similarly, analyzing human sperm for α-gustducin staining also revealed a strong labeling of the midpiece of the flagellum whereas the principle piece remained unstained. The observation that α-gustducin is expressed in the tail of mammalian spermatozoa may now motivate to identify the corresponding signaling cascade, probably defining the functional role of α-gustducin in spermatozoa.

P638

Delayed puberty with extreme uterine hypotrophy: do not conclude too early to the absence of the uterus

Edwige Yollin, Sophie Catteau-Jonard, Anne-Céline Reyss, Christine Cortet & Didier Dewailly
Lille University Hospital, Lille, France.

Objective
To emphasize the difficulties to distinguish between uterine agenesis and extreme uterine hypotrophy in the context of primary amenorrhea with delayed puberty.
Patients and methods
Among adolescents who consulted our center because of primary amenorrhea, from 1997 to 2005, three patients were referred for a suspicion of Mayer-Rokitansky-Kuster-Hauser Syndrome, after ultrasonography had failed visualizing the uterus. The 3 patients underwent endocrine and genetic evaluations. Pelvic examination was performed by transabdominal ultrasonography and MRI. Patients were placed under estrogen treatment.
Results
Endocrine evaluation indicated Primary Ovarian Failure for patient 1, and Hypogonadotrophic Hypogonadism for patients 2 and 3. Karyotype was 46,XX in all patients. Initial pelvic ultrasonography revealed the absence of uterus. MRI allowed vizualizing prepubertal uterus for patient 1, a hypotrophic uterus for patient 3 and concluded to uterine agenesis for patient 2. In all cases estradiol substitutive therapy induced uterine growth and confirmed retrospectively the diagnosis of extreme uterine hypotrophy.
Conclusion
Pelvic ultrasonography can be misleading in the evaluation of primary amenorrhea. No visualization of uterus on ultrasonography can occur in the context of delayed puberty and should not induce a premature diagnosis of Mayer-Rokitansky-Kuster-Hauser Syndrome. Indeed, such a diagnosis has therapeutic, reproductive and psychological consequences.

P639

Screening and treatment of gestational diabetes and impaired glucose tolerance in Georgia

Natalia Asatiani[1], Ramaz Kurashvili[2], Mzia Dundua[3], Elena Shelestova[1], Liana Tsutskiridze[1], Moche Hod[1], Sergey Smirnov[1] & Victor Vlasov[1]
[1]Georgian Diabetes Center, Tbilisi, Georgia; [2]Dep.of Obstetrics and Gynecology, Rabin Medical Center, Tel-Aviv, Israel; [3]Novo Nordisk A/S, Copenhagen, Denmark.

Background and Aim
One of the greatest problem in pregnancy, complicated by diabetes is screening and appropriate care for women, whose diabetes is manifested during pregnancy. The study is aimed at gathering epidemiological data on gestational diabetes mellitus (GDM) and evaluating the of appropriate therapy in this condition.
Materials and Methods
Screening for GDM was carried out in – 692 pregnant women. GDM was observed in 3.3% (22 women) and impaired glucose tolerance (IGT) - in 5.05% (35 women). 57 women with GDM and IGT comprised the Group 1 (Gr.). Fifteen pregnant women, who were not screened timely and attended the Center at 10-34 week of gestation with fasting hyperglicemia and ketoacidoses comprised the Gr.2.
Results
In Gr.2 HbA1c ($9.5 \pm 1.7\%$) levels at entry were statistically higher, than in Gr.1 ($6.3 \pm 0.93\%$)($P=0.000$). By the end of the 3rd trimester those indices dropped (($6.3 \pm 0.72\%$; $5.45 \pm 0.74\%$ respectively). In Gr.1 following pathologies were observed: pre-eclampsia had 2(3.5%), preterm delivery – 2(3.5%), macrosomia – 9(15.7%), perinatal deaths – 0. In Gr.2: pre-eclampsia had 4(26.6%), preterm delivery – 3(20%), macrosomia – 3(20%), perinatal deaths – 4(26.6%). Mean infants' birth weight (g) was 586 ± 323 (Gr.1) and $3\,260 \pm 445$ (Gr2).
Conclusion
Following epidemiological data were obtained for Georgia: IGT (5.05%) and GDM (3.3%). Good glycemia control during pregnancy not always prevents macrosomia, though significantly reduces the risk of pre-eclampsia, preterm delivery and perinatal deaths.

P640

The effects of cycloheximide, actinomycine D and indomethacin on progesterone release stimulated by PACAP 38 from cultured rat ovarian granulosa cells

Elzbieta Wasilewska-Dziubinska, Magdalena Chmielowska, Ewa Wolinska-Witort, Lidia Martynska, Wojciech Bik & Boguslawa Baranowska
Medical Centre of Postgraduate Education; Neuroendocrinology Department, Warsaw, Poland.

Neuropeptide PACAP 38 expressed in steroidogenic ovarian cells of rat could be an auto- or paracrine regulator of progesterone synthesis. Moreover, it has been shown that PACAP38 can affect ovarian secretion of prostaglandins. The first and rate-limiting step in the biosynthesis of progesterone is the transfer of cholesterol into mitochondria which is facilitated by the cycloheximide-sensitive steroid acute regulator (StAR) protein. StAR protein has been established as an essential factor required for the acute response of steroidogenic cells to trophic stimulation. It seems that PACAP 38 stimulates ovarian progesterone synthesis directly or indirectly influencing on StAR protein activity. In the present study we examined the effects of cycloheximide (an inhibitor of StAR protein synthesis), actinomycine D (an inhibitor of RNA synthesis) and indomethacin (an inhibitor of prostaglandins synthesis) on progesterone release stimulated by PACAP38 from primary culture of ovarian granulosa cells obtained from adult cyclic rat (diestrus). As exogenous substrate for progesterone synthesis 20-hydroxycholesterol, which can readily diffuse across the mitochondrial membranes to the P450 scc was used. Progesterone concentrations in supernatants were assayed by RIA method. After 2 h incubation progesterone release stimulated by PACAP38 was totally inhibited by cycloheximide and partially inhibited by actinomycine D. After 24 h incubation progesterone release stimulated with PACA38 was totally inhibited by actinomycine D and also by indomethacin. These data suggest that ongoing StAR protein synthesis is partially inhibited by actinomycine D during 2 h incubation, but that during 24 h incubation continuing synthesis requires transcriptional activity.
Conclusion
in primary culture of rat ovarian granulosa cells stimulatory effect of PACAP38 on progesterone release is connected with stimulation of StAR protein synthesis and may be mediated by local synthesis of prostaglandins.
This study was supported by CMKP grant 501-1-1-28-32/05

P641

XX-male syndrome: clinical, hormonal and molecular genetic findings in comparison to Klinefelter patients and normal men

Elena Vorona, Michael Zitzmann, Joerg Gromoll, Manuela Simoni & Eberhard Nieschlag
Institute of Reproductive Medicine of the University, Muenster, Germany.

Background
The rare 46, XX-male syndrome has to be distinguished from more frequent forms of hypogonadism, especially the Klinefelter syndrome (47, XXY). We report 11 cases of SRY-positive XX-males in comparison to 101 age-matched Klinefelter patients and 78 age-matched normal men in a case-control study.
Methods
The comparison included results from the physical examination, endocrinological data, semen analysis, cytogenetic and molecular genetic findings. X-chromosome inactivation analysis with inactivation of the androgen receptor (AR) alleles was performed in 10 heterozygous XX-male patients and the findings were compared to the X-chromosome inactivation pattern in Klinefelter patients and in women.
Results
The XX-males were significantly smaller than Klinefelter patients or normal men. The incidence of maldescended testes and gynecomastia was significantly higher than in both control groups. Most XX-male patients were hypogonadal and require testosterone replacement therapy. All investigated XX-males were infertile. The absolute X-chromosome inactivation in XX males was significantly different from random. Seven out of ten XX-male patients showed skewed X-chromosome inactivation ratios ($<20\%$ or $>80\%$) with an equal proportion (distribution) of the X-inactivation on the short and on the long AR alleles. Two had highly skewed ratios of 2:98 and 99:1. There was no preference towards the longer or the shorter AR allele. The patients with skewed inactivation ratios showed no tendencies towards any special diseases.
Conclusions
Our study demonstrates that XX-males are distinct from other patients with hypogonadism due to chromosome disorders with two X chromosomes, such as Klinefelter patients. This is reflected by decreased body height and increased rate of maldescended testes. Two thirds of XX males in our group had non-random X-chromosome inactivation ratios. A reason for the skewed X-chromosome inactivation in these patients may be an X-chromosome abnormality, namely the translocated SRY gene.

P642

Expansion of $CD4^+CD25^+$ regulatory T cells during murine pregnancy is not driven by pregnancy-associated hormones

Anne Schumacher, Catharina Thüre, Juliane Rau, Hans-Dieter Volk & Ana Claudia Zenclussen
Institut für Medizinische Immunologie, Berlin, Germany.

The physiological state of pregnancy is characterised by the tolerance of the maternal immune system towards the paternal alloantigens expressed by the foetus. Recently, $CD4^+CD25^+$ regulatory T cells (Treg) were described to play an essential role for the generation and maintenance of the tolerance state. Several research groups showed that normal pregnancy in humans and mice is associated with an augmentation in the number of Treg in different organs whereas females suffering from abortion displayed diminished numbers of Treg. We showed that the adoptive transfer of Treg from normal pregnant CBA/J ($H2^k$) females previously mated with BALB/c ($H2^d$) males into abortion-prone mice (DBA/2J-mated CBA/J females) is able to protect the semiallogeneic ($H2^d/H2^k$) foetus from maternal immune rejection. In addition, we could confirm that Treg from virgin mice could not rescue from abortion. In the light of these results, we postulated that the expansion of Treg is either driven by the presence of paternal/fetal antigens or by pregnancy-associated hormones. We therefore mated CBA/J females either with BALB/c- or DBA/2J males and determined the levels of progesterone and estradiol by chemiluminescence at different time points of pregnancy (day 0, 2, 5, 8, 10 and 12). In addition, we defined the levels of progesterone in Treg-treated mice on day 14 of pregnancy. We observed comparable levels of progesterone, estrone and estradiol in both, normal and abortion-prone animals. Treg treatment, which was effective in diminishing the abortion rate, did not modify the hormonal levels. Our data suggest that pregnancy-associated hormones are not crucial for the expansion of the Treg population and that this is rather driven by specific paternal alloantigens.

P643

The clinical outcomes of stimulation of ovulation in patients with idiopathic hypogonadotropic hypogonadism (IHH) caused by mutations of GnRH receptor

Slawomir Wolczynski, Monika Zbucka & Katarzyna Jarzabek
Department of Reproduction and Gynecological Endocrinology, Medical University, Bialystok, Poland.

We report pregnancies obtained after stimulation by gonadotropins in 3 patients with IHH caused by homozygous or compound heterozygous mutations of GnRH receptor: (R139H/R262Q, R139C/ R139C, R139H/ Phe 308 del.);

Gonadotropin response to GnRH was observed in the first patient. All the patients were stimulated with gonadotrophins according to the protocole step up with the initial dose of 150IU FSH and 75 IU LH a day. The luteal phase was suported by hCG and progesterone. After 14 days of stimulation in the patient with R139H/R262Q mutated receptor, the estradiol concentration was 540 pg/ml and two mature follicles were observed. That patient was pregnant and gave birth. Patient with R139C/ R139C mutated receptor required higher doses and much longer stimulation, 225 IU FSH and 150 IU LH for 21 days. Compare to the estradiol concentration (620 pg/m) she developed three mature follicles and lot of small follicules. She conceived with triple pregnancies. The first trimester was complicated with OHHS. She miscarried at 22 weeks. In the second stimulation with the same doses for 21 days the estradiol concentration was 580 pg/ml, she was pregnant, the first trimester was also complicated with OHHS and she had twins.

The patient with R139H/ Phe 308 del required 225IU FSH and 150IU LH for 22 days and the estradiol concentration was 560 pg/ml and in the ovary three mature follicles and lots of small follicules was observed. She was pregnant,the first trimester was complicated with OHHS. Right now she is in 27 weeks of amenorrhea.

Conclusions

Patients with the mutations of GnRH receptor type loss off require much longer stimulation with higher doses comparing to IHH patients without GnRH receptor mutations. Despite low estradiol concentration the risk of OHSS and multiple pregnancy is high

P644

Atherogenic indexes in women with premature ovarian failure-POF

Ljiljana Marina, Svetlana Vujovic, Miomira Ivovic, Milos Stojanovic, Milina Tancic-Gajic, Marija Barac & Milka Drezgic
Institute of endocrinology, Belgrade, Serbia.

POF is a disease of unclear origin affecting women under age 40. They are at high risk of cardiovascular diseases (CVD).

The objective of this study was to compare the lipid status and atherogenic indexes - total cholesterol/high density lipoprotein (TC/HDL) and low density lipoprotein/HDL (LDL/HDL) - of women with POF with healthy women of the same age and the same BMI.

We evaluated 54 women in two groups. 1st group: 31 women with POF, mean age 29.55 ± 4.3 years, mean BMI 22.4 ± 2.78 kg/m^2, with laboratory proven menopausal levels of FSH and LH and on hormone replacement therapy (HRT). 2nd group: 23 healthy women, mean age 26.73 ± 6.08 years, mean BMI 20.95 ± 2.66 kg/m^2. Statistical analysis was performed with *t*-Test.

There was no difference in age and BMI between the groups, $P > 0.05$. Mean TC in the 1st group was 5.33 ± 0.71 mmol/l and in the 2nd 4.34 ± 0.58 mmol/l. Mean HDL in the 1st group was 1.34 ± 0.35 mmol/l and 1.42 ± 0.29 mmol/l in the 2nd. Mean LDL in the 1st group was 3.53 ± 0.47 mmol/l and 2.6 ± 0.55 mmol/l in the 2nd. In the 1st group mean triglycerides were 1.21 ± 0.53 mmol/l and in the 2nd 0.83 ± 0.27 mmol/l. Mean TC/HDL in the 1st group was 4.21 ± 1.09 and in the 2nd 3.16 ± 0.57 and mean LDL/HDL in the 1st group was 2.83 ± 0.87 and 1.89 ± 0.51 in the 2nd. The difference between the 1st and the 2nd group was highly significant for TC, LDL and triglycerides as well as for atherogenic indexes TC/HDL and LDL/HDL, $P < 0.01$. There was no difference between the groups for HDL.

Our results show that women with POF are at higher risk of CVD than healthy women of the same age and the same BMI. HRT is of essential importance for these women and according to our study it is necessary to check their lipid status on regular basis.

P645

Effect of age and testosterone on sleep related erections

Antonio R M Granata, Vincenzo Rochira, Bruno Madeo, Valeria Pugni, Lucia Zirilli & Cesare Carani
Integrated Department of Medicine, Endocrinology, Metabolism and Geriatrics, University of Modena and Reggio Emilia, Modena, Italy.

Introduction

In order to study the effect of age and testosterone on sleep related erections, we enrolled 209 men (122: age 30–49 years; 87: age over 50 years), including mild and severe hypogonadal subjects (129) and eugonadal subjects (80).

Subjects and methods

The subjects were assigned to four groups, according to their testosterone serum levels. All the subjects underwent nocturnal penile tumescence and rigidity monitoring (NPTRM). The following sleep-related erection parameters were analyzed: total number of valid erections, total duration of rigidity $> 60\%$, total duration of increase in penile tumescence > 30 mm, maximum rigidity and maximum increase in penile tumescence.

Results

Total number of valid erections, total duration of rigidity $> 60\%$ and total duration of increase in penile tumescence > 30 mm showed constant lower values in the 4 groups of men over 50 years, when compared with the 4 groups of men with age range 30–49 years and with the same testosterone level. Moreover, when comparing groups of men with same age but different testosterone levels, a threshold was identified still for the previous 3 parameters: the more the T is lower than 8 nmol/L, the more sleep-related erections are impaired, but this pattern is lost when T is higher than 8 nmol/L. On the other hand, maximum rigidity and maximum increase in penile tumescence showed the same trend of the other parameters when groups with different age range are compared, but these 2 parameters were uninfluenced by testosterone levels.

Conclusions

Aging has an impairing role on sleep-related erections both in hypogonadal and eugonadal men, while testosterone has an higher effect only on some of the parameters we investigated.

P646

Hormonal and seminal parameters in patients with testicular neoplasia or lymphoproliferative disorders: two year follow up

Cataldo Di Bisceglie, Angela Bertagna, Fabio Lanfranco, Matteo Baldi, Anna Maria Barberis, Lucrezia Zurzolo & Chiara Manieri
Division of Endocrinology and Metabolic Diseases, Department of Internal Medicine, University of Turin, Turin, Italy.

Semen quality and reproductive hormones may be affected in men with testicular neoplasia (TN) and lymphoproliferative disorders (LD). We evaluated these

parameters before, 6, 12 and 24 months after the end of the oncological treatments in 60 patients with TN, and in 35 patients with LD. The patients were divided on the bases of the basal sperm concentration (A< and B≥ 10 million/ml). FSH, LH, testosterone (T) and inhibin B levels and sperm parameters were evaluated in all patients. The patients with TN showed a significant reduction of inhibin B levels and a significant increase of FSH levels 6 and 12 months after the end of the oncological treatments; LH levels showed a significant increase after 6 and 12 months only in patients of group A; T levels showed no variations after the treatment. Sperm concentration showed a significant reduction after 6 and 12 months only in patients of group B. The patients with LD showed a significant reduction of inhibin B levels after 6 and 12 months and a significant increase of FSH levels after 6, 12 and 24 months; LH and T levels showed no variations after the treatment. Sperm concentration showed a significant reduction after 6 and 12 months only in patients of group B. After 24 months reproductive hormones, except for FSH levels in LD, and sperm concentration showed no significant differences compared to basal levels. The other sperm parameters were not significantly affected by the treatment in all patients (TN and LD). In conclusion, the effect of the oncological treatments on sperm concentration is less evident in patients of group A, probably due to a predominant influence of the neoplastic condition. After 24 months we observed an improvement of the hormonal and seminal parameters in TN, except for a persistent iatrogenic effect in LD.

P647

Immunohistochemical evaluation of ghrelin expression in polycystic ovaries in patients with PCOS.

Hanna Komarowska[1], Ryszard Wasko[1], Katarzyna Iwanik[2], Przemyslaw Majewski[2], Alina Warenik Szymankiewicz[3], Ludmila Rafinska[4], Magdalena Jaskula[1] & Jerzy Sowinski[1]
[1]Departament of Endocrinology, Metabolism and Internal Medicine, Poznan University of, Poznan, Poland; [2]Depatment of Clinic Pathomorphology, Poznan University of Medical Sciences, Poznan, Poland; [3]Department of Gynecological Endocrinology, Poznan University of Medical Sciences, Poznan, Poland; [4]Department of Histology and Embryology, Poznan; University of Medical Sciences, Poznan, Poland.

Ghrelin is an endogenous ligand of the GH secretagogue receptor. The influence of ghrelin on different organs has been studied recently e.g. in the regulation of pituitary hormone release, regulation of energy homeostasis, glucose metabolism and insulin secretion, cell proliferation and reproductive function.

The etiology of PCOS has not been firmly explained, althought several pathways have been implicated – the regulatory pathways of steroid hormone synthesis, regulatory pathways of gonadotropin and GH-IGF-1 axis action, the insulin signaling pathway and pathways regulating body weight. Ghrelin seems to link these pathways.

The aim of our study was to estimate the presence of ghrelin in polycystic ovaries cells and evaluation of the relationship betwen ghrelin occurence and cells proliferation.

Methods

Ten polycystic ovaries and ovaries without pathology as the control group were compared. The ghrelin was detected using two different immunohistochemical metods with the polyclonal rabbit anti-ghrelin antibodies (Phoenix Pharmaceuticals Inc.). The cells proliferation was estimated by Ki 67 proliferation index.

Results

Ghrelin immunostaining was demonstrated in cytoplasm of ovarian secondary interstitial cells and in regressing corpora lutea. The cell nuclei were ghrelin positive in granulosa and theca layers of follicular cyst in both groups and in luteal cells of young corpora lutea in healthy ovaries. Ki 67 immunostaining was observed in granulosa and theca layers of follicular cyst in polycystic and healthy ovaries.

Conclusions

It is possible that local ghrelin expression plays an important role in the direct control of ovarian development and function and ghrelin may participate in patomechanism of PCOS.

The local Ethical Committee approved the study.

P648

Ovarian hyperstimulation syndrome during IVF induction revealing a gonadotroph adenoma

Pierre Lecomte[1], Christine Chabrolle[1], Peggy Pierre[1], Michel Jan[1] & Jacqueline Trouillas[2]
[1]Chru Bretonneau, Tours, France; [2]Université Laennec, Lyon, France.

Gonadotroph adenomas are usually detected by their local mass effects. Spontaneous ovarian hyperstimulation syndrome (OHS) has rarely been described as the main manifestation of gonadotroph adenomas in young women. We present a case with a prolonged OHS occurring during IVF ovarian induction leading to the discovery of a FSH pituitary tumour.

Case report

A 36 year-old, normal weight woman with 2 years primary infertility linked to oligomenorrhea and anovulation was included in an IVF program. PRL, androgens and gonadotrophins evaluation before ovulation induction was normal. She had presented a few weeks before a mild OHS after a five days single tablet of clomifene citrate. Before IVF induction, FSH and LH levels were 5 and 3 UI/l. Daily Decapeptyl treatment was started on January 7th for 12 days. Then long-acting Decapeptyl 3 mg was injected on January 18th after hormonal control. E2 level was very high (7300 ng/l) and enlarged ovaries were discovered with transvaginal u.s.: right 87×60 mm and left 69×50 mm with follicles and cysts (15–35 mm). Two days later, pelvic pain and more enlarged ovaries were treated with puncture but cysts quickly reappeared. One month after long-acting GnRH analog injection, E2 and inhibin B were elevated (2300 ng/l and 343 ng/l) and FSH and LH still detectable: 3 and 1.1 UI/l. Since OHS persisted, a gonadotroph adenoma was suspected. A 10 mm adenoma was found in the right part of the pituitary with MRI. Before surgery, FSH and αSU were elevated with no response after GnRH test, in contrast to LH which increased. At the end of March, the surgeon removed a right microadenoma and the pathologist confirmed a gonadotroph adenoma: all cells stained for βFSH and 5% reacted with anti- αSU and anti-βLH antisera. Shortly after surgery, hormone levels normalized and an ovulatory cycle was observed but ovarian size was persistently increased (30 mm cysts) 4 months after initial stimulation.

Conclusion

This case is unusual: OHS observed during an IVF program persisted and elevated FSH after GnRH long-acting analog allowed discovering a FSH pituitary adenoma cured by surgery. Enlarged ovaries were still detected 4 months after initial ovulation induction.

P649

Insulin levels and lipid profile in lean women with polycystic ovary syndrome

Mirjana Stojkovic, Milos Zarkovic, Jasmina Ciric, Biljana Beleslin, Slavica Savic, Djordje Marina, Bozo Trbojevic & Milka Drezgic
Institute of endocrinology, Belgrade, Serbia.

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. Insulin resistance is one of the features of PCOS and potentially affect lipid metabolism.

Objectives

The aim of this study was to compare basal insulin levels and lipid profile in lean women with PCOS with weight matched healthy controls.

Methods

The study group consisted of 64 women divided in two subgroups (1. PCOS group, $n=48$; age 25.7 ± 6.2; BMI 21.3 ± 1.9 kg/m^2. 2. group of healthy controls, $n=16$, age 26.8 ± 6.4; BMI 20.3 ± 1.6 kg/m^2). Data were analyzed by the t test.

Results

Mean basal glucose levels were 4.38 ± 0.46 mmol/L vs. 4.54 ± 0.23 mmol/L, without statistically significant difference between groups. Mean basal insulin levels were significantly higher in PCOS group than in healthy controls (24.82 ± 16.34 mIU/L vs. 6.47 ± 3.19 mIU/L; $P=0{,}001$;). Cholesterol, HDL and LDL cholesterol levels did not reach statistically significant difference between groups, while triglyceride levels were significantly higher in PCOS group than in healthy controls (1.05 ± 0.44 mmol/L vs. 0.73 ± 0.22; $P=0{,}009$;).

Conclusions

These data suggest that PCOS per se, without obesity, affects insulin secretion and lipid metabolism, mainly in triglyceride levels which enhances atherogenic potential in this subjects.

P650

Demonstration of estrogen receptor–β in human gonadotropin-releasing hormone neurons

Erik Hrabovszky[1], Imre Kallo[1], Nora Szlavik[2], Eva Keller[2], Istvan Merchenthaler[3] & Zsolt Liposits[1]

[1]Laboratory of Endocrine Neurobiology, Institute of Experimental Medicine, Hungarian Academy of Sciences, Budapest, 1083 Hungary, Budapest, Hungary; [2]Department of Forensic Medicine, Semmelweis University, Budapest, Hungary; [3]Department of Epidemiology and Preventive Medicine, University of Maryland, Baltimore, MD, United States.

The gonadotropin-releasing hormone (GnRH) neurosecretory system represents the final common hypothalamic pathway in the neuroendocrine control of reproduction. Changing levels of the ovarian sex steroid hormone 17β-estradiol (E_2) tightly regulate the activity of GnRH cells via feedback actions. Recently, our group has localized the second isoform of estrogen receptors (ER-β) within GnRH neurons of the rat brain, indicating that GnRH cells are capable of directly sensing circulating estrogens. To address the issue of whether GnRH neurons of the human hypothalamus also contain ER-β, we have carried out dual-label immunocytochemical studies on autopsy samples. Research protocols to obtain and handle tissues were reviewed and approved by the Regional Committee of Science and Research Ethics (TUKEB 49/1999). Combined technical efforts that minimized *post-mortem* interval (<24 h), optimized fixation conditions (use of a mixture of 2% paraformaldehyde and 4% acrolein) and sensitized the immunocytochemical detection (application of silver-intensified nickel-diaminobenzidine chromogen) allowed the visualization of nuclear ER-β immunoreactivity in 10.8–28.0% of GnRH neurons in the preoptic/hypothalamic area of male human individuals. The demonstration of ER-β in human GnRH cells, which lack the classical ER-α receptor isoform, indicate that estrogens may exert direct actions upon GnRH cells selectively through ER-β. In the light of the differing ligand binding characteristics of ER-β from those of ER-α, this discovery offers a potential novel approach to influence estrogen feed-back mechanisms to GnRH neurons through the recently available ER-β-selective ligands.

P651

Asymmetric dimethylarginine levels and carotid intima media thickness in patients with polycystic ovary syndrome

Baris Pamuk, Mustafa Kulaksizoglu, Ayse Nur Torun, Derun Taner Ertugrul, Ozgur Ciftci, Emel Ozcimen, Ayla Uckuyu, Sevsen Kulaksizoglu, Nilufer Bayraktar & Nilgun Guvener Demirag
Baskent University, Ankara, Turkey.

Increased plasma concentration of asymmetric dimethylarginine (ADMA), an endogenous inhibitor of nitric oxide synthase, has been associated with cardiovasular risk factors. The aim of this study was evaluate plasma ADMA levels and subclinical atherosclerosis in patients with polycystic ovary syndrome (PCOS) and healty controls.

Thirty-five patients with PCOS and age, body mass index (BMI) matced thirty-one healty subjects were included in the study.PCOS was defined according to Rotterdam criterion. Serum levels of follicle stimulating hormone (FSH), luteinizing hormone (LH), dehydroepiandrosterone sulfat (DHEAS), free testosterone and total testosterone were measured. Serum insulin and plasma glucose levels measured at baseline before the oral glucose tolerance test. Insulin resistance was evaluated by homeostasis model assesment (HOMA IR). Also serum concentrations of total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL C), triglycerides (TG), homocysteine, fibrinogen, C reactive protein(CRP) were determined. Plasma ADMA levels were measured. Intima media thickness (IMT) assessment of the common carotid artery (CCA) were performed ultrasonographically.

The PCOS group had higher levels of androgens, TG, homocysteine, insulin, and HOMA IR. ($P<0.05$). There were no significant differences in ADMA levels and IMT between two groups. Also FPG, TC, HDL C, LDL C, fibrinogen and CRP levels were not different among the groups ($P>0.05$). IMT was significantly correlated with DHEAS but no association was determined with ADMA.

To our knowledge, this is the first study that assessed ADMA levels in patients with PCOS. The results of the study showed that ADMA levels and IMT were not different in PCOS patients from healty controls.

P652

Ghrelin levels in obese patients with polycistic ovary syndrome

Danica Stamenkovic-Pejkovic, Dragan Micic, Mirjana Sumarac-Dumanovic, Aleksandra Kendereski, Goran Cvijovic, Svetlana Zoric & Maja Georgiev
Institute of Endocrinology, Diabetes and Diseases of Metabolism, Belgrade, Serbia.

It was shown that obesity and insulin resistance may influence ghrelin levels. Contraversial results were observed considering ghrelin levels in women with polycystic ovary syndrome (PCOS). The aim of our study was to investigate the basal ghrelin levels in obese patients with PCOS and to evaluate possible correlations between ghrelin levels, insulin resistance and, androgen levels. In 10 obese PCOS patients (BMI 32.50 ± 1.57 kg/m^2, age: 21.4 ± 0.85 years) and 8 obese controls (BMI 32.54 ± 1.95 kg/m^2, age: 28.12 ± 1.51 years) basal ghrelin (RIA, Linco, USA, pg/ml) and testosteron (RIA, INEP, nmol/l) levels were determinated. Insulin sensitivity was determinated using euglycemic hyperinsulinemic clamp (M index).

Results

There was significant difference in ghrelin levels between PCOS patients and controls (42.65 ± 26.91 vs 96.33 ± 37.34, $P<0.05$), while M index was lower in PCOS patients but there was no significant difference (2.39 ± 0.59 vs 3.46 ± 0.92, $P>0.05$). There was negative correlation between ghrelin and testosterone levels ($r=-0.78$, $P<0.05$) and there was no correlation between ghrelin levels and M index ($r=-0.12$, $P>0.05$). In conclusion, obese PCOS patients have lower ghrelin levels than obese heathy women. In addition, a negative correlation between ghrelin and testosteron levels might suggest an interaction between ghrelin and steroid synthesis or action.

P653

Ghrelin levels in lean patients with polycistic ovary syndrome

Maja Georgiev[1], Dragan Micic[1], Mirjana Sumarac-Dumanovic[1], Aleksandra Kendereski[1], Danica Stamenkovic-Pejkovic[1], Goran Cvijovic[1], Svetlana Zoric[1] & Nebojsa Zecevic[2]
[1]Institute of endocrinology, diabetes and diseases of metabolism, Belgrade, Serbia; [2]Clinic for Gynecology and Obstetric NARODNI FRONT, Belgrade, Serbia.

It was speculated that androgen levels and insulin resistance may have influence on ghrelin levels. Elevated, normal and low ghrelin levels were reported in women with polycystic ovary syndrome (PCOS). The aim of our study was to investigate the basal ghrelin levels in lean patients with PCOS and to evaluate possible correlations between ghrelin levels, insulin resistance and, androgen levels. In 10 lean PCOS patients (BMI 20.45 ± 0.51 kg/m^2, age: 21.4 ± 0.85 years) and 8 lean controls (BMI 20.92 ± 0.69 kg/m^2, age: 25.37 ± 2.41 years) basal ghrelin (RIA, Linco, USA, pg/ml) and testosteron (RIA, INEP, nmol/l) levels were determinated. Insulin sensitivity was determinated using euglycemic hyperinsulinemic clamp (M index).

Results

There was significant difference in ghrelin levels (51.82 ± 26.83 vs 120.11 ± 58.42, $P<0.05$) and M index values (3.68 ± 0.66 vs 7.84 ± 1.28, $P<0.05$) between PCOS patients and controls. There were no significant correlations between ghrelin and testosterone ($r=0.40$, $P<0.05$), as well between ghrelin and M index values ($r=-0.12$, $P>0.05$). In conclusion, we observed lower lower ghrelin levels in lean PCOS patients than in camparative controls. Insulin resistance might have influence on low ghrelin levels in this group of patients.

P654

Adrenal morphology on CT-scan in patients with congenital adrenal hyperplasia

Anne Bachelot[1], Fabio Roffi[2], Agnès Rouxel[1], David Eiss[2] & Philippe Touraine[1]
[1]Pitié-Salpétrière Hospital, Paris, France; [2]Necker Hospital, Paris, France.

Congenital adrenal hyperplasia (CAH) due to 21-hydroxylase deficiency is one of the most common autosomal recessive diseases. Decreased production of cortisol leads to increased secretion of CRH and ACTH, resulting in overproduction of androgens and hyperplastic adrenals. 21-OH deficiency has thus been speculated to predispose for the formation of morphological adrenal abnormalities. However, studies are rare, the most relevant showing a high incidence of adrenal masses in 82% CAH patients. We then decided to evaluate adrenal morphology on CT-scan in CAH patients. We performed adrenal helicoidal CT scan with contiguous 3-mm-thick slices in 42 patients (33 females and 9 males; mean age, 27.6 yr (14–47 yr)). Twenty one had a salt-wasting form (SW), 11 a simple virilizing one (SV) and 10 a non-classical form (NCF). We found adrenal hyperplasia in 17 patients (40%), 12 with SW and 5 with SV form. Bilateral adrenocortical adenomas were observed in 2 of them. Subjects with adrenal hyperplasia were older (31.4 ± 1.7 years versus 26.5 ± 1.5 years, $P=0.04$), and had higher levels of 17OHprogesterone (105.2 ± 24.5 ng/ml versus 11.1 ± 4.9 ng/ml, $P<0.0001$) androstenedione

(10.7±2.7 ng/ml versus 2.3±0.6 ng/ml, $P=0.0006$) and renin levels (116± 45 ng/ml versus 18±2 ng/ml, $P=0.008$) than subjects with normal adrenal CT-scan. Total glucocorticoids dose is in current evaluation in both groups. Our results suggest that morphological adrenal abnormalities are not so common in CAH patients; however, they seem to be associated with increasing severity of the enzymatic defect and undertreatment may play an important role in their development. CT-scan should then be proposed in adult CAH patient, but to avoid radiation exposure, adrenal MRI should then be proposed in the follow-up of patients with adrenal abnormalities. Moreover, we propose that CAH should always be ruled out in the case of incidentally detected adrenal masses.

P655

Anti-oxidant activity of seminal plasma in fertile and infertile men

Salah Fayed & Ashraf Ahmed
Mansoura Faculty Of Medicine, El-Mansoura, Egypt, Urology & Nephrology Center, El-Mansoura, Egypt.

To evaluate the seminal plasma anti-oxidant activity in normal and infertile men and its relation to semen quality. 58 men with idiopathic infertility problems were selected, were divided according to their sperm count into two subgroups, infertile asthenospermic group ($n=31$) and infertile oligo-asthenospermic group ($n=27$). 14 proved fertile men were selected as a control group. Semen samples were collected by masturbation, examined by conventional method. Then free seminal plasma samples were separated by centrifugation and stored at −20 C till analyzed for total anti-oxidant activity (Rice-Evans & Miller1994), total thiol concentration (Hu 1994) and the thiobarbituric acid reactive substances (TBRs) by the metod of Walker & Shah (1988).

In the present study, the seminal plasma anti-oxidant activity in infertile groups was significantly higher than in control group (P 0.014), asthenospermic versus controls (P 0.016), oligoasthenospermic versus controls (P 0.036). No significant changes were observed in total thiol concentration and thiobarbituric acid reactive substances in the seminal plasma among the different groups. TBRs showed a positive significant correlation with semen volume and a negative significant correlation with percentage of abnormal forms. It could be concluded from the present study that there is a well developed system of anti-oxidants in the seminal plasma which is activated by increased levels of reactive oxygen species and products of semen lipid peroxidation. So the high levels of seminal plasma anti-oxidant activity observed in infertile groups of our study has been considered as a compensatory protective mechanism to minimize the spermatozoa membrane damage caused by the hazardus effects of free radicals on the membrane high content of polyunsaturated fatty acids.

P656

Reproductive health of women born to bromocriptine-treated mothers

Evgenia Rashidova, Valentina Kokolina, Tatiana Romantsova & Alexej Bobrov
Moscow Medical Academy, Moscow, Russia.

A retrospective cohort study was undertaken using a reproductive health survey of 25 girls aged 14–27 years (Me 20,5 (16;23)) born to bromocriptine-treated mothers. The control group consisted of 25 women born after spontaneous pregnancy of the same age, mother age, region of residence. They were all seen in the clinic for health and psychological interviews (Multiscale Personality Assessment Test- MMPI). All of them were blood analysed for LG, FSG, prolactin, TSH, anti-TPO, testosterone and DHEAS levels; ultrasonography of the mammary gland, internal genitalia with calculation of ovarian volume was presented. Pearson Chi-Square and Fisher's Exact test were used for comparing results in two groups. No difference between two groups ($P>0,05$) was found in hormonal levels, the incidence of menstrual cycle disorders and gynaecological desease, all women had normally developed internal and external genitalia. One of them has prolactin-secreting microadenoma, receives parlodel. Women born to bromocriptine-treated mothers had earlier menarche (Me 12 (12;13)) comparing to control group (Me 13 (12;14))($P=0.046$). We found a high frequency of primary hypothyroidism in women born to bromocriptine-treated mothers -20% (5 women- 3 with subclinical and 2 with overt). The early age of manifestation (9 to 18 years) and absence of anti- thyroid antibodies are their remarkable features. Different pscopatological syndroms and psychosomatic disorders were found in 9 from 16 women (62.5%) who underwent psicological testing using MMPI compairing to 3 from 18 (16.6%) in control group ($P=0.015$) 8 women born to bromocriptine-treated mothers had spontaneous pregnancies and 7 of them have healthy children. 6 were born in term, one child was born pretermed because of intrauterine infection. The study provides additional evidence that in utero exposure to bromocriptine doesn't have severe adverse effects on later health outcomes including reproductive function. The prevalence of psycopatological syndromes may be due to specific family education.

P657

The difference in the level of lipids, CRP, androgens and prolactins between PCOS women with metabolic syndrome and PCOS women without metabolic syndrome

Zelija Velija-Asimi
University Clinical Centre of Sarajevo, Bosnia and Herzegovina.

The polycystic ovary syndrome (PCOS) is characterized by insulin resistance with compensatory hyperinsulinemia. Insulin resistance also plays a role in the metabolic syndrome. PCOS women with metabolic syndrome have more hyperandrogenism and menstrual cycle irregularity than women with PCOS only.

The aim of the study was to determine the difference in the level of lipids, CRP, androgens and prolactins between PCOS women with metabolic syndrome and PCOS women without metabolic syndrome.

Methods

The study included 47 women with PCOS evaluated in our clinic. The women were divided into two groups: 1) women with PCOS and the metabolic syndrome ($n=26$, age 30.9±8 yr, BMI = 30.7±2.1 kg/m^2; WHR = 0.9) and 2) women with PCOS without metabolic syndrome ($n=20$, age 29.5±7.5 yr, BMI = 23.7± 1.7 kg/m^2; WHR = 0.8). Laboratory evaluation included lipids, CRP, TSH, PRL, FSH, LH, E2, progesterone, testosterone, androstendion, DHEAS, insulin levels during OGTT.

Results

PCOS women with metabolic syndrome had significantly higher levels of serum testosterone (3.23±0.81 *vs*. 2.2±0.67 nmol/l, $P<0.05$) than women with PCOS without the metabolic syndrome. Levels of total cholesterol (6.56±0.91 vs. 5.6± 0,9 mmol/l), LDL cholesterol (4.63±1.2 vs. 3.3±0.7 mmol/l), CRP (5.6±1.2 vs. 2.7±1.1 mg/l) and prolactin (623±179 vs. 373±121 uIU/ml) were also higher in PCOS women with metabolic syndrome. Menstrual cycle irregularity was frequently in group PCOS women with metabolic syndrome.

Conclusion

The high level of lipids, CRP, androgens and prolactin suggest that the metabolic syndrome in women with PCOS increased risk for cardiovascular disease.

P658

Presented as S23.2

P659

Presented as S18.2

P660

Presented as S18.4

P661

Expression of p63 and Notch system in the rat testis and vaso-epididymal system during postnatal development

T Hayashi, R Ohno, N Ishii, T Matsuda, T Terao, M Morozumi & T Yamada
Saitama Medical Center, Saitama Medical University, Kawagoe, Saitama, Japan.

The testis and epididymis collaborate in the male gamete development. The testis has the specific function to generate spermatozoa and spermatozoa undergo numerous changes passing through the epididymis. p63 in the basal layer of epithelium plays a key role in maintaining cellular populations, whereas Notch 1 and its ligand Jagged 2 have an important role in the cell differentiation and

Jagged 2 is up-regulated by TAp63, which transactivates p53 target genes and induces apoptosis. However, the role of p63 and its relationship with Notch system in the testis have not been examined. Therefore, we investigated the postnatal expression of p63, Jagged 2 and Notch 1 in the testis in comparison with the vaso-epididymal epithelium by Northern blot analysis and immunohistochemistry. In the testis, TAp63 mRNA expression increased at day 14 after birth and the expressions of Jagged 2 and Notch 1 mRNAs increased at day 16, whereas p63 protein was detectable in spermatocytes and Jagged 2 and Notch 1 proteins were in spermatids, suggesting TAp63-mediated Jagged 2 induction activates the Notch system. On the other hand, deltaNp63 mRNA expression was already recognized in the vas deferens at day 0 and advanced chronologically along the duct to the caput epididymis, whereas Jagged 2 and Notch 1 mRNAs were maintained at a low level. The current study has identified that testis and vaso-epididymal system express different p63 isoforms. Moreover, our data raises the probabilities that TAp63 has an important role for maintenance of germ cell numbers, triggering or balancing the development, differentiation and apoptosis of germ cells by activating both Notch system and p53 target genes, and that the chronological differences of deltaNp63 expression result in the morphological and functional differences in the mesonephric tubule.

P662

Transcripts expressed in the mouse testis during sex-determining period

T Hayashi[1], KL Loveland[2], T Yamada[1] & DM de Kretser[2]
[1]Saitama Medical Center, Saitama Medical School, Kawagoe, Saitama, Japan; [2]Monash Institute of Medical Research, Monash University, Melbourne, Victoria, Australia.

In order to understand the mechanisms that underpin gonadal development, we have conducted a subtractive screen to identify transcripts expressed differentially during the sex-determining period. Suppression subtractive hybridization PCR was performed on cDNA derived from 12.5 dpc male and female gonadal ridges. Clones were tested for differential expression by RNA whole mount *in situ* hybridization. Those localizing to testis cords were further tested on germ cell-depleted testes, and we examined the pattern of expression of four clones with male germ cell dependent expression by *in situ* hybridization in postnatal mouse testes. Four clones showed germ cell dependent expression during sex determining period, and we examined their pattern of expression in postnatal mouse testes by *in situ* hybridization. One of these, K1, encodes a protein closely related to the kinesin-like protein, KIF2. At the onset of spermatogenesis, the transcript signal was intense in the gonocyte cytoplasm and weak in Sertoli cells. This continued until the first onset of meiosis when the signal gradually shifted from spermatogonia to spermatocytes and then to spermatids; the Sertoli cell signal disappeared entirely during the first wave of spermatogenesis. The other three clones, H21 (encoding ADP-ribose polymerase), K22 (cleavage & polyadenylation specificity factor 1) and A12 (KIAA0890) were recognized in gonocytes and Sertoli cells with strong intensity at the onset of spermatogenesis. Although the signals persisted in germ cells throughout the first wave of spermatogenesis and into adulthood, the Sertoli cell signals were lost. In adult testis, all three mRNAs were detected in spermatogonia and spermatocytes. This is the first report that demonstrates the highly regulated expression of these male germ cell dependent gene products in both somatic and germ cells throughout testis development and in adulthood.

Index of Authors

Endocrine Abstracts

Volume 14

April 2007

9th European Congress of Endocrinology

28 April–2 May 2007, Budapest, Hungary

Abstract Book

The ESE would like to thank the kind generosity of their corporate members:

ESE Corporate Members:
CMM Global
Eli Lilly
Ferring
Ipsen
Novartis
Novo Nordisk
Pfizer
Roche
Sanofi-aventis
Serono

ESE Secretariat
Euro House
22 Apex Court
Woodlands
Bradley Stoke
Bristol BS32 4JT, UK

Contact: Andrea Davis
Tel: +44 (0)1454 642247
Fax: +44 (0)1454 642222
E-mail: info@euro-endo.org
Web site: www.euro-endo.org

ESE 2007 Secretariat
Blaguss Ltd. Congress Bureau
PO Box 42, 1364 Budapest 4
Hungary

Tel: +36 1 371 7030
Fax: +36 1 312 1582
E-mail: ece2007@blauguss-congress.hu
Web site: www.ece2007.com

CONTENTS

9th European Congress of Endocrinology 2007

PRIZE LECTURES AND BIOGRAPHICAL NOTES

PLENARY LECTURES

SYMPOSIA

ORAL COMMUNICATIONS

Prize Lectures and Biographical Notes

European Journal of Endocrinology Prize Lecture

Brian Walker, UK

Brian Walker is Professor of Endocrinology at the University of Edinburgh in Scotland where he is administrative head of a 60-strong multidisciplinary research group in the Centre for Cardiovascular Science.

He graduated in medicine in Edinburgh in 1986 and completed his clinical training in Glasgow and Edinburgh. Since 1996 he has practised as an honorary consultant in Diabetes & Endocrinology at the Western General Hospital in Edinburgh, recently transferring his activities to the Royal Infirmary following the opening of the Queen's Medical Research Institute at the new Little France campus.

Brian's research interest in cortisol and cardiovascular disease began in 1989 as an MRC Training Fellow, and developed as a Lecturer and British Heart Foundation Senior Research Fellow. The focus of his group has been on translating findings in animal models into detailed mechanistic experiments in humans. He published the original studies elucidating the role of 11β-HSD type 1 as a therapeutic target in obesity and diabetes, and was influential in the studies linking activation of the HPA axis with low birthweight and adult cardiovascular risk factors.

Previous awards include the Dorothy Hodgkin Lecture from Diabetes UK, the 'Hot Topic' Plenary Lecture at the Nutrition Society, and the Society for Endocrinology Medal.

Cortisol and cardiovascular disease

Brian R Walker
Endocrinology Unit, Centre for Cardiovascular Science,
University of Edinburgh, UK

Similarities between the metabolic syndrome and Cushing's syndrome, and reversibility of the features of Cushing's syndrome, suggest that cortisol may contribute to pathophysiology in both conditions and that reducing cortisol action may provide a novel therapeutic approach in metabolic syndrome.

There is substantial evidence that circulating cortisol concentrations are higher in people with hypertension and glucose intolerance. The basis for this activation of the hypothalamic-pituitary-adrenal (HPA) axis remains uncertain, but it may be attributable to 'programming' effects of events in early life since it is associated with low birth weight.

In people who become obese, intracellular cortisol levels within adipose tissue are further amplified by increased local re-generation of cortisol by the enzyme 11β-HSD type 1. Recent evidence highlights the role of nutrition and inflammation in regulating 11β-HSD1 in rodents and in humans. In mice, transgenic manipulations of 11β-HSD1 have potent effects on obesity and associated features of the metabolic syndrome. Promising pre-clinical data suggest that novel 11β-HSD1 inhibitors will have a role in lowering intra-cellular cortisol levels as a treatment for metabolic syndrome.

In addition to their metabolic effects, glucocorticoids act in the blood vessel wall. Pharmacoepidemiological studies suggest that glucocorticoid excess is an independent risk factor for cardiovascular disease. Recent data in rodents suggest that 11β-HSD1 within the blood vessel wall influences vascular remodelling and angiogenesis, for example in the myocardium following coronary artery occlusion.

Thus, HPA axis hyperactivity may provide a lifelong susceptibility to metabolic syndrome which is amplified by altered cortisol metabolism in obesity. Glucocorticoid signalling provides a potentially tractable system to influence both risk factors for, and the outcome of, type 2 diabetes and cardiovascular disease.

Geoffrey Harris Prize Lecture

Hubert Vaudry, France

Dr Hubert Vaudry is Director of Research at the Institut National de la Santé et de la Recherche Médicale (INSERM), the French National Institute for Health, and Director of the Laboratory of Cellular and Molecular Neuroendocrinology at the University of Rouen. He was born in February 1946 in Le Havre, Normandy, and obtained his PhD at the University of Rouen in 1974. He then worked in Canada for two years as a post-doctoral fellow, at Queen's University (Kingston, Ontario) and Laval University (Quebec). He obtained a Doctor of Science degree in 1979 at the University of Rouen and has developed one of the most productive groups in the field of neuroendocrinology.

Dr Vaudry is involved in a number of International Committees and Advisory Boards. He is the author of 800 publications in first rank scientific journals and has presented over 1450 communications or lectures in international congresses. Previous awards include the Descartes-Huygens Prize for scientific cooperation between France and the Netherlands, and the Prize of the Académie Nationale de Médecine. He has been appointed as Invited Professor in several Universities including the Catholic University of Nijmegen, Netherlands (1982–1983), Waseda University in Tokyo, Japan (1986), and the University of Turin, Italy (1989).

Dr Vaudry is the Chairman of the European Institute for Peptide Research, a major multidisciplinary institute working in the field of biologically active peptides. He is also the Chairman of the Research and Education Network for Neuroscience (LARC-Neuroscience network). He is a former President of the International Federation of Comparative Endocrinology Societies (1997–2001), the European Society for Comparative Endocrinology (1998–2002) and the Société de Neuroendocrinologie (2001–2004).

Neuroendocrine control of steroid biosynthesis within the hypothalamus

Hubert Vaudry[1], Jean-Luc Do Rego[1], Delphine Beaujean[1], Ludovic Galas[1], Dan Larhammar[2], Jae Young Seong[3], Van Luu-The[4], Georges Pelletier[4] & Marie-Christine Tonon[1], [1]INSERM U413, Univ. Rouen, France, [2]Dept Neuroscience, Univ. Uppsala, Sweden, [3]Lab. G Protein-Coupled Receptors, Korea Univ. College of Medicine, Seoul, Korea, [4]Lab. Molecular Endocrinology and Oncology, Laval Univ. Medical Center, Quebec, Canada

Neuroactive steroids synthesized in the brain, referred to as neurosteroids, have gained particular attention as they appear to be involved in the modulation of various neuroendocrine, behavioral and pathophysiological processes. Thus, the distribution of steroidogenic enzymes and the identification of the biochemical pathways leading to neurosteroid formation have now been almost completely elucidated in various groups of vertebrates. In contrast however, the neuronal mechanisms controlling the activity of neurosteroid-producing cells in the brain have received little attention. Therefore, we have investigated the effects of neurotransmitters and neuropeptides on the biosynthesis of neurosteroids, using the frog brain as an experimental model. We have first observed that steroid-synthesizing neurons express several subunits of the $GABA_A$/central-type benzodiazepine receptor (CBR) complex, and we have found that GABA, acting through $GABA_A$ receptors, inhibits the synthesis of neurosteroids. We have shown that glial cells containing the octadecaneuropeptide (ODN; endogenous ligand of CBR)-like immunoreactivity make contact with neurosteroid-producing neurons, and that ODN stimulates steroid biosynthesis in hypothalamic neurons in a dose-dependent manner through activation of CBR. Steroid-producing neurons are also innervated by vasotocin (VT)-containing fibers, and they are gathered in hypothalamic regions which actively express the V1a receptor subtype and mesotocin (MT) receptor (MTR). We have found that VT and MT, acting on V1a and MTR respectively, are potent stimulators of neurosteroidogenesis. Finally, we have shown that steroidogenic neurons are innervated by NPY and GnRH fibers, and that the nuclei where these neurons are located are enriched with NPY Y_1 and Y_5 receptors, and GnRHR1/3 receptors. We have observed that NPY, acting through Y_1 receptors, inhibits neurosteroid biosynthesis, while GnRH stimulates the production of neurosteroids probably via GnRHR1/3 receptors. Taken together, these data suggest that some of the activities exerted by neurotransmitters and neuropeptides in the brain may be mediated via the regulation of neurosteroid production.

Supported by INSERM (U413), the Regional Platform for Cell Imaging, a France-Québec exchange program (INSERM-FRSQ), a France-Korean exchange program (STAR) and the Conseil Régional de Haute-Normandie.

Symposia

Hypopituitarism–S1

S1.1

Traumatic brain injury-induced hypopituitarism: whom and when to test

Chris Thompson
Beaumont Hospital/RCSI Medical School, Dublin, Ireland.

A large body of evidence has accumulated to indicate that between 20–30% of survivors of acute traumatic brain injury (TBI) develop permanent pituitary dysfunction. Growth hormone (GH) deficiency is the commonest abnormality documented in most studies followed by ACTH and gonadotrophin deficiency and hyperprolactinaemia, with TSH deficiency least common. In contrast to other forms of pituitary disease, the classical hierarchy of pituitary hormone failure is not always seen and there is a higher proportion of single hormone defects. Many of the symptoms of chronic TBI are similar to those of untreated hypopituitarism, which suggests that identification and appropriate treatment of hypopituitarism offers a valuable service to survivors of TBI. Who should be tested? Most studies have been confined to survivors of moderate to severe TBI and the rationale for investigation is currently confined to this subgroup. There is little relationship between severity of TBI, neuro-imaging studies or operative intervention and the likelihood of hypopituitarism so until better guidance is available from prospective studies, all survivors should be tested. The choice of dynamic stimuli for ACTH and GH are centre-dependent. The timing of testing is important.
In the acute phase of TBI the key deficiency to identify is ACTH; patients who develop hypotension, hypoglycaemia or hyponatraemia should be systematically screened for ACTH deficiency. Studies of the natural history of pituitary dysfunction after TBI suggest a dynamic process, with many acute abnormalities recovering within 3–6 months of TBI, while new deficiencies may manifest in this period. New deficiencies are rare after 6 months. Most authorities therefore recommend formal dynamic testing at 3–6 months following TBI. Clinicians should be aware of occasional late recovery of function. Prospective studies are needed to better identify those at greatest risk of hypopituitarism, in order to improve the logistics of post-TBI pituitary hormone assessment.

S1.2

Morbidity and mortality

BA Bengtsson
Sweden.

Abstract unavailable

S1.3

Hypocorticotropism

Stylianos Tsagarakis
Department of Endocrinology, Athens Polyclinic, Athens, Greece.

Hypocorticotropism refers to ACTH insufficiency, which may be partial or complete, isolated or combined, genetic or acquired, pituitary or hypothalamic in origin. As a result it leads to secondary adrenal failure. Adrenal secretion of cortisol and of adrenal androgens is mainly affected; aldosterone secretion is normal. Symptoms of hypocorticotropism include progressive malaise and weight loss. Because aldosterone secretion is intact salt wasting, volume contraction and hypokalemia are not present. Hypoglycemia due to defective neoglucogenesis may occur and is particularly common in children. Symptoms may be more dramatic in the case of abrupt onset. Laboratory assessment includes baseline measurements of ACTH and cortisol and dynamic tests. A low morning value of cortisol (i.e. <3 μg/dl) associated with a low ACTH is diagnostic of severe hypococorticotropism. A value of 9am cortisol over 18 μg/dl excludes hypococorticotropism. For all other values dynamic tests are required. The gold standard test is the insulin stress test (IST). Alternative tests are the glucagon, the metyrapone and the standard (SST) and low Synacthen (LST) tests. The Synacthen tests because of their simplicity and safety have superseded the gold standard IST. Although the LST was considered as a more sensitive test than the SST recent data suggest that there is no difference. A cut-off of 18 μg/dl is considered as a "pass" and is safe assuming that assessment is not close to recent pituitary failure. Confirmation of hypocorticotropism requires replacement therapy. Hydrocortisone is the preferred medication. It is better given in 3 divided doses to a total daily dose of 10–20 mg. Co-administration of GH may increase the dose requirements of hydrocortisone. The dose should also be increased during stress and surgery. The question of whether adrenal androgen replacement is beneficial is still debated. Some studies showed positive effects on the quality of life of 50 mg of DHEA but others fail to confirm this. A therapeutic trial of 3–6 months in patients, particularly women, with relevant symptoms may be justified.

S1.4

Familial neurogenic diabetes insipidus

Soren Rittig
Skejby University Hospital, Aarhus, Denmark.

Although molecular research has contributed significantly to our knowledge of familial neurohypophyseal diabetes insipidus (FNDI) for more than a decade, the genetic background and the pathogenesis still is not understood fully. FNDI is, in 87 of 89 kindreds known, caused by mutations in the arginine vasopressin (AVP) gene, the pattern of which seems to be largely revealed as only few novel mutations have been identified in recent years. The mutation pattern, together with evidence from clinical, cellular, and animal studies, points toward a pathogenic cascade of events, initiated by protein misfolding, involving intracellular protein accumulation, and ending with degeneration of the AVP producing magnocellular neurons. Molecular research has also provided an important tool in the occasionally difficult differential diagnosis of DI and the opportunity to perform presymptomatic diagnosis. Although FNDI is treated readily with exogenous administration of deamino-D-arginine vasopressin (dDAVP), other treatment options such as gene therapy and enhancement of the endoplasmic reticulum protein quality control could become future treatment modalities.

Hormones and the brain – S2

S2.1

Thyroid hormone regulation of neural and oligodendrocyte precursors in the mature brain: a possibility for remyelination and neuroprotection

Laura Calzà
University of Bologna, Ozzano Emilia, Bologna, Italy.

Re-myelination in the adult CNS has been demonstrated in different experimental models of demyelinating diseases. However, there is no clear evidence that re-myelination is effective in multiple sclerosis (MS), the most diffuse demyelinating disease. Moreover, chronic disabilities in MS are believed to be due to remyelination failure and consequent neuron damage and degeneration. Due to the presence of numerous oligodendrocyte precursors inside demyelination plaques, reasons for remyelination failure are unknown. Data from embryonic development and *in vitro* studies supports the primary role of thyroid hormone in oligodendrocyte formation from neural precursors and maturation. We have obtained positive results in promoting re-myelination and neuroprotection in chronic experimental allergic encephalomyelitis (EAE), a widely used experimental model of MS, by recruiting progenitors and channelling them into oligodendroglial lineage through administration of thyroid hormone. Experiments performed in rats and confirmed in the primate Callithrix Jacchus have generated a phase 2 clinical trial that is in progress. We have also explored the role of thyroid hormone in regulating neural precursors cells in the subventricular zone of mature brain by *in vivo* and *in vitro* experiments (neurosphere assay), with regard to cell cycle and lineage regulation. Finally, we are exploring the possibility that prenatal events disturbing thyroid function, like endocrine disruptors exposure (dioxin family), might affect oligodendrocyte development and susceptibility to demyelinating agents.

S2.2

Neuroprotective actions of estrogens in the central nervous system

Luis Garcia-Segura & Iñigo Azcoitia
Instituto Cajal, CSIC, Madrid, Spain.

Sex hormones act both as endocrine signals as well as local paracrine or autocrine factors in the nervous system. In addition to target to classical endocrine and reproductive brain areas, sex hormones and its metabolites affect learning and cognition and regulate the development and plasticity of brain regions that are not directly related to reproduction. Estrogen and progesterone exert neuroprotective

effects in the central nervous system and may affect the onset and progression of several neurodegenerative and affective disorders, as well as the recovery from traumatic neurological injury. Recent studies have shown that the brain up-regulates both estradiol synthesis and estrogen receptor expression in reactive astroglia at sites of injury. Genetic or pharmacological inhibition of brain aromatase, the enzyme involved in estradiol synthesis, results in marked neuronal death after different forms of mild neurodegenerative stimuli that do not compromise neuronal survival under control conditions. This finding strongly suggest that local formation of estradiol in the CNS is neuroprotective and that the induction of aromatase and the consecutive increase in the local production of estradiol are part of the program triggered by the neural tissue to cope with neurodegenerative insults. Proteins involved in the intra-mitochondrial trafficking of cholesterol, the first step in steroidogenesis, such as the peripheral-type benzodiazepine receptor (PBR) and the steroidogenic acute regulatory protein (StAR), are also up-regulated in the brain after injury, together with the first enzyme in the steroidogenic pathway (P450scc). This suggests that brain steroidogenesis may be modified in adaptation to neurodegenerative conditions and to the brain aging process. Recent studies have shown that Ro5-4864, a PBR ligand that increases brain steroidogenesis is neuroprotective. Therefore, StAR, PBR and aromatase are attractive pharmacological targets to promote neuroprotection in the aged brain.

Supported by MEC, Spain (SAF 2005-00272) and the European Union (EWA project: LSHM-CT-2005-518245).

S2.3

Estrogen receptor signalling and cerebrovascular disease

Tommy Olsson, Magnus Strand & Ingegerd Söderström
Department of Medicine, Umeå University Hospital, Umeå, Sweden.

The transition to the postmenopausal stage is associated with an increased risk for vascular diseases, including myocardial infarction and stroke. This has been linked to a decrease in estrogen production. Estrogens mediate their effects on the brain to a major extent through binding to nuclear receptors, estrogen receptor alpha and beta. It is possible that positive and adverse effects of estrogens are related to interactions between receptor genotypes and hormones. Notably, the estrogen receptor alpha polymorphism c 454-397T/T is associated with increased risk of hemorrhagic stroke, with a synergistic relationship between this genotype and hypertension. In experimental stroke settings estrogens influence recovery of cognitive functions, possibly via induction of neurotrophic factors and specific transcription factors including NGFI-A. This may be related to increased neuroplasticity in the hippocampal formation, a key area for memory processing. Individualized treatment with estrogen receptor medulators may be beneficial for individuals with an increased risk for stroke. Estrogens may also improve recovery after stroke.

S2.4

Immunesenescence and steroid hormones

Wiebke Arlt[1] & Janet M Lord[2]
[1]Division of Medical Sciences, University of Birmingham, Birmingham, United Kingdom; [2]MRC Centre for Immune Regulation, University of Birmingham, Bimringham, United Kingdom.

Ageing is associated with a decline in immunity, also termed immunesenescence. This is paralleled by a decline in the production of several hormones as typically illustrated by the menopausal loss of ovarian oestrogen production. This lecture will give a brief overview of the physiology and pathophysiology of steroid hormones that decline with ageing. Therein a specific focus will be laid on the ageing-associated decline in adrenal dehydroepiandrosterone (DHEA) production, an event commonly termed as "adrenopause". However, this term is rather imprecise as the other major outputs of adrenal corticosteroid production, cortisol and aldosterone secretion, do not change with ageing. The regulatory processes involved in the initiation and progression of "adrenopause" still remain elusive. Current research efforts importantly aim at clarifying whether "adrenopause" contributes to immunesenescence, also addressing the issue of an altered glucocorticoid/DHEA balance that necessarily occurs if cortisol remains unchanged while DHEA steadily declines. Previous research has shown that an increased cortisol/DHEA ratio increases the likelihood of early postoperative infections requiring hospitalisation in elderly patients with hip fracture and that these changes are associated with an impairment of neutrophil function. The lecture will summarise most recent results on differential effects of DHEA and cortisol on components of the immune response, including neutrophil and natural killer cell function, including first conclusive data on underlying mechanisms. Further understanding of immune-endocrine links in the pathophysiology of immunesenescence will hopefully help to develop clinical tools for improving health in our rapidly ageing population.

Signaling and regulation of G-protein-coupled hormone receptors – S3

S3.1

Trafficking and signaling of angiotensin receptors

László Hunyady, Eszter Karip, Gábor Turu & László Szidonya
Department of Physiology, Semmelweis University, Budapest, Hungary.

The octapeptide hormone angiotensin II (Ang II) exerts its major biological effects via angiotensin AT_1 receptors (AT_1Rs). Signaling of AT_1Rs is regulated by ß-arrestins, which bind to activated AT_1Rs, uncouple them form G proteins, and initiate their internalization via clathrin-coated pits and cause G protein independent MAP kinase activation. It has been shown previously that AT_1Rs internalize via ß-arrestin-dependent and independent mechanisms, whereas angiotensin AT_2 receptors, which are unable to internalize, do not bind ß-arrestins. To study the role of G protein independent MAP kinase activation in cells, which endogenously express AT_1Rs, a mutant receptor (S109Y) was created, which is unable to bind candesartan. On the other hand, the Ang II binding and Ang II-induced functional responses of the S109Y mutant receptor are completely normal. This mutation was combined with a mutation (DRY/AAY), which can bind to ß-arrestin2, but its G protein coupling is completely impaired. The receptors were expressed in C9 cells, which express endogenous AT_1Rs. In the presence of candesartan the Ca^{2+} signal and MAP kinase activation of the endogenous AT_1R was completely eliminated. However, the Ca^{2+} signal generation and MAP-kinase activation of the S109Y mutant receptor was readily detectable. in the presence of candesartan, which inhibits the endogenous AT_1Rs, the combined S109Y and DRY/AAY mutant receptor was unable to induce Ca^{2+} signal generation, whereas it mediated Ang II-induced MAP kinase activation with a slow kinetics. These data suggest that G protein independent MAP kinase activation can occur in C9 cells.

This work was supported by OTKA T46445 and ETT 447/2006.

S3.2

Pharmacological chaperones rescue the membrane expression and function of a mutant of the vasopressin V1b/V3 receptor

Eric Clauser, Jessica Robert, Colette Auzan & Marie Ange Ventura
Institut Cochin, INSERM U567, Paris, France, CNRS UMR8104, Paris, France; University Paris V, Paris, France.

The majority of loss-of function mutations of G protein coupled receptors, leading to diseases, such as diabetes insipidus (V2 vasopressin receptor) or retinitis pigmentosa (rhodopsin) are consecutive to retention of the receptor in the endoplasmic reticulum (ER). Cell surface expression and biological function can be restored by membrane-permeable ligands called pharmacological chaperones. The V1b/V3R, one of the 3 subtypes of vasopressin receptors, is involved in the regulation of the corticotroph axis during stress. Using an original assay for cell surface expression of the receptor, we have demonstrated that a mutation of the hydropobic 341FNX2LLX3L350 motif in the C-terminus of the human pituitary V1b/V3R (MUT V3R) leads to its retention in the ER. The precise role of this motif was further investigated using SSR149415, a nonpeptide V1b/V3R antagonist.

The absence of the mutated receptor at the plasma membrane is linked to its prolonged association with the molecular chaperone, calnexine, in the ER and to its intensive degradation by the ubiquitin-proteasomal machinery. However, this ER retention is not a consequence of a lack of oligomerization of the mutant, which can be identified as dimers in the ER with BRET technique.

Treatment with SSR149415 restores expression of the mutated receptor at the cell surface and its correct maturation, resulting into the functional recovery of its signaling properties. SSR149415 acts by stabilizing the native-like conformation of the V1b/V3R, reducing its association with calnexin and favoring a secretory pathway rather than the proteasomal degradation pathway.

In conclusion, the 341FNX2LLX3L350 sequence is an important motif for the V1b/V3R conformation and the misfolfding resulting from its mutation alters the receptor export but can be reverted by SSR149415, which behaves as a pharmacological chaperone.

S3.3

Functional impact of GPCR heterodimerzation

Heike Biebermann
Institute of Experimental Pediatric Endocrinology, Charité Campus Virchow, Universitätsmedizin Berlin, Berlin, Germany.

For over two decades the hypothesis of dimerization of G protein coupled receptors (GPCR) exist. But only in 1999 it became clear by investigation of GABA B receptors that dimerization is the prerequisite for function. Since then our understanding of GPCR function is widened by the fact that nearly all GPCRs form dimers or higher order oligomers. This formation of GPCR homodimers or heterodimers influence the functional properties of a GPCR from that we know of a monomer in its ability to traffic to the cell surface, to bind one or even a variety of ligands, to initiate one or several signalling pathways, to be internalized, or not, and at least to be a therapeutical target. This receptor cross-talk seems to be crucial for fine-tuning of receptor function in controlling physiological processes of a cell. A very large number of GPCRs are known that form heterodimers/oligomers but the functional impact for a lot of these dimers still remains unclear and functional consequences of GPCR heterodimerization are nor predictable. For some GPCR dimers the functional impact is solved, e.g. for GABA B1 and GABA B2 receptors it is known that dimerization is necessary for cell surface expression and function; the taste sensation of sweet or umami is dependent on the formation of taste receptor T1R1, T1R2 and T1R3 complexes and the formation of dopamine 2 receptor/cannabinoid 1 receptor heterodimers result in activation of the Gs instead of Gi when expressed alone.

What do these data contribute to our overall understanding of physiological processes? As long as we have no other hints we have to accept that all possible interactions of GPCRs that are expressed on a given cell type are possible and therefore have to be investigated. to clarify their physiological significance. Especially this counts for the estimation of drug pharmacology targeting a GPCR.

Our group is interested in understanding the physiological processes of hypothalamic weight regulation. Therefore we set out to investigate the interaction of GPCR that are expressed on neurons of the nucleus arcuatus and nucleus paraventricularis. For example we are able to show that the MC3R forms dimers with the ghrelin receptor both are expressed on NPY/AGRP neuron of the nucleus arcuatus. The functional consequences of these dimers have to be investigated.

The determination of GPCR heterodimer function is a great challenge and will provide explanation for so far not understood cellular processes.

S3.4

Ago-allosteric effects of agonist drugs on 7TM receptors and their endogenous hormones – example from the ghrelin receptor

Thue W. Schwartz
University of Copenhagen, Copenhagen, Denmark.

Conventionally, an allosteric modulator is neutral in respect of efficacy and binds to a receptor site distant from the orthosteric site of the endogenous agonist. However, recently compounds being ago-allosteric modulators have been described i.e. compounds acting both as agonists on their own and as enhancers for the endogenous agonists in both increasing agonist potency and often providing additive efficacy - superagonism. The additive efficacy can also be observed with agonists, which are neutral or even negative modulators of the potency of the endogenous ligand. Based on the prevailing dimeric dogma for 7TM receptors, it is proposed that the ago-allosteric modulators often bind in the orthosteric binding site, but – importantly – in the "other" or allosteric protomer of the dimer. Hereby, they can act both as additive co-agonists, and through inter-molecular cooperative effects between the protomers, they may influence the potency of the endogenous agonist. It is of interest that at least some endogenous agonists can only occupy one protomer of a dimeric 7TM receptor complex at a time and thereby they leave the orthosteric binding site in the allosteric protomer free, potentially for binding of exogenous, allosteric modulators. If the allosteric modulator is an agonist, it is an ago-allosteric modulator; if it is neutral, it is a classical enhancer. Molecular mapping in hetero-dimeric class-C receptors, where the endogenous agonist clearly binds only in one protomer, supports the notion that allosteric modulators can act through binding in the "other" protomer. It is suggested that for the in vivo, clinical setting a positive ago-allosteric modulator should be the preferred agonist drug.

T.W. Schwartz & B. Holst: Ago-allosteric modulation and other types of allostery in 7TM dimeric receptors. **J. Recept. Signal. Transduct. Res.** (2006) 26: 107–128.

Gastroenteropancreatic endocrine tumors (GEP ET) – S4

S4.1

Pathological classification of GEP neuroendocrine tumors

Mauro Papotti, Luisella Righi & Marco Volante
University of Turin, Torino, Italy.

In the gastroentero-pancreatic tract, a spectrum of neuroendocrine tumors (NET) exists, including low grade tumors (carcinoid), intermediate grade malignant carcinoid, and high grade poorly differentiated carcinomas of the small and large cell types. In year 2000, the WHO presented a classification scheme for all NETs, but mostly applied to gastroentero-pancreatic tumors. This term carcinoid was replaced by (neuro)endocrine tumor, malignant carcinoid by well differentiated (neuro)endocrine carcinoma, and the term "small cell carcinoma" was confirmed for poorly differentiated NE neoplasms. The new terminology induced some confusion in the routine application and interpretation of some NETs, especially those of intermediate grade (which underwent major changes in the new classification). New diagnostic criteria pose problems to the pathologist (e.g. correct diagnosis on scarce biopsy or cytological material) and to the clinician (choice of the appropriate therapy for single histological types). The characterization of NETs includes the immunoprofiling of NE differentiation markers and hormonal products, but also the analysis of prognostic (i.e. Ki67) and therapeutic factors. The latter include somatostatin receptor expression profile (possible in surgical, biopsy or cytology specimens by immunohistochemistry), to identify possible targets of somatostatin analogs. Finally, apart from pure endocrine tumors, NE differentiation occurs also in non-endocrine tumors (*see review in Volante M, Virchows Arch 449:499, 2006*). "Mixed endocrine-exocrine carcinomas", as well as gastric, colorectal and pancreatic adenocarcinomas with foci of NE differentiation have been described. These latter tumors can account for up to 20% of cases, depending on the method used to assess the NE phenotype (eg chromogranin A immunostaining), but to date they were not found to bear any prognostic significance (as opposed to the well established prognostic role of NE differentiated prostate cancer), with the possible exception of gastric cancer, according to a recent study by Japanese authors (*Jiang SX, Am J Surg Pathol 30:945, 2006*).

S4.2

Biological, morphological work-up and screening for inherited disease

Britt Skogseid
Uppsala University, Uppsala, Sweden.

Pancreatic endocrine tumors (EPT) may occur sporadically or in association with the rare autosomal dominantly inherited tumor syndromes; multiple endocrine neoplasia type 1 (MEN1) and von Hipple Lindau (VHL). The genes causing these syndromes have been identified, and genotyping is possible which enables the laborious clinical investigations for diagnosis of lesions to be restricted to 50% of family members. For MEN1, no clinically useful genotype-phenotype correlation as been discovered and in a majority of patients the EPT will undergo malignant transformation. Timely identification and intervention by surgery before development of metastases currently represents the only cure of the disease. Thus, repeated extensive biochemical and radiological investigations for early recognition of small EPT *in situ* should be considered in asymptomatic gene carriers. Efficacies of genetic and hormonal screening programs as well as imaging will be discussed.

S4.3

Prognosis of GEP ET

E. Baudin
France.

Abstract unavailable

S4.4

Therapeutic management of GEP ET

P. Ruszniewski
France.

Abstract unavailable

Novel bioactive peptides – lessons from animals – S5

S5.1

Discovery of novel bioactive peptides: the uniquely important contribution of amphibians to mammalian neuropeptidology

Hubert Vaudry[1], Hervé Tostivint[1], Isabelle Lihrmann[1], Nicolas Chartrel[1], Alain Fournier[2], Jérôme Leprince[1], Marie-Christine Tonon[1] & J. Michael Conlon[3]
[1]INSERM U413, Laboratory of Cellular and Molecular Neuroendocrinology, IFRMP23, University of Rouen, Mont-Saint-Aignan, France; [2]INRS – Institut Armand-Frappier, Univeristy of Québec, Montreal, Canada; [3]Department Biochemistry, Faculty Medicine and Health Sciences, UAE University, Al Ain, United Arab Emirates.

The concentration of many neuropeptides in the brains of ectothermic vertebrates is several orders of magnitude higher than in the brains of mammals. We have taken advantage of this singular situation to isolate from the brain of the European green frog, *Rana esculenta*, a number of regulatory peptides that are orthologous to mammalian neuroendocrine peptides. These include α-MSH, γ-MSH, two tachykinins, two GnRH variants, CRH, PACAP, NPY, CGRP, CNP, GRP, and ODN. This peptidomics project has also led to the discovery of several novel neuroendocrine peptides that were first isolated from frog brain tissue but have subsequently been identified in mammals. In particular, we have characterized (1) the somatostatin-14 (S-14) isoform [Pro^2, Met^{13}]S-14 as well as authentic S-14, thereby providing the first evidence for the occurrence of two somatostatin variants in the brain of a single species, (2) the first tetrapod urotensin II, a peptide that had long been thought to be produced only in the caudal neurosecretory system of fish, (3) secretoneurin, a peptide derived from the post-translational processing of secretogranin II, and (4) 26RFa, a novel member of the Arg-Phe-NH_2 family of biologically active peptides. Orthologs of all these frog neuropeptides have now been identified in man and have been shown to exert important regulatory effects in mammals.

Supported by grants from INSERM (U413), the European Institute for Peptide Research (IFRMP23), the Platform for Cell Imaging of Haute-Normandie (PFRRICHN), the Conseil Régional de Haute-Normandie and the Laboratoire International Associé Samuel de Champlain.

S5.2

Comparative approaches to resolve the complexities of human appetite regulation

Dan Larhammar
Uppsala University, Uppsala, Sweden.

The regulatory processes of appetite and metabolism have turned out to be exceedingly complex and involve numerous hormones and neurotransmitters, particularly peptides. Evolutionary studies in our laboratory have shown that many genes encoding peptides and receptors were duplicated in the early stages of vertebrate evolution through chromosome duplications. Thus, many of the components have existed for 400–500 million years, for instance the various members of the families of NPY-like peptides, opioid peptides, tachykinins, glycoprotein hormone beta subunits (FSH, LH and TSH) and others. The chromosome duplications also explain the origin of many peptide receptors, for instance the NPY-family receptors, opioid receptors, oxytocin-vasopressin receptors, tachykinin receptors and CRF receptors. Also the glucocorticoid-mineralcorticoid receptors arose through a chromosome duplication. These observations of ancient chromosome duplications explain a great deal of the complexity of the vertebrate endocrine and neuronal networks. Duplication of complete genes in this manner means that the duplicates initially had identical gene regulation. This makes it particularly intriguing that some duplicates now have opposing functional roles. One striking example is the peptide hormone PYY, released from gut endocrine cells after meals, which acts as an appetite inhibitor on the Y2 receptor in the hypothalamus. In contrast, the related peptide NPY is the body's most potent stimulator of appetite, acting on receptor subtypes Y1 and Y5. Probably the switch in function occurred when the duplicated genes became expressed in different cell types. We have functionally studied the roles of the NPY-family peptides in a herbivorous species with frequent meals, the guinea-pig, and a carnivore with rare meals, the dog. The role of NPY appears to be the same in the guinea pig as in intermittent feeders like rats. We are presently evaluating the role of PYY as an appetite inhibitor in dogs. Functional studies in different species will provide a firmer basis for predicting and testing the functions of these peptides in humans as well as their possible roles in states of obesity and anorexia.

S5.3

Bioactive peptides in invertebrate model organisms

Liliane Schoofs, Inge Mertens, Geert Baggerman, Peter Verleyen & Elke Clynen
K.U.Leuven, Leuven, Belgium.

Genome sequence projects in combination with advances in mass spectrometry and bioinformatics have created several new possibilities for comparative endocrinology. In 2001 we introduced the peptidomics technology that allows the identification of the complement of native (neuro)peptides in cells, tissues, organs and organisms. Especially when genome sequence information is available (*D. melanogaster, A. mellifera, C. elegans*…), neuropeptidomes were successfully identified and compared in different physiological conditions.

Synthetic libraries of newly sequenced peptides can be used to screen orphan neuropeptide G-protein coupled receptors in cell-based assays that express the receptor. This has boosted receptor identification in insects and other invertebrates. One of the advantages of model organisms, such as *C. elegans* and *Drosophila* is their amenability for genetic manipulations and the availability of knockouts as a result of (ongoing) gene disruption programs.

In this presentation, we show how all these technological developments contributed to the discovery of novel neuropeptide signalling systems in *Drosophila* and in *C. elegans*. In the nematode worm, we will focus on the functional characterisation of neuropeptide processing enzymes and two neuropeptide GPCR signalling systems, respectively related to the mammalian GnRH receptor and the VPAC receptor in vertebrates. We will discus the implications of these findings with respect to the evolutionary conservation of these signalling systems.

S5.4

Somatostatin, cortistatin and their new and old receptors: from comparative to translational endocrinology

Justo P. Castaño, Mario Durán-Prado, Rafael Vázquez-Martínez, Antonio J. Martínez-Fuentes, Manuel D. Gahete, Raul M. Luque & Maria M. Malagón
Department Cell Biology, Physiology and Immunology. Univ. Córdoba, Cordoba, Spain.

omatostatin, originally isolated from ovine hypothalami in 1973, and cortistatin, identified a decade ago in amphibians and then in human and rodents, are two highly related peptides thought to derive from a common ancestor gene. Owing to their high structural homology, both peptides bind with similar affinity to the five so-called somatostatin receptors (sst1-sst5), and exert virtually undistinguishable effects on several physiological targets, including inhibition of endocrine secretions. Yet, each peptide also shows distinctive, specific functions, which should involve different receptors and/or signalling mechanisms still to be defined, and also display divergent patterns of expression in normal and tumoral tissues. In particular, cortistatin selectively regulates locomotion- and sleep-related processes and exerts potent antiinflammatory effects with a promising therapeutic potential. In this context, recent work from our group has aimed at characterizing the response of pituitary somatotrope cells to cortistatin and somatostatin, and to isolate sst receptors in a domestic species, the pig. This led to us to demonstrate that both peptides similarly exert a dual, inhibitory and stimulatory effect on GH release in vitro, which likely involve sst1/sst2 and sst5, respectively. Furthermore, while cloning porcine sst5, we discovered two new truncated isoforms of this receptor, termed psst5B and psst5C, which display distinct tissue distribution and, when expressed in clonal cell lines, show selective functional responses to somatostatin (psst5B) and cortistatin (psst5C). Interestingly, FRET studies revealed that these novel receptors functionally interact with their full-length counterpart psst5A, as well as with the rest of pig sst. Moreover, we recently cloned two similar human sst5 truncated isoforms (hsst5B and hsst5C) that also show selective functional response to somatostatin and cortistatin, functionally interact with and modulate hsst5A and hsst2, and are differentially distributed in normal and tumoral human tissues, suggesting a possible pathophysiological role for these novel receptors.

Support: CVI-139&P06-CTS-01705-J.Andalucia, BFU2004-03883-MEC/FEDER

Diabetes and insulin – S6

S6.1

Perspectives of islet cell transplantations

B Keymeulen
Belgium.

Abstract unavailable

S6.2

Cytokines as pathogenetic effectors in type 1 and type 2 diabetes

Thomas Mandrup-Poulsen
Steno Diabetes Center, Gentofte, Denmark; Karolinska Institute, Stockholm, Sweden.

The pro-inflammatory cytokine interleukin-1 is selectively cytotoxic to rodent and human beta-cells *in vitro*, and anti-IL-1 therapies reduce diabetes incidence in animal prevention models: (1) IL-1 alone or in combination with other inflammatory cytokines causes beta-cell destruction in rodent and human islets and in perfused pancreas via MAPK and NFkB signaling, (2) IL-1 given i.p. to non-diabetes prone animals causes transient insulinopenic diabetes (3) IL-1 is expressed early in islets of the non-obese diabetic (NOD) mouse, a model of spontaneous autoimmune diabetes (4) anti-IL-1 intervention prevents diabetes development in animal models of Type 1 diabetes and islet graft destruction and (5) transgenic mice with knock-out of the IL-1 receptor reduces diabetes incidence.

We recently completed a 13-week clinical study of IL-1 Receptor Antagonist (IL-1Ra, anakinra, Kineret^{R}, Amgen) therapy in Type 2 diabetics based on the rationale that *in vitro* glucotoxicity to human beta-cells can be prevented with IL-1Ra, and that glucose induces islet IL-1 production, which causes beta-cell apoptosis by pathways similar to those believed to operate in Type 1 diabetes. This study provided proof-of-principle that inhibition of IL-1 signalling can improve glycemia and beta-cell function in humans. Interestingly, maximal effect on glycosylated hemoglobin with anakinra was seen after 4 weeks, and fasting blood glucose was significantly reduced already after 1 week, suggesting rapid effects on beta-cell secretory capacity. These preclinical and clinical studies warrant studies to investigate the effect of IL-1 blockade in patients with recent-onset Type 1 diabetes mellitus.

S6.3

GLP-1 as a drug target

JJ Holst
Denmark.

Abstract unavailable

S6.4

Engineering beta cells to recover insulin function

Bernat Soria, A Hmadcha, JR Tejedo, FJ Bedoya & F Martin
Andalusian Center for Molecular Biology and Regenerative Medicine, Seville, Spain.

Stem cells are clonogenic cells capable of both self-renewal and multilineage differentiation. Therefore, these cells have the potential to proliferate and differentiate into any type of cell and to be genetically modified '*in vitro*', thus providing cells which can be isolated and used for transplantation. Moreover, these derived cells have proven to be useful in different animal models. Using a combination of several directed differentiation methods (nicotinamide, sonic hedgehog signalling inhibition, soluble factors from pancreatic buds] and a 'cell trapping' system, we have obtained insulin-secreting cells from undifferentiated embryonic stem cells. Lineage-trapping constructs used allows the expression of a neomycin selection system under the control of the regulatory regions of insulin gene and other B-cell genes, such as Nkx6.1. Selection of differentiated cells exclude non-differentiated cells which use to be present and are teratogenic. Transplanted animals correct hyperglycaemia within 1 week and restore body weight in four weeks. Graft removal rescued the diabetic condition. Glucose tolerance test (IPGTT) and blood glucose normalization after a challenge meal was similar in control and in transplanted mice. More recently, progenitors from peripheral human blood cells (PCMO) have been convinced to acquire an insulin-producing phenotype which normalize blood glucose of immunocompromised (SCID) diabetic mice, an option with tentative applications in regenerative medicine. This approach opens new possibilities for tissue transplantation in the treatment diabetes mellitus.

References:
1. Soria B *et al.* (2000) *Diabetes* **49**: 157–162.
2. Soria B *et al.* (2001) *Diabetologia* **44**: 407–415.
3. León-Quinto T *et al.* (2004) *Diabetologia* **47**(8): 1442–1451.
4. Runhke M *et al.* (2005) *Gastroenterology* **128**(7): 1774–1786.
5. Vaca P *et al.* (2006) *Stem Cells* **24**(2): 258–265.

Thyroid cell biology – S7

S7.1

New insights from zebrafish: the molecular and cellular base of thyroid development

Burkhard Alt, Dejan Adzic, Osama Elsalini & Klaus Rohr
University of Köln, Institute for Developmental Biology, Köln, Germany.

Due to experimental advantages such as its rapid development, transparent embryos, and accessibility for genetic analysis as well as embryonic manipulation, the zebrafish is a useful model organism for research on organogenesis. My lab has established that the basic mechanisms of thyroid development are essentially conserved on the morphological as well as on the molecular level between fish and mammals. We use zebrafish to identify as yet unknown factors involved in thyroid development. In this talk, I will give an overview about new, unique approaches to understand thyroid development in zebrafish. I will touch different aspects such as genetics of early induction, the molecular base of cellular behaviour in primordial relocalisation, and morphogenesis of the gland. Concentrating on selected molecules, I will exemplify how research on zebrafish contributes to a general understanding of thyroid development that sheds new light on the causes of congenital hypothyroidism.

S7.2

Involvement of cardiovascular development and non-cell autonomous signaling in mouse thyroid organogenesis

Henrik Fagman
Institute of Biomedicine, The Sahlgrenska Academy at Göteborg University, Göteborg, Sweden.

Thyroid dysgenesis (comprising agenesis, hemiagenesis or ectopic localization) is the major cause of congenital hypothyroidism in humans. Recent experimental observations indicate that thyroid dysgenesis may be a polygenic disease with variable penetrance depending on genetic background. Also, thyroid dysgenesis might be one manifestation of syndromic malformations. The molecular mechanisms of thyroid dysgenesis in humans are largely unknown; so far genes encoding thyroid transcription factors that are required for normal thyroid development in mouse, i.e. Titf1/Nkx2.1 (also known as TTF-1), Foxe1 (also known as TTF-2) and Pax8, have been found to be mutated only in a minority of patients. The underlying molecular mechanism is in most cases unknown, but the frequent co-incidence of cardiac anomalies (3–12%) suggests that the thyroid morphogenetic process may be linked to cardiovascular development.

I will give an overview about critical steps in murine thyroid morphogenesis. Emphasis will be put on proliferative patterns and the possible relationship between shaping of the thyroid and development of the of pharyngeal arch artery system. In this context, recent results from our laboratory providing a mechanistic explanation to thyroid dysgenesis incidentally reported to occur in children with the DiGeorge syndrome will be discussed. The role of non-cell-autonomous factors (Shh, Tbx1) in thyroid development will be put in relation to other transgenic models where thyroid dysgenesis has been described. Finally, possible clinical implications of the findings will be discussed.

S7.3

Thyroglobulin deposition and cathepsin-dependent Tg mobilization

Klaudia Brix[1], Sasa Jenko-Kokalj[2], Dusan Turk[2], Dieter Brömme[3], Nicole Kühl[1] & Silvia Jordans[1]
[1]Jacobs University Bremen, School of Engineering and Science, Bremen, Germany; [2]Jozef-Stefan Institute, Ljubljana, Slovenia; [3]University of British Columbia, Vancouver, BC, Canada.

Thyroid hormones thyroxine and triiodothyronine are essential for development, growth and metabolism. The prohormone thyroglobulin (Tg) is stored in high concentrations and in covalently cross-linked form within the lumen of thyroid follicles. Thyroid hormones are liberated from Tg in a regulated manner in that TSH triggers the secretion of lysosomal enzymes into the extracellular follicle lumen where they solubilize covalently cross-linked Tg and liberate thyroxine by partial Tg degradation. Using mice deficient in cysteine cathepsins B, K, and/or L, we showed that liberation of thyroid hormones from within Tg is based on the concerted action of a protease network. Cathepsins B and L are key players in conversion of cross-linked Tg-globules to soluble Tg. Moreover, assessment of thyroid morphology and serum thyroxine levels of cathepsin K- and L-deficient mice revealed impaired mobilization of Tg. The respective mice exhibited a phenotype reminiscent of hypothyroidism, proving the importance of cathepsins K and L for the liberation of thyroid hormones. Tg storage and Tg mobilization both occur extracellularly. Hence, the conditions for Tg processing are non-favorable for the proteolytic activity of lysosomal cysteine cathepsins. Therefore, we set-up an *in vitro* degradation assay that simulates the *in vivo* situation. Indeed, in such assays the cysteine cathepsins B, K, L and S were able to partially degrade their natural substrate Tg even at neutral pH and oxidizing conditions. Analysis of the cleavage sites of cysteine cathepsins under extracellular conditions revealed that sub-cellular and sub-follicular localization of the proteases as well as the timing of proteolysis are crucial steps in the regulation of thyroid hormone liberation from Tg. Any interference with the delicate protease network in the thyroid may result in impaired function.

S7.4

Role of the complex Megalin-RAP in thyroglobulin trafficking

M Marino
Italy.

Abstract unavailable

Advances in adrenal hypersecretory disorders – S8

S8.1

Autocrine-paracrine pathways in primary adrenal disorders

Hervé Lefebvre[1], Vincent Contesse[1], Dorthe Cartier[1], Véronique Perraudin[1], Catherine Delarue[1], Hubert Vaudry[1], Jérôme Bertherat[2], Pierre-François Plouin[3], Jean-Marc Kuhn[4] & Estelle Louiset[1]
[1]INSERM U413, IFRMP23, Laboratory of Cellular and Molecular Neuroendocrinology, University of Rouen, Mont Saint Aignan, France; [2]Department of Endocrinology, CHU Cochin & Institut Cochin, INSERM U567, CNRS UMR8104, IFR 116, University of Paris V-René Descartes, Paris, France; [3]Hypertension Unit, European Hospital Georges Pompidou, University of Paris V-René Descartes, Paris, France; [4]Department of Endocrinology, University Hospital of Rouen, Rouen, France.

It is now well demonstrated that, in the human adrenal gland, aldosterone and cortisol productions are stimulated by autocrine/paracrine factors, like serotonin (5-HT) and arginine vasopressin (AVP). Several data indicate that these signals may also be involved in the regulation of corticosteroidogenesis in adrenocortical hyperplasias and tumors. 5-HT is detected in clusters of steroidogenic cells in aldosterone-producing adrenocortical adenomas (APAs), and in both ACTH-independent macronodular adrenal hyperplasias (AIMAHs) and adenomas responsible for Cushing's syndrome. In these lesions, 5-HT stimulates steroidogenesis through activation of overexpressed eutopic 5-HT_4 and/or ectopic 5-HT_7 receptors. Immunohistochemical studies have shown the occurrence of AVP in a subpopulation of steroidogenic cells in APAs and AIMAHs. In APAs, AVP activates aldosterone production through the eutopic V_{1a} receptor whereas its stimulatory effect on cortisol secretion from AIMAH tissues is mediated by both overexpressed V_{1a} and/or ectopic V_{1b} and V_2 receptors. Interestingly, administration of V_{1a} antagonists to patients with APA induces an aldosterone response to the upright stimulation test, indicating that, in these tumors, inhibition of the vasopressinergic tone sensitizes the tissues to the action of posture-responsive hormones. Finally, the presence of ACTH has been observed in AIMAH tissues and the ACTH receptor antagonist corticostatin inhibits basal cortisol secretion from AIMAH explants, demonstrating that glucocorticoid production is dependent on the paracrine action of intraadrenal ACTH in some primary adrenal disorders causing Cushing's syndrome. In conclusion, autocrine/paracrine regulatory factors are produced within adrenocortical hyperplasias and tumors in which they play an important role in the control of steroidogenesis. These local factors may therefore represent promising targets for the treatment of primary adrenal disorders. *This work was supported by INSERM, the University Hospital of Rouen, the Conseil Régional de Haute-Normandie and the COMETE network (PHRC AOM 02068).*

S8.2

Carney complex and primary pigmented nodular adrenocortical diasease

Jérôme Bertherat
Institut Cochin, INSERM U567, Paris, France.

The Carney complex (CNC) is a dominantly inherited syndrome characterized by spotty skin pigmentation, endocrine overactivity and myxomas. The most common endocrine gland manifestations are acromegaly, thyroid tumors, testicular tumors, and ACTH-independent Cushing's syndrome due to primary pigmented nodular adrenocortical disease (PPNAD). PPNAD, a rare cause of Cushing's syndrome, is due to primary bilateral adrenal defect that can be also observed in some patients without other CNC manifestations nor familial history of the disease. Myxomas can be observed in the heart, skin and breast. Cardiac myxomas can develop in any cardiac chamber and may be multiple. One of the putative CNC genes located on 17q22-24, (*PRKAR1A*), has been identified to encode the regulatory subunit (R1A) of protein kinase A. Heterozygous inactivating mutations of *PRKAR1A* were reported initially in 45 to 65% of CNC index cases, and may be present in about 80% of the CNC families presenting mainly with Cushing's syndrome. PRKAR1A is a key component of the cAMP signaling pathway that has been implicated in endocrine tumorigenesis and could, at least partly, function as a tumor suppressor gene. More recently, germline inactivating mutations of PDE11A4 have been identified in patients with isolated primary nodular adrenocortrical disease. This underlines the importance of the cAMP signalling pathway in the pathophysiology of secreting endocrine tumors. Somatic *PRKAR1A* mutations have been observed in adrenal adenomas responsible for Cushing syndrome. *In vitro* and transgenic models have been developped to study the consequences of PRKAR1A inactivation. In these models dysregulation of the cAMP pathway, but also others signalling pathways, have been observed. The new insights coming from the genetics of CNC and these experimental models in the pathophysiology of endocrine tumorigenesis will be discussed.

S8.3

Diagnosis of primary aldosteronism

GP Rossi
Italy.

Abstract unavailable

S8.4

Adrenocortical carcinoma: current and future therapeutic options

Martin Fassnacht[1], Stefanie Hahner[1], Sarah Johanssen[1], Ann-Cathrin Koschker[1], Marcus Quinkler[2] & Bruno Allolio[1]
[1]Dept. of Medicine, University Hospital Wuerzburg, Wuerzburg, Germany; [2]Dept. of Medicine, Charite University, Berlin, Germany.

Adrenocortical carcinoma (ACC) is a rare and heterogeneous malignancy with incompletely understood pathogenesis and poor prognosis. Recent data from the German ACC Registry ($n=377$) demonstrate an overall 5-year survival of 46%. Survival is clearly stage-dependent ($P<0.01$) with a 5-year survival of

85% in stage 1, 56% in stage 2, 42% in stage 3, and 16% in stage 4, respectively.

In stages I –III open surgery by an expert surgeon aiming at a R0 resection is the treatment of choice. However even after R0 resection, only 37% of the patients are disease-free after 5 years. Therefore, adjuvant treatment options are urgently needed. In a recent series including 177 patients from Italy and Germany, adjuvant mitotane prolonged significantly disease-free survival compared to observational follow-up. In addition, adjuvant radiotherapy of the tumor bed is a promising option to prevent local recurrence.

In tumor recurrence and metastatic disease, surgery should be considered if complete resection is feasible. In patients not amenable to surgery, mitotane (alone or in combination with cytotoxic drugs) remains the treatment of choice. Monitoring of drug levels (therapeutic range 14–20 mg/l) is mandatory for optimum results. In advanced disease, the most promising therapeutic options (etoposide, doxorubicin, cisplatin plus mitotane and streptozotocin plus mitotane) are currently compared in an international phase III trial (www.firm-act.org). In 2006, we have administered EGFR inhibitors and VEGF antibody as salvage therapy in a small series of patients – so far without significant success. However, in 2007 the Collaborative group for Adrenocortical Carcinoma Therapy (CO-ACT) will initiate three new trials for salvage therapies investigating two multi-targeted tyrosine kinase inhibitors and an IGF-1 receptor antibody, respectively, leading hopefully to improved clinical outcome. Future advances in the management of ACC will depend on a better understanding of the molecular pathogenesis of ACC facilitating the use of new targeted therapies.

Imaging in endocrinology – S9

S9.1

PET in diagnostics of metabolic alterations and endocrinological tumours

Pirjo Nuutila
University of Turku, Turku, Finland.

Positron emission tomography (PET) is an imaging technique that enables direct observation of tissue radioactivity concentration over time in vivo. Unlimited number of natural substrates (e.g. glucose, fatty acid), substrate analogs can be labelled for use with PET. PET combined with tracer kinetic models measures blood flow, membrane transport, metabolism, ligand receptor interactions and recently also gene expression noninvasively and quantitatively.

An abnormal action of insulin and handling glucose and fatty acids in muscle, heart, liver, brain and visceral and subcutaneous adipose tissue have been studied *in vivo* in humans. This tissue specific assessment has increased the understanding of the pathophysiology of metabolic syndrome, obesity and diabetes and the differences in tissue specific action *in vivo*. The effects of insulin, free fatty acids, exercise and diet have been evaluated. PET is powerful tool for the assessment of tissue specific action of drugs targeted to metabolic disorders. The hybrid PET/CT scanners enable correlation of anatomic and functional information.

The clinical use of PET is rapidly expanding. In addition to (18)F-labelled deoxyglucose (FDG) which is routine used in oncology for diagnosis of cancer, many more specific tracers have been shown to improve diagnostics of neuroendocrinological tumours (NETs). The most promising of those is (18)F-fluorodihydroxyphenylalanine (FDOPA). It appears to be more useful in carcinoid tumours than scintigraphic imaging and might replace it. It is more sensitive than CT or MRI in detection of insulinomas and has quickly replaced other methods in the assessment of focal form of congenital hyperinsulinism of infancy (CHI). FDOPA-PET improves diagnostics and prediction of prognosis, and be used to assess patients' response to treatment for NETs.

S9.2

Macro-, micro-, and molecular imaging of bone

Claus-C. Glüer
Medizinische Physik, Klinik f. Diagnostische Radiologie, Universitätsklinikum Schleswig-Holstein, Campus Kiel, Germany.

The clinical diagnostic examination of patients and the research-oriented investigation of the pathophysiology of skeletal disorders require imaging techniques that allow to visualize the skeleton at different scales: from the organ level to tissue, cellular, and subcellular levels, depicting morphology and function. Progress in the field of imaging technologies resulted in methods suited for clinical investigation of patients in vivo, non-invasive methods for preclinical animal studies and sophisticated functional and molecular imaging methods for both *in vivo* and *ex vivo* characterization of bone status have been introduced.

At the macroscopic scale the mechanical function of individual bones can now be assessed by 3-D volumetric spiral CT approaches. The image-data collected can be analyzed using Finite Element Models to calculate breaking strength under simulated impacting forces. This allows more accurate identification of subjects at risk for fracture and the monitoring of progress in fracture healing.

At the microscopic scale micro-CT has seen impressive advances with ever increasing image resolution – some devices are now suited for nano-CT imaging. This technology allows studies on the effects of bone turnover in normal and diseased tissue, including metabolic bone disorders such as osteoporosis but also of arthritis and skeletal tumours and metastases. Examinations of living animals enable the non-invasive longitudinal monitoring of skeletal effects of therapeutic interventions.

Finally, molecular imaging, i.e. the visualization of molecular, biochemical or cellular processes with radiological methods: to date this method is mostly restricted to animal studies. However, the achievements seen here are impressive: localized visualization of molecular and physiological information, e.g. imaging of labelled osteoblasts and their precursors, monitoring of the effects of hormones or gene therapy, or an earlier identification of skeletal metastases. Substantial research is still required to bring these advances to the clinic but the prospects for better individualized patient care based on combined molecular imaging and therapy are most exciting.

S9.3

New tracers for neuroendocrine tumors

Marcel Stokkel
LUMC, Leiden, Netherlands.

Neuroendocrine tumors (NET) comprise a wide variety of neoplasms that have certain characteristics in common. However, they are defined not by site but by molecular characteristics. The most common forms arise in the gastrointestinal tract, but there are NET which are not directly related to this site, such as medullary cell carcinoma or small-cell lung cancer. Different secretory syndromes have also resulted in certain subtypes receiving names as carcinoid if they produce serotonin or insulinoma if they produce insulin, etc. It has to be realized however, that 50% are described as non-secretors. With respect to nuclear medicine techniques available, many reports have focussed on the use of meta-Iodobenzylguanidine (MIBG) and radiolabelled somatostatin analogs, such as Indium-111-octreotide. Especially In-111-octreotide has a reported sensitivity ranging from 65% for medullary thyroid cancer to almost 100% in small-cell lung cancers and pancreatic neuroendocriene tumors. Many other potential receptors other than the somatostatin receptors, such as GRP-R, CCK2, GLP-1-R, NK1 and VPAC1, have been developed and studied over the past years. It has been suggested that the simultaneous expression of multiple of these peptide receptors in NET provide the molecular basis for in vivo multireceptor targeting, thus improving the efficacy of radiolabelled peptides for diagnosing, staging and treating NET. Most of the peptides under study directed against the previously mentioned receptors were labelled with Indium-111 or Technetium-99m, both easily applicable in clinical practice. Despite optimal results of positron emission tomography (PET) using F18-deoxyglucose in many malignant tumors, its role in NET is still limited. In contrast, PET using F18-DOPA and Ga-68-DOTA octreotate has shown promise. C11-5-hyroxytryptophan (C11-5-HTP) has demonstrated specific and irreversibly entrapment by serotonin-producing tumors, but it has been shown that non-functioning or poorly differentiated tumors or necrotic ones cannot be detected accurately. Highly important improvements have been made by the introduction of hybrid cameras such as SPECT/CT or PET/CT. The combination of both techniques allows whole body imaging quickly providing functional and anatomic information. A close clinical relation between imaging and treatment with radiolabelled peptides has been established over the past decades. Many studies have reported good and/or promising results with respect to Lutetium-177 (Lu-177) and Yttrium-90 (Y-90) labelled peptides, such as Y-90 DOTATOC or Lu-177-lanreotide. In current presentation, an overview is given on the nuclear medicine diagnostic and therapeutic options and developments in neuroendocrine tumors.

S9.4

Echoendoscopy for the diagnosis of pancreatic endocrine tumors

Claudio De Angelis
Gastrohepatology Department, San Giovanni Battista Hospital, University of Turin, Turin, Italy.

Pancreatic neuroendocrine tumors (NET) have always represented a complex dilemma for diagnostic imaging. This is mainly due to their small size and brought during the years to a complex range of diagnostic proposals. A correct preoperative detection and staging are mandatory in order to choose management options and to optimize surgical treatment. Endoscopic Ultrasound (EUS) has been claimed to be the best technique for imaging the pancreas, it allows.

High resolution images of the main pancreatic duct and surrounding parenchyma. One of the more relevant advantages of EUS compared with US, CT and MRI was indeed the superior parenchymal resolution, that gives reason for the results of several studies that established the superior sensitivity of EUS (98%) for the diagnosis of pancreatic tumors in comparison to all the other imaging modalities. The results of EUS were even better in small tumors, less than 3 or 2 cm, where sensitivity of US and CT decreased to only 29%. However the introduction of multidetector helical CT has today revolutionized the field of pancreatic imaging. More recent data on pancreatic NETs confirmed that the distance between helical-CT and EUS has nearly been annulled. EUS remains the best method for the detection of small pancreatic insulinomas and gastrinomas, but the first imaging modality to be used today in the suspicion of a pancreatic NET must be a multislice CT. EUS is needed as a second step in the diagnostic algorithm when CT shows negative or doubtful results. So the most effective method for revealing pancreatic NET is a combined imaging protocol that consists of both CT and EUS. The endosonographic pattern of these tumors is mainly represented by small focal hypoechoic, omogeneous, round lesions, with sharp margins, often hypervascular. Several studies have shown the high sensitivity and specificity of EUS in localizing endocrine tumors of the duodeno-pancreatic area. We demonstrated a correct localisation of pancreatic tumors in 86.7% of 23 cases surgically confirmed. In conclusion EUS is highly accurate in the detection of pancreatic neuroendocrine tumors and is cost effective when used early in the preoperative localization strategy. EUS decreased the need for additional invasive tests and avoided unnecessary morbidity and resource consumption.

EUS should play a primary role in preoperative localization and staging of these tumors.

GH and prolactin at their targets – S10

S10.1

Cellular control mechanisms for GH sensitivity

Ger Strous, Peter van Kerkhof, Monique van den Eijnden & Joyce Putters
University Medical Center, Utrecht, Netherlands.

The growth hormone (GH) receptor is a key regulator of cellular metabolism. Using model cell systems we have investigated how GH-induced signaling is regulated, both in paracrine and autocrine conditions.

Three features render GHR unique: (a) an active ubiquitination system is required for both endocytosis and degradation in lysosomes; (b) uptake of receptor is a continuous process, independent of GH binding and Jak2 signal transduction; (c) only cell surface expression of *dimerised* GHRs is controlled by the ubiquitin system. Despite recent progress, molecular mechanisms underlying GHR endocytosis and degradation are unknown. Evidence from research on the interferon and prolactin receptors has identified SCF^{TrCP} as a positive factor for their degradation. This E3 is known for its regulatory role in cell division and various signal transduction pathways. Our results show that the ubiquitin ligase SCF^{TrCP} is required for GHR endocytosis: removal of its mRNA by small interference RNA results in inhibition of GH uptake. In addition, we demonstrate that interaction between GHR and beta-TrCP is independent of a canonical DSGxxS motif. These results show the involvement of a SCF E3 ligase in endocytosis, thereby regulating GH-sensitivity of cells. In cells that produce both GH and GHR, the situation is basically the same. In these cells we investigated how GH affects GHR receptor degradation, and how the Jak/Stat signaling pathway is regulated. The consequences of these studies are important for understanding autocrine-activated GHR in fetal and peri-natal, and cancer tissues.

S10.2

GH receptor signalling

Gunnar Norstedt, Petra Tollet Egnell & Amilcar Flores Morales
Karolinska Institutet, Stockholm, Sweden.

GH receptor stimulation changes intracellular protein phosphorylation and activates the JAK-STAT signalling pathway. The JAK2 - STAT5 components of this pathway seem critical for growth. Factors of essence for cellular effects of GH include the duration of GH receptor stimulation and in different species there are sex differences in GH secretion where males have an episodic and females have a more continuous mode of GH secretion. At the cellular level, these two types of GH secretion cause different gene expression patterns to emerge and this is in particular the case for GH effects on the liver. GH controls important aspects of liver metabolism and it is interesting to note that some of these seem to depend on the secretory GH pattern. Another aspect of GH signaling is that the duration of GH receptor signals is related to changes in SOCS (suppression of cytokine signaling) expression. The SOCS proteins seem to be part of an intracellular feed back loop that silence GH signals. In our studies, SOCS2 appears to be a key intracellular regulator of GH sensitivity since elimination of SOCS2 creates a situation of increased GH sensitivity. Our working hypothesis is that SOCS2 ubiqitinates the GH receptor and thereby causes its proteasomal degradation. The concept that SOCS2 is a part of an ubiquitin ligase complex is substantiated by structural and biochemical findings. Furthermore, the gene targets for GH induced signals include the SOCS2 gene. In this gene we have characterized STAT 5 DNA binding elements in proximity to another transcription factor binding site that is unique for SOCS2 the SOCS protein family. In summary our data suggest that the liver is an important tissue for GH to exert metabolic regulation and that SOCS2 is a component that determines GH sensitivity.

S10.3

Gene expression profiling of the antiangiogenic factor 16K human prolactin (hPRL) on endothelial cells underlines the key role of NF-κB and reveals novel mechanisms of action

Sébastien Tabruyn[1], Céline Sabatel[1], Ngoc-Quynh-Nhu Nguyen[1], Catherine Verhaeghe[1], Karolien Castermans[2], Ludovic Malvaux[1], Arjan Griffioen[2], Joseph Martial[1] & Ingrid Struman[1]
[1]University of Liège, GIGA-Research, B-4000 Sart Tilman, Liège, Belgium; [2]University and Hospital of Maastricht, P.O. Box 5800, NL-6202 Maastricht, The Netherlands.

The 16-kDa N-terminal fragment of human prolactin (16K hPRL) is a potent angiostatic factor described to prevent tumor growth in mouse models. Using microarray experiments, we have dissected how the endothelial-cell genome responds to 16K hPRL treatment. Of the 23.000 transcripts present on the chips, 210 are regulated by 16K hPRL. Bio-informatic analysis and experiments performed on endothelial cells with various chemical inhibitors clearly suggest that NF-κB is crucial for the direct regulation of the majority of theses genes. In addition, our results reveal that the angiogenesis inhibitor 16K hPRL regulates apoptosis and proliferation in endothelial cells by numerous non-previously identified targets. Unexpectedly, a large proportion of 16K hPRL-regulated genes turned out to be associated with the process of immunity. 16K hPRL induces expression of various chemokines and endothelial adhesion molecules. These expressions, under the control of NF-κB, result in an enhanced leukocyte-endothelial cell interaction. Furthermore, analysis of B16-F10 tumor tissues reveals a higher expression of adhesion molecules (ICAM-1, VCAM-1 or E-selectin) in endothelial cells and a significantly higher number of infiltrated leukocytes within the tumors treated with 16K hPRL than in the untreated ones. In conclusion, this study describes a new anti-tumor mechanism of 16K hPRL. Since cellular immunity against tumor cells is a crucial step in therapy, the discovery that treatment with 16K hPRL overcomes tumor-induced anergy may become important for therapeutic perspectives.

Work supported by grants from the F.R.I.A. (“Fonds pour la formation à la recherche dans l’industrie et l’agriculture”), Télévie, F.N.R.S. (“Fonds national pour la recherche scientifique”), the Fédération Belge contre le Cancer and the Université de Liège (Fonds Spéciaux).

S10.4

Development of human prolactin receptor antagonists

Vincent Goffin[1], Vincent Rouet[1], Jean-Baptiste Jomain[2], Estelle Tallet[2], Christine Kayser[1] & Paul A. Kelly[2]
[1]Inserm, Paris, France; [2]University René Descartes Paris 5, Paris, France.

Experimental, clinical and/or epidemiological evidence points to a role for prolactin (PRL) in the promotion of benign and malignant tumors of the breast

and the prostate. However, therapies reducing PRL levels (dopamine agonists) are currently not considered for the treatment of these pathologies. Dopamine agonists only target PRL secretion from the pituitary, while recent observations suggest that the involvement of autocrine PRL is perhaps even more relevant than circulating PRL in the growth of breast/prostate tumors. Therefore, alternative strategies targeting locally-produced PRL warrant investigation.

For many years, we have been working on the development of PRL receptor antagonists, by introducing mutations into appropriate regions of human PRL. Ideal antagonists should be high-affinity ligands that bind but do not activate the PRL receptor, leading to competitive inhibition of endogenous PRL actions. This presentation will describe the most representative antagonists we have designed, including their structure-function relationships based on cell and animal studies. We will also discuss the pros/cons of our lead compound, the pure antagonist del1-9-G129R-hPRL. Finally, we will address the potential therapeutic indications of this novel class of molecules.

Polycystic ovary syndrome – S11

S11.1

The CAG repeat polymorphism of the androgen receptor gene is an independent risk factor for polycystic ovary syndrome (PCOS)

Andreas Schuering[1], Andrea Jurgens[1], Jorg Gromoll[2], Michael Zitzmann[2], Barbara Sonntag[1], Eberhard Nieschlag[2], Robert Greb[1] & Ludwig Kiesel[1]
[1]Department of Obsterics and Gynecology Muenster University Hospital, Muenster, Germany; [2]Institute of Reproductive Medicine, Muenster University Hospital, Muenster, Germany.

Introduction
Polycystic ovary syndrome (PCOS) is a frequent disorder with a variable phenotype and a suspected genetic background. Androgenic effects constitute the central mechanism for the clinical, biochemical and sonographic features of PCOS. Androgenic effects are transported by the androgen receptor, whose activity can be modulated by a genetic polymorphism. We investigated the role of the CAG repeat polymorphism of the androgen receptor in PCOS.
Patients and methods
In the infertility unit of a university clinic 126 patients fulfilling the Rotterdam criteria of PCOS were compared with 184 controls undergoing a standardized diagnostic work-up prior to infertility treatment. Individuals were assessed regarding clinical, endocrine and sonographic parameters indicating the presence of PCOS. The number of CAG repeats was determined by PCR, labelling with IR-800 and PAGE. X-chromosome inactivation was assessed by a methylation-sensitive assay. CAG repeat length was compared between groups and correlated with the extent of oligomenorrhoea. In a regression analysis CAG repeat length was tested including established risk factors of PCOS.
Results
PCOS patients displayed a shorter mean CAG repeat length compared to controls ($P=0.001$). CAG repeat length correlated inversely with the extent of oligomenorrhea, a central androgen dependent feature of PCOS ($P=0.007$). In a binomial regression analysis including BMI, LH and testosterone, CAG repeat length was identified as a novel independent risk factor for PCOS ($P=0.001$).
Conclusion
The CAG repeat polymorphism was identified as a novel independent risk factor for PCOS. It could constitute a factor in the familial background, convey the phenotypic variability and transport metabolic consequences of the syndrome.

S11.2

Genetic markers of polycystic ovary syndrome (PCOS)

Joop Laven
Div Reproductive Medicine, Rotterdam, Netherlands.

Polycystic ovary syndrome (PCOS) represents the most common cause of anovulatory infertility and its etiology is still unknown. Gene expression profiles from human PCOS ovaries have identified dysregulated expression of genes encoding components of several biological pathways or systems such as Wnt signaling, extracellular matrix components, immunological factors and androgens which, seem to play a key role in the pathogenesis of PCOS.
Candidate genes have been extensively studied using Single Nucleotide Polymorphisms (SNP's). The impact of functional SNP's on Gonadotrophins, growth factors and their receptors as well as the consecutive enzymes of the steroid biosynthesis pathways have been assessed in PCOS. Up till now only two functional SNP's have been consistently associated with PCOS. An FSH receptor and an aromatase polymorphism seem to be more prevalent in PCOS and are both associated discrete changes in the endocrine environment in PCOS.
Family studies and linkage analysis is hampered by the lack of large well phenotyped family cohorts. Recently we have studied PCOS patients from an isolated population aiming to map gene(s) involved in PCOS susceptibility. The genome wide association analysis revealed only weak evidence of association for some markers scattered over the genome. Taken these findings into account it seems that PCOS constitutes a complex genetic disease with multiple genetic contributors which, might in turn be modified through different environmental factors. The individual contribution of these genetic components to the phenotype of PCOS seems to be very limited and hence, detection of genetic factors is far from easy.

S11.3

Hyperandrogenism and metabolic syndrome (MBS) in polycystic ovary syndrome (PCOS)

Andrea Dunaif
The Feinberg School of Medicine, Northwestern University, Chicago, United States.

PCOS is a complex genetic disease resulting from the interplay between susceptibility genes and environmental factors. The syndrome is characterized by hyperandrogenism, disordered gonadotropin secretion, profound insulin resistance and, frequently, obesity. It is a leading risk factor for type 2 diabetes mellitus and MBS in adolescent and young adult women. In PCOS, MBS risk increases with increasing androgen levels, independent of insulin resistance and obesity, and antagonizing androgen action ameliorates features of MBS. Obese premenarchal girls have elevated androgen levels. Hyperandrogenemia is the major reproductive phenotype in families of women with PCOS, including mothers and brothers. First-degree relatives also have metabolic phenotypes, including MBS. We have now mapped a genetic variant conferring PCOS susceptibility to an allele of a dinucleotide repeat in an intron of the fibrillin-3 gene on chromosome 19p13.2 that is both linked and associated with the reproductive phenotype. Further, the PCOS susceptibility allele is associated with metabolic phenotypes in women with PCOS and their first-degree relatives. These observations suggest that the cardinal reproductive defect in PCOS, hyperandrogenemia, itself contributes to metabolic risk. *In utero* testosterone excess can reproduce features of the PCOS reproductive and metabolic phenotypes in rodents, sheep and non-human primates. We propose that hyperandrogenemia resulting from variation in a gene(s) regulating steroidogenesis causes many of reproductive and metabolic features of PCOS by programming actions at critical periods of development as well as by ongoing actions in the adult. Additional environmental factors, such as obesity, modify these phenotypes.

S11.4

Individual pharmacological therapy for polycystic ovary syndrome: lessons from the phenotype

Renato Pasquali
Division of Endocrinology, S. Orsola-Malpighi Hospital, University of Bologna, Bologna, Italy.

Hyperandrogenism, hyperinsulinemia and insulin resistance are the cardinal features of most women with the polycystic ovary syndrome (PCOS). They contribute in different ways to its phenotypic expression, including hirsutism, menses abnormalities, oligo-anovulation, metabolic disturbances, and susceptibility to develop type 2 diabetes. From the theoretical point of view, individual pharmacotherapy of PCOS should be planned in order to counteract the main pathophysiological mechanisms, with the aim of producing an overall benefit on all clinical and biochemical aspects of the disorders, after the major complaints of each individual have been considered. Dietary-induced weight loss and life style modifications should however represent the first line therapeutic advice for every obese woman with PCOS. Whether this applies in otherwise normal weight PCOS women has not yet been demonstrated, although the scientific basis for such an

approach is appealing, particularly in those with abdominal fatness and insulin resistance.
Since almost all obese PCOS women and more than half of lean PCOS women are insulin resistant, therefore presenting some degree of hyper-insulinemia, the use of insulin sensitizers should be suggested in most patients with PCOS. Their use has been associated with a reduction in androgen levels, improvement of insulin and insulin resistance, and reversal of serum lipid abnormalities and PAI-1. This therapy has also been associated with a decrease in hirsutism and acne, although the main benefit should be expected on menses abnormalities, anovulation and infertility. In our experience, at least one third of obese PCOS women improve menses and ovulation after a short period of treatment with metformin and life style changes. Antiandrogens have been used for a long-time in the treatment of hirsutism and hyperandrogenemia, We have recently performed pilot studies to investigate potential additional effects of long-term treatment with antiandrogens, and we have found that they can selectively improve visceral fatness, lipid abnormalities and even insulin resistance, although their main effect was on hirsutism and hyperandrogenemia.
The dual approach with insulin sensitizers and/or antiandrogens may provide a rationale for targeting different therapeutical options according to the required outcomes.

Hypothalamic network controlling food intake – S12

S12.1

Processing of metabolic signals in the hypothalamus: the integrative role of the paraventricular nucleus

Zsolt Liposits[1], Ronald Lechan[2] & Csaba Fekete[1]
[1]Institute of Experimental Medicine, Budapest, Hungary; [2]Tufts-New England Medical Center, Boston, MA, United States.

The hypothalamic paraventricular nucleus (PVN) is a major regulatory centre of energy homeostasis by possessing the unique capability of simultaneously controlling endocrine axes, water balance and autonomic functions. It receives neuronal information form orexigenic and anorexigenic cell groups of the basal hypothalamus that monitor peripheral metabolic signals (leptin, insulin, ghrelin, glucose, glucocorticoids) and also from brainstem centers relaying sensory information from visceral organs. In the regulation of energy homeostasis, the hypophysiotrophic corticotropin-releasing hormone (CRH) and thyrotropin-releasing hormone (TRH) neuronal systems play a key role and both neuron populations are wired to neuronal circuits of the basal hypothalamus and the brainstem. The lecture provides information about the structural organization, functional domains and major neuronal connections of the PVN, introduces the novel glutamatergic phenotype of hypophysiotrophic CRH and TRH systems, elucidates the diverse chemical nature of their synaptic afferents and describes the structural correlates of retrograde endocannabinoid signalling acting upon inhibitory and excitatory presynaptic terminals in the nucleus. The presentation also reveals distinct hypothalamic and extrahypothalamic sources of neuronal afferents carrying orexigenic (NPY) and anorexigenic (CART) peptides to TRH and CRH neurons and demonstrates the impact of the released neuropeptides on the postsynaptic targets. In addition to rodent data, the interrelationship of NPY and α-MSH neuronal systems and the features of their projections to CRH and TRH neurons will be presented in *post-mortem* human hypothalamic samples.
Supported by grants from the National Science Foundation of Hungary (OTKA T046492, T046574), NKFP 1A/002/2004 and the Sixth EU Research Framework Programme (LSHM-CT-2003-503041).

S12.2

Neurotransmitter content of orexigenic and anorexigenic neurones

Björn Meister, Katrin Dürr & Ebba Norsted
Karolinska Institutet, Stockholm, Sweden.

During the last two decades attention has been focused on the role of different neuropeptides in hypothalamic control of feeding behavior. Se-veral hypothalamic peptides that participate in the control of ingestive behavior are produced in neuronal cell bodies of the arcuate nucleus and/or the lateral hypothalamic area. Apart from producing orexigenic or anorexigenic compounds of peptidergic nature, it has recently become apparent that these neurons also produce several classical neurotrans-mitters. The role of classical transmitters in regulating energy ba-lance has received less attention in comparison to neuropeptides. The arcuate nucleus-median eminence area, a region with a weak blood-brain barrier (BBB), contains at least two neuronal cell populations that exert oppo-sing actions on energy balance. The majority of the neurons located in the ventromedial aspect of the arcuate nucleus, which pro-duce the or-exigenic peptides neuropeptide Y (NPY) and agouti-related peptide (AGRP), in addition contain the GABA-synthesizing enzyme glutamic acid decarboxylase (GAD) and the vesicular GABA transporter (VGAT), thereby supporting their GABAergic nature. Subpopulations of anorexi-genic neurons producing proopiomelanocortin (POMC)- and cocaine- and amphetamine-regulated transcript (CART), located in the ventro-lateral division of the arcuate nucleus have recently been reported to contain the vesicular acetylcholine (ACh) transporter (VAChT) and cho-line acetyltrans-ferase (ChAT), markers for cholinergic neurons, or the vesicular glutamate transporter 2 (VGLUT2), a marker for glutamatergic neurons. In addition, two new neuropeptides in have been identified in arcuate POMC neurons. In the lateral hypothalamic area, hypocretin-/orexin neurons express VGLUT1 or VGLUT2, but not GAD, whereas some melanin-concentrating hormone (MCH) cells contain GAD. These observations support the view that ACh, GABA and glutamate, relatively neglected feeding transmitters, are present in neurons that regulate body weight and consequently may represent important orexigenic-/anorexigenic mediators that convey information from the hypo-thalamus to other brain regions that participate in regulation of energy balance.

S12.3

The picture of the hypothalamus is becoming clearer: new concept of cross-talk

Tamas Horvath, Qian Gao & Sabrina Diano
Yale University School of Medicine, New Haven, CT, United States.

Significant advancements have been made in the last century regarding the neuronal control of feeding behavior and energy expenditure. The effects and mechanism of action of various peripheral metabolic signals on the brain have become clearer. Molecular and genetic tools for visualizing and manipulating individual components of brain homeostatic systems in combination with neuroanatomical, electrophysiological, behavioral and pharmacological techniques have begun to elucidate the molecular and neuronal mechanisms of complex feeding behavior and energy expenditure. This talk will attempt to highlight some of these advancements that have lead to the current understanding of the brain's involvement in the acute and chronic regulation of energy homeostasis. The case will also be made to suggest that the hypothalamic circuitry, which governs feeding behavior, is an appropriate model to examine in order to yield the experimental proof for the causal relationship between synaptic plasticity and behavior.

S12.4

Whom is insulin in the brain speaking to?

J Bruening
Germany.

Abstract unavailable

Glucocorticosteroids – S13

S13.1

Recent developments in nuclear receptor action

JA Gustafsson
Sweden.

Abstract unavailable

S13.2

Evaluation of steroid receptor function by gene targeting in mice

Gunther Schuetz
German Cancer Research Center, Dept. of Molecular Biology of the Cell I, Heidelberg, Germany.

Germline and somatic gene targeting of genes for steroid hormone receptor allows the characterization of their functions as well as their molecular modes of action. For the glucocorticoid receptor (GR) multiple modes of action have been identified. The receptor activates expression of genes, e.g. for gluconeogenic enzymes in hepatocytes, by binding as a dimer to glucocorticoid response elements (GRE) as well as by interaction with Stat5, functioning as a coactivator for DNA-bound Stat5. This functional interdependence of GR and Stat5 is reflected by sharing one third of their target genes. The receptor is able to repress AP-1/NF kB-dependent expression of genes involved in inflammation by protein-protein interaction and inhibits proopiomelanocortian and prolactin expression via binding to negative GREs. Cre/loxP-mediated generation of somatic mutants of the mineralocorticoid receptor (MR) circumvents the early lethality observed after germline inactivation. Inactivation of MR in the forebrain leads to impaired hippocampal-dependent learning as evidenced in Morris water- and radial maze analyses. Normal circadian corticosterone levels indicate that the limbic MR is dispensable for the maintenance of basal hypothalamic-pituitary-adrenal axis activity. The mechanisms underlying the critical actions of estrogen in the secretion of the gonadotropin-releasing hormone (GnRH) are unknown. A neuron-specific ERa mutation in the forebrain leads to infertility and loss of the positive feedback effects of estrogen upon GnRH neurons. As GnRH neurons do not express ERa, these results indicate that ERa-expressing neuronal afferents to GnRH neurons are critical for the preovulatory GnRH/LH surge. These genetic approaches to evaluate steroid hormone receptor activity not only reveal novel neural functions of these regulatory molecules in gene expression, but also unprecedented modes of their activity.

S13.3

The 11β-hydroxysteroid dehydrogenase story

Jeremy Tomlinson
University of Birmingham, Birmingham, United Kingdom.

The global epidemic of obesity has heightened the need to understand the mechanisms that contribute to its pathogenesis and also to design and trial novel treatments. Patients with glucocorticoid (GC) excess, 'Cushing's syndrome' share many phenotypic similarities to patients with simple obesity. GC availability to bind and activate the glucocorticoid receptor (GR) is controlled by the type 1 isoform of 11β-hydroxysteroid dehydrogenase (11β-HSD1) that converts inactive cortisone to cortisol and therefore amplifies local GC action. We have previously shown that expression of 11β-HSD1 is crucially important in both adipocyte differentiation and proliferation; Furthermore, over-expression specifically within adipose tissue leads to obesity and insulin resistance in rodent models. In addition, we have recently been able to show that inhibition of 11β-HSD1 in human adipose tissue can limit GC induced lipolysis. Selective 11β-HSD1 inhibitors (selective in that they block the activity of 11β-HSD1 and not 11β-HSD2 which inactivates cortisone to cortisol in mineralocorticoid target tissues) are currently in development although not yet available for use in clinical studies. Rodent studies utilizing these compounds have shown dramatic improvements in insulin sensitivity as well as improvements in lipid profiles and atherogenesis. The most fundamental question is whether these observations in rodents will translate to the clinical setting. It is likely that within the very near future, data from the first human studies will be available. If these compounds prove to be as efficacious in humans, then they may well represent an entirely novel, additional therapeutic strategy in the treatment of obesity, insulin resistance and type 2 diabetes.

S13.4

Glucocorticoid sensitivity: consequences for the clinic?

Jan W Koper
Internal Medicine / Endocrinology, Erasmus MC, Rotterdam, Netherlands.

Glucocorticoids (GCs) exert a wide variety of functions throughout the human body, including mediation of the stress response, regulation of lipid and glucose metabolism, immunosuppressive and anti-inflammatory actions, vascular effects, increase of bone resorption, as well as effects on the development and function of numerous organs. The immuno-suppressive effects of GCs are routinely used in the treatment of chronic inflammatory or immune diseases (e.g. inflammatory bowel disease, asthma). However, severe side effects (including diabetes and osteoporosis) are associated with GC-treatment, limiting its therapeutic usefulness.

Within the normal population, there exists a considerable inter-individual variation in GC sensitivity. Whereas some patients develop side effects on relatively low doses of topically administered GCs, others appear to be less sensitive to GCs, as they do not show an adequate improvement in response to treatment even on high doses. Some patients are even resistant to the anti-inflammatory effects of GCs while at the same time showing side effects known to reflect normal sensitivity to GCs, including suppression of the hypothalamic-pituitary-adrenal axis. Variability in GC sensitivity can be divided into GC resistance and GC hypersensitivity.

The signaling pathway of GCs is a complex process, in which distinct pathways are involved that can influence GC sensitivity. Also, other mechanisms such as the transport, local conversion and degradation of GCs play a role in the intracellular bioavailability of GCs.

Here we will discuss the possible consequences for the clinic of genetic variation in genes involved in the GC signalling pathway, and resulting in inter-individual differences in glucocorticoid sensitivity.

Trojan horses for hormones – S14

S14.1

Alpha-fetoprotein protects the developing female brain from estrogens

Julie Bakker[1], Christelle De Mees[2], Jacques Balthazart[1] & Claude Szpirer[2]
[1]University of Liège, Liège, Belgium; [2]Université Libre de Bruxelles, Gosselies, Belgium.

The classic view of sexual differentiation in mammalian species holds that sex differences in the brain and behavior develop under the influence of estrogens derived from the neural aromatization of testosterone: the brain develops as male in the presence of estrogens and as female in their absence. In agreement with this view, it has been proposed that the female brain needs to be protected from estrogens produced by the placenta and that alpha-fetoprotein (AFP) - a major fetal plasma protein present in many developing vertebrate species and produced transiently in great quantities by the hepatocytes of the fetal liver– is the most likely candidate to achieve this protection because of its estrogen-binding capacity. However, the idea that the female brain develops in the absence of estrogens and the role of AFP in protecting the brain against the differentiating action of estrogens have been challenged. First, there is accumulating evidence that the normal development of the female brain might actually require the presence of estrogens. Second, the presence of AFP within neurons in the absence of any evidence for local AFP synthesis suggests that AFP is transported from the periphery into the brain. It was thus proposed as well that AFP acts as a carrier, which actively transports estrogens into target brain cells and, by doing so, has an active role in the development of the female brain. The availability of AFP mutant mice (AFP-KO) now finally allowed us to resolve this longstanding controversy concerning the role of AFP in brain sexual differentiation, and thus to determine whether prenatal estrogens contribute to the development of the female brain. We showed that the brain and behavior of female AFP-KO mice were masculinized and defeminized. However, when estrogen production was blocked by fetal treatment with an aromatase inhibitor, the feminine phenotype of these mice was rescued. These results clearly demonstrate that the principal action of prenatal estrogen exposure is to defeminize the brain and that AFP normally binds estradiol circulating in the female fetus and thereby protects the developing brain from defeminization.

S14.2

Role of endocytic receptors in cellular uptake of steroid hormones

Thomas Willnow[1], Annette Hammes[1], Thomas Andreassen[2] & Anders Nykjaer[2]
[1]Max-Delbrueck-Center for Molecular Medicine, Berlin, Germany; [2]University of Aarhus, Aarhus, Denmark.

Androgens and estrogens are transported bound to the sex hormone binding globulin (SHBG). SHBG is believed to keep sex steroids inactive and to control the amount of free hormones that enter cells by passive diffusion. Contrary to the free hormone hypothesis, we demonstrate that megalin, an endocytic receptor in reproductive tissues acts as a pathway for cellular uptake of biologically active androgens and estrogens bound to SHBG. In line with this function, lack of receptor expression in megalin knockout mice results in impaired descent of the testes into the scrotum in males and in blockade of vaginal opening in females. Both processes are critically dependent on sex steroid signaling and similar defects are seen in animals treated with androgen or estrogen receptor antagonists. Thus, our findings uncover the existence of endocytic pathways for protein-bound androgens and estrogens, and their crucial role in development of the reproductive organs.

S14.3

Hepatic deiodinase activity is dispensable for the maintenance of normal thyroid hormone levels in mice

Ulrich Schweizer
Charité Universitätsmedizin Berlin, Berlin, Germany.

The main product of the thyroid is thyroxine (T4). However, the physiological ligand of nuclear thyroid hormone receptors is triiodothyronine, T3. Deiodination of T4 to yield T3 is achieved by 5′-deiodinase activity. Type I-deiodinase (Dio1) was the first deiodinase cloned and its strong expression in liver and kidny, together with the size of these organs, suggested a role for Dio1 in peripheral conversion of T4 to T3. Later, Dio2 and Dio3 were cloned, enzymes with a more restricted pattern of expression that mediate 5′- and 5-deiodination, respectively. A model emerged in which activation and inactivation of thyroid hormones is governed by the concerted action of tissue-specific deiodinase expression. One aspect of this familiar textbook model, a central role of hepatic Dio1 in T3 production, was recently challenged. Since all deiodinase enzymes are selenoproteins, targeted removal of the gene encoding selenocysteine tRNA (Trsp) allowed the liver-specific inactivation of Dio1 activity. Using Albumin-Cre; Trsp fl/fl mice we showed that loss of hepatic deiodinase did not disturb circulating thyroid hormone levels. Moreover, deiodinase activities in other organs did not show compensatory up-regulation. Data derived from the conventional Dio1 knockout mice suggest that hepatic Dio1 is involved in the re-cycling of iodine from iodothyroines. Since the targeted inactivation of Dio2 perturbed pituitary feedback regulation, but did not reduce serum T3 levels, the question remains which deiodinase provides circulating T3. We have taken these investigations further and will present data regarding the effects of thyroid-specific Trsp inactivation in transgenic mice.

S14.4

IGF-independent actions of IGFBPs

Jean-Marc Ricort
[1]INSERM U515, Paris, France; [2]ENS de CACHAN, Paris, France.

The discovery that IGF binding proteins (IGFBPs) are capable of action independently of ligand binding opened up a broad scope of investigation into the mechanisms by which the IGFBPs elicit their intrinsic cellular effects. Numerous studies have demonstrated the special role of IGFBPs in as diverse processes as cell proliferation, migration and survival/apoptosis. However, the pathways by which these actions occur have not been completely defined but interactions of IGFBPs with other proteins or biomolecules must be involved.

IGFBPs can bind to many partners other than IGFs, although the relationship between most of these binding interactions and IGFBP actions remains uncertain. Several studies have identified membrane proteins that bind IGFBPs with relatively high affinity. These include proteins known to be involved in other signalling pathways (such as integrin receptor and TGFβ receptor) and putative receptors, the precise nature of which remains to be determined. Moreover, IGFBPs can also bind to intracellular (even nuclear) proteins.

Therefore, an exciting challenge in identifying the signalling pathways modulated by such interactions between IGFBPs and their partners is currently open.

Novel bone hormones and regulators – S15

S15.1

Sclerostin, an osteocyte-produced regulator of bone formation

Socrates Papapoulos
Leiden University Medical Center, Leiden, Netherlands.

Sclerosteosis and van Buchem disease are closely related, rare sclerosing disorders characterized by substantial increase in bone mass of good quality which is due to increased bone formation. Both diseases have been linked to deficiency of the *SOST* gene product sclerostin, which in the adult is localized exclusively in osteocytes, the most abundant bone cell. In particular sclerostin is localized in mature osteocytes in mineralised cortical and cancellous bone, it inhibits the activity of osteoblasts and prevents them from promoting excessive bone formation. It is, thus, a negative regulator of bone formation. Sclerostin may be transported by the canaliculi to the bone surface where it inhibits the bone-forming activity of osteoblasts.. In this respect it serves the function of the unknown inhibitory factor proposed by Marotti and Martin that is secreted by mature osteocytes and communicates with osteoblasts at a forming surface causing the adjacent osteoblast to slow osteoid formation.

Because of its structural similarity to the DAN family of glycoproteins, it was originally thought that sclerostin is a BMP antagonist. Whilst sclerostin inhibits BMP-stimulated bone formation, it does not affect BMP signaling and is distinct from classical BMP antagonists. Instead it antagonizes Wnt signaling in osteoblastic cells.

The human high bone mass (HBM) phenotype is an autosomal dominant condition that, like sclerosteosis and van Buchem disease, is characterized by increased bone mass due to enhanced bone formation in the presence of normal bone resorption. It is due to mutations of the *LRP5* gene that make it resistant to the inhibitory action of Dkk1, thereby increasing Wnt signalling. The observations that sclerostin antagonizes Wnt signaling rather than BMP signaling raises the possibility that these skeletal diseases are due to increased activity of the same signaling pathway: LRP5-mediated canonical Wnt signaling.

The restricted expression pattern of sclerostin and the exclusive bone phenotype of good quality of patients with sclerosteosis and van Buchem diseases provide a basis for the design of therapeutics that specifically stimulate bone formation, an action of primary importance for the management of patients with osteoporosis. As sclerostin is a secreted protein, one approach to achieve this is to develop humanized monoclonal antibodies capable of inhibiting the biological activity of sclerostin, mimicking, thus, the absence of sclerostin in sclerosteosis. Preliminary results of such approaches in animal models have been very encouraging.

S15.2

Hormonal regulation of periosteal bone growth

Dirk Vanderschueren & Katrien Venken
Unversity Hospitals, Leuven, Belgium.

In light of the gender differences in bone geometry, sex steroids have been proposed as key regulators of pubertal periosteal bone formation. Sex steroids may affect periosteal bone apposition following activation of sex steroid receptors [androgen receptor (AR), estrogen receptor alpha (ERα) or beta (ERβ)]. Traditionally, it has been assumed that AR-mediated androgen action stimulates periosteal bone formation and thereby determines the larger bone size in males, whereas estrogens suppress periosteal bone formation resulting in a smaller bone size in females. However, optimal periosteal growth in the male is only obtained in the presence of both AR and ER activation as demonstrated in mice with a disruption of the AR gene and in an adolescent man with a mutation in the gene encoding the aromatase enzyme. Moreover, the bone phenotypes of ERα, ERβ and double knock-out mice indicate that the presence of ERα and ERβ increase and decrease periosteal bone expansion, respectively (the former is observed in males and females, the latter only in females). Furthermore, administration of an aromatase inhibitor that blocks the conversion of androgens into estrogens also limits periosteal bone expansion in growing male mice and rats. Beside sex steroids, growth hormone (GH) and insulin-like growth factor-I (IGF-I) are also major determinants of radial skeletal growth. Moreover, sex steroids and GH-IGF-I closely interact in pubertal life in order to obtain optimal stimulation of periosteal bone formation. In this context, targeted disruption of ERα in mice or pharmacological inhibition of aromatization of androgens in mice and rats reduce serum IGF. Such finding raises the question to what extent sex steroids are able to affect periosteal bone formation independently from the GH-IGF-I axis. We therefore studied periosteal bone formation following androgen or estrogen administration in orchidectomized male mice with disrupted growth hormone receptor (GHR). GHR activation appears the main determinant of radial bone expansion, but both GHR signaling and androgen action are independently and cooperatively needed for optimal stimulation of periosteal growth in the male during puberty. Interestingly, estrogen treatment rescued periosteal bone formation in mice with disrupted growth hormone receptor which was explained by a stimulation of IGF-I synthesis in the liver independently from GHR activation.

In conclusion, optimal periosteal bone formation in the male during puberty primarily depends on a functional GH-IGF-I axis, followed by activation of the AR. However, both GH/IGF-I and androgens are independently needed for optimal stimulation of radial bone growth. Moreover, part of the androgen action on periosteal bone may be explained by aromatization and subsequent ERα activation. The latter may interact with GH/IGF-I and may influence periosteal growth by estrogen-related changes in serum IGF-I.

S15.3

Wnt signaling and LRP 5/6 regulation of bone mass

Peter Bodine
Wyeth Research, Collegeville, PA, United States.

Wnts are a large family of carbohydrate- and lipid-modified growth factors that mediate essential biological processes such as embryogenesis, morphogenesis and organogenesis. These proteins bind to a membrane receptor complex comprised of a

frizzled (FZD) G-protein-coupled receptor and a low-density lipoprotein (LDL) receptor-related protein (LRP). The formation of this ligand-receptor complex initiates a number of signaling cascades that includes the canonical/beta-catenin pathway as well as several noncanonical pathways. In recent years, canonical Wnt signaling has been reported to play a significant role in the control of bone formation and remodeling. Clinical studies have found that mutations in LRP-5 are associated with bone mineral density and fractures. Investigations of knockout and transgenic mouse models of Wnt pathway components including Wnt-10b, LRP-5 and -6, secreted frizzled-related protein-1 and -4, dickkopf-1 and -2, Sclerostin, axin-2, beta-catenin and T-cell factor-1 have shown that canonical signaling modulates almost all aspects of osteoblast physiology including proliferation, differentiation, function, mineralization, apoptosis and mechanosensory perception as well as coupling to osteoclasts. In addition, preclinical studies with pharmacologic compounds such as those that inhibit glycogen synthase kinase-3beta support the importance of the canonical pathway in modulation bone formation. Moreover, well-established bone forming agents like bone morphogenetic proteins and parathyroid hormone have been demonstrated to intersect and utilize components of Wnt signaling pathways. Future research in this swiftly expanding area of skeletal biology should focus on understanding Wnt/FZD specificity in the control of bone cell physiology, the role of noncanonical pathways in bone remodeling, the interplay between Wnt signaling and other bone metabolic pathways and direct actions of Wnts on cells of the osteoclast lineage.

S15.4

Thyroid hormones/TR and bone

Graham R Williams
Molecular Endocrinology Group, MRC CSC, Hammersmith Hospital, Imperial College London, London, United Kingdom.

Childhood hypothyroidism results in severely delayed skeletal development whereas adult thyrotoxicosis is associated with a 3–4 fold increase in osteoporotic fracture. To investigate molecular mechanisms underlying these abnormalities we characterized the skeletal phenotypes of mice harboring dominant negative mutations (TRα1PV/+, TRα1R384C/+, TRβPV/PV) or deletions (TRα0/0, TRβ−/−) of the genes encoding TRα and TRβ. Endochondral ossification, linear growth and bone mineralization were retarded in TRα0/0 mice and more severely delayed in TRα1 dominant-negative mutants. In contrast, these parameters were all advanced in TRβ knockout and PV-mutant mice. In adults, 3D bone micro-architecture and micro-mineralization densities were analyzed by quantitative backscattered electron scanning electron microscopy. TRα mice displayed increased cortical bone width, and an 8–9 fold increase in trabecular bone volume with increased thickness of individual trabeculae and greater micro-architectural complexity. In contrast, analysis of all these parameters including quantitation of bone micro-mineralization density revealed TRβ mutants were markedly osteoporotic. Studies of T3-target gene expression revealed phenotypes of skeletal hypothyroidism in TRα mutant mice but skeletal thyrotoxicosis in TRβ mutants. We further demonstrated that TRα is expressed at 15-fold higher levels in bone than TRβ, whereas TRβ is predominantly expressed in hypothalamus and pituitary and controls negative feedback regulation of TRH and TSH. Accordingly, TRα mutant mice were euthyroid whereas TRβPV/PV and TRβ−/− displayed pituitary resistance to thyroid hormone with elevated circulating thyroid hormone levels. This analysis of a series of TR mutant mice with differing genetic backgrounds unequivocally demonstrates that TRα is the predominant TR isoform in bone, and shows that skeletal responses to disrupted TRβ signaling result from effects of the mutation on systemic thyroid status.

Immune-endocrine turmoil of pregnancy – S16

S16.1

Endocrine diseases during pregnancy

Risto Kaaja
Helsinki University Hospital, Helsinki, Finland.

Successful pregnancy depends on the ability of the maternal immune system to tolerate a genetically incompatible feto-placental unit. One of the important adaptations leading to this immuno-tolerance is the shift, at implantation, of Th 1 dominance to Th 2 dominance. Successful pregnancy is a Th2 dominant immune state, therefore, it is not surprising that women with a Th 1 dominant immune disease such as rheumatoid arthritis, thyroiditis or multiple sclerosis improve during pregnancy, while patients suffering from Th 2 dependent immune disease, such as SLE, fare worse during pregnancy.

Interestingly, three autoimmune diseases, rheumatoid arthritis, multiple sclerosis and thyroiditis, that are reported to ameliorate or stabilize during pregnancy in the majority of women, are more likely to relapse during the year after delivery. The postpartum period can be regarded as a time of ongoing heightened inflammatory activity. The onset of rheumatoid arthritis is five times more likely in the puerperal period than at any other time. Multiple sclerosis is known to ameliorate during the last trimester of pregnancy. After delivery, the relapse rate is higher than that before pregnancy. Importantly, the decrease in the relapse rate during pregnancy was more marked than any drug mediated therapeutic effect reported to date. Of the acute endocrine emergencies an acute form of Sheehan's may go unrecognized, leading to unnecessary maternal deaths. Cushing's syndrome has very bad consequences for the fetus and must be diagnosed and treated urgently, if not emergently. Pheochromocytomas are always endocrine emergencies requiring urgent and sometimes emergent treatment. Hyperparathyroidism is usually mild, but severe hypercalcemia can be a true endocrine emergency.

Recognition of the interactions of these endocrine conditions and their specific treatments with the complicated maternal-fetal unit makes their diagnosis and treatment simultaneously both difficult and extremely rewarding.

S16.2

Estetrol (E4), the forgotten fetal steroid

Herjan Coelingh Bennink & Monique Visser
Pantarhei Bioscience B.V., Holland, Netherlands.

Estetrol (E4) is a natural human steroid, produced exclusively during pregnancy by the fetal liver. Estetrol has been discovered in 1965 by Diczfalusy at the Karolinska Institute in Stockholm.

The role of E4 in embryonic physiology and/or human pregnancy is not known. During human pregnancy E4 is detectable from 9 weeks pregnancy onwards. Estetrol reaches the maternal blood circulation via the placenta. Maternal and fetal E4 concentrations increase exponentially during pregnancy and peak at high levels at term with fetal levels about 10–20 times higher than maternal levels as confirmed by data obtained by Pantarhei Bioscience. Based on low receptor binding compared to estradiol (E2), E4 was thought to be a weak estrogen. Since the early eighties the molecule has been neglected.

The pharmacological properties of oral E4 as investigated by Pantarhei Bioscience can be summarised as follows. Estetrol is orally bioavailable in the rat and acts as an estrogen on bone, brain, vagina and endometrium. Estetrol suppresses hot flushes and inhibits ovulation. Surprisingly, E4 acts as an estrogen antagonist on the breast since it was shown to prevent development of breast tumors and to remove pre-existing breast tumors in the DMBA rat model.

In phase I studies in early postmenopausal women E4 showed high oral absorption, full dose linearity, high bioavailability, low inter-subject variability and a long elimination half-life with a mean of 28 hours. Estetrol appeared to be efficacious, safe and without side-effects up to a dose of 20 mg per day for 28 days.

Estetrol will be developed further for the treatment of breast cancer, prostate cancer, osteoporosis and for those Th2-mediated auto-immune diseases, that are known to improve during pregnancy.

S16.3

Regulatory T cells in pregnancy

Ana Claudia Zenclussen[1], Juliane Rau[1], Catharina Thuere[1], Anne Schumacher[1], Gerolf Zimmermann[2], Hans-Dieter Volk[1] & Henry Alexander[2]
[1]Charite, Institute of Medical Immunology, Berlin, Germany; [2]University of Leipzig, Leipzig, Germany.

The survival of the semiallogeneic fetus within the mother is thought to be due to mechanisms of immunological tolerance. Regulatory T cells (Treg) are believed to have a crucial role in maintaining pregnancy by creating a transient tolerant microenvironment within the maternal uterus as former studies confirmed. We have evidences that Treg expand in lymph nodes from normal pregnant mice already on day 2 of pregnancy. Abortion-prone mice present diminished numbers of Treg in immune organs throughout pregnancy. As both pregnancy combinations (normal pregnant and abortion-prone mice) present similar levels of progesterone, estriol and estrone, hormones do not seem to be involved in Treg expansion. However, they may be involved in their recruitment into the vaginal lumen.

An enormous augmentation in the number of $TCR\alpha\beta^+CD4^-CD8^-foxp3^+$ cells in vaginal mucus from normal pregnant animals already on day 0.5 after conception, followed by an increase in Treg numbers in lymph nodes, suggest that Treg need to be activated by male antigens for being protective. The antigen presentation would take place in the periphery e.g. in vaginal mucus, the first site

of contact with paternal antigens, directly after insemination as we could confirm by identifying paternal antigens and paternal APCs at this site. This explains previous observations on Treg transfer being effective in preventing abortion if done on days 0–2 of pregnancy but not later. Interestingly, mating CBA/J females with vasectomized BALB/c males generated foxp3$^+$ cells in lymph nodes draining the uterus, while pseudopregnancy induced by mechanical stimulation did not. Treg-induced tolerance is transient, as Treg came back to the normal levels after the disappearance of the paternal/fetal antigens, 14 days post-partum.

The molecules responsible for Treg recruitment immediately after copulation are being currently studied in our laboratory. Besides, running clinical studies will help us clarifying whether similar pathways are taking place in humans.

S16.4

The effect of pregnancy on immune disease
M. Hazes
The Netherlands.

Abstract Unavailable

Somatostatin receptors in health and disease – S17

S17.1

Pro and contra of SRIF analogue therapy in pituitary tumors
AJ van der Lely
Erasmus MC, Rotterdam, Netherlands.

Long-acting somatostatin analogues normalize serum IGF-I levels in about 65% of acromegalic patients. Somatostatin analogs reduce GH secretion but also induce GH resistance of the liver because of low portal insulin levels; i.e. patients have a relative high GH level and a GH resistance of the liver which results in a relative low IGF-I action because of high IGFBP1 levels, but the other tissues still have normal GH sensitivity. One might predict that long-term follow-up of treated acromegalic patients is mandatory for find out the potential differential effects of the various medical treatment modalities. Especially as nowadays, the combination of somatostatin analogues and GH-R antagonists will be used by clinicians more frequently in order to decrease administration interval of the GH-R antagonist, as well as reduce its dose that is necessary to control disease activity in those acromegalic patients that do not respond to long-acting somatostatin monotherapy. The novel multiligand analogue SOM230 might increase the number of patients that can be biochemically controlled. SOM230 inhibits free IGF-I in a more sustained fashion compared to octreotide, implying longer duration of action. The superior action of octreotide compared with SOM230 in stimulating IGFBP-1 levels in acromegalic patients, suggests direct regulation of IGFBP-1 by somatostatin analogues *via* the somatostatin subtype 2 receptor. In summary, somatostatin analogs are the only compounds of which, at least in acromegaly, it has been shown that they reduce tumor size in those subjects that express sst on their pituitary tumors. However, the expression of sst on other tissues, involved in glucose metabolism, might have a negative influence on glucose metabolism on some patients

S17.2

Somatostatin receptors in neuroendocrine tumors
Kjell Oberg
Department of Endocrine Oncology, Uppsala University Hospital, Upppsala, Sweden.

A unique feature of neuroendocrine tumors is that they express peptide hormone receptors. All five subtypes of somatostatin receptors are expressed in neuroendocrine tumors with dominance for receptor type 2 (SST2). Stimulation of SST2 can not only inhibit hormone release from the tumor, but also tumor cell growth. Both SST2 and 3 are involved in apoptosis of neuroendocrine tumor cells. SST's in intratumoral blood vessels might implicate a role of anti-angiogenesis of somatostatin and somatostatin analogues. Midgut carcinoids express about 80% of the tumors SST2. The same is true for most endocrine pancreatic tumors, except for benign insulin producing tumors that has a lower expression (50%). Signaling through SST2 inhibit hormone release and causes antiproliferation, whereas stimulation of SST2 and 3 causes apoptosis. ^{111}Indium-DTPA-octreotide (Octreoscan®) can be applied for localisation and staging of neuroendocrine tumors. Labelling of octreotide with either ^{177}Lutetium or ^{90}Yttrium is used for tumor targeted radioactive treatment (PRRT). The use of somatostatin analogues, Octreotide and Lanreotide, has been a real break-through in the management of functioning neuroendocrine tumors. Symptomatic and biochemical improvement has been noticed in 50-60% of the patients and tumor reduction in 5–10%. A new somatostatin analogue – SOM230 – has been applied in phase-2-trials. This analogue is binding with high affinity to receptor 1, 2, 3 and 5, but not 4. It has already demonstrated significant symptomatic effects in patients with functioning neuroendocrine tumors, resistant to octreotide treatment. In the future analysis of the expression pattern of different somatostatin receptors in neuroendocrine tumors will be important, particularly if new somatostatin analogues will be developed.

S17.3

Peptide receptor therapy
DJ Kwekkeboom
The Netherlands.

Abstract unavailable

S17.4

Cortistatin, a multi-functional somatostatin receptor analog
Luis de Lecea
Stanford University, Palo Alto, CA, United States.

Cortistatin is a neuropeptide that belongs to the somatostatin family, and shares 11 of its 14 amino acid residues with somatostatin. Studies in the central nervous system have shown that cortistatin has activities different from somatostatin, including enhancing slow wave sleep and selective conductances. However, in the periphery cortistatin appears to act as a somatostatin receptor analog. We have generated cortistatin ko mice and have analyzed the molecular, behavioral and immunological consequences of cortistatin deficiency. Our data suggest that cortistatin is a parallel system to somatostatin in the central nervous system, and may have specific and relevant functions in the immune system.

Puberty and hypogonadism – S18

S18.1

Endocrine disorders of puberty
Marek Niedziela
Poznan University of Medical Sciences, Poznan, Poland.

Puberty is a process in humans that leads to the development of secondary sexual characteristics and reproductive capabilities. The physical changes of puberty result from two separate and independent but overlapping processes: gonadarche and adrenarche. The activation of hypothalamic-pituitary-gonadal (HPG) axis plays a key role in gonadarche whereas body weight and body mass index are postulated as triggering the adrenarche. The impairment of this cascade will result in temporary or permanent disorders of reproductive endocrine function. This primarily endocrine process can be disrupted by genetic and environmental factors. The timing of pubertal onset is defined as normal if occurs between the ages of 8 and 13 years in girls and 9 and 14 years in boys. However the controversies concerning the age limit of onset of puberty have been raised. Precocity can be central (GnRH-dependent) or peripheral (GnRH-independent) in its etiology and iso- or heterosexual (consistent or inconsistent with gender). Central precocious puberty in girls is rather idiopathic whereas in boys has predominantly pathologic cause. Peripheral precocious puberty occurs rarely. The most common cause of delayed puberty is constitutional delay of growth and puberty, especially in

boys. However the other common etiologies should be considered: 1. Functional hypogonadotropic hypogonadism; 2. Permanent hypogonadotrophic hypogonadism and 3. Permanent hypergonadotrophic hypogonadism. The treatment strategy is highly specific for each single disorder. Genetic studies on newly detected factors regulating HPG axis (eg. KiSS-1 and GPR54 as gatekeepers of gonadotropin-releasing hormone release neurons or FGFR1) may improve understanding of normal variation in pubertal timing and provide further directions for treatment.

S18.2

Role of sex steroids and nitric oxide in male sexual function

Vincenzo Rochira
Department of Medicine, Endocrinology and Metabolism, Geriatrics, University of Modena and Reggio Emilia, Modena, Italy.

Nitric oxide (NO) is the main final effector for penile erection achievement and maintenance in men and it constitutes a crucial target for therapeutical strategies addressed to the treatment of erectile dysfunction. The role of sex steroids penile NO pathway is still unclear, but some data suggest a positive role of androgens. In order to study the effects of sildenafil on human sleep-related erections according to the state of androgenization, we recently evaluated the effects of sildenafil (S) or placebo (P) on sleep-related erections in hypogonadal (H) men with very low testosterone levels: <200 ng/dl (6.93 nmol/L), before (H−T) and during (H+T) testosterone replacement treatment (T) and in control (C) subjects. Sleep-related erections were impaired in hypogonadal men before testosterone treatment (H−T+P) when compared with control subjects taking placebo (C+P). Testosterone alone (H+T+P) and sildenafil alone (H−T+S) restored normal sleep related erections, however, the combined treatment (sildenafil + testosterone) resulted in the maximum positive effect on sleep-related erections parameters. The effects of testosterone plus sildenafil resulted higher than the sum of the effects of both drugs used alone. Sildenafil administered at bedtime improves sleep-related erections in hypogonadal men, suggesting that the nitric oxide pathway may be pharmacologically enrolled and enhanced despite low serum testosterone. Furthermore, these data strongly support the idea of a synergic effect of sildenafil and testosterone on sleep-related erections. In clinical practice this concept is supported by the evidence that testosterone treatment restores sildenafil efficacy in subjects with erectile dysfunction and low to low-normal serum testosterone, who were non-responder to sildenafil alone. The combined treatment seems to be efficacious also in subjects with metabolic diseases such as diabetes mellitus. Whether or not estrogens are able to modulate NO pathway within the penile tissue remains to be ascertained in detail, but an androgen-estrogen cross-talk seems to be involved in the pathophysiology of male penile erection, but concerning estrogens dose-response and in vivo studies are lacking.

S18.3

Clinical management of premature ovarian failure

S Christin-Maitre
France.

Abstract unavailable

S18.4

Gonadal function in ageing men

Jean M Kaufman
Ghent University Hospital, Ghent, Belgium.

Involutional changes of gonadal function in healthy ageing men are progressive and mostly of modest amplitude with considerable between-subject variability. Albeit some men may remain relatively spared, the occurrence of age-related changes are nevertheless well documented at the population level in both cross-sectional and longitudinal observational studies. These changes affect Sertoli cell function and spermatogenesis as well as Leydig cell function and testosterone (T) production and they are the consequence of both primary testicular changes and alterations in neuro-endocrine regulation of gonadotropin secretion. Men retain fertility until old age, but there are age-related reductions of testicular size, Sertoli cell mass and spermatogenic activity with moderate decline in semen volume, sperm motility and morphology with maintained sperm concentration. This is reflected in a progressive increase of FSH levels and marked decrease of the ratio of serum levels of inhibin over FSH. More than 20% healthy men over 60 yr present with serum T levels below the range for young men. This is the consequence of decreased Leydig cell mass and altered hypothalamic regulation of LH secretion. Together with a progressive age-related increase in serum SHBG levels, this results in a 50% reduction of the serum bioavailable fractions of serum T (i.e. free and non SHBG-bound T) between age 20 and 75 yr. Although the clinical changes of ageing in men are reminiscent of signs and symptoms of hypogonadism in young men, clinical relevancy of the decline in sex steroid levels in ageing men has not been unequivocally established and minimal androgen requirement for elderly men remain poorly defined and are likely to vary between individuals. Therefore, borderline androgen deficiency cannot be reliably diagnosed in the elderly and differentiation between "substitutive" and "pharmacological" androgen administration is not possible. Awaiting documentation of long-term risk-benefit ratio, a conservative approach to androgen treatment in elderly men seems appropriate.

Pituitary cell biology – S19

S19.1

Role of folliculo-stellate cells in the anterior pituitary: a historical review

Wilfried Allaerts
Nijverdal, Netherlands.

Cell developmental studies have frequently used the hypophysis as a model for complex differentiation pathways. Nevertheless, many of this work has been focused on the hormone-producing cell types of the anterior pituitary (AP), whereas the so-called folliculo-stellate cells (FS cells) have often been ignored in these studies. FS cells form an enigmatic, non-hormone-secreting cell group. Initially designated as supportive cells, they were soon found to be the putative source of many, newly discovered peptides and growth factors. They were also shown to be involved in paracrine communication with other pituitary cell types and in communication through electrically coupled syncytia. Moreover, several authors have provided evidence for their possible role in pituitary cell regeneration and processes of cell transdifferentiation.

So far, little is known about the precise embryological origin of the mature FS cells. Since the discovery of adult stem cell populations in various organs, several authors have indicated a possible role of FS cells in this respect too. Also new evidence relating FS cells to the production of cytokines, their involvement in nitric oxide signaling and an *in vitro* immune accessory function were added to the list of physiological roles of the FS cells. The question however is whether these multiple functions can be ascribed to one, homogeneous but pluripotent cell type, or whether the pituitary FS cells represent a heterogeneous cell group consisting of various subtypes (unrelated or related to a common ancestor cell type).

We previously demonstrated the partial overlap between immunocompetent MHC-class II-positive dendritic cells (DC) and S100 protein-positive FS cells. In a transgenic mouse model for conditional DC ablation, we showed that early macrophages could be prevented from colonizing the AP. Also, around embryonic day 12 of chick development, early macrophages were detected in the anterior pituitary before pituitary cell differentiation was completed and well before FS cells obtained their mature phenotype.

The present historical review of FS cell research highlights the importance of conceptual frameworks in cell lineage studies. Cell biological systems from the past, like the reticulo-endothelial system or the more recent mononuclear phagocyte system, nowadays are considered obsolete and incomplete. Still there is a need for theoretical frameworks in new annotation studies and for the clinical applications of contemporary research. The FS cell model not only is very interesting for the study of development of organs with two or more embryonic Anlagen. Also, questions related to the therapeutic usefulness of pituitary cell regeneration are envisaged in cases of pituitary dysfunctioning or hypopituitarism.

S19.2

Signalling in pituitary tumours: the roles of Akt, BRAF, AIP and other novel agents

Márta Korbonits
Department of Endocrinology, Barts and the London Medical School, London, United Kingdom.

Numerous growth factors, oncogenes, tumour suppressor genes and hormonal influences have been implicated in pituitary tumorigenesis. We have demonstrated that the PI3K-Akt pathway is upregulated in pituitary tumours and since Akt is a major downstream signalling molecule of growth factor-liganded tyrosine kinase receptors it is possible that an abnormality at this level could be the primary driver of pituitary tumorigenesis. The serine/threonine kinase B-Raf functions as a downstream effector of Ras, interpolated between the tyrosine kinase receptor and the mitogen-activated protein kinase (MAPK) pathway and acting in parallel to the Akt pathway. We have found significant over-expression of B-Raf mRNA in pituitary adenomas, specifically NFPAs and a positive correlation between mRNA and protein expression. B-Raf overexpression could lead to increased activation of the MAPK pathway. Using microarray we have found that the Bcl-associated athanogene (BAG1) mRNA is overexpressed in somatotroph adenomas and NFPAs; this oncogene binds to and activates Raf-1, which can potentiate B-Raf activity by heterodimerisation. In a pituitary protein array we have identified several over- and underexpressed proteins and one of the prominent differentially expressed proteins with potential importance in tumorigenesis was the heat shock protein 110 (HSP110). This showed significant overexpression in NFPAs and prolactinomas. Interestingly, another molecular chaperone, the aryl hydrocarbon receptor interactive protein (AIP) has been recently identified as a cause for pituitary adenomas in families with isolated pituitary tumours. We have identified 5 different mutations in 19 familial acromegalic families causing autosomal dominant disease with incomplete penetrance. We have also observed prominent differences in AIP mRNA and protein expression between normal pituitary cells and sporadic pituitary tumours. Previous data suggest that AIP acts as a tumour suppressor gene but the exact mechanism leading to pituitary tumorigenesis when AIP is lacking remains to be identified.

S19.3

Oncogene gsp and Gsα overexpression in pituitary cell biology

Anne Barlier[2], Corinne Gérard[1] & Enjalbert Alain[1]
[1]Laboratory ICNE UMR6544 CNRS Université de la Méditérranée, Marseille, France; [2]Laboratory of Biochemistry anf Molecular Biology, CHU Conception, Marseille, France.

Somatic mutations of the αs subunit of G proteins were initially reported by Landis and collaborators in 1989 in somatotroph tumors characterized by markedly high cAMP levels. These mutations are localized at two critical sites concerning the intrinsic guanosine triphosphatase activity of the protein leading to a constitutive activation of the adenylyl cyclase. The mutated protein has been named the *gsp* oncogene. On the other hand, Gsα mRNA level varied among human somatotroph adenomas, the highest expression being observed in *gsp*- tumors (not bearing the active Gsα mutant). We previously showed that *gsp* oncogene impacted tumoral phenotype, *gsp*+ tumors being smaller and more sensitive to octreotide treatment. We have recently showed that high Gsα expression impacted also tumoral phenotype of *gsp*- tumors.

Gsα is coded from GNAS gene which is imprinted in a tissue-specific manner. Gsα is paternally silenced in normal pituitary, but a Gsα imprinting relaxation is found in some tumoral tissue. Unexpectedly, we found that the loss of Gsα imprinting did not induce the expected Gsα overexpression and was not associated with a modification of methylation status of exon1A DMR (a differentially methylated region controling the Gsα imprinting) in human pituitary tumors.

To explore the impact on transduction pathways of mutated or overexpressed Gsα protein, we obtained somatolactotroph GH4C1 cell lines by performing doxycycline-dependent conditional overexpression of the wild type Gsa protein and expression of the gsp oncogene. Although the resulting adenylyl cyclase and cAMP levels were ten-fold lower in the wild type Gsa overexpressing cell line, a sustained MAPKinase ERK1/2 activation was observed in both cell lines. Overexpression of the wild type Gsα protein as the *gsp* oncogene initiated chronic activation of endogenous PRL synthesis and secretion, as well as chronic activation of ERK1/2-sensitive human PRL and GH promoters.

S19.4

Adipocytokines and pituitary function

Maria M. Malagon[1], Francisca Rodríguez-Pacheco[1], Antonio J. Martínez-Fuentes[1], Rafael Vázquez-Martínez[1], Manuel Tena-Sempere[1], Carlos Diéguez[2] & Justo P. Castaño[1]
[1]Dept. Cell Biology, Physiology and Immunology. Univ. Córdoba, Cordoba, Spain; [2]Dept. Physiology, Univ. Santiago de Compostela, Santiago de Compostela, Spain.

It is widely accepted that, in addition to serving as a repository for energy reserves, adipose tissue is an active endocrine organ that secretes a variety of signalling molecules, the adipokines, which play important roles in the regulation of metabolism, energy balance, feeding behaviour, vascular homeostasis and immunity. In particular, leptin, resistin and adiponectin have been implicated in energy and glucose homeostasis. Additional neuroendocrine functions have also been recognized for leptin as it regulates the secretion of pituitary GH and LH. In order to elucidate whether adiponectin, as leptin, may be involved in the regulation of pituitary cell function, we investigated the effect of this adipokine on somatotrophs and gonadotrophs and analyzed its interaction with major stimulatory regulators of these cells (ghrelin, GHRH, GnRH), as well as with their corresponding receptors (GHS-R, GHRH-R, and GnRH-R, respectively). Results show that adiponectin inhibits GH and LH secretion as well as both ghrelin-induced GH release and GnRH-stimulated LH secretion in rat pituitary cell cultures, wherein the adipokine also increases GHRH-R and GHS-R mRNA content while decreasing that of GnRH-R. Additionally, we have demonstrated that the pituitary expresses both adiponectin and the adiponectin receptors, AdipoR1 and AdipoR2, under the regulation of the adipokine. Taken together, these data indicate that adiponectin, either locally produced or from other sources, may play a neuroendocrine role in the control of both somatotrophs and gonadotrophs. These results will be further discussed on the context of adiponectin expression in pituitary tumoral cells and its interaction with other adipokines present in the pituitary.

Financial-support: CVI-139-J.Andalucia, BFU2004-03883-MEC/FEDER, and CIBER Obesity&Nutrition-ISCIII. Spain.

Thyroid – S20

S20.1

Updated guidelines for the follow-up of thyroid cancer.

Martin Schlumberger
Institut Gustave Roussy, 94805b Villejuif, France.

Follow-up of thyroid cancer patients is aimed at controlling the adequacy of thyroid hormone treatment and at the early diagnosis of recurrent disease.

Long term thyroxine treatment is given at suppressive doses only in the few patients with persistent or recurrent disease. When cure has been assessed, serum TSH should be maintained in the normal range (around 1 μU/ml).

The absence of disease is first controlled by the total body scan (TBS) performed 3 to 5 days after the post-operative administration of radioiodine. When the TBS is informative and does not show any focus of uptake outside the thyroid bed, a subsequent routine diagnostic TBS is usually not necessary.

Cure is assessed at 9–12 months with a neck ultrasonography and a serum Tg determination obtained 3 days after rhTSH stimulation (0.9 mg im, on 2 consecutive days). The quality of life of thyroid cancer patients is improved with the use of recombinant human TSH (rhTSH) that avoids hypothyroidism, provides an effective stimulation of any thyroid tissue and does not increase the global cost of follow-up.

Low risk patients with a normal neck US and an undetectable rhTSH stimulated serum Tg are considered cured. This reliable assessment of cure permits reassurance of patients, the subsequent use of replacement doses of thyroxine and the simplicity of the subsequent yearly follow-up with serum TSH and Tg determinations. There is a close relationship between basal and TSH-stimulated serum Tg levels, and the benefits of TSH stimulation may decrease with Tg methods with an improved functional sensitivity. At the present time, there is however no firm evidence that TSH-stimulated Tg determination can be obviated.

When serum Tg is detectable at a low level following TSH stimulation, another TSH stimulation should be performed 1 or 2 years later, and the trend will indicate either irradiated thyroid cells (with decreasing Tg level) or neoplastic cells (with an increasing Tg level).

S20.2

Congenital hypothyroidism with gland *in situ*

Luca Persani
Dept of Medical Sciences, University of Milan and IRCCS Ist Auxologico Italiano, Milan, Italy.

Congenital hypothyroidism (CH) is the most frequent endocrine congenital defect affecting about 1:3000 newborns. In economically/ socially advanced counties, CH is routinely screened by means of TSH (and/or T4) measurement on dry blood spot (dbs) since more than 20 years. Neonatal screening allows early recognition and treatment of affected newborns. Upon data collected in years <2000 by the Italian CH Registry, the newborns with confirmed CH and gland-in-situ constituted about 20% of total CH cases. However, in more recent years the technical improvements in TSH determination in the Center for Neonatal Screening of Milan region have lead to a progressive lowering of dbs TSH cutoff value for newborn recall down to 10 mU/l. This has resulted in a significant increase of the recall rate for CH (CH incidence 2003: 80/91,948 newborns), with gland-in-situ cases nowadays accounting for more than 55% of total CH cases. This phenomenon has several important implications concerning the correct diagnosis and adequate management of these babies. One of the most important questions raised by this new picture concerns the necessity to treat babies with mild TSH elevations. The possibility to give correct answers to these questions is complicated by the extreme heterogeneity of this CH category, highlighted by the variable thyroid phenotype as well as by the multiple possibilities of association with non-thyroid malformations/disorders. Relevant advancements have been done in recent years with the discovery of new genetic causes and the description of their underlying molecular mechanisms and related phenotypic presentation. Nevertheless, the cause of several gland-in-situ CH cases remains still unsolved justifying further efforts in this research field. These efforts will contribute to reach a more complete pathogenic classification of CH with gland-in-situ which represents one of the major steps toward an improved and evidence-based clinical management of CH patients.

S20.3

Thyroid and ageing

Istvan Szabolcs
Semmelweis University, Budaoest, Hungary.

In the healthy elderly there seems to be an age dependent decrease of TSH and FT3 but not FT4. The prevalence of TPOAb positivity increases with age but surprisingly it has been found to be decreased in centenarians. Antibody positivity is not predictive for future thyroid dysfunction in old age. The upper range of normal TSH for the healthy elderly living in sufficient iodine intake areas is higher than in case of iodine deficiency. In iodine deficient areas there is a high prevalence of nodular goiter and hyperthyroidism is mainly caused by toxic nodules. Radioiodine should be prefered for therapy of Graves' disease in old age, long term thyrostatic therapy is not safe. TAO is more severe in old age and there is a less favourable outcome of the therapeutical options. In an elderly subject subclinical hyperthyroidism with suppressed TSH is a risk factor for progression to overt disease, for atrial fibrillation, osteoporosis and may be associated with increased cardiovascular and all-cause mortality, thus we believe that it should be treated. The clinical significance of subnormal but measurable TSH is less clear, but in old age treatment may be considered in case of heart disease or osteoporosis. Subclinical hypothyroidism is a risk factor for atherosclerosis but slightly elevated TSH in old age should not be treated: it may even be favourable to have a longer life. In any case, TSH levels outside the reference intervals should first be controlled before considering treatment. The cancer risk in cold thyroid nodules increases with advanced age. According to most but not all studies, in older differentiated thyroid cancer-patients poor prognostic features are more frequent, total thyroidectomy and radioablation are recommended and additional treatment of progressive disease should not be denied because of advanced age.

S20.4

Thyroid autoimmunity: genes and environment

Pia Skov Hansen
[1]Odense University Hospital, Odense, Denmark; [2]University of Southern Denmark, Odense, Denmark.

Autoimmune thyroid diseases (AITD) comprise two clinical phenotypes, Graves' disease and Hashimoto's thyroiditis. These conditions share distinct immunological features such as autoreactivity against the key thyroid autoantigens thyroglobulin and thyroid peroxidase. Considering Graves' disease as well as Hashimoto's thyroiditis, twin studies have revealed a higher concordance rate among monozygotic (MZ) as compared to dizygotic (DZ) twins, suggesting a relative strong genetic influence in the aetiology. According to the endophenotypic approach, it might be useful to subdivide a clinical phenotype into a set of variables thought to represent more basic processes. The presence of thyroid autoantibodies in euthyroid individuals can be regarded as a central phenotypic anchor point and, using the twin design, the relative contributions of genetic as well as environmental effects in the aetiology of AITD, at this early stage of the disease process, has been clarified as well.

The genetic contribution to autoimmune disease (AID) has been intensely investigated, and a slow progress towards identification of AITD susceptibility genes is seen. There is evidence of association and, in some cases, even linkage between AITD and several genetic loci. However, one problem is often the very pronounced discrepancy between the initial and subsequent reports. On the other hand, epidemiological studies aim at identifying specific measurable environmental exposures of importance for the development of AITD. So far only a few environmental factors (e.g. iodine intake and smoking habits), with a clear detectable effect on the disease, have been characterized. The underlying challenges in trying to understand a complex phenotype, such as AITD, will be discussed.

Pheromones, odorant and taste receptors – S21

S21.1

Odorant receptors and reproduction

Hanns Hatt
Ruhr-University Bochum, Bochum, Germany.

Fertilization is still one of the nature's best-kept secrets. Despite a century of research we still lack a comprehensive understanding how mammalian sperm cells navigate inside the female body, locate, and finally fertilize the egg. More than a decade ago, the unexpected finding of olfactory receptor expression in human testicular tissue led to speculation about a potential role of these chemoreceptors in various aspects of mature sperm behavior, especially sperm chemotaxis. We could obtain first evidence in favor of this hypothesis by the identification of hOR17-4, a testicular olfactory receptor that mediates human sperm chemotaxis. We showed that *in vitro* activation of the receptor hOR17-4 by a variety of floral odorants (e.g. bourgeonal, cyclamal) mediates both chemotaxis and chemokinesis in human sperm cells. A detailed characterization of the receptor's molecular receptive range as well as the first description of a potent receptor antagonist could provide the basis for future applications in fertility treatment with important consequences in contraception. Very recently we reported cloning, recombinant expression and functional characterization of another human testicular olfactory receptor (hOR17-2). Using a combination of imaging behavioral assays, we showed activation of sperm by cognate receptor ligands and described a specific receptor-mediated motility pattern. Comparative analysis of different OR-induced signaling pathways as well as cell-specific receptor expression profiles are subject of current research. Given an estimated number of up to 40 different testiculary expressed odorant receptors, an identification of the stimulatory ligands of further members of this "unconventional" group of ORs is critical to gain new insight in their role in reproduction.

S21.2

Molecular architecture of pheromone sensing in mammals

Catherine Dulac
HHMI, Cambridge, MA, United States, Harvard University, Cambridge, MA, United States.

The neuronal processing of pheromone signals within distinctive brain structures leads to marked changes in animal behaviour and endocrine status. The highly reproducible and species-specific character of the response to pheromones offers a unique opportunity to uncover the neural basis of genetically pre-programmed behaviours. Molecular and genetic investigation of the mechanisms underlying pheromone pheromone-evoked responses in the mouse nose and brain have revealed a neural strategy that is strikingly different from that used in other chemosensory modalities such as taste and olfaction. Our studies have provided novel insights into the sensory coding of pheromone signals leading to gender identification and aggressive behaviour, and into the developmental mechanisms leading to the emergence of distinct olfactory pathways. Our most recent

experiments using conditional and GFP-expressing viral vectors are aimed at visualizing entire brain circuits responsible for innate behaviours.

S21.3

Endocrine and behavioural responses to pheromones

Peter Brennan
University of Bristol, Bristol, United Kingdom.

According to the original definition, pheromones are substances released by an individual that have definite behavioural or physiological effects on another individual of the same species. For example, male mouse urine contains a complex mixture of chemosignals, some of which, such as brevicomin and thiazole are testosterone-dependent and signal the presence of a reproductively active male. These have powerful effects as releaser pheromones to elicit aggression from other males, as well as having effects as primer pheromones on female reproductive state, such as puberty acceleration and induction of oestrus. However, as the complexities of vertebrate chemosensory communication have become evident, the original definition of pheromones has begun to appear too restrictive. For instance, peptide chemosignals related to the major histocompatibility complex convey information about individual identity, which as signaller pheromones can influence behaviour or physiology without eliciting a definite response.

In addition to mediating individual recognition in social contexts, these individuality chemosignals enable female mice to recognise the urinary pheromones of their mate, to which they are exposed at mating. This chemosensory memory is vital for their reproductive success, as it prevents the pre-implantation pregnancy failure that is induced by exposure to urinary pheromones from an unfamiliar male. This pregnancy block effect (Bruce effect) is mediated by the vomeronasal system, via the dopaminergic suppression of prolactin production by the pituitary. A range of evidence suggests that memory formation to the mating male's pheromones involves synaptic changes in the accessory olfactory bulb at the first stage of the vomeronasal pathway. This results in a selective inhibition of the mate's pheromonal signal, preventing it from activating neural circuits in the corticomedial amygdala and hypothalamus that mediate the endocrine changes responsible for pregnancy block. This is just one example of the way that learning can reinforce or inhibit innate pheromonal responses.

S21.4

Bitter taste receptors and food intake

Wolfgang Meyerhof, Maik Behrens, Bernd Bufe, Anne Brockhoff, Susann Förster, Claudia Reichling, Christina Kuhn & Marcel Winnig
German Institute of Human Nutrition, Potsdam-Rehbruecke, Germany.

Taste is an overriding determinant of food choice and leads to the development of preferences impacting on nutrition and eventually health. To investigate the molecular basis of gustation and its link to nutritional behaviour, we isolated all 25 members of the human bitter taste receptor gene family, TAS2Rs, and established their expression profile on the tongue. Using functional assays we identified the cognate bitter compounds for ~half of the encoded receptors. Our data suggest that TAS2Rs appear to be broadly tuned to detect compounds with common structural motifs, explaining how humans are capable of perceiving thousands of bitter substances with a small set of receptors. This broad tuning is likely caused by the presence of multiple binding sites for various bitter compounds on the TAS2Rs. Our experiments also revealed that the biochemical properties of the receptors define perceptual sensitivity of individuals. Moreover, frequently occurring polymorphisms in TAS2R genes determine numerous receptor variants, which can differ in the sensitivities for their cognate bitter compounds up to 1000 fold, thereby generating perceptual variability in the population. How far receptor mechanisms determine tasting is shown for saccharin, a compound that taste sweet through activation of the sweet taste receptor at low and moderate concentration, with an off-taste caused by its ability to activate two TAS2R bitter taste receptors simultaneously and to block the sweet taste receptor at higher concentrations.

To date direct evidence is still missing that convincingly proves or disproves the impact of gustation on intake behaviour. However, strong circumstantial evidence comes from the phylogenetic analysis of human TAS2R genes and from the analysis of TAS2R polymorphisms and taster phenotypes that evolved independently in chimanzees and humans as well as from an association study identifying a TAS2R16 allele as a risk factor of alcohol dependence. Taken together, our data strongly suggest that genetics and peripheral taste receptor mechanisms govern gustatory perception and perceptual variability in the population with a probable impact on nutrition and health.

Bone – S22

S22.1

Bisphosphonates: molecular mode of action and adverse effects

Michael J Rogers
University of Aberdeen, Aberdeen, United Kingdom.

Bisphosphonates are the mainstay of treatment for metabolic bone diseases such as post-menopausal osteoporosis and Paget's disease. Enormous progress has been made over the last few years in understanding how these drugs act at the molecular level. After targeting bone and selective internalisation by osteoclasts, simple bisphosphonates are incorporated into cytotoxic, non-hydrolysable analogues of ATP. By contrast, the more potent nitrogen-containing bisphosphonates inhibit FPP synthase (an enzyme of the mevalonate pathway), which disrupts the synthesis of the isoprenoid lipids FPP and GGPP. These lipids are required for the carboxy-terminal modification (prenylation) of small GTP-binding proteins such as Ras, Rho, Rac and Rabs. Prenylated small GTPases act as molecular switches, regulating processes fundamental to osteoclast function, including membrane ruffling, vesicular trafficking, cytoskeletal organisation and cell survival. Inhibition of FPP synthase by bisphosphonates prevents the prenylation of small GTPases and causes the accumulation of the unprenylated (and, in some cases, inappropriately activated) forms of the proteins, thus disrupting osteoclast function and causing osteoclast apoptosis.

The most common adverse effect of intravenous bisphosphonate therapy is a brief, 'flu-like acute-phase reaction. We have recently demonstrated that this effect appears to be due to inhibition of FPP synthase in peripheral blood mononuclear cells, which causes an accumulation of the upstream isoprenoid lipid IPP. The latter is known to stimulate the Vgamma9Vdelta2 subset of gamma,delta-T cells, causing the release of TNFalpha and IFNgamma and hence the rapid onset of 'flu-like symptoms. Esophageal irritation by oral bisphosphonates may also be caused by inhibition of FPP synthase in GI epithelial cells, however the exact cause of recently-described, rare cases of osteonecrosis of the jaw remains unclear.

Thus, the ability of nitrogen-containing bisphosphonates to inhibit the mevalonate pathway explains their well-known, potent inhibitory effects on bone-destroying osteoclasts as well some of their adverse effects.

S22.2

Calcimimetics in the management of hyperparathyroidism

Munro Peacock
Indiana Universtity, Indianapolis, United States.

The cell surface calcium receptor (CaR) in the parathyroid gland plays a central role in the regulation of serum calcium homeostasis. Activation and inactivation mutations in the CaR lead to chronic hypocalcemia and hypercalcemia states (Brown, EM. Mutations in the calcium-sensing receptor and their clinical implications. *Horm Res* 1997 **48** 199–208). Type 11 calcimimetics are a novel class of compounds that directly reduce PTH secretion from the parathyroid cell by binding to the CaR and increasing its sensitivity to extracellular ionized calcium, thus causing a left-shift in the Ca-PTH setpoint (Nemeth, EF *et al*. Calcimimetics with potent and selective activity on the parathyroid calcium receptor. *PNAS USA* 1998 **95** 4040–4045). Cinacalcet is an oral calcimimetic that has been shown to reduce serum PTH and calcium in secondary hyperparathyroidism of renal failure (Block, GA *et al*. Cinacalcet for secondary hyperparathyroidism in patients receiving hemodialysis. *N Engl J Med* 2004 **350** 1516–1525), in primary hyperparathyroidism (Peacock *et al*. Cinacalcet Hydrochloride maintains long-term normocalcemia in patients with hyperparathyroidism. *J Clin Endocrinol* 2005), and in parathyroid cancer (Silverberg, SJ *et al.* Cinacalcet reduces hypercalcemia in patients with parathyroid carcinoma. *J Bone Min Res* 2006 **21** Suppl. 1 S440).

Cinacalcet therapy is well tolerated long-term, and current studies indicate that it may play a valuable role in the medical management of diseases of hyperparathyroidism.

S22.3

Primary hyperparathyroidism: surgical approach and benefits

Svatopluk Adamek
3rd Dpt of Surgery, First Fac of Medicine, Charles University, Prague, and Teaching Hospital Motol, Czech Republic, Prague, Czech Republic.

Surgical therapy of the primary hyperparathyroidism (PHP) offers a definite and curative treatment. The cooperation with an experienced endocrinologist is necessary, because the confidence that the patient has a PHP, is the primary presumption for the proper surgical therapy of the PHP. The result of a parathyroidectomy depends mainly on preoperative localization of hyperfunctional tissue and the experience of the surgeon. The parathyroidectomy remains curative approach in 97% of patients if provided by an experienced surgeon. The neck ultrasonography and MIBI scintigraphy of parathyroid glands remain the gold standards in preoperative imaging. The surgeon must but be able to perform a parathyroidectomy in case where preoperative localizing methods are not successful. In addition, we require an indication to surgical approach in patient with concomitant thyreopathy. The basic technique of a parathyroidectomy is the bilateral exploration of the neck with the examination of all locations of the parathyroid glands, including ectopic ones, usually from the collar skin incision above the jugulum.

In terms of a minimalization of surgical approach, unilateral, radionavigated and miniinvasive approaches were developed. In case of intrathoracic-mediastinal localization of parathyroid glands, the partial median sternotomy is the basic approach. In 3.5% of 680 our patients, the neck approach was not sufficient. The complications of parathyroidectomy are not common. They include the hypoparathyroidism and the recurrent laryngeal nerve injury with following vocal cord paralysis. Benefits To date, the parathyroidectomy is a short, one-day surgery operation in surgical centers. The improvement of surgical technique offers a surgical treatment to "asymptomatic" patients. In case of a clear localization of parathyroid adenoma by sonography or MIBI scintigraphy, the operation is short, safe and does not stress the patient. In these patients, the so-called small symptoms (fatigue, musculoskeletal pain, weakness, dyspepsia, polydypsia, constipation, polyuria, pruritus, depression) are ameliorated.

Supported by IGA MZ ČR č. 8308-5/2005

S22.4

Absolute risk prediction for fracture

H Pols

Abstract unavailable

Reproductive endocrinology/andrology – S23

S23.1

Androgen regulation of spermatogenesis

Philippa Saunders[1], Lee Smtih[1], Robert Hooley[1], Karel De Gendt[2], Evi Denolet[2], Guido Verhoeven[2] & Richard Sharpe[1]
[1]MRC Human Reproductive Sciences Unit, Edinburgh, United Kingdom; [2]Catholic University of Leuven, Leuven, Belgium.

Spermatogenesis is a complex process involving interactions between the somatic cells (Sertoli, Leydig, peritubular) and germ cells within the adult testis. Androgens are key regulators of spermatogenesis and intra-testicular concentrations of testosterone (T), produced by the Leydig cells, are higher than that in blood. Androgen action is mediated by the androgen receptor (AR), an X-chromosome-encoded, ligand-activated, transcription factor. The mechanisms by which androgens regulate testis function have been explored by determining the pattern of expression of AR, by manipulating androgen concentrations, by performing studies *in vitro* on isolated tubules/cells and most recently by studying mice with cell-specific deletion of the *Ar* gene.

In adult testes AR have been immunolocalised to the nuclei of Sertoli, Leydig and peritubular myoid cells as well as the cells lining blood vessels. Expression in adult Sertoli cells is stage-dependent and *in vitro* studies have demonstrated that it is T-regulated. In rats, ablation of Leydig cells with ethane dimethane sulphonate results in an acute reduction in intra-testicular T and germ cell loss; germ cell demise is first observed in the stages of spermatogenesis in which AR expression in Sc is highest. The impact of Sertoli cell-specific ablation of Ar on testicular function has been investigated in three independent laboratories. In all cases Ar ablation resulted in a reduction in testicular size, germ cell loss and infertility. Expression of rHox5, a Sertoli cell protein previously shown to be T-regulated, was reduced as was expression of proteins involved in formation of junctional complexes. Leydig cell function was altered even though expression of Ar was maintained in these cells confirming the existence of paracrine interactions between the seminiferous and interstitial compartments. In conclusion, testicular function and male fertility are androgen dependent; expression of AR in Sertoli cells is essential for normal germ cell maturation and fertility.

S23.2

The experimental mouse model for men with Klinefelter syndrome

YanHe Lue, Christina Wang, David Jentsch, Krista Erkkila, Peter Liu, Monica Schwarcz & Ronald Swerdloff
Dept of Medicine, Division of Endocrinology, LABiomed at Harbor-UCLA Med Ctr, Torrance, CA, USA.

Klinefelter syndrome (XXY males) is the most common sex chromosome aneuploidy, occurring in about 1 per 500 men. To study the underlying molecular mechanisms caused by the extra X chromosome, we have developed an experimental mouse model for men with Klinefelter's syndrome. We have demonstrated that adult XXY mice have absence of germ cells, decreased serum testosterone levels, and elevated gonadotropin levels. Testicular failure begins early as a result of massive germ cell loss that precedes the initiation of meiosis. Loss of germ cells is mediated through apoptosis. Gene microarray with testicular RNA samples from 1-day-old mice showed inactive X specific transcripts (Xist) expression increased 4.14-fold, indicating the extra X chromosome is inactivated in XXY testes. Proapoptotic Bcl2-interacting killer-like and caspase 7 have 1.59- and 1.68-fold increase, and antiapoptotic transcripts IAP and Bcl2-like-10 have 3.73- and 2.08-fold decrease respectively in XXY mice. By immunohistochemistry, we found c-kit expression in gonocytes occurred earlier in XXY than XY siblings, suggesting early differentiation of gonocytes may contribute to germ cell loss in XXY mice. In addition to germ cell defect, androgen receptor expression in Sertoli cells is nearly depleted in adult XXY mice, suggestive of Sertoli cell dysfunction. By transplantation of XY germ cells into adult XXY testes, we found a few donor XY spermatogonia were able to survive for 10 weeks without further differentiation. Leydig cells in adult XXY mouse testes are both hypertrophic and hyperplastic. Testosterone production from XXY Leydig cells is impaired. Besides reproductive dysfunction, we have demonstrated that XXY mice have impaired learning, memory, and social interaction. By giving testosterone implants to adult XXY mice, we demonstrated that testosterone treatment significantly improves the learning ability of adult XXY mice.

S23.3

Genes involved in male infertility: sorting facts from fiction

Ewa Rajpert-De Meyts
Copenhagen University Hospital (Rigshospitalet), Copenhagen, Denmark.

Male infertility is a common disorder and a growing health problem. A large proportion of unexplained cases have been summarily categorised as idiopathic infertility. The majority of idiopathic cases, especially those with severely impaired spermatogenesis incl. azoospermia, are presumably caused by genetic defects. Genetics of male infertility has been a largely unexplored area, until quite recently, when new molecular tools unabled discovery of a growing number of genes involved in spermatogenesis and gamete maturation, e.g. genes mapped to the AZF region of the Y-chromosome and some genes on the X-chromosome. In addition, several pathways related to hormonal regulation of reproductive function contain polymorphic genes, which may affect the function of a given gene in a discrete manner, such as the CAG and GGN repeats on androgen receptor, or polymorphisms in *CYP*, *INSL3* genes. Finally, polymorphisms of genes seemingly unrelated to the reproductive function, have been associated with male infertility, e.g. mitochondrial gene polymerase, *POLG*. A rush to analyse polymorphic genes in various populations, often with poorly characterised cases and controls, created a lot of confusion in the literature as to the real pathogenetical involvement of the studied genes in male infertility. There is a need for large and well-controlled studies, underpinned by basic functional studies of the investigated genes. A great care must be taken to use proper control groups, which must be selected with fertility, ethnicity, and age of the subjects in mind. A very important point is having in mind that environmental exposures and/or lifestyle factors frequently exert their influence primarily in genetically predisposed individuals. A good description of the reproductive parameters (outcomes), preferably with the analysis of the reproductive function on children, is also essential for the analysis of the consequences of studied polymorphism/gene aberration, and for an early prognosis as to the future fertility problems.

S23.4

Genetic basis of testicular tumors

Leendert Looijenga
Erasmus MC, Rotterdam, Netherlands.

Various types of human germ cell tumors (GCTs) can be found, referred to as type I, II and III. The type I are the teratomas and yolk sac tumors of neonates and infants. No genomic aberrations have been identified in teratomas, while yolk sac tumors show chromosomal imbalances related to chromosomes 1, 6 and 20. Type II GCTs are the seminomas and nonseminomas, derived from carcinoma *in situ* (CIS)/intratubular germ cell neoplasia unclassified (ITGCNU). CIS/ITGCNU and seminoma cells mimic primordial germ cells/gonocytes, amongst others characterized by expression of the diagnostic marker OCT3/4-POU5F1. All invasive tumors show gain of the short arm of chromosome 12. The type III GCTs, i.e. spermatocytic seminomas, occur predominantly in elderly, and only in the testis. They originate from primary spermatocytes, and show consistent gain of chromosome 9, of which DMRT1 is a candidate. GCTs show specific patterns of mRNA and microRNA expression, of possible diagnostic and prognostic value. Besides familial predisposition and infertility, disorders of sex differentiation (DSD) is a risk factor for type II GCTs. This specifically forms of hypovirilization and gonadal dysgenesis, in the presence of part of the GBY region. Besides CIS/ITGCNU, gonadoblastoma can be the precursor in DSD patients. Gonadoblastoma is the earliest developmental stage in the genesis of GCTs. TSPY (testis specific protein on the Y chromosome) is a likely candidate to explain the requirement of the GBY region for malignant transformation of germ cells. A significant limiting diagnostic factor in DSD is lack of specific markers for CIS/ITGCNU in case of maturation delay of germ cells. The type II GCTs are in fact an embryonic cancer in adult patients. This explains a number of specific characteristics, like their histology (totipotency), overall sensitivity to DNA-damaging agents, as well as their chromosomal and genetic constitution.

Obesity – S24

S24.1

Altering adipocyte metabolism as a way to counteract obesity and insulin Resistance

Sven Enerbäck
Medical Genetics, Dept of Medical Biochemistry, Göteborg University, Medicinareg, 9A, Box 440, SE 405 30 Göteborg, Sweden.

Advances over the last two decades in our understanding of the adipocyte have clarified its role as a key regulator of both energy balance and intermediary metabolism. It is now known that in addition to being an insulator and energy depot, the adipocyte is a highly active cell, secreting a wealth of factors, including leptin, that play a part in CNS and appetite regulation. There is also a much greater understanding of how fat cells themselves develop from precursor cells FOXC2, pRb, PGC-1and RIP140 has been discussed as genes influencing adipocyte cell fate. By increasing the already existing pool of brown adipocyte in human adipose tissue, as a way to dissipate excess energy through uncoupling, this would help conserve ample triglyceridestorage capacity in white adipocyte and hence counteract ectopic lipid depositions in tissues like liver and muscles. Since ectopic lipid deposition is intimately connected to the development of insulin resistance and the metabolic syndrome, factors affecting white versus brown fat partitioning constitutes an interesting approach to this health problem.

S24.2

Triglyceride-lowering effect of metabolic switch in white adipose tissue

Jan Kopecky
Institute of Physiology, Academy of Sciences of the Czech Republic, Prague, Czech Republic.

High level of triglycerides (TG) in plasma is a risk factor for cardiovascular disease. Various treatment strategies aimed at decreasing plasma TG concentrations affect synthesis of lipoproteins in the liver and/or increase clearance of TG by peripheral tissues. Lipid-lowering effects of fibrates reflects modulation of the liver metabolism. Antidiabetic agents thiazolidinediones (TZD) lower plasma TG by enhancing lipoprotein lipase activity in white adipose tissue (WAT). Long-chain polyunsaturated fatty acids of *n*-3 series, namely eicosapentaenoic (EPA; 20:5 *n*-3) and docosahexaenoic (DHA; 22:6 *n*-3) acids, that are abundant in sea fish, act as hypolipidemics, while decreasing the production of lipoproteins. EPA and DHA may also affect the TG clearance. Most of the above mentioned treatments induce expression of mitochondrial uncoupling proteins (UCPs) in WAT. The aims of our studies were to characterize: (i) the potency of WAT to decrease plasma TG levels; and (ii) the involvement of WAT in the hypolipidemic effects of EPA and DHA. A large potency of WAT to decrease plasma TG was demonstrated using transgenic mice with ectopic expression of UCP1 in WAT (aP2-*Ucp1* mice). The ectopic UCP1 induces respiratory uncoupling in WAT, hence stimulating *in situ* lipid oxidation and mitochondrial biogenesis, and clearance of plasma TG. Moreover, aP2-*Ucp1* mice were resistant to high-fat diet induced obesity and showed higher whole body lipid oxidation. The obesity in wild type mice was also prevented by replacing only 9% of the dietary lipids by EPA and DHA. This dietary treatment lowered plasma TG, while inducing lipid oxidation and mitochondrial biogenesis in WAT. These results supported a possibility to induce a metabolic switch in WAT, which may change whole body phenotype, including the lowering of plasma TG. Further studies are required to assess the importance of this switch for the effectiveness of the lipid-lowering treatments.

S24.3

Adipokines and insulin sensitivity in humans

José Manuel Fernández-Real
Hospital of Girona, Girona, Spain.

Decreased insulin action has been proposed as the common factor that is in the background of the different components of the metabolic syndrome. Insulin resistance is also associated with a chronic activation of the innate immune system. The innate immune system constitutes the first line of body's defence and it is constituted by different barriers (epithelia, adipose tissue), and different blood and tissue components as macrophages, and neutrophils. Once activated, the acute phase response is activated, with generation of different acute phase proteins and cytokines that are produced in order to struggle against different aggresions, as infections and traumatisms. The aim of this response is to eradicate these agents, to repair the harmed tissues, and, through increased insulin resistance, to optimize the energetic substrates, which will be drained to vital tissues and organs (i.e. brain and the immune system). Evolution pressures have led to survival of the fittest individuals, those with genetics that allows the best defence against infection and periods of famine. The initial evolutive advantages of increased inflammatory responses, hypersecretion of proinflammatory cytokines (TNF-α, interleukin 6, interleukin 18), counterbalanced by antiinflammatory molecules (adiponectin, sCD14, BPI, MBL), turn into chronic inflammation conditions, such as obesity and type 2 diabetes. Increasing evidence is reported according to which chronic inflammation precedes these conditions. The knowledge of how these metabolic pathways interact with the inflammatory cascade will facilitate new therapeutic approaches.

S24.4

Lipodistrophy and abdominal fat accumulation: new therapeutic alternatives

LF Van Gaal
Department of Diabetology, Metabolism and Clinical Nutrition, University Hospital Antwerp, Antwerp, Belgium.

Lipodystrophy (LD) is a well-recognised clinical syndrome of peripheral fat atrophy and central adiposity, often associated with laboratory abnormalities such as dyslipidemia and glucose intolerance, and probably linked to insulin resistance. The long-term consequences of LD and its potential association with cardiovascular disease remain unknown. The visceral fat accumulation is characterised by the increased, abundant secretion of a number of peptides such as leptin, insulin-like growth factor (IGF), adiponectin and the recently reported resistin and visfatin hormones. Elevated resistin and tumour necrosis factor (TNF-alpha) levels and low levels of adiponectin secretion may have implications for the risk of development of type 2 diabetes and cardiovascular disease. LD is observed not only in rare autosomal syndromes, but also in patients positive for the human immunodeficiency virus (HIV) who have been treated with protease inhibitors. Both the origin of LD and its treatment deserve more attention and further research in clinical settings.

Potential treatment options with leptin and human growth hormone can be considered to reduce the burden and cardiovascular risk of lipodystrophy.

Novel hormones – S25

S25.1

Hormones help you live longer - the threat of Klotho

M Kuro-o
The University of Texas Southwestern Medical Center, Dallas, Texas, USA.

A defect in *klotho* gene expression in mice leads to a syndrome resembling aging, including a shortened life span, hypogonadism, growth arrest, hypoactivity, skin atrophy, muscle atrophy, hearing loss, premature thymic involution, cognition impairment, motor neuron degeneration, arteriosclerosis, osteopenia, soft tissue calcification, and pulmonary emphysema among others. In contrast, overexpression of the *klotho* gene extends life span in the mouse. Thus, the *klotho* gene functions as an aging suppressor gene. The *klotho* gene encodes a single-pass transmembrane protein and is expressed in limited tissues, notably in the kidney and brain. The extracellular domain of Klotho is shed and secreted in the blood, raising the possibility that Klotho protein itself may function as a humoral factor.

Extended life span in transgenic mice that overexpress Klotho is associated with increased resistance to insulin/IGF1 and oxidative stress, mechanisms for the suppression of aging evolutionarily conserved from worms to mammals. Klotho may affect aging processes partly through its ability to inhibit insulin/IGF1 signaling and to reduce oxidative stress.

Mice defective in fibroblast growth factor-23 (FGF23) exhibit aging-like phenotypes similar to those observed in Klotho-deficient mice, suggesting that Klotho and FGF23 may function in a common signal transduction pathway(s). My laboratory has shown that Klotho binds to multiple FGF receptors (FGFRs) and enhances the ability of FGF23 to activate FGF signaling. FGF23 was originally identified as a hormone that inhibited phosphate reabsorption in the kidney. In fact, both Klotho-deficient mice and FGF23-deficient mice exhibit elevated serum phosphate levels. In addition, many aging-like phenotypes in these mice are rescued by restriction of dietary phosphate or ablation of vitamin D activity. These findings imply a novel concept that FGF signaling and phosphate metabolism may participate in the regulation of aging in mammals.

S25.2

Phosphatonins and the regulation of renal phosphate transport

P Kumar
USA.

Abstract unavailable

S25.3

Correlation of desoxypyridinolin and c– terminal telopeptide of collagen type I within different patient collectives

Walter Fassbender, M Goedde, Vincent Brandenburg, Klaus-Henning Usadel & Ulla Stumpf
Hospital z. Hl. Geist, Kempen, Germany; University Hospital, Frankfurt/Main, Germany; University Hospital, Aachen, Germany; Endokrinologikum, Frankfurt/Main, Germany; University Hospital, Duesseldorf, Germany.

Bone metabolism can be measured indirectly with specific biochemical markers. Desoxypyridinolin (DPD) is a derivate of hydroxypyridinium, which is discharged by bone resorption and is totally excreted urinary. A further marker of collagen resorption is the c-terminal telopeptide of collagen type I, which is liberated to blood circulation within the bone's degradation and undergoes renal elimination. The aim of our investigation was to look after a correlation of these parameters in healthy subjects ($n=28$), patients with type 1 diabetes mellitus (DM) ($n=65$), and female patients with diagnosed postmenopausal osteoporosis (PMO). For the labaratory analysis of DPD we used a solid phase chemiluminescence enzymimmunoassay and for assessment of c-terminal telopeptide of type I – collagen a quantitative ELISA was used. We found correlations of both parameters within the main group ($n=181$), and all the other subgroups. The strongest correlation could be found in the group with DM type 1 ($r=0.79$, $P<0.05$) followed by the group of healthy subjects ($r=0.75$, $P<0.05$). In the group of female patients (PMO) a weaker, but significant positive correlation could be verified ($r=0.58$, $P<0.05$). The arithmatic average of DPD was in the group of healthy subjects about 15,4 nM DPD/mM Krea (95%KI: 11.1–19.72), in the group of type 1 DM patients 21.02 (11.23–30.82) and about 38.51 (28.32–48.7) nM DPD/mM Krea in the group of the female patients (PMO). Both parameters reflect the diverse amount of bone turnover and correlated significantly positive to each other. In comparison to the healthy subjects an enhanced bone turnover could be measured consistently in the group of type 1 DM patients. The hihgest values but concurrent the widest statistic spread with weaker correlation was measured in the group of female patients (PMO). This may indicate, that the results found before therapy are of limited diagnostic value, unlike in the course of antiresorptive therapy the observed significant alterations of bone resorption parameters are of specific diagnostic value.

S25.4

Hormonal regulation of iron homeostasis by hepcidin

Sophie Vaulont
Institut Cochin, Paris, France.

Hepcidin is a small circulating 25-amino-acid cysteine-rich peptide first identified in human blood and urine. The hepcidin gene is expressed mainly in the hepatocytes, secreted in the circulation and cleared by the kidney. In mammals, convincing evidence indicates that hepcidin constitutes the master regulator of iron homeostasis; the circulating peptide acts to limit gastrointestinal iron absorption and serum iron by inhibiting dietary intestinal iron absorption and iron recycling by the macrophages. To limit iron egress, hepcidin binds to ferroportin, a transmembrane iron exporter, thereby inducing its internalization and subsequent degradation, leading to decreased export of cellular iron.

As befits an iron-regulatory hormone, hepcidin synthesis is induced by iron stores and inflammation and inhibited by anemia and hypoxia. The mechanisms regulating hepcidin expression are only beginning to be understood. Recent studies have highlighted two regulatory cascades: BMP/Smad signaling of hemojuvelin (a transmembrane protein whose mutation is leading to juvenile hemochromatosis) and IL-6/STAT3 signaling of inflammation.

Dysregulation of hepcidin is involved in the pathogenesis of a spectrum of iron disorders. Most of the iron overload syndromes known to date (Hereditary Hemochromatosis and secondary iron overloads) imply a reduction of hepcidin secretion. In contrast, excessive cytokine-induced hepcidin expression causes hypoferremia and contributes to the anemia of inflammation.

The emergence of hepcidin as the pathogenic factor in most systemic iron disorders should provide important opportunities for improving their diagnosis and treatment.

Oral Communications

Thyroid clinical - OC1

OC1.1 – ESE Young Investigator Award

Prevalence of inactivating TSH receptor (TSHR) mutations in a large series of pediatric subjects with non-autoimmune mild hyper-thyrotropinemia (hyperTSH)

Daniela Cordella[1], Alessandro De Marco[2], Davide Calebiro[1], Tiziana de Filippis[2], Giorgio Radetti[3], Giovanna Weber[5], Maria Cristina Vigone[5], Marco Cappa[4], Alessandro Sartorio[2], Marta Busnelli[2], Marco Bonomi[2], Bice Chini[6], Paolo Beck-Peccoz[7] & Luca Persani[1]
[1]Dept of Medical Sciences, University of Milan and Ist. Auxologico Italiano, Milan, Italy; [2]Lab of Experimental Endocrinology, IRCCS Ist Auxologico Italiano, Milan, Italy; [3]Pediatric Unit, Bolzano, Italy; [4]Pediatric Unit, Bambin Gesù Hosp, Rome, Italy; [5]Pediatric Unit, HSR, Milan, Italy; [6]CNR, Milan, Italy; [7]Endocrine Unit, Fondazione Policlinico IRCCS, Milan, Italy.

Mild hypothyroidism is a heterogeneous and frequent disorder in the general population that is due to autoimmune disease in most of the cases. TSH resistance is considered a rare genetic disease due to germline loss-of-function TSHR mutations. However, TSHR mutations have been mainly searched in patients with large TSH elevations and their actual prevalence among patients with mild TSH elevations (as those found in mild hypothyroidism) is so far unknown. In this study, we evaluated the involvement of TSHR mutations in a large pediatric series of unrelated cases of hyperTSH ($n=48$, 26 W and 22 M; age 0–12 yrs) selected in various collaborating centers. All subjects had high TSH (4–15 mU/ml), normal freeT4 concentrations, no antithyroid antibodies and normal thyroid volume and structure at ultrasound. Through dHPLC (WAVE apparatus, Transgenomic) and direct sequencing of abnormal PCR products (ABI Prism), we analyzed TSHR coding sequence, proximal promoter and intron-exon boundaries. These investigations lead to the disclosure of 11 carriers of heterozygous TSHR mutations among the 48 patients with hyperTSH (frequency: 22.9%). Seven of these 11 carriers had at least another first-degree relative with known hyperTSH and 4/11 were positive at neonatal TSH screening. Three TSHR mutations are novel (P162L, T607I, R609Q), never found in other patients with TSH resistance and in 150 internal control alleles, and 4 mutations had been previously reported (C41S, P162A, L467P, 655delAC). The mutations C41S, P162A, T607I, 655delAC have been found in 2 unrelated cases. In conclusion, the prevalence of heterozygous TSHR mutations in a pediatric series of hyperTSH is surprisingly elevated. The diagnosis of TSH resistance by means of TSHR gene analysis retains a primary role for appropriate clinical management of subjects with hyperTSH and genetic counseling of their families.

OC1.2

Expression gene profile may be useful for the diagnosis of thyroid malignancies

Pamela Piampiani[1], Cristina Romei[1], Barbara Cosci[1], Agnese Vivaldi[1], Raffaele Ciampi[1], David Viola[1], Valeria Bottici[1], Riccardo Giannini[2], Fulvio Basolo[2], Aldo Pinchera[1] & Rossella Elisei[1]
[1]Department of Endocrinology, University of Pisa, Pisa, Italy; [2]Department of Oncology, University of Pisa, Pisa, Italy.

Although 20% of follicular neoplasms are papillary thyroid carcinoma (PTC), their cytological diagnosis is not diagnostic. A different profile of gene expression between malignant and benign thyroid tumors has been reported. Aim of this study was to identify a gene expression profile to be used in distinguishing malignant from benign thyroid neoplasms. By real-time RT-PCR we analyzed mRNA expression of 6 thyroid differentiation genes (TTF-1, PAX8, TPO, TSHr, NIS and Tg) and 5 genes known to be involved in thyroid tumorigenesis [PPARγ, Gal3, EGFR, MET and oncofibronectin (onfFN)] in 174 human thyroid tissues (87 tumor samples and 87 corresponding normal tissues) belonging to 72 patients affected with PTC and 15 patients affected with benign nodular disease (BND). Our results indicate that thyroid differentiation genes and PPARγ were significantly less expressed in PTC samples than in normal tissue (TPO, 61/72 cases, $P<0.0001$; NIS, 64/72 cases, $P<0.0001$; Tg, 59/72 cases, $P=0.0002$; TSHr, 57/72 cases, $P=0.0169$; TTF1, 47/72 cases, $P=0.002$; PAX8, 55/72 cases, $P=0.0001$; PPARγ, 57/72 case, $P<0.0001$). On the contrary, 3 genes were more expressed in the tumor than in normal tissue (onfFN, 64/72 cases, $P<0.0001$; MET, 55/72 cases, $P=0.0018$; Gal3, 53/72, $P<0.0001$). No statistically significant difference was observed for the mRNA expression of EGFR between tumoral and normal tissues. In BND a statistically significant difference between mRNA expression in tumoral and normal tissue was observed only for PPARγ as observed in PTC specimen. Summarising, our data show that 10/11 selected genes are differentially expressed in the tumor tissue with respect to normal. On the contrary only 1/11 was differentially expressed in BND with respect to its normal tissue. In conclusion, 9/11 of these genes are characterized by a gene expression profile that was specific for the malignant neoplasms. The analysis of the levels of expression of these genes in Fine Needle Aspiration material might represent a helpful and innovative method for the presurgical diagnosis of citologically indeterminate thyroid nodules.

OC1.3

Persistence of decreased peripheral B-lymphocytes after Rituximab treatment is associated to inactive disease in patients with thyroid-associated ophthalmopathy

Irene Campi[1], Guia Vannucchi[1], Danila Covelli[1], Paola Bonara[2], Nicola Currò[3], Davide Dazzi[4], Giacinta Pirola[3], Stefania Rossi[5], Claudio Guastella[6], Paolo Beck-Peccoz[1] & Mario Salvi[1]
[1]Endocrine Unit Department of Medical Science; [2]Internal Medicine; [3]Ophthalmology; Fondazione Policlinico IRCC, University of Milan, Milan, Italy; [4]Internal Medicine, Ospedale di Fidenza, Fidenza, Italy; [5]Pathology Unit, Department of Medicine, Surgery and Dentistry, University of Milan, Ospedale S. Paolo and Fondazione Policlinico IRCCS, Milan, Italy; [6]Otolaryngology, Fondazione Policlinico IRCC, University of Milan, Milan, Italy.

The anti-CD20 antibody Rituximab (RTX) induces peripheral B cells depletion. Aim of the present study was to evaluate changes of lymphocytes after RTX therapy, administered at the dosage of 1000 mg twice at 2-week interval, in 10 patients with Graves' disease, 8 of whom had associated ophthalmopathy (TAO). In all patients, we studied the standard immunophenotypic panel before therapy and monthly for up to 2 years. Total CD20+ (and CD19+) cell depletion was observed after the first infusion in 9 patients while one patient had persistence of <5% CD19+CD5+ lymphocytes. 8/10 patients were depleted for 4–6 months after RTX, while 1 and 1 patients after 2 and 10 months respectively. A reduction of CD20+ cells of about 50% from baseline was observed in 6 patients at 18 months and in 3 at 26 months. While after RTX there was no significant change of serum thyroid autoantibodies levels, nor correlation with CD20+ depletion, we observed a stable improvement of TAO with a significant decrease of the clinical activity score. Although progression to inactive TAO did not correlate with CD20+ cells, since at 5 months they began repopulating, we did not observe relapse of active TAO even after B cell return. In contrast, in the patient with persistence of CD19+5+, severe TAO relapsed at the time of CD20+ cells return. Another cycle of RTX (1000 mg) was then administered but again we observed persistence of <7% CD19+5+ with no definite improvement of the clinical signs of TAO. At subsequent orbital decompression we were able to detect CD19+5+ in the orbital tissues. In conclusion, in patients with TAO a reduction of CD20+ of about 50% from the baseline is still present at 18–24 months after RTX treatment. This may explain the consistent improvement of TAO and the lack of relapse, in patients after total B-cells depletion. Persistence of CD19+5+ lymphocytes in the peripheral blood and, perhaps, in the orbit, may associate to a not completely satisfactory therapeutic response.

OC1.4

A novel tyrosine-kinases selective inhibitor with anti-tumoral efficacy (Sunitinib) induces a block in iodine uptake and transient hypothyroidism

Deborah Mannavola[1], Guia Vannucchi[1], Marco Carletto[2], Virgilio Longari[2], Rossella Bertuelli[3], Paola Coco[3], Paolo Casali[3], Paolo Beck-Peccoz[1] & Laura Fugazzola[1]
[1]Endocrine Unit, Dept. of Medical Sciences, University of Milan, Fondazione Policlinico IRCCS, Milan, Italy; [2]Nuclear Medicine Unit, Fondazione Policlinico IRCCS, Milan, Italy; [3]Dept. of Oncology, Istituto Nazionale dei Tumori, Milan, Italy.

Sunitinib (SU11248) is a multitarget inhibitor of tyrosine-kinases (RTK) recently tested in clinical trials for the treatment of some human cancers. Side effects are mostly represented by asthenia and appear in a dose and time

correlated manner. After the unexpected observation of a myxedematous coma in a patient affected with GIST and treated with Sunitinib, we evaluated the effect of this drug on thyroid function in 24 patients treated for GISTs Imatinib resistant. Patients received the following cycles of therapy: 4 weeks of daily treatment at the dose of 50 mg/day orally (ON) and 2 weeks of withdrawal (OFF). On days 1 and 28 of each cycle TSH, FT3, FT4, thyroglobulin, anti-Tg and anti-TPO autoantibodies were measured. Eleven patients (46%) treated with SU11248 developed a transient hypothyroidism between the 1st and the 6th cycle of treatment (median 3rd cycle). Hypothyroidism was subclinical in 10 cases and overt in 1 patient. During the OFF periods TSH normalized, but a progressive increase of TSH levels was observed. After a variable number of cycles, the lack of normalization during the OFF periods was observed. In order to elucidate the possible mechanism underlying Sunitinib-induced hypothyroidism, *in vivo* morpho-functional examinations were performed. Neither ultra-sonographic alterations (in particular destructive-like), nor variations in thyroglobulin and anti-thyroid autoantibodies, were observed during the ON and OFF phases even after several cycles. On the contrary, ^{123}I uptake was normal in basal conditions and largely reduced after the 4 weeks of treatment, with partial or total normalization after the 2 weeks of withdrawal. In conclusion, SU11248 determines hypothyroidism in the 46% of patients. The absence of anti-thyroid autoantibodies and the normal echographic pattern allow to exclude autoimmune and/or destructive mechanisms. Interestingly, hypothyroidism seems to be correlated with a defect in the uptake of iodine. The possibility to perform a selective and temporary block of thyroid function could be useful in the treatment of some thyroid diseases.

OC1.5

CTLA- 4 gene polymorphisms and autoimmune thyroid diseases: meta-analyses of published and individual-level data

Fotini Kavvoura[1], Takashi Akamizu[2], Takuya Awata[3], Yoshiyuki Ban[4], Dimitry Chistiakov[5], Irena Frydecka[6], Abbas Ghaderi[7], Stephen Gough[8], Yuji Hiromatsu[9], Rafal Ploski[10], Pei-Wen Wang[11], Yoshio Ban[4], Tomasz Bednarczuk[12], Emma Chistiakova[13], Marcin Chojm[10], Joanne Heward[8], Hitomi Hiratani[2], Suh-Hang Hank Juo[14], Lidia Karabon[18], Shigehiro Katayama[3], Susumu Kurihara[3], Rue-Tsuan Liu[11], Ikuyo Miyake[9], Gholam-Hossein Omrani[15], Edyta Pawlak[18], Matsuo Taniyama[16], Teruaki Tozaki[17] & John PA Ioannidis[1]

[1]Department of Hygiene and Epidemiology, University of Ioannina School of Medicine, Ioannina, Greece; [2]Translational Research Center, Kyoto University Hospital, Kyoto University School of Medicine, Kyoto, Japan; [3]Department of Medicine, Saitama Medical University, Saitama, Japan; [4]Third Department of Internal Medicine, Showa University School of Medicine, Tokyo, Japan; [5]Department of Pathology, University of Pittsburgh Medical Center, Pittsburgh, PA, United States; [6]Department of Hematology, Medical Academy, Wroclaw, Poland; [7]Shiraz Institute for Cancer Research, Medical School, Shiraz University of Medical Sciences, Shiraz, Iran; [8]Institute of Biomedical Research, The Medical School, University of Birmingham, Birmingham, United Kingdom; [9]Kurume University School of Medicine, Kurume, Fukuoka, Japan; [10]Human Molecular Genetics Laboratory of the Department of Forensic Medicine, Department of Medical Genetics, Medical University of War, Warsaw, Poland; [11]Chang Gung Memorial Hospital-Kaohsiung Medical Center, Chang Gung University College of Medicine, Kaohsiung, Taiwan; [12]Department of Endocrinology, Medical Research Center, Polish Academy of Science, Warsaw, Poland; [13]Department of Molecular Diagnostics, National Research Center GosNIIgenetika, Moscow, Russia; [14]Graduate Institute of Medical Genetics, Kaohsiung Medical University Hospital, Kaohsiung, Taiwan; [15]Endocrine and Metabolism Research Center, Namazee Hospital, Shiraz, Iran; [16]Division of Endocrinology and Metabolism, Showa University Fujigaoka Hospital, Yokohama, Kanagawa-ken, Japan; [17]Department of Medical Information, Showa University School of Pharmaceutical Science, Showa, Japan; [18]Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wroclaw, Poland.

Background

CTLA-4 polymorphisms have been widely examined for their associations with autoimmune thyroid diseases (Graves' disease [GD] and Hashimoto thyroiditis [HT]) but their relative population effect remains unclear.

Methodology/Principal findings

Meta-analyses of group-level data from 32 ($n=11.019$ subjects) and 12 ($n=4.479$) published and unpublished studies were performed for the association of the A49G polymorphism with GD and HT, respectively. Fifteen ($n=7.246$) and 6 ($n=3.086$) studies were available for the CT60 polymorphism, respectively. Meta-analyses of individual-level data from 10 ($n=4.906$ subjects) and 5 ($n=2.386$) collaborating teams for GD and HT, respectively, using haplotypes of both polymorphisms were also performed. Group-level data suggested significant associations with GD and HT for both A49G (odds ratio 1.49, $P=6\times10^{-14}$ and 1.29 [$P=0.001$] per G allele, respectively) and CT60 (OR 1.45, [$P=2\times10^{-9}$] and 1.64 [$P=0.003$] per G allele, respectively). Results were consistent between Asian and Caucasian descent subjects. Individual-level data showed that compared with the AA haplotype the risk conferred by the GG haplotype was 1.49 (95% CI: 1.31–1.70) and 1.36 (95% CI: 1.16–1.59) for GD and HT, respectively. The AG haplotype also increased the risk of GD (1.35, 95% CI: 1.16–1.55) but not of HT (1.02, 95% CI: 0.71–1.47). The results for the GA haplotype were inconclusive. Data were consistent with a dose-response effect for the G-allele of CT60.

Conclusions/Interpretation

The CT60 polymorphism of CTLA-4 maps an important genetic determinant for the risk of both GD and HT across diverse populations.

OC1.6

Sensitization against Soybean may induce an increase in the levels of anti-thyroid peroxidase antibodies in thyroid autoimmunity

Ildikó Molnár

Kenézy County and Teaching Hospital, Debrecen, Hungary.

Soybean diet could involve in the development of goitre according to antithyroid effects of isoflavones. Isoflavones from Soybean could induce not only inhibition of thyroid peroxidase (TPO) catalyzed reactions but they are allergens for patients suffering from atopic or autoimmune diseases. Two hundred-sixty patients with thyroid autoimmunity (150 with Graves' disease, 110 with Hashimoto's thyroiditis) were investigated for the sensitization against Soybean. Allergen-specific IgE levels were measured by Western blot Allergy Screen panels and the levels of thyroid hormones (TSH, FT_4, FT_3) and anti-TPO, anti-Htg (thyroglobulin), TSH receptor (TRAK) antibodies were detected by immunoassay. The data were presented as mean $\pm$ SE.

Allergic sensitization against Soybean was as follows: 24 cases in Graves' disease and 16 cases in Hashimoto's thyroiditis. Graves' patients with Soybean allergy showed increased anti-TPO levels compared to patients who were negative for allergen (567.33 ± 82.88 IU/ml vs 264.88 ± 30.77 IU/ml, $P<0.001$). However, in patients with Soybean allergy, the elevation in anti-TPO levels was higher in hyperthyroid cases than in those without allergy (736.6 ± 138.87 IU/ml ($n=7$) vs 296.15 ± 50.81 IU/ml ($n=41$), $P<0.011$). Surprisingly, higher FT_3 (and FT_4) levels were demonstrated in sensitized hyperthyroid cases compared to nonsensitized ones (15.92 ± 4.7 pg/ml vs 5.44 ± 0.56 pg/ml, $P<0.001$ for FT_3 (and $P<0.049$ for FT_4)). The increase in anti-TPO levels for sensitized euthyroid Graves' patients strongly associated with ophthalmopathy in comparison with nonsenzitized ones (669.98 ± 162.38 IU/ml ($n=6$) vs 156.81 ± 48.46 IU/ml ($n=29$), $P<0.003$).

In conclusion, the presence of Soybean allergen-specific IgE levels in thyroid autoimmunity could contribute to the elevation in anti-TPO levels for Th2 dominant Graves' disease. The sensitization against Soybean may induce thyroid autoimmunity due to increased anti-TPO levels in disease susceptible patients.

OC1.7

Pregnant women on thyroxine substitution are often dysregulated in early pregnancy

Bengt Hallengren[1], Mikael Lantz[1], Bengt Andreasson[2] & Lars Grennert[3]

[1]Department of Endocrinology, Malmö University Hospital, Malmö, Sweden; [2]Department of Pediatrics, Malmö University Hospital, Malmö, Sweden; [3]Department of Obstetrics and Gynecology, Malmö University Hospital, Malmö, Sweden.

Background

Thyroid hormones are important for normal fetal development. The aim of this prospective study was to explore whether thyroxine treated pregnant women with hypothyroidism are adequately thyroxine substituted during pregnancy.

Material and method
During the years 1997–2002 119 pregnancies in 101 females with thyroid diseases were followed at the Department of Endocrinology. The diagnoses were autoimmune thyroiditis (AIT) with or without hypothyroidism $n=46$, hypothyroidism (non AIT) $n=9$, status post Graves' thyrotoxicosis (GD) $n=33$, active GD $n=8$, multinodular toxic goitre (MNTG) $n=2$, atoxic goitre with or without autonomous function $n=20$, operated thyroid cancer $n=1$ ($+1$ in the group status post GD).
Results
64 patients were on thyroxine due to hypothyroidism at the first visit: 50% (32/64) had serum TSH values within the reference range (0.4–4.0 mIE/l) at first laboratory control. 20% (13/64) had TSH <0.40 mIE/l, 14% (9/64) ≤ 0.1 mIE/l, 30% (19/64) had TSH >4.0 mIE/l, 14% (9/64) >10 mIE/l. 67% (44/66) had to increase the dose during pregnancy, 2/66 could stop thyroxine medication when finishing antithyroid drugs, 30% (20/66) did not have to change the dose.16 miscarriages, 1 late miscarriage, 1 intrauterine fetal death occurred. Of these 18/119 (15%) patients 78% (14/18) had TSH outside the reference range at first control. 44% (8/18) had TSH <0.40 mIE/l, 33% (6/18) had TSH >4.0 mIE/l.
Summary
In 50% of pregnant women on thyroxine substitution the serum TSH values were outside the reference range at first control. A majority had to increase the thyroxine substitution during pregnancy. In pregnant women with miscarriage a great majority had TSH values outside the reference range at first control. The study demonstrates that pregnant women with thyroxine substitution should be carefully checked and the thyroxine dose increased early in pregnancy to avoid hypothyroidism.

OC2.1 Bone and calcium – OC2

Effect of once-yearly infusion of zoledronic acid 5 mg in postmenopausal women with osteoporosis

Richard Eastell[1], Peter Lakatos[2], Dennis Black[3], Steven Boonen[4], Felicia Cosman[5], Steven Cumings[3], Pierre Delmas[6], Erik Fink Eriksen[7], Ping Chung Leung[8], Zulema Man[9], Peter Mesenbrink[10], Ian Reid[11] & Jane Cauley[12]
[1]University of Sheffield, Sheffield, United Kingdom; [2]Semmelweis University, Budapest, Hungary; [3]University of California, San Francisco, San Francisco, CA, United States; [4]Leuven University Center for Metabolic Bone Diseases, Leuven, Belgium; [5]Helen Hayes Hospital, West Haverstraw, NY, United States; [6]INSERM Unit 403, Hopital Edouard Herriot, Lyon, France; [7]Novartis Pharma AG, Basel, Switzerland; [8]The Chinese University of Hong Kong, Hong Kong, China; [9]Centro TIEMPO, Buenos Aires, Argentina; [10]Novartis Pharmaceuticals Corporation, East Hanover, NJ, United States; [11]University of Auckland, Auckland, New Zealand; [12]University of Pittsburgh, Pittsburgh, PA, United States.

Background and methods
The HORIZON-PFT is a multinational, 3-year, randomized, double-blind, placebo-controlled trial evaluating the potential of once-yearly zoledronic acid (ZOL) 5 mg, infused over 15 minutes, to decrease risk of fracture in 7736 postmenopausal osteoporotic women 65–89 years of age.
Results
Treatment with ZOL 5 mg resulted in significant relative risk reductions in morphometric vertebral fracture of 70% vs PBO (3.8% vs 12.8%; 95% CI [62%, 76%]) and in hip fracture of 41% vs PBO (1.4% vs 2.5%; 95% CI [17%, 58%]). Secondary endpoints, non-vertebral (excluding finger, toe, and facial), clinical vertebral, and any clinical fracture (including non-vertebral, hip, and clinical vertebral), were significantly reduced by 25%, 77%, and 33% (all $P<.0001$), respectively. Bone mineral density increased significantly in ZOL vs PBO at total hip (6.0%), lumbar spine (6.9%), and femoral neck (5.0%) ($P<.0001$). While transient increases in serum creatinine ≥ 0.5 mg/dl over pre-infusion levels were seen in a small fraction (1.3%) of patients in the ZOL 5 mg group, no cumulative impact on renal function was demonstrable. Hypocalcemia (serum calcium $<$ 2.075 mmol/l) was observed in 2.3% of patients. Virtually all events occurred after the first infusion of ZOL and all were asymptomatic and transient. Adverse events occurring ≤ 3 days after infusion were more frequent after first infusion (44.7% ZOL vs 14.7% PBO) but declined markedly on subsequent infusions. There were more atrial fibrillation serious adverse events in ZOL vs PBO (1.3% vs 0.5%). Two cases of osteonecrosis of the jaw (1 in PBO, 1 in ZOL) were identified on adjudication; both resolved with antibiotic therapy and limited debridement.
Conclusion
Once-yearly infusion of ZOL 5 mg over 3 years achieves a highly significant decrease in vertebral, hip, and other fracture risk and is generally safe and well tolerated.

OC2.2

Role of IGF system on the regulation of osteoblast aromatase activity in vitro

Claudia Palermo, Marica Arvigo & Francesco Minuto
Department of Endocrinological & Metabolic Sciences and Center of Excellence for Biomedical Research, University of Genoa, Genoa, Italy.

Several studies demonstrated that IGFs stimulate aromatase activity in ovary but no data on bone are available. In the present study the role of IGF system components on aromatase action has been characterized during osteogenic differentiation in a model of rat tibial osteoblasts. At confluence (day 0) cells have been transferred to differentiating medium supplemented with 1% FCS and delta4androstenedion with or without IGF-II 3 nM, IGFBP-2 1 nM and IGFBP-3 1 nM. Cells have been treated with test substances continuously for 9 days or at intervals corresponding to the stages of osteogenesis: Stage 1 = proliferation; Stage 2 = extracellular matrix deposition; Stage 3 = mineralization of extracellular matrix. The aromatase activity has been evaluated by measuring in the conditioned medium the concentration of estradiol by a competitive chemiluminescent enzyme immunoassay. The differentiating effect has been evaluated by the measurement of alkaline phosphatase, which is an early marker of osteogenesis and of calcium incorporation, which is a late marker of osteogenesis. The secretion of the metalloproteinases by means of zymogram has been evaluated in different stages. The results showed that the continous treatment for 9 days with IGF-II and IGFBP-2 alone or in combination, inhibits the physiologic decrease of aromatase and stimulates the differentiation markers, including the metalloproteinase activation. Conversely, treatment with IGFBP-3 inhibits both aromatase activity and cellular differentiation. IGF-II, IGFBP-2 and IGFBP-3 exerted their action on aromatase activity and cellular differentiation also when added in S1 stage. IGF-II resulted ineffective when added alone in S2 or S3 but in S2 addition of IGFBP-2 restored the effect. IGFBP-3 and IGFBP-2 exerted their action also in S2 but not in S3.

In conclusion, these preliminary data suggest that in our cell system the aromatase activity is related to the osteogenic differentiation stages. Moreover, the IGF system plays an important role in the regulation of both bone aromatase activity and osteogenesis.

OC2.3

Clinical and biochemical differences in patients affected with sporadic and type 1 multiple endocrine neoplasia (MEN) related primary hyperparathyroidism

Cristina Eller-Vainicher[1], Sara Massironi[2], Maddalena Peracchi[2], Luca Persani[3], Paolo Beck-Peccoz[1], Anna Spada[1] & Sabrina Corbetta[4]
[1]Endocrine Unit, Medical Sciences Department, University of Milan, Fondazione Ospedale Maggiore, Mangiagalli, Regina Elena IRCCS, Milan, Italy; [2]Gastroenterology Unit, Department of Medical Sciences, University of Milan, Fondazione Ospedale Maggiore, Mangiagalli, Regina Elena IRCCS, Milan, Italy; [3]Lab of experimental endocrinology, Department of Medical Sciences, University of Milan, Istituto Auxologico Italiano IRCCS, Cusano Milanino, Milan, Italy; [4]Endocrinology and Diabetology Unit, Department of Medical-Surgical Sciences, University of Milan, Policlinico San Donato IRCCS, San Donato, Milan, Italy.

Primary hyperparathyroidism (PHPT) may occur sporadically or within MEN syndromes. It is classically thought that PHPT in MEN occurs at earlier ages than sporadic PHPT without significant differences in clinical and biochemical presentation. The aim of the study was to compare clinical and biochemical parameters between sporadic PHPT and MEN1 patients. The study included 41 genetically diagnosed MEN1 patients (14M, 27F) and 88 sporadic PHPT patients (24M, 64F) matched for age at diagnosis. All PHPT patients were studied for calcium metabolism parameters and renal and bone complications and evaluated

for familial history and the presence of signs or symptoms possibly related to MEN1. Young (<50 ys) MEN1 patients showed significantly lower serum PTH (71.23±50.89 *vs* 224.42±220.20 mg/dl, mean±SD, $P=0.019$), total (11.05±0.56 *vs* 12.02±1.22 mg/dl, $P=0.015$) and ionized calcium levels (1.48±0.07 *vs* 1.62±0.19 mmol/l, $P=0.021$) compared with age-matched sporadic PHPT patients, while such differences were not detected in old (51–70 ys) MEN1 *vs* sporadic PHPT patients. Despite the low PTH and calcium levels in MEN1, the prevalence of nephrolithiasis and osteoporosis was similar in the two PHPT forms. A female to male ratio of 1:1 was observed both in MEN1, as expected, and young sporadic PHPT patients. Moreover, young sporadic PHPT patients showed significantly higher serum calcium levels than the old patients (12.0±1.2 *vs* 11.2±0.9 mg/dl, $P=0.008$), in contrast to the pattern observed in MEN1. Our data suggested that milder hypercalcemia and PTH levels within the normal range were not uncommon in young MEN1 with respect to young sporadic PHPT patients, though both groups of patients did not differ for renal and bone complications. In conclusion, young symptomatic hyperparathyroid patients with slightly elevated serum calcium and PTH levels should be carefully screened for MEN1 diagnosis.

OC2.4

Assessment of prevalent vertebral deformities in morphometric X-ray absorptiometry

Elzbieta Skowronska-Jozwiak[1], Pawel Pludowski[2], Elzbieta Karczmarewicz[2], Andrzej Lewinski[1] & Roman Lorenc[2]
[1]Department of Endocrinology & Metabolic Diseases, The Medical University of Lodz, Lodz, Poland; [2]Department of Biochemistry and Experimental Medicine, The Children's Memorial Health Institute, Warsaw, Poland.

Vertebral morphometric X-ray absorptiometry (MXA) is a new tool developed to evaluate the presence of vertebral deformities. Low dose of radiation, fan-beam and the centerline scan technique are believes more advantageous than the classic morphometry using conventional lateral radiograms. We assessed the prevalence of vertebral fractures by MXA in adult population of Łódź region as a part of Polish population studied in EPOLOS epidemiological study.

Patients and methods

362 subjects without history of osteoporosis in anamnesis were examined [244 women, mean age 53±16 years (x±SD) and 97 men, mean age 53±14 years]. MXA lateral scans were performed using DXA system Expert-XL. Six point digitization were used to calculate the anterior (Ha), central (Hc), and posterior (Hp) height of the vertebral bodies Th_4-L_4. Vertebra were defined as having prevalent deformities when at least one ratio value (Ha/Hp, Hc/Hp, Hp/Hp up, or Hp/Hp low) fell 3 SD below or even more than the reference mean of that ratio at any vertebral level.

Results

3969 vertebrae were analyzed. 126 (3.17%) vertebrae in 863 subjects (22.7% of examined individuals) were classified as deformed. In 56 subjects (69.13%) one deformity and in 25 subjects multiple deformities were detected. In 89% of fractures, mild deformities (grade 1) were observed. The prevalence of vertebral fractures was higher in women and increased with age. Th_8 and Th_{12} were the most frequently deformed.

Conclusions

Bone studies indicated that, as in other regions of Poland, also in Łódź region vertebral osteoporotic fractures are common. Thus, the morphometric X-ray absorptiometry (MXA) seems to be a useful and safe tool in the diagnostics of vertebral fractures.

OC2.5

Effect of gonadal status on baseline and after rhGH treatment prevalence of spinal deformities in adult patients with growth hormone deficiency (GHD)

Gherardo Mazziotti[1], Antonio Bianchi[2], Stefania Bonadonna[1], Monica Nuzzo[1], Vincenzo Cimino[2], Alessandra Fusco[2], Laura De Marinis[2] & Andrea Giustina[1]
[1]Department of Internal Medicine, University of Brescia, Brescia, Italy; [2]Department of Endocrinology, Catholic University, Rome, Italy.

Adult GHD patients may have reduced BMD, which is thought to be reverted by long-term rhGH replacement therapy. We have recently reported high prevalence of vertebral osteoporotic deformities in untreated adult GHD patients. Gonadal status is the main determinant of bone loss in patients with primary form of osteoporosis.

In this cross-sectional study, we investigated whether the prevalence and degree of spinal deformities in adults with treated or untreated GHD was in relation to the gonadal status of the patients. Seventy-six adult hypopituitary patients (46 males and 30 females; mean age 46.8 years, range: 16–81) with severe GHD were evaluated for BMD (dual-energy X-ray absorptiometry) and vertebral deformities (T4-L5 quantitative morphometric analysis according to Genant score). At the study entry, 41 patients were eugonadic (21 patients with preserved gonadal function and 20 patients in adequate replacement therapy), whereas 35 patients were hypogonadic.

Vertebral deformities (>20%) were found in 48 patients (63.2%), with higher prevalence in untreated (42 cases) vs. treated patients (24 cases) [76.9% vs. 33.3%; $P<0.001$]. Eugonadic and hypogonadic patients with untreated GHD showed comparable fracture rate (78.6% vs. 75.0%; $P=0.8$). rhGH replacement therapy was accompanied by a significant decrease in fracture rate as compared to untreated patients [eugonadic: 35.3% vs. 75.0%, $P=0.01$; hypogonadic: 28.6% vs. 78.6%, $P=0.01$]. Eugonadic patients had slightly but significantly higher BMD than hypogonadic patients. Multivariate logistic regression analysis demonstrated that no treatment with rhGH was the only factor significantly influencing the occurrence of spinal deformities in adult GHD patients (odds ratio: 5.8, CI 95% 1.9–18.1) whereas no significant correlation was found with gonadal status, BMD, sex and age.

Gonadal status of adult patients with GHD may be not critical for the prevalence of vertebral radiological deformities which is instead mainly affected by the replacement treatment with rhGH.

OC2.6

Sunlight exposure and vitamin D supplementation at the institutionalized elderly – effects on calcium and bone metabolism

Peter Bottermann[2], Dumitru Branisteanu[1], Voichita Mogos[1] & Eusebie Zbranca[1]
[1]University of Medicine and Pharmacy "Gr.T.Popa", Iasi, Romania; [2]Technical University, Munchen, Germany.

We investigated calcium and bone metabolism in a group of 123 institutionalized volunteers between 60 and 98 years old, 73 females and 50 males. 25OH-D_3 was measured by an indoor RIA technique. 1.25$(OH)_2D_3$ was measured by HPLC, serum calcium by photocolorimetry, bone alkaline phosphatase by immunoenzymatic technique, whereas serum PTH and urinary deoxypyridinoline (DPD) were measured by IRMA. Almost all volunteers (92.6%) had low 25OH-D_3 values, but normal or even increased levels of the active hormone, 1.25$(OH)_2D_3$. High PTH was found in 40 cases (32.5%), of which three were primary hyperparathyroidism, whereas the others had low or low-normal calcium levels (secondary hyperparathyroidism). PTH-induced 1α hydroxylation in the elderly with undamaged kidney function seems to compensate the paucity of vitamin D substrate. More than half of the cases had high DPD levels, suggesting high bone turnover. Bone turnover parameters were higher in females than in males ($P<0.05$). A positive correlation between PTH and urinary DPD was noticed ($R^2=0.351$), suggesting the role of secondary hyperparathyroidism in high turnover bone loss. We further supplemented the vitamin D intake in 42 volunteers with a daily dose of 2000 IU of 25-OHD_3 for three months in the summer period, whereas other 42 volunteers received placebo (vitamin B). Normalization of 25-OHD_3 levels was seen in both groups, suggesting that even mild sun exposure increases skin resources of vitamin D. A more significant increase in both 25OH-D_3 and 1.25$(OH)_2D_3$ was however observed in the vitamin D-treated group. Normalization of serum PTH, but not of turnover parameters was observed in both groups. Mild hypercalcemia and increase in serum creatinine were noticed in the vitamin D-treated group. Vitamin D supplementation might therefore be accompanied by hypercalcemic and nephrotoxic effects at doses higher than 2000 IU/day. Sunlight exposure seems efficient to replenish vitamin D reserves at institutionalized patients.

OC2.7

Vitamin K2 induces phosphorylation of protein kinase A and expression of novel target genes in osteoblastic cells

Kuniko Horie-Inoue[1], Tomoe Ichikawa[1] & Satoshi Inoue[2]
[1]Research Center for Genomic Medicine, Saitama Medical University, Hidaka-shi, Saitama, Japan; [2]Graduate School of Medicine, the University of Tokyo, Bunkyo-ku, Tokyo, Japan.

Vitamin K is known as a critical cofactor in blood coagulation and bone homeostasis by helping the function of vitamin K-dependent gamma-carboxylase. We have recently shown that vitamin K2, one of the natural vitamin Ks, has a novel function to regulate the transcription of extracellular matrix-related genes in osteoblastic cells and increase collagen accumulation by activating the steroid and xenobiotic receptor, SXR. In the present study, we searched for novel vitamin K target genes up-regulated specifically by menaquinone-4 (MK-4), a potent vitamin K2 isoform, using oligonucleotide microarray analysis in human osteoblastic MG63 cells. Among these genes, growth differentiation factor (GDF15) and stanniocalcin 2 (STC2) were characterized as MK-4-specific targets, as their mRNA expression was not induced by vitamin K1, another vitamin K2 isoform MK-7, or the MK-4 side chain structure geranylgeraniol. The MK-4-specific induction of GDF15 and STC2 was also observed in murine MC3T3-E1 cells and shown to be independent of either gamma-carboxylation or SXR signaling. As a possible mechanism for MK-4-specific gene regulation, we investigated the contribution of protein kinase A (PKA), one of the key regulators of transcription in osteoblasts. We found that MK-4 enhanced PKA phosphorylation, and the MK-4-specific induction of GDF15 and STC2 genes was reduced by treatment with the PKA inhibitor H89 or siRNA against PKA alpha-catalytic subunit. In conclusion, vitamin K2 has novel functions beside its activity as a coenzyme and plays a significant role in regulating various gene expression and modulating collagen production in osteoblastic cells.

Endocrine tumors and neoplasia – OC3

OC3.1

Multiple somatostatin receptor subtypes activation reduces cell viability in non-functioning pituitary adenomas by inhibiting Vascular Endothelial Growth Factor secretion

Maria Chiara Zatelli[1], Daniela Piccin[1], Federico Tagliati[1], Maria Rosaria Ambrosio[1], Antonio Bianchi[2], Marta Bondanelli[1], Herbert A Schmid[3], Massimo Scanarini[4], Laura De Marinis[2], Giulio Maira[5] & Ettore C degli Uberti[1]
[1]Section of Endocrinology, Department of Biomedical Sciences and Advanced Therapies – University of Ferrara, Ferrara, Italy; [2]Institute of Endocrinology, Catholic University of Rome, Rome, Italy; [3]Novartis Institutes for Biomedical Research, Oncology, Basel, Switzerland; [4]Division of Neurosurgery – Hospital of Padova, Padova, Italy; [5]Department of Neurosurgery, Catholic University of Rome, Rome, Italy.

Somatostatin (SRIF) analogs have been employed in medical therapy of non-functioning pituitary adenomas (NFA), with contrasting results. Previous evidence showed that SRIF can exert its antiproliferative effects by reducing Vascular Endothelial Growth Factor (VEGF) secretion and action, and that VEGF expression may be related to pituitary tumor growth. The aim of our study was to clarify the possible effects of a multireceptor SRIF ligand on VEGF secretion and cell proliferation in human NFA primary cultures, we assessed SRIF receptors (SSTR1-5) expression and the *in vitro* effects on VEGF secretion and on cell viability of SRIF and of the stable SRIF analogue pasireotide (SOM230) which activates SSTR1, 2, 3 and 5. Twenty-five NFA were examined by RT-PCR for expression of α-subunit, SSTR, VEGF, and VEGF receptors 1 (VEGF-R1) and 2 (VEGF-R2). Primary cultures were tested with SRIF and with pasidreotide. All NFA samples expressed α-sub, VEGF and VEGFR-1 and 2, while SSTR expression pattern was highly variable. Two different groups were identified according to VEGF secretion inhibition by SRIF. VEGF secretion and cell viability were reduced by SRIF and pasireotide in the "responder" group, but not in the "non responder" group, including NFA expressing SSTR5. SRIF and pasireotide completely blocked Forskolin-induced VEGF secretion. In addition, SRIF and pasireotide completely abrogated the promoting effects of VEGF on NFA cell viability. Our data demonstrate that pasireotide can inhibit NFA cell viability by inhibiting VEGF secretion, and suggest that the multireceptor-SSTR agonist pasireotide might be useful in medical therapy of selected NFA.

OC3.2 – ESE Young Investigator Award

Adrenal lesions in multiple endocrine neoplasia type 1: data from the French Group for the Study of Endocrine Tumors (GTE)

Blandine Gatta[1], Maud Monsaingeon[1], Pierre Goudet[2], Arnaud Murat[3], Patricia Niccoli-Sire[7], Alain Calender[5], Vincent Rohmer[6], Olivier Chabre[4] & Antoine Tabarin[1]
[1]Dpt Endocrinology, Bordeaux, France; [2]Dpt Surgery, Dijon, France; [3]Dpt Endocrinology, Nantes, France; [4]Dpt Endocrinology, Grenoble, France; [5]Lab Genetics, Lyon, France; [6]Dpt Endocrinology, Angers, France; [7]Dpt Endocrinology, Marseille, France.

The characteristics of adrenal involvement in Multiple Endocrine Neoplasia type 1 (MEN1) have been defined from studies involving a limited number of patients. We have assessed retrospectively the prevalence, characteristics and evolution of adrenal involvement from the French group for the study of endocrine tumours (GTE) registry, involving 688 patients with MEN1. In our series, adrenal tumours identified at abdominal imaging occurred in 130 patients (18.9%). The mean age of patients at the discovery of the adrenal lesion was 46.1 yr (range, 2–78 yr). Adrenal lesions were bilateral in 32% of cases and the mean tumor diameter was 27.6 mm (range, 7–70 mm). Hormonal hypersecretion was found in 16% of patients with adrenal involvement (10 cases of Cushing's syndrome, 7 cases of primary hyperaldosteronism, 2 cases of hyperandrogenism and 1 pheochromocytoma). Among adrenal lesions that were removed, histopathologic examination revealed benign lesions (adenoma and hyperplasia) in 87.5% of cases, adrenal carcinoma in 7.5% and adrenal metastasis in 5%. Overall, malignancy of adrenal lesions was documented in 3.8% of the whole series. Adrenal lesions were associated with enteropancreatic tumors in 66.4% of cases. In patients in whom follow-up imaging was available (mean 6.6 years), 15% demonstrated significant tumoral progression and 13% developed controlateral lesions. No case of adrenal malignancy was found during the follow-up. No correlation was found between genotypic lesions of the *menin* gene and the presence or the type of adrenal lesion. In our series, adrenal tumours are a less frequent than previously reported. Most of adrenal lesions are small in size benign and not responsible for hormonal hypersecretion. Our series do not support the hypothesis of a physiopathologic link between pancreatic tumours and adrenal lesions in MEN1.

OC3.3

BMP dependent effects on adrenal tumorigenesis and function

Inga Johnsen[1], Sarah Kaufmann[1], Lilia Spady[1] & Felix Beuschlein[2]
[1]Institute of Molecular Medicine and Cell Research, Albert-Ludwigs-University Freiburg, Freiburg, Germany; [2]Medical Clinic, University Hospital Innenstadt, Ludwig Maximilians University, Munich, Germany.

Members of the TGFb family of ligands - including bone morphogenic proteins (BMPs) - have been demonstrated to profoundly impact tumorigenesis in a variety of tumor entities. As for the adrenal cortex, BMP6 has been implicated as an important modulator of aldosterone secretion. To screen for alterations of TGFß dependent pathways in adrenal tumorigenesis we performed gene profiling experiments. By comparing human adrenal carcinoma (ACC) against normal adrenal tissue samples (Co) we detected a down-regulation of various BMPs (e.g. BMP2 and BMP5) which was further validated by Real Time analysis (BMP5, ACC vs. Co $6.1 \pm 1.4\%$ vs. $100 \pm 29.7\%$, $P < 0.01$; BMP2, ACC vs. Co $35.1 \pm 1.2\%$ vs. $100 \pm 17\%$, $P < 0.01$). As similar expression pattern with loss of BMP5 expression was evident in NCIh295 cells, this cell line was used as an *in vitro* model to assess potential impact of BMP dependent pathways. Incubation with recombinant hBMP5 induced phosphorylation of SMAD 1/5/8 and subsequent increase of ID protein expression levels in a dose dependent manner, while co-incubation with the physiological BMP antagonist Noggin neutralized these effects. Thus, these findings demonstrated the integrity of the pathway in NCIh295 cells. Notably, BMP5 treatment resulted in a decrease in cellular viability ($68.3 \pm 1.1\%$ vs. $100 \pm 2.7\%$, $P < 0.01$) but increase in the expression levels of steroidogenic enzymes such as StAR ($225 \pm 9.6\%$ vs. $100 \pm 2.3\%$, $P < 0.01$) and SCC ($460.3 \pm 58.8\%$ vs. $100 \pm 0.53\%$, $P < 0.01$). The BMP5 dependent reduced viability was accompanied by concomitant changes in the cell cycle possibly through an increased rate in apoptosis. Taken together, we demonstrate that loss of BMP expression is a common finding in ACC. Moreover, we provide first evidence that BMP dependent pathways might be involved in modulation of the malignant phenotype of adrenocortical cancer.

OC3.4

RET mutation – Tyr791Phe – the genetic cause of different diseases derived from neural crest

Eliska Vaclavikova[1], Sarka Dvorakova[1], Petr Vlcek[2], Richard Skaba[3], Radovan Bilek[1] & Bela Bendlova[1]
[1]Dept. of Molecular Endocrinology, Institute of Endocrinology, Prague, Czech Republic; [2]Dept. of Nuclear Medicine and Endocrinology, 2nd Faculty of Medicine, Charles University and Hospital Motol, Prague, Czech Republic; [3]Dept. of Pediatric Surgery, 2nd Faculty of Medicine, Charles University and Hospital Motol, Prague, Czech Republic.

Familial medullary thyroid carcinoma (MTC), multiple endocrine neoplasia types 2A and 2B (MEN2A, 2B) and Hirschsprung disease (HSCR) are inherited neurocristopathies linked to germline mutations in the RET proto-oncogene. Activating germline RET mutations are presented in patients with FMTC, MEN2A and MEN2B, on the other hand, inactivating germline mutations in patients with HSCR. Nevertheless, there is an overlay in specific mutations in the exon 10 of the RET proto-oncogene. The aim of this study was to screen 6 exons (10,11,13,14,15 and 16) of the RET proto-oncogene by fluorescent sequencing method in three different groups of patients - 174 families with MTC (including MEN2A, 2B), 73 families with HSCR and 20 patients with only pheochromocytoma. In this report, we show that the point mutation Tyr791Phe in exon 13 of the RET proto-oncogene can cause different diseases derived from neural crest. We found Tyr791Phe mutation in 5 families with MTC (3%), 2 families with HSCR (3%) and 1 family with pheochromocytoma (5%). All these patients with the mutation have also a silent polymorphism Leu769 (T/G) in exon 13. In addition, in 2 families with MEN2 double germline mutations were detected: MEN2A family Tyr791Phe + Cys620Phe (exon 10) and MEN2B family Tyr791Phe + Met918Thr (exon 16). Tyr791Phe mutation had not been previously observed in HSCR patients. Detection of Tyr791Phe mutation in MEN2/MTC and HSCR families leads to a question whether this mutation has dual "Janus" character (gain-of-function as well as loss-of-function) as mutations described in exon 10 in HSCR/MEN2A patients. This study shows other character of this strange and frequently discussed Tyr791Phe mutation. On the basis of our genetic finding total thyroidectomy was recommended for all patients with Tyr791Phe mutation.

The work was supported by IGA MZ CR NR/7806-3, GACR 301/06/P425 and IGA MH CR NR/8519-3 and was approved by local Ethical committee.

OC3.5 – ESE Young Investigator Award

[^{123}I]Iodometomidate as a radiotracer for adrenal scintigraphy – first clinical experience

Stefanie Hahner[1], Andrea Stuermer[1], Martin Fassnacht[1], Michael Kreissl[2], Christoph Reiners[2], Felix Beuschlein[3], Martina Zink[1], Ilse Zolle[4], Andreas Schirbel[2] & Bruno Allolio[1]
[1]University of Wuerzburg, Dept. of Endocrinology and Diabetology, Wuerzburg, Germany; [2]University of Wuerzburg, Dept. of Nuclear Medicine, Wuerzburg, Germany; [3]University of Munich, LMU, Division of Endocrine Research, Munich, Germany; [4]University of Vienna, Ludwig-Boltzmann-Institute of Nuclear Medicine, Vienna, Austria.

Adrenal masses are highly prevalent tumours comprising of a variety of entities. Therefore, therapeutic consequences also vary considerably. The CYP11B-specific PET-tracer [^{11}C]metomidate has been shown to be suitable to characterize adrenal lesions. However, its availability is restricted to PET-centers with an on-site cyclotron. Also imaging is hindered by the short tracer half-life (20 min). Therefore, we have developed [^{123}I]iodometomidate as a tracer for adrenal imaging. Pharmakokinetics and biodistribution after i.v.-injection of 40 MBq of [^{123}I]iodometomidate were analyzed in mice using small animal single photon emission computed tomography (SPECT). A 49 year old woman with bilateral adrenal tumors (hounsfield units >10 suggesting a non-adenoma lesion) and borderline urinary catecholamines (patient 1) and a 22 year old man after adrenalectomy for adrenocortical carcinoma with a lesion suspicious for metastasis in the os sacrum (patient 2) were investigated with [^{123}I]iodometomidate-SPECT. Adrenals were excellently visualized in mice with high tracer uptake and little background activity. In patients, adrenals were first detected 60 min p.i. with a maximum uptake in the adrenals after 5–6 hours indicating slow pharmacokinetics of the tracer. At 24 h.p.i. high uptake was detected exclusively in the adrenals. In patient 1 both tumours exhibited high tracer uptake confirming the adrenocortical origin of the lesions. In patient 2 the remaining hyperplastic adrenal was clearly visible. However, no uptake was detected in the os sacrum lesion. Subsequent biopsy revealed a periostal chondroma. For both patients calculated whole body radiation exposure was 3.2 mSv. This is the first description of [^{123}I]Iodometomidate as a radiotracer in patients. Iodometomidate is a highly suitable tracer combining specific uptake in adrenocortical tissue with far lower radiation exposure compared to norcholesterol scintigraphy. Availability and pharmacokinetics are superior to [^{11}C]metomidate-PET. Furthermore, radiotherapy of adrenocortical carcinoma using [^{131}I]iodometomidate appears to be feasible.

OC3.6

ERß-specific transcriptional profile in colon cancer

Valentina Martineti[1], Massimiliano Mascherini[4], Carmelo Mavilia[1], Silvia Carbonell Sala[1], Isabella Tognarini[1], Chiara Azzari[3], Federico Mattia Stefanini[4], Francesco Tonelli[2] & Maria Luisa Brandi[1]
[1]Department of Internal Medicine, School of Medicine, University of Florence, Florence, Italy; [2]Department of Clinical Physiopathology, School of Medicine, University of Florence, Florence, Italy; [3]Department of Pediatrics, School of Medicine, University of Florence, Florence, Italy; [4]Department of Statistics, University of Florence, Florence, Italy.

Epidemiological data clearly evidence a protective role of estrogens against the development of colon cancer and ERβ has been identified as the predominant ER subtype in human colon. More recently it has been identified as a favourable prognostic marker in this disease, possibly explaining the protective effect of estrogens against colon cancer development. To understand the specific role and mechanism of action of ERβ in colon tumorigenesis we developed an *in vitro* engineered cell model through transfection and cloning of HCT8 human colon cancer cell line for stable over-expression of wild type human ERβ (HCT8β8), providing the first direct evidence that ERβ plays an important role in colon cancer as a regulator of cell proliferation through induction of G1-S phase transition arrest. To investigate the molecular events underlying growth arrest we analyzed specific ERβ-regulated genes by comparing expression profile of HCT8β8 cells versus its non-engineered counterpart using Agilent's Human 1A Oligo Microarray (V2) chips harbouring over 22,000 human genes and ESTs. A list of 189 reproducibly ERβ-regulated targets, comprising 64 up-regulated and 125 down-regulated genes, emerged indicating that ERβ over-expression heavily affects different aspects of HCT8 cell function regarding both its intracellular metabolism and relationship with the extracellular milieu. According to their function, ERβ-modulated genes have been grouped into 16 categories, our interest for further validation (by quantitative real time RT-PCR and Western blotting) focused on cell cycle and mitosis genes category and this technique confirmed 50% of gene modulations. On the whole a trend to the slowing down of the cell cycle is demonstrated and one of the up-regulated genes is E4F transcription factor 1 (E4F1), which is already known to be an estrogen-modulated transcription factor. Two of their downstream targets are $p21^{waf1}$ and cyclin E whose altered expression has already been documented in our cell model. We hypothesize that E4F1- $p21^{WAF1}$-cyclin E is an ERβ specific pathway in colon cancer cells.

OC3.7

Fasting insulin levels are predictors of colonic lesions in patients with acromegaly: an observational, open, prospective study in 189 patients

Annamaria Colao[1], Rosario Pivonello[1], Mariano Galdiero[1], Renata S. Auriemma[1], Diego Ferone[2], Paolo Marzullo[3] & Gaetano Lombardi[1]
[1]Department of Molecular and Clinical Endocrinology and Oncology, section of Endocrinology, University "Federico II" of Naples, Naples, Italy; [2]Department of Endocrine and Metabolic Diseases, University of Genova, Genova, Italy; [3]Section of Endocrinology, Auxologic Institute of Verbania, Verbania, Italy.

Elevated insulin levels are correlated with colonic adenomas and carcinomas in the general population. Patients with acromegaly are considered at high risk to develop colonic lesions and have a high insulin levels. To evaluate the role of insulin levels on colonic polyps (hyperplastic, adenomatous, single or synchronous) or adenocarcinoma in acromegaly we designed this analytical, observational, open, prospective, study enrolling 189 patients (100 women, 89 men, age 20–82 yrs) undergoing pan-colonoscopy at diagnosis. Age, gender, estimated disease duration, body mass index, GH and IGF-I levels, fasting glucose and insulin

levels, HOMA-index [R (resistance) and β (β-cell function)] were considered as predictors. Colonic lesions were found in 74 patients (39.1%): hyperplastic polyps in 31 (16.4%), adenomatous polyps in 24 (12.7%), both hyperplastic and adenomatous polyps in 14 (7.4%) and adenocarcinoma in 6 patients (3.2%); polyps were single in 22 patients (29.8%) and synchronous in 52 (70.3%). Colonic lesions were positively correlated with patients' age, insulin levels, HOMA-R and HOMA-β ($P<0.0001$), negatively with GH levels ($P=0.006$) but not with estimated disease duration, IGF-I levels, BMI or glucose levels. Compared to patients with normal glucose tolerance, patients with impaired glucose tolerance had a prospective risk (RR) to develop colonic lesions 2 times higher (95% CI 1.2–3.3) while those with diabetes 2.9 times higher (95% CI 1.8–4.6). Serum fasting insulin levels were the strongest predictor of the presence of colonic lesions. The best cut-off of insulin levels to predict the presence of colonic lesions was 20.6 mUI/liter [sensitivity = 73.8% (61.5–84%); specificity = 81.1% (72.5–87.9%); positive predictive value = 69.6%, negative predictive value = 84.1]. The patients with fasting insulin levels > 20.6 mU/liter at the diagnosis of acromegaly had a RR to develop colonic lesions 5.1 times higher than those with levels ≤ 20.6 mU/liter (95% CI 3.1–8.5). In conclusion, high fasting insulin levels predict the presence of adenomas and adenocarcinomas.

Neuroendocrinology basic – OC4

OC4.1

Organismal, cellular and molecular evolution of water balance regulation in vertebrates: the amphibian hinge

Roger Acher, Jacqueline Chauvet & Yves Rouillé
University Paris VI, Paris, France.

Amphibia, through metamophosis, recapitulate the evolution of water homeostasis from aquatic life to terrestrial one. Whereas the tadpole has the status of a freshwater fish, the adult has developed a three osmoregulatory organ system, including kidney, bladder and skin, for facing terrestrial dehydration. Amphibia have differentiated epithelial hydroosmotic cells in each organ: principal cells in nephron collecting duct, granular cells in urinary bladder, principal cells in ventral skin. These cells, equipped with hormone receptors and effectors (aquaporins, ion channels, urea transporter) are largely controled by neurohypophysial hormones. Each vertebrate possesses two similar neurohypophysial nonapeptides. From the 13 peptides chemically characterized in the laboratory, we have traced two main evolutionary paralog lines: vasotocin (nonmammalian vertebrates) – vasopressin (mammals) involved in osmoregulation, and isotocin (bony fish) – mesotocin (nonmammalian tetrapods) – oxytocin (mammals) possibly implicated in reproduction.

Twelve amphibian species originating from Europa, North- and South-America, Africa and Asia have been investigated. Neurohypophysial secretory granules have been isolated from the neurointermediate pituitary by sucrose gradient centrifugation and their components, purified by HPLC, identified by aminoacid sequencing and/or coelution with synthetic peptides. Along with vasotocin ([Ile^3]-vasopressin) and mesotocin (([Ile^8]-oxytocin), vasotocinyl-Gly (hydrin2) has been identified in all species. This peptide results from a limited processing of the 141-residue provasotocin. A 4-enzyme cascade operating in secretory granules on vasotocinyl-Gly-Lys-Arg sequence leads usually to the alpha-amidated vasotocin but down-regulation of the last amidating enzyme gives, in amphibians only, vasotocinyl-Gly. Vasotocin and hydrin2 have different conformations and act on distinct receptors. Whereas vasotocin shows a water (re)absorption activity in kidney, bladder and skin, hydrin2 is devoid of antidiuretic activity and is more active than vasotocin on the skin. Hydrin2 is twice more abundant in species living in arid countries. Evolution has synchronized a new osmoregulatory organ (skin) with a new specific hormone, making two hormones from a single precursor.

OC4.2

Growth Hormone-Releasing Hormone (GHRH) exerts protective effects on adult rat hippocampal progenitor cells

Silvia Destefanis[1], Inger Johansson[2], David Aberg[2], Riccarda Granata[1], Ezio Ghigo[1] & Jorgen Isgaard[2]
[1]University of Turin, Turin, Italy; [2]Sahlgrenska University, Goteborg, Sweden.

Growth hormone releasing hormone (GHRH) is a neuropeptide mainly synthesised in the hypothalamus, known to exert a stimulatory effect on the synthesis and release of growth hormone (GH) from the pituitary via the activation of specific receptors. New data indicate that GHRH is also produced in both extrahypothalamic brain areas and in peripheral tissues. GHRH-receptor splice variants (SVs) have been found in several peripheral normal and neoplastic human tissues and mediate effects on cell proliferation and differentiation. At present, central non-endocrine effects of GHRH in extra-pituitary brain tissues have not yet been characterised. The aim of the present study was to investigate the effects of GHRH on cell survival in rat adult hippocampal progenitor cells (AHP) and to study the intracellular pathway involved. Cell viability was assessed by the Alamar blue assay. RT-PCR was performed to detect the presence of GHRH receptor mRNA. The results showed that GHRH receptor is expressed in AHP cells. GHRH dose dependently increased cell survival on AHP cells compared to control. After GHRH administration a significant increase of cAMP levels analyzed by ELISA was observed, suggesting a GHRH-induced activation of cAMP pathway. Consistently, western blot analysis showed a significant activation of Akt and ERK 1/2 survival pathway after GHRH administration. Activation of these signalling pathways preceded CREB phosphorylation, which plays an important role in the differentiation and maturation of newborn neurons in hippocampus. In conclusion, this study shows that GHRH has a protective effect on AHP cells. Moreover, in these cells GHRH is able to activate the cAMP-CREB pathway. Akt and ERK1/2 seem to be involved in this survival signalling. Thus, GHRH and its receptor may play an important role for hippocampal progenitor cells survival.

OC4.3

Absence of germline AIP mutations in early onset sporadic somatotropinomas

Leonor Gomes[1], Hugo Prazeres[2], Isabel Paiva[1], Cristina Ribeiro[1], Olinda Rebelo[3], Teresa Martins[2], Manuela Lacerda[2] & Manuela Carvalheiro[1]
[1]Serviço de Endocrinologia, Diabetes e Metabolismo dos Hospitais da Universidade de Coimbra, Coimbra, Portugal; [2]Laboratório de Patologia Molecular do Serviço de Anatomia Patológica do Centro Regional de Oncologia de Coimbra do Instituto Português de Oncologia, EPE, Coimbra, Portugal; [3]Serviço de Anatomia Patológica dos Hospitais da Universidade de Coimbra, Coimbra, Portugal.

Objective
The pathogenesis of pituitary tumours is still incompletely understood. Somatotropinomas occur both sporadically and in the context of familial syndromes, such as multiple endocrine neoplasia type 1 (MEN1), Carney complex (CNC) and isolated familial somatotropinoma (IFS). Recently, germline mutations were reported in *AIP* (aryl hydrocarbon receptor interacting protein) gene in Finish and Italian families and in Finish patients with apparently sporadic pituitary tumours. The aim of this study was to determine if *AIP* gene mutations influence individual susceptibility to develop sporadic pituitary somatotropinomas in a group of young patients originating from the central region of Portugal.
Methods
Blood samples were obtained from 20 patients (8 males and 12 females) with sporadic somatotrophinomas, including 6 plurihormonal for GH and PRL, who were diagnosed when they were younger than 35 years of age (mean age 25.7±4.97, 16-33 years). Detection of the *AIP* germline mutations was carried out by PCR amplification of genomic DNA, followed by direct sequencing of the entire gene coding sequence and intron-exon boundaries as previously described.
Results
In this series of patients, with early onset sporadic oversecreting-GH pituitary adenomas, no *AIP* germline mutations were found. A heterozygous synonymous C→T polymorphism (Asp45Asp) was found in a single patient.
Conclusions
Our results provide evidence that *AIP* germline mutations are not associated with sporadic pituitary tumours. We studied patients diagnosed at young ages, with a hypothetically higher probability of harbouring occult germline mutations. The absence of germline mutations in this group of patients suggests that *AIP* germline mutations probably do not play an important role in the pathogenesis of sporadic pituitary somatotropinomas. Similar observations have been made by other groups. Further studies are needed in order to identify other genetic factors underlying early onset sporadic pituitary tumours.

OC4.4

Is there a role for dopamine D_2 receptor gene polymorphisms in determining cabergoline sensitivity in prolactin-secreting pituitary adenomas?

Marcello Filopanti, Anna Maria Barbieri, Rita Angioni, Andrea Lania, Giovanna Mantovani, Anna Spada & Paolo Beck-Peccoz
Endocrine Unit, Dept. Medical Sciences, University of Milan; Fondazione Ospedale Maggiore Policlinico IRCCS, Milano, Italy.

Dopamine agonist cabergoline (CB) is the first-choice treatment in prolactin-secreting adenomas (PRL-omas). It is effective in reducing PRL secretion and tumour size in about 90% of patients by binding dopamine D_2 receptor (DRD2). Although no mutations in DRD2 were found, it has been reported that several polymorphisms of this locus associate with alcoholism and schizophrenia, diseases in which dopaminergic system plays an important role. To assess the possible association of DRD2 gene polymorphisms (i.e. TaqIB, HphIG/T, NcoIC/T and TaqIA) with the sensitivity to CB, a multicentric retrospective study was carried out including 252 patients with PRL-oma and 211 healthy controls. Genotyping was carried out by restriction fragment length polymorphism analysis (RFLP) on blood DNA. Pituitary MRI and PRL assay were performed at diagnosis and during CB therapy follow-up (median 17 months, range 5–49). Patients were defined as resistant when they failed to normalize PRL levels and/or to reduce tumor size with a CB dosage higher than 3 mg/week. According to this definition, in our series the overall prevalence of resistant patients was 8% and 3.4%, respectively. As far as DRD2 genotypes were concerned, no differences in allele frequencies between patients and normal subjects were observed. Moreover, any polymorphism correlated with clinical presentation, biochemical data and tumor size. Conversely, we observed an higher frequency of NcoIT+ allele in subjects defined as resistant to CB in term of both normalization of PRL levels [$(\chi^2)P=0.038$] and tumor size reduction [$(\chi^2)P=0.006$]. Finally, [A1-/B1-/T-/T-] haplotype was found to be associated with a greater sensitivity to CB in term of PRL normalization. In fact, this haplotype was found in 34% of patients taking less of 3 mg/week of CB vs 11% of resistant patients [$(\chi^2)P=0.021$]. In conclusion, further studies are required to assess the mechanisms underlying the involvement of DRD2 gene polymorphisms in determining the CB sensitivity.

OC4.5

Lack of nuclear Hes1 expression coincides with transformation of endocrine pancreatic cells in Men1 knock out mice

Térèse Johansson, Margareta Halin Lejonklou & Britt Skogseid
Institution of Medical Sciences, Uppsala, Sweden.

Background
Homozygous inactivation of the *MEN1* tumor suppressor gene frequently occurs in endocrine pancreatic tumors (EPT); however, a heterozygous germ line inactivation of the gene seems to lead to development of an increase amount of endocrine pancreatic cells. The Notch signaling cascade plays a vital role in sustaining the balance between cell proliferation, differentiation and apoptosis during pancreatic development. Whether Notch signaling is *MEN1* dependent is unknown.
Aim
To explore the Notch pathway by means of the transcription factors *Hes1*, *Hey1* and *Mash1* expression pattern and their role in endocrine tumour progression by in $Men1^{+/-}$ mice.
Methods
Notch1, *Hes1*, *Hey1*, *Mash1*, and *Men1* mRNA expression were investigated by qPCR. Fifteen mice (10 $Men1^{+/-}$, five Wt, 12 or 18 month,) were used; the endocrine tissue was divided according to size: small islets, islets, small tumors and larger tumors. Protein expression were assessed by immunohistochemistry (13 $Men1^{+/-}$ and 12 Wt, 9–22 month)
Results
Men1, *Notch1*, *Hes1,* and *Hey1* mRNA expression was found in endocrine tissue of all sizes; *Mash1* was found in 28/55 samples. Variable degree of loss of menin (the *Men1* protein) expression was observed in tumors of $Men1^{+/-}$ mice age 14–22 month. $Men1^{+/-}$ and Wt mice showed no difference in *Notch1*, *Hey1*, and *Mash*1 immunoreactivity. Wild type mice of all ages expressed nuclear *Hes1*, whereas only the younger $Men1^{+/-}$ mice displayed nuclear *Hes1* immunoreactivity. The tumors of the heterozygous mice age 14–22 month had lost nuclear *Hes1* expression.
Conclusions
Mash1 immunoreactivity was invariably and abundantly displayed. The lack of *Hes1* in tumor cell nuclei in elderly $Men1^{+/-}$ mice indicates that *Hes1* might be of importance in endocrine pancreatic tumorigenesis.

OC4.6

Metabolic abnormalities in patients with adrenal adenomas may be associated with BclI polymorphism in the glucocorticoid receptor (GR) gene and expression of tumor-specific hsp70 isoforms

Svetozar Damjanovic, Jadranka Antic, Bojana Beleslin, Sanja Ognjanovic, Jovana Vignjevic, Milan Petakov, Djuro Macut, Ivan Paunovic, Tatjana Isailovic, Bojana Popovic & Ivana Bozic
Institute for Endocrinology, Diabetes and Metabolic Diseases, Belgrade, Serbia.

Introduction
Intronic *Bcl*I polymorphism of glucocorticoid receptor (GR) gene and adrenal adenomas of incidental discovery are frequently associated with metabolic syndrome. We studied in these patients metabolic and hormonal parameters, the sequence alteration in *Bcl*I polymorphism of the GR gene from constitutive DNA and the expression of Hsp70 within the tumor and in tissue adjacently to tumor.
Patients and methods
We assessed 114 healthy subjects (79 men; age range 21–68 years) and 30 patients operated due to non-functioning adrenal mass (5 men; age range 36–76 years). Besides clinical and anthropometrical assessment, morning cortisol and fasting insulin levels were determined. DNA was obtained from leucocytes and after amplification, PCR fragments were digested with *Bcl*I enzyme. Subsequently, the sequence of the fragments was analyzed. Equal amounts of protein obtained from total cell lysates of adenomatous and adjacent normal adrenal tissue was resolved by 9% SDS-PAGE and transferred to nitrocellulose membranes (Western blot analysis).
Results
We found a G-to-C transition in the second intron of GR gene in 24 of 26 (92%) patients that is significantly higher frequency of the larger allele within patients than in normal population (42% vs 4.2%; $P<0.001$). Patients and controls had similar BMI and morning cortisol levels. However, the frequency of diabetes type 2, and hypertension were significantly higher in patients with adrenal tumor ($P=0.002$ for both) and these patients had significantly higher HOMA index than controls (6.8 ± 1.9 vs 2.9 ± 0.1; $P<0.001$). In all tumor tissues two isoforms of Hsp70 co-expressed while only higher molecular weight isoform was detected in adjacent normal tissue.
Conclusion
Increased sensitivity to glucocorticoids associated with specific *Bcl*I polymorphism of GR gene and altered trafficking of GR by the Hsp90/Hsp70-based chaperone machinery within adrenal adenoma seems to play role in the development of insulin resistance in these patients.

OC4.7

Function and evolution of GHRH, PACAP and PRP in vertebrates

Billy KC Chow, Francis KY Siu, Janice KV Tam & Leo TO Lee
University of Hong Kong, Pokfulam, HK, SAR, China.

In mammals, GHRH is the most important neuroendocrine factor that stimulates the release of GH from the anterior pituitary. In non-mammalian vertebrates, however, the previously named GHRH-like peptides were unable to demonstrate robust GH-releasing activities. In this report, we provide evidence that these GHRH-like peptides are homologues of mammalian PACAP-related peptides (PRP). Instead, GHRH peptides encoded in cDNAs isolated from goldfish, zebrafish and African clawed frog were identified. Moreover, receptors specific for these GHRHs were characterized from goldfish and zebrafish. These GHRHs and GHRH-Rs are phylogenetically and structurally more similar to their mammalian counterparts than the previously named GHRH-like peptides and GHRH-like receptors. Information regarding their chromosomal locations and organization of neighbouring genes confirmed also that they share the same origins as the mammalian genes. Functionally, the goldfish GHRH activates cAMP production in receptor-transfected CHO cells as well as GH release from goldfish pituitary cells. Tissue distribution studies by real-time PCR showed that the goldfish GHRH is expressed almost exclusively in the brain, while the goldfish GHRH-R is actively expressed in brain and pituitary. In addition, specific receptors for PRPs (formerly GHRH-like peptides) were cloned from goldfish, zebrafish and Xenopus, clearly suggesting a function of PRP in these species. By phylogenetic and chromosomal syntenic studies, we found PRP receptors only in non-mammalian vertebrates but not in mammals, indicating that the receptor was lost in the mammalian lineage. Based on these data, a comprehensive evolutionary scheme for GHRH, PRP-PACAP, PHI-VIP genes in relation to 3 rounds of genome duplication early on in vertebrate evolution is proposed. Finally, the newly discovered GHRHs, also found in flounder, Fugu, medaka, stickleback, Tetraodon and rainbow trout, provide new research directions regarding the neuroendocrine control of growth in vertebrates.

Thyroid basic – OC5

OC5.1

Structure-function of glycoprotein hormones using site-directed mutagenesis and gene transfer: designing new agonists and antagonists

Fuad Fares, Naiel Azam, Rinat Bar-Shalom & Zaki Kraiem
Carmel Medical Center and the Faculty of Medicine, Technion-israel Institute of Trechnology, Haifa, Israel.

Objectives
The main goal of the present study is to investigate the role of N-linked oligosaccharides on the structure and function of human thyrotropin (hTSH). A second aim of the present study is to design new analogs of hTSH.
Methods
Overlapping PCR technique was used to convert hTSH heterodimer to a biologically active single chain by fusing the α subunit to the carboxyl terminal end of hTSHβ subunit in the absence (hTSH$\beta\alpha$) or presence of a $\sim$30 aminoacid peptide from hCGβ (CTP) as linker (hTSHβCTPα). hTSH mutants lacking the sequence site of N-linked oligosaccharides were prepared using site-directed mutagenesis. hTSH variants were expressed in CHO cells. The TSH receptor binding activities of the variants were determined by radioligand receptor assay using CHO cells stably transfected with hTSH receptor. *In vitro* bioactivity was tested using cultured human thyroid follicle cells and *in vivo* longevity and bioactivity were tested in mice animal model.
Results
The single-peptide variants of hTSH were biologic active *in vitro* and *in vivo* with a longer half-life. Variants lacking the N-linked oligosaccharides were expressed and secreted from CHO cells. Interestingly, the deglycoselated variants were significantly less potent than TSH wild type. Moreover, the deglycoselated variants blocked cAMP formation and T_3 secretion stimulated by hTSH or by hTSI in the receptor level. The variants were found to bind the hTSH receptor with high affinity. In addition, deglycoselated hTSH variants had a partial activity *in vivo* and significantly inhibited TSH bioactivity
Conclusions
Human TSH single peptides are biologically active. Deglycosylated variants inhibit the activity of hTSH and hTSI. These variants may offer novel therapeutic strategies in the treatment of Thyroid diseases.

OC5.2

Tyroglobulin (Tg) depletion in receptor associated protein (RAP) KO mice is due to a reduction of Tg aggregates

Simonetta Lisi, Roberta Botta, Aldo Pinchera, Claudio Marcocci & Michele Marinò
Department of Endocrinology, University of Pisa, Pisa, Italy.

RAP KO mice have a reduction of colloidal Tg resulting in subclinical hypothyroidism and histological signs of goiter. The difference in colloidal Tg between RAP KO and WT mice was striking by immunohistochemistry, but could not be detected in thyroid extracts. To explain this discrepancy, we hypothesized that the reduction of Tg reflected a reduction of Tg aggregates discarded during tissue extraction. To investigate this possibility, pellets obtained by thyroid homogenization were solubilized with 6M guanidine and analyzed by Western blotting. Tg resolved into two bands at 660 and 330 kDa, which were found in WT, but not in RAP KO mice, supporting a reduction of Tg aggregates in the latter. We then investigated the effects of detergents, denaturation and pH on homogenates separated into membrane-associated and cytoplasmic fractions. The Tg bands were detected in all samples from RAP KO and WT mice. Detergents and high pH increased the intensity of the bands in the cytoplasmic fractions from WT mice, suggesting the presence of Tg aggregates of high molecular mass. Under denaturing conditions the Tg bands were less intense, probably due to Tg degradation. In RAP KO mice, cytoplasmic Tg was less sensitive to detergents and pH, possibly because of a reduced number of Tg aggregates compared with WT mice. Higher amounts of Tg were found in the membrane-associated than in the cytoplasmic fractions, regardless of the extraction procedure and the genotype, representing Tg-containing vesicles within the colloid, Tg within intracellular organelles, and cell membrane-bound Tg. In RAP KO mice the amounts of membrane-associated Tg were greater than in WT mice, in agreement with immunohistochemical findings. In conclusion, the absence of RAP in the thyroid gland results in a reduction of colloidal Tg aggregates, which are known to represent the major storage form of thyroid hormones.

OC5.3

Polarized plasma membrane targeting of the Na^+/I^- symporter (NIS) is regulated by its carboxy terminus

Orsolya Dohan[2], Carla Portulano[1], Chris Ginter[1] & Nancy Carrasco[1]
[1]Albert Einstein College of Medicine, Bronx, New York, United States; [2]Institute of Experimental Medicine Hungarian Academy of Sciences and Szent Rokus Hospital and Institutions, Budapest, Hungary.

The Na^+/I^- symporter (NIS), a glycoprotein expressed at the basolateral plasma membrane of thyroid epithelial cells, mediates active I^- uptake for the biosynthesis of thyroid hormones and radioiodide transport for diagnosis and treatment in thyroid cancer. Our cloning of the NIS cDNA and generation of anti-NIS antibodies provided the basis to investigate the decrease in I^- transport in thyroid cancer relative to healthy thyroid cells. Instead of finding only the expected lower NIS expression, we have reported that in the majority of thyroid cancers, NIS is surprisingly overexpressed as compared to the surrounding tissue but retained intracellularly. Therefore, it is of considerable interest to elucidate the mechanisms underlying NIS plasma membrane targeting, a pursuit that could lead to new therapeutic interventions to increase the sensitivity of radioiodide diagnostic imaging and the effectiveness of radioiodide therapy. We report that the NIS carboxy terminus contains crucial information for NIS trafficking and that the length of the carboxy terminus correlates linearly with functional cell surface expression of the transporter. We also demonstrate that whereas the last four amino acids ($E_{615}TNL_{618}$) are not necessary for NIS trafficking, even though they comprise a PDZ binding motif, the 602–614 sequence carries essential determinants for NIS basolateral targeting.

OC5.4

$BRAF^{V600E}$ mutations but not RET/PTC rearrangements are correlated with a lower expression of NIS mRNA expression in papillary thyroid cancer (PTC)

Cristina Romei[1], Pamela Piampiani[1], Pinuccia Faviana[3], Fulvio Basolo[3], Valeria Bottici[1], Mariangela Sculli[1], Piero Berti[2], Gabriele Materazzi[2], Aldo Pinchera[1] & Rossella Elisei[1]
[1]Department of Endocrinology, University of Pisa, Pisa, Italy; [2]Department of Surgery, University of Pisa, Pisa, Italy; [3]Department of Oncology, University of Pisa, Pisa, Italy.

Several studies have identified a relationship between oncogene activation and dedifferentiation of PTC. Mutations of RAS, RET/PTC and BRAF modulate the expression of thyroid genes. An impaired NIS expression has been demonstrated in PTCs harboring the $BRAF^{V600E}$ mutation.Aim of this study was to analyze BRAF and RET/PTC1-3 alterations and their influence on the expression of thyroid differentiation genes. Seventy-one PTC samples were studied. Quantitative analysis of TPO, Tg, TSH-R, TTF1 and NIS were performed by real time RT-PCR. Our results indicate that 44/71 cases (62%) were positive for one genetic alteration and 7/71 (9.8%) showed the simultaneous presence of 2 gene mutations. In particular $BRAF^{V600E}$ and RET/PTC rearrangements were present in 32.2% and 19.7% of cases respectively. $BRAF^{V600E}$ was more frequently found in the classical than in the follicular variant ($P=0.02$). At variance no correlation was identified between RET/PTC rearrangement and clinico-pathological features of PTCs. Genetic alterations were correlated with mRNA expression (ΔCt) of Tg, TPO, TSH-R, TTF-1, NIS. mRNA expression of NIS gene was significantly lower ($P=0.0001$) in PTCs harbouring the BRAF mutation with respect to not mutated samples. By immunohistochemistry we did not find any relationship between $BRAF^{V600E}$ and NIS protein. No difference in NIS mRNA expression was found in PTC with or without RET/PTC rearrangements. We did not observe any significant difference in the expression of thyroid differentiation genes neither when compared with BRAF mutation or RET/PTC rearrangements. Furthermore no relationship was found between serum TSH and the expression of NIS mRNA in thyroid tumors. In conclusion our data indicate that (a) the frequency of $BRAF^{V600E}$ mutations and RET/PTC rearrangements was 35% and 20% respectively; (b) in our series 10% of PTC cases harbored 2 different genetic alterations; (c) NIS mRNA expression was significantly lower in PTCs harboring a BRAF mutation but not a RET/PTC rearrangement; (d) the expression levels of other thyroid differentiation genes were not correlated with the presence of gene alterations.

OC5.5

Transcriptional regulation of human type 2 deiodinase and chorionic gonadotropin genes in human placenta: emerging evidence of a common promoter code

Gianluca Canettieri, Maria Giulia Santaguida, Antonella Franchi, Michele Della Guardia, Alberto Gulino & Marco Centanni
University of Rome "La Sapienza"- Polo Pontino, Latina, Italy.

Human type 2 deiodinase (hD2) regulates T3 production in placenta during trophoblast development. hD2 mRNA and protein levels are elevated only during the first trimester of gestation then becoming barely detectable. These variations are similar to those of chorionic gonadotropin (hCG), a well-known marker of early gestation secreted by the cytotrophoblast. A peculiar promoter architecture of the gene encoding the alpha subunit of hCG allows a CRE-mediated synergism between cAMP and EGF, leading to elevated levels of hCG-mRNA only during early pregnancy. In addition, hCG promoter contains several CCAAT boxes, that are likely to confer tissue specificity to this gene. Similarly, in our previous studies we have demonstrated that *Dio2* promoter is synergistically stimulated by cAMP and mitogens. These signals are integrated and converge to the Dio2 CRE, which recruits a transcription factor complex including CREB, c-Jun and c-Fos. Here we show that CCAAT enhancer binding proteins (C/EBPs), are master regulators of Dio2 expression in JEG3 cells, a cell line similar to early trophoblast. RT-PCR studies have demonstrated that C/EBPs significantly increases hD2 mRNA levels. With functional assays of micro-deletion mutant constructs we have shown that C/EBPs robustly enhanced the transcriptional activity of hD2 gene through a highly conserved CCAAT element, located nearby the TATA box. Biochemical evidence confirmed the binding of C/EBPs to this regulatory site. Remarkably, the inducibility was dramatically increased in promoter constructs lacking the CRE or when CREB/CRE interaction was prevented by an acidic dominant negative inhibitor. This latter observation suggested that CREB and C/EBP regulates transcription of *Dio2* gene in an antagonistic fashion. In conclusion we have found that αCG and Dio2 genes seem to share a common promoter code, represented by CCAAT, CREs, TATA/TSS units, that imparts tissue specificity and inducibility to both genes in early trophoblast.

OC5.6

A crucial role of interleukin-6 in the pathogenisis of thyrotoxicosis-related disturbances of bone turnover in mice

Janusz Mysliwiec[1], Robert Zbucki[2], Maria Malgorzata Winnicka[2], Boguslaw Sawicki[3], Agnieszka Nikolajuk[1], Karol Kaminski[4], Wlodzimierz Musial[4], Piotr Mysliwiec[5], Zofia Bondyra[6], Jerzy Walecki[6] & Maria Gorska[1]
[1]Dept. of Endocrinology, Diabetology and Internal Diseases, Bialystok, Poland; [2]Dept. of General and Experimental Pathology, Bialystok, Poland; [3]Dept. of Histology and Embryology, Bialystok, Poland; [4]Dept. of Cardiology, Bialystok, Poland; [5]II Dept. of General Surgery, Bialystok, Poland; [6]Dept. of Radiology, Bialystok, Poland.

Interleukin-6 (IL-6) has been shown to be involved in the pathogenesis of several bone diseases characterized by a negative balance between bone resorption and formation.

The aim of the study was to estimate serum markers of bone turnover: osteoclast-derived tartrate-resistant acid phosphatase form 5a (TRACP 5b) and osteocalcin in IL-6 knock-out mice to assess the role of IL-6 in the pathogenesis of thyrotoxicosis-related disturbances of bone metabolism.

Material and methods

C57BL/6J (wild-type; WT) and C57BL/6J$^{IL6-/-Kopf}$ (IL-6 knock-out; IL6KO) mice randomly divided into 4 groups with 10 in each one: 1/WT mice in thyrotoxicosis (WT-thx), 2/WT controls (WT-ctrl), 3/IL6KO mice with thyrotoxicosis (IL6KO-thx) and 4/ IL6KO controls. Experimental model of hyperthyroidism was induced by intraperitoneal injection of levothyroxine. The serum levels of TRACP 5b and osteocalcin were determined by ELISA.

Results

Serum concentration of TRACP 5b (median and interquartile ranges) were significantly increased in both groups of mice with thyrotoxicosis: WT (28.2 (18.8–41.6) U/l) and IL6KO (26.4 (23.0–31.2) U/l) as compared to the respective controls. Osteocalcin serum levels in IL6KO-thx mice (111.9 (103.1–175.6) ng/ml) were significantly elevated in comparison to WT-thx animals (46.1 (32.5–58.9) ng/ml).

Conclusions

The results of the present study suggest that IL-6 plays a crucial role in thyrotoxicosis-related disturbances of bone turnover in mice, determining the imbalance between bone resorption and bone formation caused by excess of thyroid hormones predominantly by inhibition of bone formation.

Acknowledgements

This work was supported by AMB Grant 3-50693L (to JM) and KBN Grant 2P05B01826 (to KK).

OC5.7

The 1188A/C polymorphism of IL-12 gene in Graves' disease

Natalia Wawrusiewicz-Kurylonek, Maria Gorska, Janusz Mysliwiec & Adam Jacek Kretowski
Department of Endocrinology, Diabetology and Internal Medicine, Medical University of Bialystok, Bialystok, Poland.

Background and aims

Interleukin-12 (IL-12) is a pro inflammatory cytokine, which was suggested to play a key role in the pathogenesis of Th1-cell-mediated autoimmune diseases. The aim of our study was to estimate the association of 1188A/C polymorphism of IL12B gene with the predisposition to Graves' disease (GD) in Polish population.

Materials and methods

The study was performed in the group consisting of 245 individuals with GD sequentially recruited from the endocrinology outpatient clinic. GD was confirmed on the basis of clinical observation, biochemical criteria of thyrotoxicosis and the presence of TSH receptor antibodies. Two hundred and one healthy volunteers served as the control group. In all subjects A1188C polymorphism in the 3′-UTR region of the IL-12B gene was determined by direct sequencing of the appropriate fragment of IL-12B gene.

Results

In our study the frequencies of 1188C allele and 1188CC genotype were significantly higher in patients with GD in comparison to healthy subject (respectively, 22.1% vs. 16.2%, $P=0.027$ and 7.7% vs. 1.5%, $P=0.003$). There were no differences in the distribution of 1188AA and 1188AC genotype IL-12B gene between the studied groups. Furthermore we also observed that frequency of 1188CC genotype was higher in patient with ophtalmopathy in comparison to subject without ophtalmopathy and healthy controls. The frequency of 1188CC IL-12 genotype was also higher among patients, who developed GD before the age of 40 years, when compared to subjects with Graves' disease onset before age of 40.

Conclusions

We observed that the frequency of 1188CC genotype of IL-12B gene is higher in patients with GD and with ophtalmopathy in comparison to subject without ophtalmopathy and healthy controls. This suggests that 1188A/C polymorphism in IL-12B gene could have a role in predisposition to Graves' ophthalmopathy.

Cardiovascular endocrinology – OC6

OC6.1

Growth hormone-releasing hormone prevents cardiomyocyte apoptosis and activated PI3K/AKT, ERK1/2 and CREB signaling pathways

Riccarda Granata, Letizia Trovato, Silvia Destefanis, Fabio Settanni, Marta Annunziata, Davide Gallo & Ezio Ghigo
Dept. Internal Medicine, Div. Endcocrinology and Metabolism, University of Turin, Turin, Italy.

The hypothalamic hormone growth hormone-releasing hormone (GHRH), has been shown to function via its receptor splice variants as an autocrine/paracrine growth factor in normal and malignant cell lines and tissues, besides positively regulating growth hormone (GH) synthesis and secretion from the pituitary. Moreover, GHRH antagonists are known to suppress the proliferation of a wide variety of cancer cells through mechanisms yet to be fully elucidated. Aim of this study was to investigate the effect of GHRH on cell death and apoptosis induced by either serum deprivation or by the β adrenergic agonist isoproterenol (ISO) in rat H9c2 cardiomyocytes and in isolated adult rat cardiac myocytes. H9c2 cells and cardiac myocytes were cultured in serum-deprived medium for 48 h in the presence or absence of either ISO (100 μM) or GHRH (0.5 μM). RT-PCR analysis revealed the presence of GHRH receptor (GHRH-R) mRNA in both H9c2 cells and rat cardiac myocytes. GHRH (0.5 μM) significantly counteracted serum starvation- and ISO-induced cell death and apoptosis in both cell models. Further, either GHRH or isoproterenol induced ERK1/2 phosphorylation, whereas only GHRH activated Akt survival signaling pathway. Interestingly, both GHRH and ISO induced cAMP increase and phosphorylation of its down-stream transcription factor cyclic AMP response element-binding protein (CREB) in H9c2 cells. Finally, the GHRH-R antagonist JV-1-36 completely abolished the survival effects of GHRH in H9c2 cells, under both serum starvation- and ISO-induced cell death and apoptosis.

These results indicate that GHRH is a survival factor for cardiac myocytes. Moreover, they suggest that this molecule may play a role in the prevention of cardiac cell loss in pathological conditions that ultimately lead to the development of heart failure.

OC6.2

Testosterone replacement attenuates fatty streak formation and improves the HDLC profile in the Tfm mouse: an effect which is independent of the classical androgen receptor

Joanne E Nettleship[1], Richard D Jones[1], Kevin S Channer[2] & Hugh T Jones[1]
[1]Hormone and Vascular Biology Group, Academic Unit of Diabetes, Endocrinology & Metabolism, Division of Genomic Medicine, University of Sheffield Medical School, Beech Hill Road, Sheffield, S10 2RX, United Kingdom; [2]Department of Cardiology, Royal Hallamshire Hospital, Sheffield Teaching Hospitals, NHS Trust, Glossop Road, Sheffield, S10 2JF, United Kingdom.

Research indicates that low testosterone is associated with CAD in men. Evidence suggests that men with hypotestosteronemia and concomitant CAD may benefit from physiological testosterone replacement therapy (PTRT). The mechanism by which testosterone produces these cardio-protective effects and the role of the androgen receptor remains largely unknown. The aim of this study was to determine whether testosterone modulates atheroma formation via its classical signalling pathway, via conversion to 17β-estradiol or via an alternative-signalling pathway. Group 1: 8-week-old Tfm (exhibiting a dysfunctional androgen receptor and testosterone deficiency) and control mice were castrated or sham-operated. Group 2: 9-week-old Tfm and controls were administered either placebo, PTRT, PTRT in conjunction with ERα-antagonist or Anastrazole. At 10-weeks both groups were administered a cholesterol-enriched-diet. Mice were sacrificed at 28-weeks. Sections through the aortic sinus were stained using oil-red-O, and lipid-stained areas quantified via digital analysis, and expressed as percentage of medial area. Total cholesterol, HDLC, testosterone and 17β-estradiol were quantified via ELISA. Low endogenous testosterone was associated with fatty-streak formation following feeding on cholesterol-enriched-diet. PTRT prevented aortic fatty streak formation in the Tfm mouse, and increased levels of HDLC. Fatty-streak formation was less marked in PTRT-treated mice, in conjunction with ERα-antagonist or Anastrazole, although this was still significantly lower than that of placebo-treated Tfm mice. Improvement in HDLC was completely attenuated by co-treatment with these agents. PTRT in the Tfm mouse is associated with a reduction in aortic fatty-streak formation. The majority of this action is due to a direct non-genomic action of testosterone, with a component of the response being medicated via conversion to 17β-estradiol and subsequent activation of ERα. The beneficial effect of PTRT upon HDLC appears to be solely mediated by conversion of testosterone into 17β-estradiol, via modulation of genomic ERα-dependent pathways.

OC6.3

Plasma brain natriuretic peptide (BNP) levels predict acute right ventricular dysfunction in pulmonary embolism – prospective study on 70 patients

Alina Mihaela Pascu[1], Mariana Rădoi[2] & Mihail Coculescu[3]
[1]"Transilvania" University, Faculty of Medicine, Department of Patho-physiology, Cardiology Clinic, Brasov, Romania; [2]"Transilvania" University, Faculty of Medicine, Department of Internal Medicine, Cardiology Clinic, Brasov, Romania; [3]"Carol Davila" University of Medicine and Pharmacy, Department of Endocrinology, "C.I. Parhon" Institute of Endocrinology, Bucuresti, Romania.

Background
Acute right ventricular dysfunction (RVD) on echocardiography (ECHO) is critical for risk stratification in pulmonary embolism (PE). Plasma BNP, a consecrated marker of left ventricular dysfunction, could represent a valuable biomarker of RVD in PE.
Aim and objective
Assessment of plasma BNP levels in patients with PE in relationship with right ventricular (RV) function evaluated by ECHO.
Methods
Prospective study of 70 patients with confirmed PE, 42 men (60%), mean age 52.5 ± 8.8. Plasma BNP levels were measured on admission using a quantitative fluorescence immunoassay (Triage BNP). ECHO evaluation of the RV function was performed in the first hour after admission. Study protocol was approved by local Ethical Committee. Patients were divided in two groups: group 1 – with acute RVD on ECHO, $n = 24$ patients (34.3%); group 2 – without acute RVD on ECHO, $n = 46$ patients (65.7%).
Statistics
SPSS 14.0; MedCalc 8.1.
Results
Plasma BNP levels were significantly higher in patients with acute RVD on ECHO (group 1), median value (25th, 75th percentiles) = 79.75 (45.77, 329.75) pg/mL vs. 7.85 (6.22, 16.07) pg/mL in patients without acute RVD on ECHO (group 2), $P < 0.0001$. BNP proved good in discriminating between patients with and without acute RVD – area under the receiver operating characteristic curve = 0.86 (95% Confidence Interval C.I. 0.77–0.94), $P < 0.0001$. The cut-off level of plasma BNP = 50 pg/mL had the best sensitivity = 0.84 (95% C.I. 0.79–0.88) and specificity = 0.80 (95% C.I. 0.75–0.85) in the same time in identifying acute RVD. Plasma BNP correlated significantly with RV end-diastolic diameter ($R = 0.74$, $P < 0.0001$), RV systolic pressure ($R = 0.77$, $P < 0.0001$). Logistic regression analysis showed that plasma BN $P > 50$ pg/mL was the best acute RVD predictor, odds ratio 21.0 (95% C.I. 5.5–79.5).
Conclusions
Plasma BNP higher than a cut-off level of 50 pg/mL could predict acute right ventricular dysfunction in patients with pulmonary embolism with a good sensitivity and specificity.

OC6.4

Carotid IMT and stiffness, aortic stiffness and pulse pressure: association with hormone therapy in postmenopausal women: baseline findings from the CASHMERE trial

T Simon[1], P Boutouyrie[2], S Christin-Maitre[1], A Gompel[3], R Joanides[4], A Kearny-Schwartz[5], Ch Kuntz[6], S Laurent[2], B Pannier[7], B Pornel[8], HH Struijker-Boudier[9], Ch Thuilliez[4], L Van Bortel[10], F Zannad[5], I Pithois-Merli[11] & P Jaillon[1]
[1]Saint Antoine-UPMC ParisVI university-Hospital, Paris, France; [2]HEGP-Paris V-University Hospital, Paris, France; [3]Hotel Dieu-Paris V-University Hospital, Paris, France; [4]CHU Rouen, Rouen, France; [5]CHU Nancy, Nancy, France; [6]CHU Strasbourg, Strasbourg, France; [7]Service de Cardiologie, Fleury-Mérogis, France; [8]Centre de Ménopause, Bruxelles, Belgium; [9]Cardiovascular Department, Maastricht, Netherlands; [10]Cardiovascular Department, Ghent, Belgium; [11]Pfizer, Paris, France.

Common carotid artery intima media thickness (CCA-IMT), aortic stiffness (carotid-femoral pulse wave velocity-PWV) and central pulse pressure (PP) are early markers of atherosclerosis. The influence of hormonal replacement treatment (HRT) on arterial parameters in menopausal women remains to be investigated.

We used baseline data of 665 menopausal women with hypercholesterolemia, screened for the CASHMERE study, a 12-month double-blind randomized trial comparing the effects of atorvastatin (80 mg/day) versus placebo, ± HRT, on the progression of CCA-IMT. CCA-IMT, PP, PWV were measured by using a high-definition echotracking device (Esaot®), aplanation tonometry (Sphygmocor®), and Complior® respectively. Mean age was 58 ± 6 years with a mean duration of menopause (M) of 8 ± 7 years. Age at M was 50 ± 5 years. Among them, 17% were smokers, 23% had hypertension and 28% were HRTusers.

	CCA-IMT (μm)			Central PP (mmHg)			PWV (m/s)		
Independent determinants	β coef.	R^2 increment	*P*	β coef.	R^2 increment	*P*	β coef.	R^2 increment	*P*
Age at M (5 yrs)	25	2.9	<0.001	3.0	4.0	<0.001	0.4	4.7	<0.001
M duration (5 yrs)	25	4.8	<0.001	3.5	7.2	<0.001	0.6	12.4	<0.001
Current use of HT (yes)	−37	2.3	0.002	−2.7	0.9	0.003	−0.3	0.9	0.01
Mean BP (10 mmHg)		–	–	7.0	32.4	<0.001	0.4	8.8	<0.001
Central PP (10 mmHg)	9	1.3	0.004	–	–	–	–	–	–
Total R^2		13.2			48.3			24.0	

R^2: % of explained variance, β coef: slope of the multivariate correlation.

Conclusions
Duration and age at menopause were associated with thickening and stiffening of large arteries. Current users of HRT had significantly thinner and more distensible arteries than non users.

OC6.5

Effects of ezetimibe and/or simvastatin on LDL receptor protein expression and on LDL receptor and HMG-CoA reductase gene expression in mononuclear blood cells: a randomized trial in healthy men

Ioanna Gouni-Berthold, Heiner Berthold, Helena Gylling, Yon Ko, Jogchum Plat & Wilhelm Krone
Department of Internal Medicine II, University of Cologne, Cologne, Germany; Drug Comission of the German Medical Association, Berlin, Germany; Department of Clinical Nutrition, University of Kuopio, Kuopio, Finland; Medical Poliklink, University of Bonn, Bonn, Germany; Department of Human Biology, University of Maastricht, Maastricht, Netherlands.

Context
Ezetimibe and simvastatin are often used in combination to lower blood lipid levels. The consequences of this combination at the molecular level are unknown.
Objective
To examine their effects on the LDL receptor (LDLR) protein expression and on the LDLR and HMG-CoA reductase gene expression in peripheral blood mononuclear cells (PBMC).
Design, setting and participants
Prospective, randomized, parallel 3-group trial. Twenty-four healthy men (mean age 32 ± 9 years) received a 14-day treatment with either ezetimibe (10 mg/day), or simvastatin (40 mg/day) or their combination. Blood was drawn before and after treatment.
Main outcome measures
LDLR protein expression, and LDLR and HMG-CoA reductase gene expression, lipid levels, non-cholesterol sterols and the ratio of precursor sterols over cholesterol concentrations, a valid marker of cholesterol synthesis and HMG-CoA reductase activity.
Results
LDL-C decreased by $22 \pm 10\%$, $41 \pm 12\%$, and $60 \pm 10\%$ in the ezetimibe, simvastatin and combination groups, respectively (all $P < 0.0001$). The HMG-CoA reductase gene expression increased significantly in the simvastatin ($+33\%$; $P = 0.032$) and combination groups ($+36\%$; $P = 0.0056$) and remained unchanged in the ezetimibe group ($+14\%$; $P = 0.27$). Similarly, the LDLR gene expression increased significantly in the simvastatin ($+72\%$; $P = 0.024$) and combination groups ($+56\%$; $P = 0.0012$), but not in the ezetimibe group ($+14\%$; $P = 0.49$). The LDLR protein expression, however, remained unchanged in all groups.
Conclusions
Unlike simvastatin, the lipid-lowering effects of ezetimibe do not involve an upregulation of the HMG-CoA reductase or LDLR gene expression. The simvastatin-induced upregulation of the LDLR gene expression did not lead to an increase in the LDLR protein. Further studies are necessary to fully clarify the posttranscriptional mechanisms regulating LDLR protein abundance.

OC6.6

The importance of the TAAAA(n) alleles at the SHBG gene promoter for the severity of cardiovascular disease in women

Katerina Saltiki[2], Nectaria Xita[3], Adriana Cimponeriu[1], Ioannis Kanakakis[2], Emily Mantzou[1], Charalambos Doukas[2], Ioannis Georgiou[3] & Maria Alevizaki[1]
[1]Endocrine Unit, Evgenidion Hospital, Athens University School of Medicine, Athens, Greece; [2]Dept Medical Therapeutics, Alexandra Hospital, Athens University School of Medicine, Athens, Greece; [3]Medical Genetics Unit, Dept of Obstetrics and Gynaecology, Medical School, University of Ioannina, Ioannina, Greece.

Objective
Androgen may be detrimental in the development of coronary artery disease (CAD) in women. We investigated possible associations between the (TAAAA)n polymorphism of sex hormone binding globulin (SHBG) gene promoter, which influences transcriptional efficiency of the SHBG gene and the severity of CAD in women.
Methods
One hundred and twenty women (37–82 yrs), undergoing coronary angiography. CAD severity, history of angina, myocardial infarction and reproductive history were recorded and hormonal parameters measured. According to the number of SHBG gene promoter repeats polymorphisms, patients were classified as short (≤ 7), medium length ($=8$) and long repeat (≥ 9) allele groups.
Results
Significant CAD was more prevalent in the group with the long-repeat allele carriers: 75% of the patients with 3 vessels with severe stenosis belonged to the long repeat allele group while only 37% of patients with mild CAD belonged to this group ($P = 0.004$). History of angina and prevalence of hypertriglyceridemia was more frequent in the long repeat allele group ($P < 0.05$). SHBG levels correlated inversely with BMI and waist perimeter ($P < 0.05$).
Conclusions
Longer (TAAAA)n repeats in the SHBG gene promoter are associated with more severe CAD in women undergoing coronary angiography, a finding not previously reported. This association may reflect the life-long tissue exposure to higher free androgens and supports the adverse cardiovascular effect of androgenic exposure in this highly selected group of women.

OC6.7

Evaluation of tolvaptan, an oral vasopressin V2 receptor antagonist, in 'asymptomatic' hyponatremia: effects on sodium concentration and patient reported health outcomes

Peter Gross[1], Joseph Verbalis[2], Guy Decaux[4], John Ouyang[3], Mary Hobart[3], Cesare Orlandi[3] & Frank Czerwiec[3]
[1]Universitätsklinikum Carl Gustav Carus, Dresden, Germany; [2]Georgetown University, Washington, DC, United States; [3]Otsuka Maryland Research Institute, Rockville, MD, United States; [4]Erasme University Hospital, Brussels, Belgium; [5]For the SALT Study Investigators, in Europe, Canada and the, United States.

Background
Hyponatremia ($Na + \leq 134$ mmol/L), the most common electrolyte derangement, is caused by inappropriate vasopressin-mediated water resorption in the kidney. Treating symptomatic hyponatremia is difficult and risky; as difficult as maintaining normal sodium levels. We tested if tolvaptan, an oral vasopressin V2 receptor antagonist, improves hyponatremia and self-reported health outcomes.
Methods
Two multicenter, randomized, double-blind, placebo-controlled trials evaluated tolvaptan in asymptomatic, non-hypovolemic hyponatremia patients. Upon obtaining local Ethics Committee approval and patients' consent, oral placebo ($n = 223$) or tolvaptan ($n = 225$) was given for 30 days. The first single daily dose (15 mg) was monitored in-hospital with optional fluid restriction. Patients were discharged and fluid intake and study drug (30 or 60 mg) were titrated as clinically indicated. Co-primary endpoints were the average daily area under the curve of serum sodium concentration change from baseline to day 4 and 30. Overall SF-12 Physical (PCS) and Mental Component Summary (MCS) score changes from baseline to day 30 were secondary endpoints. A hyponatremia disease-specific survey (HDS) was also tested.
Results
Serum sodium increased more with tolvaptan than placebo over the first 4 days ($P < 0.001$) and the entire 30-days ($P < 0.001$). On stopping tolvaptan therapy, sodium concentrations fell to placebo levels. The day 30 PCS was unchanged, however the MCS was significantly improved in the tolvaptan group ($P = 0.02$). MCS improvements correlated positively with rise in serum sodium ($r = 0.2$, $P = 0.001$). Tolvaptan differed from placebo in the HDS survey in the moderately severe hyponatremia subjects (< 130 mmol/L) in mental concentration, calculation and memory ($P < 0.05$ or better). Side effects associated with tolvaptan included increased thirst, dry mouth, and increased urination.
Conclusions
Tolvaptan, an oral V2 receptor antagonist, effectively increased and maintained serum sodium concentrations in hyponatremic patients. These changes were associated with improved perception of mental/cognitive health.

Reproduction 1 – OC7

OC7.1

Kallmann syndrome: mutations in the genes encoding prokineticin-2 (PROK2) and prokineticin receptor-2 (PROKR2)

Catherine Dodé & Jean-Pierre Hardelin
Institut Cochin, Paris, France.

Kallmann syndrome (KS) combines hypogonadotropic hypogona dism and anosmia. Anosmia is related to the hypoplasia of the olfactory bulbs and tracts. Hypogonadism is due to deficiency in gonadotropin-releasing hormone (GnRH), and probably results from a failure of the embryonic migration of GnRH-synthesizing neurons. This is a genetically heterogeneous disease, which affects 1:8000 males and five times less females. Loss-of-function mutations in *KAL1* and *FGFR1* account for the X-chromosome linked form and an autosomal dominant form of the disease, respectively. *KAL1* encodes anosmin-1, a locally restricted glycoprotein of embryonic extracellular matrices, which is likely to be

involved in FGF-signaling through FGFR1. Nearly 80% of the KS patients, however, do not carry a mutation in either of these genes.

We considered the genes, encoding the PROKR2 and PROK2, most relevant candidates because olfactory bulbs do not develop normally in *prokr2*$^{-/-}$ or in *prok2*$^{-/-}$ mice. *Prokr2*$^{-/-}$ mice have a severe atrophia of the reproductive system related to the absence of GnRH-synthesizing neurons in the hypothalamus. We sought mutations in *PROKR2* and *PROK2* in a cohort of 192 unrelated individuals affected by KS. Ten different *PROKR2* mutations were detected in 14 patients in heterozygous, homozygous, or compound heterozygous state, and heterozygous *PROK2* mutations were found in 4 KS patients. Notably, *PROKR2* and *PROK2* mutations were also present in some clinically unaffected individuals. These results shed new light on the complex genetics of KS.

OC7.2 – ESE Young Investigator Award

Neuropilin-2 and its ligands are involved in the migration of GnRH-secreting neurons

Anna Cariboni[2], Sonja Rakic[1], Jason Hickok[3], William Andrews[1], Shelley Tischkau[3], Roberto Maggi[2] & John Parnavelas[1]
[1]University College London, London, United Kingdom; [2]University of Milan, Milan, Italy; [3]University of Illinois at Urbana-Champaign, Urbana-Champaign, United States.

Reproduction in mammals is centrally regulated by neuroendocrine neurons scattered in the hypothalamus and secreting the decapeptide GnRH (gonadotropin releasing hormone). During development, GnRH-secreting neurons originate in the olfactory placode – at least in rodents – and migrate along olfactory nerves (the vomeronasal and the terminalis) to gain access to the forebrain and reach their final destinations in the hypothalamus. Defects in the migration of these neurons in humans result in infertility. The mechanisms underlying the establishment of the migration route and the movement of GnRH neurons are not very well understood and are thought to involve different classes of molecules. Candidates comprise semaphorins and their receptors (neuropilins) because of their high levels of expression in the developing olfactory system, which is intimately related with the development of the GnRH neurons. Moreover, reproductive problems and defects in the fasciculation of the vomeronasal nerves have been reported in the mutant mice for Neuropilin-2 (Npn-2), one of the class III semaphorins receptors, leading to investigate the role of these molecules in the migration of GnRH neurons. Analysis of newborn Npn-2$^{-/-}$ mice showed a significant reduction in number of GnRH neurons within the brain but an abnormal presence of such neurons stacked in the nasal regions. Expression studies performed on RNA derived from GFP-GnRH FACS-sorted cells showed presence of Npn-1, 2 and their ligands (Sema3A, 3F), suggesting the importance of these molecules in this system. *In vitro* experiments using immortalized GnRH neurons (GN11) showed that semaphorins 3A and 3F inhibit their migration, whereas VEGF, another Npn-2 ligand, reverted this effect, suggesting the possibility that *in vivo* the migration of the GnRH neurons might be influenced by a balance between positive (VEGFs) and negative (semaphorins) cues, acting through common receptors (neuropilins). These findings provide new insights into the molecular mechanisms of the migration of GnRH neurons and propose new candidate genes, likely involved in the pathogenesis of hypogonadotropic hypogonadisms.

OC7.3

Gonadotrophins regulate germ cell survival, not proliferation, in normal adult men

Saleela Ruwanpura, Robert McLchlan & Sarah Meachem
Prince Henry's Institute, Clayton, VIC, Australia; Monash University, Clayton, VIC, Australia.

Men with suppressed gonadotrophins, as induced by androgen-based contraceptive treatment, exhibit a 70% reduction in germ cell numbers (1). The mechanisms by which the germ cell populations are decreased are unknown. This study aimed to quantify the amount of germ cell apoptosis and proliferation and to identify the pathway(s) involved in gonadotrophin-induced germ cell loss in men. Testicular tissues from normal fertile men that received no treatment or testosterone (200 mg i.m. weekly) plus depot medroxyprogesterone acetate (300 mg i.m. once) for 2 or 6 weeks ($n = 5/10$ per group) to suppress gonadotrophins and consequently spermatogenesis were used (1). Apoptosis and proliferation were identified by TUNEL (a DNA fragmentation marker) and PCNA (a cell cycle marker) labelling methods, respectively. Intrinsic and extrinsic apoptotic pathways were identified by co-localisation of TUNEL-labelled germ cells with the pathway-specific proteins: activated caspase (aCaspase) 9 and 8 by confocal microscopy. The proportion of cells labelled and co-labelled by each method was quantified using stereological techniques. By 2 and 6 weeks of gonadotrophin suppression, the proportion of TUNEL-labelled spermatogonia was increased to 354% and 268% of control ($P < 0.001$), respectively. The proportion of TUNEL-labelled spermatocytes was increased (139% and 303% of control, respectively, not significant (NS)), with no TUNEL-labelled spermatids being observed. No difference in the number of PCNA-labelled cells was observed in gonadotrophin-suppressed men compared to control. By 2 and 6 weeks of gonadotrophin suppression, there was a trend that aCaspase 9 activity was increased to 130% of controls (NS), with no changes in aCaspase 8 activities. This study demonstrates for the first time that gonadotrophins act as survival factors for the spermatogonial (and possibly spermatocyte) population, possibly by regulating the intrinsic pathway of apoptosis. Understanding the mechanisms by which germ cells progress may provide important clues in infertile men where germ cells fail to progress due to hormonal pertubations.

(1) McLachlan *et al. Journal of Clinical Endocrinology and Metabolism* 2002 **87**: 546.

OC7.4

Capacitation and acrosome reaction in human ejaculated spermatozoa involve activation of a novel SRC tyrosine kinase

Gabriele Varano, Adriana Lombardi, Gianni Forti, Elisabetta Baldi & Michaela Luconi
University of Florence, Dept. Clinical Physiopathology, Florence, Italy.

Tyrosine phosphorylation of proteins is one of the main processes associated with the development of some specific functions of ejaculated human spermatozoa. Although this process, as well as the identity of the phosphorylated targets, has been well characterized, only few tyrosine kinases (TKs) have been identified so far. Moreover, their roles in regulating sperm functions are still unknown.

In the present work, we report the presence and localization of Src kinase in ejaculated human spermatozoa and investigate the role played by this TK during capacitation. Immunoprecipitation and western blot analysis of protein lysates from human spermatozoa using specific anti-p60src antibodies identified a single band of about 70 kDa molecular weight. Immunofluorescence analysis of fixed and permeabilized sperm localized positivity mainly in the post-acrosomal region of sperm head and midpiece in over 80% of the sperm population. By both immunoprecipitation and immunofluorescence techniques with antibodies recognizing tyrosine phosphorylation of Src at 416 or at 527 position, which identify the active or inactive kinase respectively, we showed an increased phosphorylation in Y416 during sperm capacitation. Blocking Src activity with its inhibitor SU6656 resulted in a significant reduction in tyrosine phosphorylation of sperm proteins, in particular in the 80–115 kDa molecular weight range. Moreover, such inhibitor completely blocked progesterone-induced acrosome reaction and interfered with calcium response to progesterone evaluated in fura-2 loaded sperm. No effects on sperm motility and hyperactivation parameters resulted from incubation of sperm with SU6656. Finally, by the use of TK and PKA inhibitors (erbstatin A and H89, respectively), we demonstrated that Src activation during capacitation is dependent on tyrosine kinase but not on protein kinase A activity. In conclusion we identified a novel Src isoform in human spermatozoa and demonstrated its involvement in capacitation and acrosome reaction.

OC7.5

Estrogens regulate epididymal contractility through RhoA/Rho-kinase signaling

Sandra Filippi[3], Annamaria Morelli[1], Linda Vignozzi[1], Rosa Mancina[1], Sara Mungai[1], Stefano Ambrosini[4], Gabriella Barbara Vannelli[4], Gianni Forti[2] & Mario Maggi[1]
[1]University of Florence, Department of Clinical Physiopathology, Andrology Unit, Florence, Italy; [2]University of Florence, Department of Clinical Physiopathology, Endocrinology Unit, Florence, Italy; [3]University of Florence, Interdepartmental Laboratory of Functional and Cellular Pharmacology of Reproduction, Dept. of Pharmacology and Clinical Physiopathology, Florence, Italy; [4]University of Florence, Department of Anatomy, Histology and Forensic Medicine, Florence, Italy.

Epididymis (epi) is a sex steroid-sensitive duct provided with spontaneous motility, allowing sperm transport. We previously demonstrated that human epi expresses a high abundance of mRNA for ER-alpha and ER-beta. We demonstrated that in epi estrogens up-regulate either oxytocin (OT) responsiveness, acting at the receptor level, and responsiveness to endothelin-1 (ET-1), another well-known stimulator of epididymal motility. However, we did not find any significant change either at gene or protein level in ET-1 and its receptors. Hence, other molecular effectors should

mediate the increased sensitivity to ET-1. In particular we hypothesized that estrogens up-regulate some contractile effectors, such as RhoA/Rho-kinase pathway, downstream to the ET-1 receptors. To investigate the effect of changing endocrine milieu on RhoA/Rho-kinase pathway, we induced hypogonadism (hypo) in rabbits with a single administration of a long-acting GnRH analog, triptorelin, and we replaced weekly hypo rabbits with different sex steroids (Testosterone, T or estradiol valerate, E2). After 8 weeks from GnRH analog administration, T plasma levels were decreased and the relaxant effect of the Rho-kinase inhibitor, Y-27632 on ET-1 pre-contracted epididymal strips, was significantly decreased. T administration restored T plasma levels, but not Y-27632 sensitivity in the epididymal strips. E2 not only completely restored Y-27632 responsiveness but even amplified it, as indicating that the RhoA/Rho-kinase calcium sensitizing pathway is up-regulated by E2. Accordingly, real time RT-PCR studies, western blot and immunohistochemistry analysis indicate that Rho kinase gene and protein was induced by E2 but not by T. To verify whether endogenous estradiol is involved in the regulation of Y-27632 responsiveness, we treated intact rabbits with an aromatase inhibitor, letrozole. Blocking aromatase activity abolished Y-27632 responsiveness in epi. In conclusion, our results support the hypothesis that epi is a male target for E2, which regulates its motility tuning up contractile hormones and local peptides responsiveness by increasing RhoA/Rho-kinase signalling and therefore calcium sensitivity.

OC7.6

Serum anti-Müllerian hormone levels in men with normo- and oligozoospermia

Frank Tüttelmann[1], Nina Dykstra[1], Axel PN Themmen[2], Jenny Visser[2], Eberhard Nieschlag[1] & Manuela Simoni[1]
[1]Institute of Reproductive Medicine of the University, Münster, Germany; [2]Department of Internal Medicine, Erasmus MC, Rotterdam, Netherlands.

Objective
Anti-Müllerian hormone (AMH) has recently been evaluated as a marker for follicle reserve and as a new marker for ovarian function in women. In the male, it is produced in Sertoli cells (SC) in the testis. We evaluated serum levels of AMH as a marker of SC function and male fertility by comparing normo- and oligozoospermic men.
Materials and methods
Serum levels of AMH were determined by enzyme immunoassay in two groups of men with normal ($n=105$) and reduced ($n=79$) sperm concentration (above or below 20×10^6/ml). These data were retrieved from the institute's database Androbase©.
Results
Significant differences ($P<0.001$) between the two groups were observed in sperm concentration (58.6 ± 37.9 in normo- vs. $9.1\pm10.6\times10^6$/ml in oligozoospermic, mean±S.D.) and count (202.6 ± 147.4 vs. $33.8\pm40.2\times10^6$) as well as in the percentage of progressively motile sperm ($50.6\pm7.0\%$ vs. $40.8\pm13.9\%$), percentage of normal morphology ($12.3\pm5.1\%$ vs. $7.2\pm4.7\%$) and testicular volume (55.8 ± 14.6 ml vs. 44.0 ± 13.8 ml), which were all lower in the oligozoospermic men as expected. Follicle-stimulating hormone (FSH) was higher in this group (4.1 ± 3.0 U/l vs. 7.0 ± 7.2 U/l), AMH showed a trend towards lower levels (7.7 ± 4.8 ng/ml vs. 6.7 ± 4.8 ng/ml, $P=0.06$), but neither LH (3.6 ± 1.9 U/l vs. 4.0 ± 2.2 U/l) nor testosterone (T, 15.2 ± 5.1 nmol/l vs. 14.2 ± 4.3 nmol/l) was different between the groups. We found a significant ($P<0.01$) negative correlation between AMH and FSH ($r=-0.48$), and relatively weak positive correlations with sperm concentration/count ($r=0.44$ and 0.39) and sperm motility ($r=0.35$). By contrast, in the normozoospermic men AMH correlated only very weakly with T and free T ($P<0.05$, $r=0.21$ and 0.22) but with no other hormone or semen parameters.
Conclusions
In contrast to normozoospermic men, AMH correlates with FSH and sperm parameters in oligozoospermic men and might serve as a new marker for reduced SC function.

OC7.7

Use of atorvastatin, but not simvastatin in men with Type 2 diabetes is associated with lower total testosterone levels with no effect on bioavailable or free testosterone

Roger D Stanworth[1], Dheeraj Kapoor[2], Kevin S Channer[3] & T Hugh Jones[4]
[1]Academic Unit of Endocrinology, University of Sheffield, Sheffield, United Kingdom; [2]Department of Diabetes and Endocrinology, Barnsley Hospital NHS Foundation Trust, Barnsley, United Kingdom; [3]Faculty for Health and Wellbeing, Sheffield Hallam University, Sheffield, United Kingdom; [4]Department of Cardiology, Sheffield Teaching Hospitals NHS Foundation Trust, Sheffield, United Kingdom.

There is a high prevalence of low testosterone levels in men with type 2 diabetes (DM2) and low testosterone predates the onset of DM2. Testosterone replacement therapy for hypogonadal men with DM2 improves insulin sensitivity and glycaemic control as well as reducing central obesity. This may lead to an increase in biochemical assessment of hypogonadism in men with DM2. Androgens and other steroid hormones are produced from cholesterol and it has been postulated that treatment with HMG-Co-enzyme A reductase inhibitors (statins) could decrease testosterone levels by reducing the availability of cholesterol and/or inhibiting steroidogenesis. Low testosterone levels in men with DM2, and the widespread use of statins in DM2 mean that any such effect would be particularly important in this group.

We compared androgen status with statin use in a group of 355 Caucasian men with DM2. Data was collected in year 2002–2003. In our group, 168 patients were treated with statins (mainly simvastatin and atorvastatin) and 187 men were untreated. There were no significant differences between treated and untreated men in terms of glycaemic control, blood pressure or obesity. Statin use was associated with lower total testosterone (TT) ($P=0.009$) and SHBG ($P=0.005$) levels but bioavailable (BioT) and calculated free testosterone (cFT) were not significantly reduced. ADAM hypogonadal symptom score was not affected.

Atorvastatin was associated with reduced TT ($P=0.006$) and SHBG ($P=0.005$) compared with no treatment and there was an apparent dose response effect with the lowest levels of testosterone seen in men treated with higher doses of atorvastatin. Simvastatin did not cause a significant reduction in testosterone or SHBG levels. Our study illustrates the importance of using measured or calculated bioavailable or free testosterone in the assessment of hypogonadism in men with DM2 treated with statins, particularly atorvastatin.

Neuroendocrinology clinical – OC8

OC8.1

Growth hormone response during OGTT: the impact of assay method, gender and BMI on the estimation of reference values in patients with acromegaly and in healthy controls

Ayman M Arafat[1], Mathias Möhlig[1], Martin O Weickert[1], Frank H Perschel[2], Johannes Purschwitz[1], Joachim Spranger[1], Christian J Strasburger[3], Christof Schöfl[1] & Andreas FH Pfeiffer[1]
[1]Department of Endocrinology, Diabetes and Nutrition, Charité-University Medicine Berlin, Campus Benjamin Franklin and the German Institute of Human Nutrition, Berlin, Germany; [2]Department of Clinical Chemistry and Pathobiochemistry, Charité-University Medicine Berlin, Campus Benjamin Franklin, Berlin, Germany; [3]Department of Clinical Endocrinology, Charité-University Medicine Berlin, Campus Mitte, Berlin, Germany.

Objectives
Besides the measurement of IGF-1, GH suppression during OGTT to assess the biochemical status in acromegaly is recommended. However, as a consequence of the development of highly sensitive and specific GH assays a critical re-evaluation of the criteria for the diagnosis and follow-up management of acromegaly is mandatory. The aim of our study was to evaluate the between-method discrepancies in GH determinations by different immunoassays considering further confounders like age, gender, and BMI.
Methods
GH was measured during a 75-g OGTT in 10 controlled and 22 uncontrolled acromegalics (12 men; age 31–62 years; BMI 21–30 kg/m^2) and in 213 apparently healthy subjects (66 men; age 20–76 years; BMI 19–62 kg/m^2) using 3 different assays (DPC Immulite 2000, Nichols and DSL-10-19100) that are calibrated against recommended standard (IS 98/574). Ethical Committee approval was obtained.
Results
There was a strong correlation between all assays ($r=0.72$–0.994, $P<0.0001$). However, the results obtained with DPC were, on average, 2.4-fold higher than those obtained with Nichols and 11-fold higher than those obtained with DSL. GH-nadir in controlled acromegalics was 0.98 ± 0.26 μg/l (DPC) and 0.5 ± 0.15 μg/l (Nichols), whereas in those with an active disease was 7.98 ± 1.7 and 4.5 ± 1.2, respectively. In controls, GH-nadir was 0.13 ± 0.01 μg/l (DPC), 0.06 ± 0.01 μg/l (Nichols) and 0.018 ± 0.004 μg/l (DSL). Both basal and nadir-GH were significantly higher in females than in males (DPC: 2.2 ± 0.28 vs. 0.73 ± 0.15 μg/L and 0.16 ± 0.013 vs. 0.08 ± 0.01 μg/L, $P<0.001$, respectively). Age, BMI and waist/hip ratio correlated negatively with both basal and nadir-GH ($r=-0.2$, -0.32 and -0.48, $P<0.01$). In multiple regression analysis age, BMI and waist/hip ratio were independent predictors for both the basal and the nadir-GH (β-values ranging from -0.2 to -0.3 and -0.14 to -0.3, respectively).
Conclusions
Post-glucose GH-nadir values are assay-, gender-, age- and BMI-specific indicating the need of individual cut-off limits for each assay.

OC8.2 – ESE Young Investigator Award

Effect of GH receptor antagonist pegvisomant on cardiovascular risk and atherosclerosis in acromegalic patients resistant to somatostatin analogues

Maria Cristina De Martino[1], Renata S Auriemma[1], Gregorio Brevetti[2], Mariano Galdiero[1], Monica De Leo[1], Gaetano Lombardi[1], Annamaria Colao[1] & Rosario Pivonello[1]
[1]Departments of Molecular and Clinical Endocrinology and Oncology, Federico II University, Naples, Italy; [2]Department of Internal Medicine, Division of Cardiovascular and Immunological Sciences, Federico II University, Naples, Italy.

Acromegaly is known to be associated to an increased cardiovascular risk, due to the increased prevalence of glucose intolerance and dyslipidemia and pre-atherosclerotic lesions. The aim of this study was to evaluate the effect of treatment with the GH receptor antagonist pegvisomant on cardiovascular risk and atherosclerosis in patients with acromegaly resistant to somatostatin analogues. Twelve patients (4 m, 8 f, 28–58 yrs) and 24 sex-, age- and BMI-matched controls entered the study. The patients were evaluated before and after 18 months of treatment with pegvisomant at the dose of 10–40 mg/day. In all patients and controls, serum total, LDL and HDL cholesterol, triglycerides, glucose, insulin and fibrinogen levels, total/HDL cholesterol ratio and HOMA index, as well as common carotid intima-media thickness (IMT) were measured and correlated with serum GH and IGF-I levels. At baseline, increased GH and IGF-I levels were confirmed in all patients. HDL-cholesterol were significantly lower ($P<0.05$) whereas total/HDL-cholesterol ratio ($P<0.001$), glucose levels ($P<0.05$), HOMA index ($P<0.001$) and fibrinogen levels ($P<0.001$) were significantly higher in patients than controls. Moreover, maximal IMT were significantly higher in patients than in controls (1.13 ± 0.55 *vs* 0.69 ± 0.1 mm; $P<0.001$). At 18-month follow-up, serum IGF-I levels were normalized in 9 (75%) patients and significantly reduced in the remaining patients. Both serum glucose levels (5.62 ± 1.33 *vs* 4.86 ± 0.73; $P<0.05$) and HOMA index (3.31 ± 2.24 *vs* 1.10 ± 0.22; $P<0.05$) were significantly decreased after treatment. A trend to a decrease in maximal IMT (1.13 ± 0.55 *vs* 0.96 ± 0.16 mm) was also found after 18 months of treatment with pegvisomant. A significant correlation was found between the changes in serum IGF-I levels and maximal IMT ($P<0.05$). The results of the current study demonstrated that the treatment with pegvisomant is able to improve the cardiovascular risk, especially through the improvement of glucose tolerance, and prevent the progression of atherosclerosis in patients with acromegaly resistant to somatostatin analogues.

OC8.3

Pituitary imaging abnormalities in patients with and without hypopituitarism after traumatic brain injury

Harald J. Schneider[1], Chiara G. Croce[2], Ginevra Corneli[2], Ezio Ghigo[2], Günter K. Stalla[1] & Gianluca Aimaretti[2]
[1]Internal Medicine/Endocrinology and Clinical Chemistry, Max Planck Institute of Psychiatry, Munich, Germany; [2]Division of Endocrinology and Metabolism, Department of Internal Medicine, Turin, Italy.

Recent evidence shows that patients with traumatic brain injury (TBI) are at substantial risk of hypopituitarism. However, the pathomechanisms are still not completely understood. Little is known about the association of morphological changes in the sella region with pituitary function in TBI. In this study, we assessed morphological abormalities of the sella region in patients with TBI and their relation to endocrine function.

We have studied MR or CT scans of 22 patients with TBI (17 men, 5 women, age [mean +/− SD] 43.5+/− 10.6 years). Of these, 15 patients had some degree of hypopituitarism.

We found abnormalities of the sella region in 80% of the patients with hypopituitarism and 29% of those without hypopituitarism ($P=0.03$). The most common abnormality was loss of volume or empty sella, followed by inhomogeneities, perfusion deficits, and lack of neurohypophyseal signal.

This is the first study to investigate the association of morphological alteration and pituitary function in TBI. Our results indicate that pituitary imaging abnormalities are more common in TBI patients with than without hypopituitarism. Possibly, necrosis and/or hemorrhage play a potential role in posttraumatic hypopituitarism.

OC8.4

Idiopathic central hypothyroidism: report of a human natural model of congenital TRH receptor (TRHR) absence

Marco Bonomi[1], Marta Busnelli[1], Alba Pilotta[2], Elena Prandi[2], Mario Maggi[3], Fabio Buzi[2], Paolo Beck-Peccoz[1] & Luca Persani[1]
[1]Dept. of Medical Sciences, University of Milan; Istituto Auxologico Italiano IRCCS and Fondazione Ospedale Policlinico IRCCS, Milan, Italy; [2]Growth and Development Unit, Children's Hospital, Brescia, Italy; [3]Dept. of Physiopathology, University of Florence, Florence, Italy.

Central Hypothyroidism (CeH) is a rare thyroid hormone production defect due to an insufficient stimulation of a normal thyroid gland. Candidate genes for isolated CeH include TSHβ (several cases reported) and TRHR (only one case reported so far). Here, we report the clinical and genetic studies in 2 males and 3 females affected with isolated CeH with normal/low TSH levels (0.05–0.95 mU/L) and low FT4 levels (3.6–4.6 pM). None of the patients was detected at neonatal screening, but came to medical attention during childhood or even adulthood (3–42 years). MRI alterations were detected only in one case (empty sella). Ultrasound showed hypoplastic/normal thyroids. None of the patients presented thyroid autoimmunity. In 3 subjects, TRH test showed absent TSH but normal PRL responses but TSHβ gene analysis was negative. The fourth patient presented CeH associated with severe obesity and type 2 diabetes mellitus and a normal TSH response to TRH. No mutations were identified in TRH as well as in Leptin and LeptinR genes. The last case presented with growth delay at 11 years. Absent TSH/PRL responses after TRH stimulation suggested TRH resistance. We identified a C to T homozygous nonsense mutation in TRHR gene resulting in a premature stop codon (R17X) and the production of a truncated receptor lacking the 7 transmembrane domains. This is the 2nd patient with TRHR mutations and represents a natural model of TRHR congenital absence associated with CeH and absent/poor neonatal manifestations. Since TRH is considered to play an essential role in postnatal adaptation to extrauterine life and maturation of thyroid axis, our findings may challenge this view or uncover the possible existence of other TRHR isoforms also in humans. The lack of mutations in 4/5 cases suggests the existence of still unknown candidate genes for CeH.

OC8.5

Inoperable pituitary tumours treated with ^{90}Y-DOTA-TATE – initial results

Grzegorz Kaminski[1], Norbert Szalus[1], Grzegorz Zielinski[1], Zbigniew Podgajny[1], Wojciech Zgliczynski[2], Anna Kasperlik-Zaluska[2], Joanna Cyperling-Kaminska[3] & Eugeniusz Dziuk[1]
[1]Military Institute of Health Services, Warsaw, Poland; [2]Medical Center of Postgraduate Education, Warsaw, Poland; [3]LIM Medical Center, Warsaw, Poland.

Introduction
The patients with inoperable hormone - secreting pituitary tumours are treated with cold somatostatin analogues, but it is not always effective. DOTA-TATE preparation is a somatostatin analogue coupled with β (−) emitter ^{90} Y. The efficacy of the treatment is based on excessive expression of somatostatin receptors (SSTR) in these tumours.
The aim of the study
To assess the feasibility of treatment of pituitary tumours with ^{90}Y-DOTA-TATE preparation.
Material and methods
^{90}Y-DOTA-TATE preparation was used in 4 patients with inoperable tumour: 3 patients with acromegaly and 1 with the Nelson's syndrome. The presence of SSTR was confirmed in scintigraphy with $^{99\,m}$Tc-HYNIC-TATE preparation earlier. Both radiopharmaceuticals are produced by POLATOM – Swierk/Poland. In 2 pts with acromegaly the dose was repeated twice. 1 pt with acromegaly and 1 pt with Nelson's syndrome were treated with the ^{90}Y-DOTA-TATE four times (3.7 GBq per dose). The renal protection was provided by 10 hours infusion of 1000 ml 10% amino acids preparation with max. speed of 120 ml/h. The local Ethical Committee approval has been obtained before the study.
Results
There were no serious adverse events observed after ^{90}Y-DOTA-TATE treatment. An insignificant, transient decrease of thrombocytes and lymphocytes was noted. In patients with the Nelson's syndrome the ACTH serum concentration decreased by 31%, in patients with acromegaly GH serum concentration decreased by about 30–40%, and clinical improvement was obtained.
Conclusions
^{90}Y-DOTA-TATE radiopharmaceutic is feasible and promising in treatment of inoperable pituitary tumours.

OC8.6

Improved glucocorticoid replacement therapy by a novel oral hydrocortisone modified-release tablet

Stanko Skrtic[2], Hans Lennernäs[3], Anna Nilsson[1], Ragnhildur Bergthorsdottir[1], Thomas Hedner[2] & Gudmundur Johannsson[1]
[1]Dept of Endocrinology, Sahlgrenska University Hospital, Goteborg, Sweden; [2]Dept of Clinical Pharmacology, Sahlgrenska Academy, Goteborg University, Goteborg, Sweden; [3]Dept of Biopharmacy and Pharmacokinetics, Uppsala University, Uppsala, Sweden.

Background
Mortality rate in patients with primary and secondary adrenal insufficiency is increased. A contributing factor could be the dose and the pattern of glucocorticoid replacement therapy. Hydrocortisone administered twice or thrice daily produces high serum peaks and low trough values in-between. A novel, once daily, hydrocortisone modified release tablet with combined immediate and extended release characteristics was developed.
Purpose
The aim was to determine single-dose pharmacokinetics and dose-proportionality of oral 5 and 20 mg modified-release hydrocortisone tablets in healthy volunteers.
Material and methods
Studies were performed with betamethasone suppression. The two first study days were blinded and randomized between the 5 and 20 mg tablet in a fasting state and the third was open with the 20 mg tablet taken 30 min after a high calorie, high fat meal. The plasma samples were assayed using a validated (GLP) LC-MS/MS method. The plasma pharmacokinetic variables were calculated using non-compartmental data analysis.
Results and discussion
The time to reach a clinically significant serum concentration of cortisol (>200 nmol/L) was within 25 minutes and a peak of 400–450 was obtained within 50 min after the 20 mg tablet. Serum cortisol levels remained above 200 nmol/L for around 6 h thereafter whereas all serum concentrations 18–24 h after intake were below 50 nmol/L. In the fed state the time to 200 nmol/L was delayed by 45–50 minutes. The 5 mg and 20 mg tablets produced almost superimposable profiles.
Conclusion
This modified-release tablet allows for a once-daily administration producing a near physiological serum cortisol profile. The time to clinically significant cortisol concentrations was short and after the peak level a slow decline occurred throughout the day allowing for a cortisol-free interval 18–24 hour after intake. This new tablet for once-daily administration may help to improve compliance and outcome in patients with adrenal insufficiency.

OC8.7

A single intravenous bolus of dexamethasone for the diagnosis of Cushing's syndrome

Gabriel Munter[1], Moriah Kirshner[2], Rosler Ariel[2], Shilo Shmuel[1], Leibowitz Gil[2] & Glaser Benjamin[2]
[1]Shaare Zedek Medical Center, Jerusalem, Israel; [2]Endocrinology Service, Hadassah-Hebrew University Medical Center, Jerusalem, Israel.

The diagnosis of Cushing's syndrome (CS) is based primarily on diagnostic tests evaluating the cortisol response to dexamethasone suppression. Tests based on oral administration of dexamethasone may be compromised by poor compliance. We evaluated the diagnostic accuracy of a novel intravenous dexamethasone suppression test (IDST). The test is performed by intravenous (iv) bolus injection of 8 mg dexamethasone, with blood cortisol determinations made before injection, then hourly during the first 6 h and finally at 24 h. ACTH is measured prior to dexamethasone injection and at 6 and 24 h following injection. We performed a retrospective analysis of patients studied for suspected CS in Hadassah, between 1994–2004. The study included 101 patients: 54 patients with pituitary CS, 22 with adrenal CS, 4 with ectopic ACTH CS (EAS) and 24 in whom the diagnosis of CS was excluded. Patients without CS showed rapid suppression of cortisol and ACTH that persisted for 24 hours. Patients with pituitary CS showed suppression of cortisol and ACTH levels at 6 hours with subsequent escape at 24 hours. Patients with adrenal CS or with EAS failed to suppress cortisol or ACTH levels. Using 60% suppression of blood cortisol at 24 h as the cutoff for the diagnosis of CS, IDST had 94% sensitivity, 95% specificity and 98% positive predictive value (PPV) for the diagnosis of CS. Similar results were obtained by using a cortisol level of 200 nmol/l at 24 hours as the cutoff for the diagnosis of CS. Adding the criteria of ACTH levels >4 pmol/l at 24 hours, the PPV of the IDST increased to 100%. Conclusions: IDST is a reliable, simple and accurate test for diagnosing hypercortisolism. Measuring cortisol levels before and 24 h after 8 mg i.v. dexamethasone administration is required to adequately diagnose patients with CS. ACTH levels at 24 h may increase the test's PPV. The sensitivity, specificity and PPV of the IDST are equal or higher than those reported for other commonly used non-invasive tests. Further studies are required to determine if IDST can discriminate effectively between pituitary disease and EAS.

Signal transduction – OC9

OC9.1 – ESE Young Investigator Award

Investigation of the role of MRAP in the functional expression of the melanocortin 2 receptor

Sadani Cooray, Lou Metherell, Mike Cheetham & Adrian Clark
William Harvey Research Institite, London, United Kingdom; Institute of Ophthalmology, London, United Kingdom.

Mutations in the ACTH receptor (Melanocortin 2 receptor/MC2R) are associated with Familial Glucocorticoid Deficiency/FGD. FGD is an autosomal recessive disorder that results from ACTH insensitivity at the adrenal cortex. However, only about 25% of FGD are caused by mutations in the MC2R suggesting the genetically heterogeneous nature of the disease. The transfection-mediated functional expression of the MC2R can only be achieved in cell lines of adrenal origin implying that the receptor may require an adrenal specific accessory factor/factors for functional expression. The causative gene for FGD type 2 (normal MC2R) was identified in our lab. It encoded a novel single transmembrane domain protein of unknown function that we subsequently named MRAP (melanocortin receptor accessory protein). We demonstrated that MRAP assists the MC2R to the cell surface as determined by confocal microscopy on CHO and SKN-SH cells. MRAP was also shown to play a role in the production of a functional MC2R in these cell lines as was indicated by the enhanced cAMP response to ACTH when co-transfected with MC2R and MRAP (Metherell L.A., *et al.*, *Nature Genetics* 2005 **37** 166–170). The knockdown of MRAP expression by transient transfection of MRAP siRNA (small interfering RNA) duplexes in Y1 mouse adrenocortical cells resulted in a reduction in MC2R signalling as determined by the significant decrease in cAMP when stimulated with ACTH. The expression and function of MRAP was restored in the clonal cell lines expressing mouse MRAP shRNAs by the transfection of the human MRAP sequence. Co-immunoprecipitation studies showed an interaction between MRAP and MC2R but not the other four melanocortin receptors. The production of cAMP through MC1R, MC3R, MC4R and MC5R was not enhanced in the presence of MRAP. In summary MRAP was found to be essential for the functional expression of the MC2R.

OC9.2

The human orexin receptor type 2 gene: Alternative promoters determining tissue-specific expression and identification of alternate splice variants and altered translational activities

Jing Chen & Harpal Randeva
University of Warwick, Medical School, Coventry, United Kingdom.

Orexins, acting via their receptors, are involved in the control of feeding, sleep-wakefulness, arousal, neuroendocrine homeostasis and autonomic regulation. However, the 5′ structure and regulation of human orexin type 2 receptor (OX2R) gene remains is not known. We present original findings regarding the 5′ structural organization of the human OX2R gene and identify four OX2R mRNA transcripts that differ in their 5′-untranslated region (UTR). The four transcripts revealed that the three alternative exons arise from alternative splicing. These exon 1 variants, arising from a single OX2R gene, were distributed over a region of 29504 bp and designated as exons 1A, 1B and 1C on the basis of their 5′ to 3′ order. In transfection studies, different transcripts exerted cell-specific effects on mRNA, but consistently reduced protein expression. Tissue-specific expression of these transcripts in human tissues has been demonstrated by RT-PCR. We show those 5′ -flanking regions to exon 1A and exon 2, but not exon 1C, drive alternative promoter activity in HEK-293 and SH-5YSY cells. Using progressive deletion analysis, a proximal promoter region between −456 and −123 (relative to the translation start site) was shown to exhibit the higher activities in HEK-293, SH-5YSY and NT2 cells. One CRE, GATA-2

and Oct-1 motif was identified within this region, which was responsible for the stimulation both by Dibutyryil-cAMP (db cAMP) and phorbol-12-myristate-13-acetate (PMA). Mutational studies demonstrated that these motifs functioned co-operatively to stimulate hOX2R gene transcription. Using the chromatin immunoprecipitation assay, we demonstrated that three motifs bind to the region of hOX2R proximal promoter. These novel data suggest that usage of alternate promoters, 5′-UTR and alternative splicing may contribute regulatory mechanisms for tissue-specific expression of the hOX2R gene.

OC9.3

Orexin-A inhibits glucagon secretion and proglucagon gene expression

Eva Göncz[1], Carsten Grötzinger[1], Marily Theodoropoulou[2], Bertram Wiedenmann[1], Mathias Strowski[1] & Ursula Plöckinger[1]
[1]Charite, Medizinische Klinik mit Schwerpunkt Hepatologie, Gastroenterologie & Interdisziplinäres Stoffwechsel-Centrum/Endokrinologie und Diabetes Mellitus, Campus Virchow-Klinikum, Berlin, Germany; [2]Max Planck Institut für Psychiatrie, München, Germany.

Background and aim
Orexin-A (OXA) increases insulin secretion and inhibits glucagon secretion, suggesting a role in regulating glucose homeostasis. The effects of OXA on pancreatic A-cells on the cellular level have not yet been demonstrated. Aim of our study was therefore to characterise the underlying signal transduction pathways and to study the OXA effects on proglucagon gene transcription.
Methods
The effects of OXA on glucagon secretion were evaluated using an *in situ* perfused rat pancreas model and clonal pancreatic A-cells (InR1-G9). OXR-1 expression in InR1-G9 cells was detected by western blot and immunofluorescence. The effects of OXA on intracellular cyclic AMP, AKT, PDK-1, CREB and EGR-1 were measured by ELISA and western blots, intracellular calcium (Ca^{2+}) by Fura-2. Proglucagon and Foxo1 mRNA levels were quantified by real-time PCR. Foxo1 was silenced using short interfering RNA (siRNA).
Results
Pancreatic A-cells express OX1R. OXA reduced glucagon secretion and proglucagon gene expression. OXA decreased intracellular cyclic AMP and Ca^{2+} concentrations, and increased the phosphorylation of AKT und PDK-1. PI-3 kinase inhibitor blocked the effects of OXA on proglucagon gene expression. OXA reduced the expression and phosphorylation of CREB, and EGR-1. Silencing of Foxo1 had no effects on basal proglucagon gene expression; however the inhibitory effect of OXA on glucagon gene expression was reversed.
Conclusions
We demonstrate for the first time the direct interaction of OXA with pancreatic A-cells and identify cAMP/AKT/PDK-1 and Ca^{2+} as intracellular target molecules for OXA action. We identify transcription factors Foxo1, CREB and EGR-1 as downstream targets for OXA signalling, suggesting a role in mediating the inhibitory effects of OXA on glucagon gene expression. We have now increasing evidence that OXA affects glucagon homeostasis.
Inhibition of glucagon secretion by OXA may have potential implication at lowering hyperglucagonemia frequently encountered in type 2 diabetes.

OC9.4

Signalling and internalisation properties of corticotrophin-releasing hormone (CRH) receptor type 2

Danijela Markovic, Hendrik Lehnert & Dimitris Grammatopoulos
Warwick Medical School, University of Warwick, Coventry, United Kingdom.

The family of urocortins (UCNs) exert important pathophysiological actions in the control of peripheral homeostatic mechanisms, through activation of the type 2-corticotropin releasing hormone receptor (CRH-R2). This G-protein coupled receptor preferentially binds urocortins (UCN, UCNII and UCNIII) than CRH. In most tissues, CRH-R2 activation leads to increased cAMP production. In this study we used HEK293 cells stably overexpressing recombinant CRH-R2β receptors to investigate intracellular events controlling receptor functional activity and their potential link to activation of distinct signalling cascades. Our results showed that agonist-induced CRH-R2β activation is followed by receptor endocytosis. Interestingly, we identified important agonist-specific temporal differences in receptor internalization kinetics; UCNII (a CRH-R2 specific agonist) induced CRH-R2β internalization within 15 min whereas the weaker agonist, CRH, induced CRH-R2β internalization only after 30–45 min of treatment. The role of intracellular molecules involved in GPCR internalization was also investigated. Confocal microscopy studies revealed that β-arrestin and clathrin were recruited to the plasma membrane as early as 2 min following UCNII treatment, and 5 min following CRH treatment. Furthermore, clathrin, but not β-arrestin, co-localize with the internalized receptor in the cytoplasm. We also investigated agonist induced ERK1/2 activation; both UCNII and CRH induced a transient ERK1/2 activation that returned to basal within 30 min. Confocal microscopy studies showed that activated ERK1/2 was uniformly distributed in the cytoplasm and nucleus. Receptor internalization inhibitors (conconavalin A and MDC) as well as expression of a dominant negative β-arrestin (319–418) markedly reduced UCNII and CRH induced ERK1/2 phosphorylation. In conclusion, we provide novel evidence of agonist-specific differences in the internalization characteristics of CRH-R2β which involve recruitment to clathrin coated-pits and β-arrestin to the plasma membrane. Receptor transport to the cytoplasm involves association with clathrin but not β-arrestin. This mechanism appears to be crucial for activation of distinct signaling cascades such as ERK1/2.

OC9.5

The third intracellular loop of human SST5 is crucial for receptor internalization after SS28 stimulation

Erika Peverelli[1], Giovanna Mantovani[1], Andrea G Lania[1], Davide Calebiro[1], Andrea1 Doni[2], Sara Bondioni[1], Paolo Beck-Peccoz[1] & Anna Spada[1]
[1]Endocrine Unit, Dpt. of Medical Sciences, Fondazione Ospedale Maggiore Policlinico Mangiagalli e Regina Elena IRCCS, University of Milan, Milan, Italy; [2]Istituto Clinico Humanitas, Rozzano, Milan, Italy.

Somatostatin (SS) is a widely distributed polypeptide that exerts inhibitory effects on hormone secretion and cell proliferation by interacting with five different receptors (SST1-SST5), that display important differences in tissue distribution, coupling to second messengers, affinity for SS and intracellular trafficking. SS analogues currently used in the treatment of acromegaly inhibit hormone secretion and cell proliferation by binding to SST2 and 5. Beta-arrestins have been implicated in regulating SST internalization but the structural domains mediating this effect are largely unknown. The aim of this study was to characterize the intracellular mechanisms responsible for internalization of human SST5 in the rat pituitary cell line GH3. To this purpose we evaluated by fluorescence microscopy SS28-mediated trafficking of receptor fused to DsRed and beta-arrestin2 fused to GFP. To identify the SST5 structural domains involved in these processes, we evaluated progressive C-terminal truncated proteins, SST5 mutants in which serine or threonine residues within the third cytoplasmic domain were mutated (S242A, T247A) and a naturally occurring R240W mutant in the third loop previously found in one acromegalic patient resistant to somatostatin analogues. We tested the ability of these mutants to associate with beta-arrestin2 and to internalize under agonist stimulation. The truncated mutants are comparable to the wild-type receptor with respect to beta-arrestin recruitment and internalization, whereas third cytoplasmic loop mutants show a significantly reduced internalization and arrestin translocation upon SS28 stimulation. Surprisingly, SST5 with both C-terminal truncation and third loop mutation exhibits normal internalization and beta-arrestin recruitment. Our results indicate SST5 third intracellular loop as an important mediator of beta-arrestin/receptor interaction and receptor internalization, while the role of the C-terminal tail would be to sterically prevent beta-arrestin/receptor interaction in basal conditions. Further elucidation of the molecular signals underlying SST5 intracellular trafficking will provide a better understanding of its function during prolonged agonist treatment.

OC9.6

Somatostatin receptor subtype-2 and -3 – selective agonists inhibit insulin secretion from INS-1 cells through modulation of the R-type Ca^{2+} channel

Stefan Mergler, Vandana Singh, Sylvia Zacharias, Bertram Wiedenmann, Ursula Plöckinger & Mathias Strowski
Hepatology & Gastroenterology, Berlin, Germany.

Objectives
Somatostatin (SST) inhibits insulin secretion from pancreatic B-cells through a reduction of intracellular free calcium ($[Ca^{2+}]_i$). The influx of Ca^{2+} is mediated by voltage-operated Ca^{2+} channels (VOCCs). The role of VOCCs of the R-type ($Ca_V2.3$) in SST-mediated processes is unknown. Therefore, we designed a study to identify SST-receptor subtypes (SSTR) in insulinoma cells (INS-1) and characterize the role of the $Ca_V2.3$ in mediating the effects of SST in these cells.
Methods
The expression of SSTRs in INS-1 cells was detected by RT-PCR. The effects of highly SSTR-selective agonists (SSTR-Ag) on cyclic AMP, insulin secretion and $[Ca^{2+}]_i$ were measured by ELISA, RIA and cell fluorescence imaging. VOCCs were characterized by patch-clamp technique.
Results
INS-1 cells express SSTR2 and SSTR3. SSTR2-selective agonist (SSTR2-Ag) more potently reduced cyclic AMP production than SSTR3-Ag. SSTR2-Ag transiently increased $[Ca^{2+}]_i$ which then rapidly decreased below the basal. Blockade of L- and R-type channels modulated $[Ca^{2+}]_i$ changes in response to SSTR2-Ag treatment. In contrast, SSTR3-Ag lowered $[Ca^{2+}]_i$ after 30 min, only. Blockade of R-type channels of cells treated with SSTR3-Ag less potently influenced $[Ca^{2+}]_i$ than SST or SSTR2-Ag. SST (EC50: 0.04 nM) and SSTR2-Ag (EC50: 0.06 nM) more potently inhibited 20 mM glucose/10 nM exendin-4-stimulated insulin secretion than SSTR3-Ag. The specific R-type channel blocker SNX-482 more potently reduced the inhibition of insulin secretion by SST and SSTR2-Ag as compared to SSTR3-Ag.
Conclusions
INS-1 cells express SSTR2 and SSTR3. SSTR2-Ag more effectively reduces intracellular cyclic AMP-accumulation and insulin secretion than SSTR2-Ag. Blockade of R-type Ca^{2+} channels prevents SSTR2- and SSTR3-induced inhibition of insulin secretion, suggesting that these agonists inhibit insulin secretion through modulation of R-type channel activity.

OC9.7

Seven transmembrane receptors mediated actin cytoskeleton rearrangement: comparison with constitutively active mutants of G protein alpha-subunits

Alenka Hrovat, Robert Frangez, Azra Pogacnik & Milka Vrecl
Veterinary Faculty, University of Ljubljana, Ljubljana, Slovenia.

Reorganization of the actin cytoskeleton could coincide with the activation of several seven transmembrane receptors (7TM receptors) (1). Stimulation of Rho family members leads to rapid remodeling of the actin cytoskeleton and subsequent stress fiber formation and certain 7TM receptors were shown to induce Rho-dependent responses via heterotrimeric G-proteins. $G\alpha_{12}$, $G\alpha_{13}$ as well as $G\alpha_{q/11}$ can link 7TM receptors to RhoA activation. However, some controversy exists over the exact role of $G\alpha_{q/11}$ (2).

The study's aim was to examine whether activation of the $G\alpha_{q/11}$- and $G\alpha_s$-coupled 7TM receptors involves changes in cell morphology and reorganization of the actin cytoskeleton. Actin cytoskeletal organization was also monitored in cells transfected with constitutively active mutants of Gprotein α-subunits and compared with the receptor-mediated redistribution pattern. Autofluorescently-tagged β-actin (pEYFP-actin) was co-expressed together with receptor constructs (neurokinin type 1 receptor (NK1-R) and $\beta_2$adrenergic receptor, β_2-AR)) or constitutively active mutants of $G\alpha_q$, $G\alpha_{12}$, and $G\alpha_s$ in the HEK 293 cells. Evaluation of the autofluorescently-labeled actin filaments was performed with the use of confocal microscope.

The acquired data shows that the $G\alpha_{q/11}$-coupled NK1-R activation caused changes in cell morphology, enhancement in the cortical actin signal and stress fiber formation. After the activation of other $G\alpha_{q/11}$-coupled receptors comparable results were also observed. Furthermore, the presence of over-expressed constitutively active $G\alpha_q$ and $G\alpha_{12}$ also lead to noticeable stress fiber formation. In contrast, neither the β_2-AR activation nor constitutively active mutant of $G\alpha_s$ caused any apparent changes in actin cytoskeleton status in the HEK-293 cells. Based on these findings it could be assumed that only $G\alpha_{q/11}$-coupled receptors activation coincides with the robust changes in the actin cytoskeleton organization.

References
1. Merrifield CJ. *Trends in Cell Biology* 2004 **14** 352–358.
2. Barnes WG, Reiter E, Violin JD, Ren XR, Milligan G, Lefkowitz RJ. *Journal of Biological Chemistry* 2005 **280** 8041–8050

Obesity and metabolism – OC10

OC10.1

The selective neuronal deletion of cannabinoid type 1 receptor is still able to provide resistance to diet-induced obesity

Cristina Cervino[1], Daniela Cota[2], Luigi Bellocchio[1], Giacomo Mancini[3], Beat Lutz[3], Giovanni Marsicano[4], Renato Pasquali[1] & Uberto Pagotto[1]
[1]Endocrine Unit and CRBA Hospital S.Orsola-Malpighi, Bologna, Italy; [2]Psychiatry Department, Cincinnati, United States; [3]Physiological Chemistry Department, Mainz, Germany; [4]Avenir INSERM, Bordeaux, France.

It is well known that cannabinoid type 1 receptor (CB1) antagonist drugs may reduce body weight and improve metabolic profiles in obese animals and humans by a double mechanism: at first, targeting mesolimbic and hypothalamic nuclei and, thereafter, peripheral organs involved in energy storage and expenditure. However, it is still unknown which of these sites of action may have a predominant role in the endocannabinoid effect on energy balance regulation. To solve this question we generated a mouse line in which the CB1 coding region is flanked by two loxP sites ($CB1^{f/f}$). By crossing this with mice that express Cre recombinase under the control of the regulatory sequences of the Ca^{2+}/calmodulin-dependent Kinase IIa gene ($CB1^{CaMKIIaCre}$ mice), we obtained $CB1^{f/f;CaMKIIaCre}$ mice in which CB1 receptor is deleted in all principal neurons of the forebrains, including those at mesolimbic and hypothalamic level modulating the positive incentive to palatable food and the orexigenic signals, respectively. Here we show that adult male $CB1^{CaMKIIaCre}$ (*n*. 15 each group, age 16–21 weeks for each diet) were still statistically significant leaner than the wild type littermates either undergoing standard diet or with high fat diet (40% kcal given by fat). However, when cumulative food intake was investigated, adult male $CB1^{CaMKIIaCre}$ mice did not show any statistically significant difference in caloric intake as compared to wild types with both diets. These data seem to indicate that other neuronal pathways may overcome the lack of the central CB1 orexigenic drive; on the other hand, it may suggest that CB1 may still play a crucial role at cerebral level as a sensor of yet unknown peripheral signals involved in energy homeostasis.

OC10.2

11beta-hydroxysteroid dehydrogenase type 1 (11beta-HSD1) mRNA expression in liver of patients with non-alcoholic steatohepatitis

Sarah Konopelska[1], Tina Kienitz[1], Matthias Pirlich[1], Jürgen Bauditz[1], Paul M. Stewart[2], Herbert Lochs[1], Christian J. Strasburger[1] & Marcus Quinkler[1]
[1]Internal Medicine, Center for Gastroenterology, Hepatology and Endocrinology, Charite Campus Mitte, Charite Universitaetsmedizin Berlin, Berlin, Germany; [2]Division of Medical Sciences, Institute of Biomedical Research, University of Birmingham, Birmingham, United Kingdom.

Background
Non-alcoholic fatty liver disease (NAFLD) is recognized as common liver disorder that represents the hepatic manifestation of the metabolic syndrome including visceral obesity, type 2 diabetes, insulin resistance and hyperlipidemia. Non-alcoholic steatohepatitis (NASH) is the progressive form of liver injury with the risk for progressive fibrosis, cirrhosis and end-stage liver disease. The pathophysiology that leads to NAFLD and NASH is not well understood. We hypothesize that an altered cortisol metabolism in the liver may be a pathogenetic factor. Hepatic 11beta-HSD1 regenerates cortisol from its inactive metabolite cortisone and requires NADPH as cosubstrate, which is supplied by hexose-6-phosphate-dehydrogenase (H6PDH).
Methods
76 patients (29 men, 48 women) underwent liver biopsy due to elevated liver enzymes. We quantified 11beta-HSD1 and H6PDH mRNA expression by real-time PCR with 18S as housekeeping gene using a BioRad iCycler. In addition, anthropometric measurements and analysis of 24 hour excretion rates of glucocorticoids using gas chromatographic-mass spectrometric (GC-MS) analysis were performed.
Results
11beta-HSD1 mRNA expression correlated significantly ($r^2=0.803$; $P<0.001$) with H6PDH mRNA expression. We detected a significant correlation between 11beta-HSD1 mRNA expression and waist-to-hip ratio ($r^2=0.211$; $P<0.05$), but not to urinary (THF+5alphaTHF)/THE ratio, total cortisol metabolite excretion, age or BMI. No gender specific differences were seen in mRNA gene expression.

Discussion
Our data suggest that 11beta-HSD1 gene expression highly depends on H6PDH gene expression. Surprisingly, 11beta-HSD1 gene expression did not correlate with any urinary glucocorticoid ratio showing the limitation of urinary analysis. In our patient's cohort a higher waist-to-hip-ratio (abdominal obesity) was associated with a lower 11beta-HSD1 mRNA expression in the liver.

OC10.3

Selective leptin resistance within the brainstem of histamine deficient mice

Anna Földes, Veronika Márkus & Krisztina Kovács
Institute of Experimental Medicine, Budapest, Hungary.

Histamine is an important anorexic factor that suppresses food intake via hypothalamic H1 receptors and increases energy expenditure by stimulating lipolysis. Mice with targeted deletion of the key enzyme of histamine biosynthesis, histidine decarboxylase (HDC-KO), are unable to synthesize histamine. These animals display a metabolic phenotype with adult onset obesity, selective increase in visceral fat depots, impaired glucose tolerance and hyperleptinemia. To test the possibility that changes in the leptin-induced signal transduction pathways are responsible for leptin resistance in histamine deficient mice, we have analyzed phosphorylation of signal transducer and activator of transcription (STAT-3) a key component of leptin action in target cells. Adult male, wild type and HDC-KO animals were injected ip with leptin and phosphoSTAT-3 (Tyr 705) immunoreactivity was revealed 30 min after injection by conventional avidin-biotin-peroxydase histochemical reaction and the number of phosphoSTAT-3 cell nuclei was counted. Wild type mice display leptin-induced phosphoSTAT-3-ir in the arcuate-, dorsomedial- and ventromedial nuclei in the hypothalamus, in the midbrain as well as in the dorsal vagal complex (DVC) of the brainstem. In histamine deficient mice, the distribution of leptin-responsive neurons and the number of pSTAT-3 ir profiles within the hypothalamus was similar to those seen in wild type animals. In contrast, cells in the dorsal vagal complex of HDC-KO mice display significantly less phospho-STAT-3 -immunoreactivity than the wild type controls in response to exogenous leptin. These data suggest that leptin action in the brainstem, but not in hypothalamus, is specifically impaired in histamine-deficient mice. Defects in leptin signaling in neurons within the DVC may contribute in the pathogenesis of leptin-resistant obesity as well as in the inability of HDC-KO animals to mobilize their energy stores.

OC10.4

Restoration of signalling capabilities in total loss of function MC4R mutations

Harald Brumm[1], Florian Bolze[2], Susann Friedel[3], Anke Hinney[3], Martin Klingenspor[2], Johannes Hebebrand[3], Annette Grüters[1] & Heike Biebermann[1]
[1]Charité Universitätsmedizin Berlin, Inst. Exp. Ped. Endocrinology, Berlin, Germany; [2]Phillipps-Universität, Inst. Biologie/Tierphysiologie, Marburg, Germany; [3]Rheinische Kliniken Essen, Universität Duisburg Essen, Klinik für Psychiatrie und Psychotherapie des Kinder- und Jugendalters, Essen, Germany.

Objectives
The melanocortin 4 receptor (MC4R) belonging to the large superfamily of G-protein coupled receptors plays a crucial role in hypothalamic weight regulation. In approximately 3–5% of investigated obese patients inactivating MC4R mutations are the underlying molecular cause for early onset obesity. Functional characterisation revealed for specific partial loss of function MC4R mutations that restoration of receptor function is possible by usage of highly potent MC4R analogs. The analogue NDP-α-MSH is capable to restore wild type signalling in some cases of partial loss of function. However, for total loss of function receptors this procedure is insufficient.
Methods
To prove functional restoration cell surface expression was determined by cell a surface ELISA approach with N-terminal HA-tagged mutant MC4R. Signalling was determined by cAMP measurement with radioisotope labelled adenine.
Results
In the present study we set out to investigate the restoration of specific total loss of function mutations by usage of bioactive agents. We are able to show that in dependence of the location and the kind of the mutation a functional rescue is possible to different degrees.
Conclusion
This study is the first to show that *in vitro* restoration of signalling properties in total loss of function MC4R is possible.

OC10.5

Mice lacking CRF receptor type 1 (CRFR1) have reduced vulnerability to diet-induced obesity

Yolanda Diz-Chaves[1], Maud Monsaingeon[1], Luc Penicaud[2], Daniel Ricquier[3], Angelo Contarino[1] & Antoine Tabarin[1]
[1]Lab Nutrition et Neurosciences, Bordeaux, France; [2]Lab Neurobiologie et Metabolisme, Toulouse, France; [3]Lab Metabolisme Mitochondrial, Paris, France.

Evidence has accumulated about the involvement of the CRF system in the regulation of energy balance. The effects of CRF are mediated by two receptors: CRFR1 and CRFR2. The role of the CRFR1 in the regulation of energy balance is not well defined. To address this issue, adult male CRFR1 KO mice and WT littermates were given low fat (LFD) or high-fat (HFD) diets for 4 months. Under LFD no differences between genotypes were seen on body weight (BW) and caloric intake. KO mice had lower fat mass ($13.6 \pm 0.6\%$ vs $19.1\% \pm 1.7$; $P < 0.01$) and increased lean mass (26.0 ± 0.4 g vs 23.9 ± 0.6 g, $P < 0.01$). During a HFD, KO mice had similar intake of calories but gained only 10% of the fat mass that the WT mice did, indicating a reduced feeding efficiency. 24-h locomotor activity was similar between genotypes. Plasma FFA and Betahydroxybutyrate levels in KO mice suggested increased fat oxidation and KO mice had a increased expression of UCP 1 in BAT. Since CRFR1 deletion impairs the HPA axis activity, KO mice were given 5 μg/ml of Cort (KO-Cort) or vehicule (KO-Veh) in drinking water. After two weeks on HFD, BW increases in KO-Cort mice and reached that of WT mice after 16 weeks. Cort supplementation decreased biological markers of fat oxidation in KO-cort mice to the levels of WT mice. No difference in muscle expression of enzymes involved in FFA oxidation was found between groups. Conclusion: CRFR1 have constitutively reduced fat mass, increased fat oxidation and BAT thermogenic activity resulting in a reduced vulnerability to diet-induced obesity. The decreased vulnerability to HFD-induced obesity in CRFR1 KO mice seems to depend mainly of their constitutively low corticosterone secretion.

Supported by Grants from ATC Nutrition, FRM and Conseil Régional d'Aquitaine

OC10.6

3-Iodothyronamine (T1AM) is a novel modulator of metabolic rate and glucose homeostasis

David K. Grandy, Katherine L. Suchland, Thomas M. Keck, Feifei Yan, Show-Ling Shyng & Thomas S. Scanlan
Oregon Health & Science University, Portland, Oregon 97239, United States.

3-Iodothyronamine (T1AM) is a novel endogenous derivative of thyroid hormone (TH), recently described by Scanlan *et al.* (*Nat. Med.* **10**: 638, 2004). *In vitro*, T1AM can stimulate the production of cAMP via activation of a heterologously expressed G protein-coupled receptor (GPCR) now referred to as trace amine-associated receptor 1 (TAAR1; Lindemann et al. *Genomics* **85**: 372, 2005). In adult, unanesthetized C57Bl6/J mice, T1AM produces profound and long-lasting anergia, bradycardia, hypophagia, and hypothermia ($-10\,^{\circ}C$ @ $T_{ambi} = 24\,^{\circ}C$). In an effort to better understand these manifestations of T1AM, we evaluated its effect on metabolic rate. In addition, experiments were performed to characterize T1AM's effect on blood sugar and the pancreatic hormones glucagon and insulin. Finally, the effect of T1AM on an *in vitro* cellular model of glucose-stimulated insulin release was investigated. Within minutes of its injection (i.p.) into male mice housed at $T_{amb} = 22\,^{\circ}C$, and prior to the development of hypothermia, T1AM (25 mg/kg) reversibly depressed metabolic rate ~50% of vehicle-injected controls, as measured by oxygen consumption (ml/g/min). Also within minutes, T1AM dose-dependently elevated blood sugar, reaching a maximum of ~320 mg/dL, almost 3 times normal, by 3.5 hrs post injection. By 2 hrs post-injection, T1AM had produced a dose-dependent increase in circulating glucagon (~400 pg/ml) that was nearly twice the vehicle controls. Furthermore, T1AM (50 mg/kg) administered to fasted mice (26 hrs) prior to their receiving a bolus of D-glucose (3 g/kg, i.p.) blocked the sugar's ability to stimulate circulating insulin levels compared to vehicle-treated mice. Finally, *in vitro* studies revealed T1AM could dose-dependently prevent glucose-stimulated insulin release from cultures of rat INS1823/13 insulinoma cells. Taken together, these results support the thesis that T1AM is a rapid-acting novel modulator of metabolism with actions opposite in direction to those of TH. As such, T1AM and its related compounds may signal via one or more GPCRs to fine-tune TH's effects and thereby help the organism efficiently meet its metabolic needs minute-to-minute.

OC10.7

Serum level of retinol binding protein 4 in obese individuals with insulin resistance and with type 2 diabetes mellitus treated by metformin

Mária Tajtáková[1], Zuzana Semanová[1], Gabriela Ivancová[2], Jozefína Petrovicová[3], Viera Donicová[2] & Eva Zemberová[4]
[1]1st Department of Internal Medicine, University of P.I.Šafárik, Košice, Slovakia; [2]Department of Diabetes, Košice, Slovakia; [3]Institute of Medical Informatics, University of P.I. Šafárik, Košice, Slovakia; [4]RIA Laboratory, Košice, Slovakia.

Objective
To reveal whether there are differences in serum level of retinol binding protein 4 (RBP4) in obese individuals with insulin resistance (IR) and without diabetes in comparison to those with 2 type diabetes mellitus (2 DM) treated by metformin and not obese controls.
Methodology
The serum level of retinol binding protein 4 was examined by RIA method in 28 obese individuals with insulin resistance, 11 patients with 2 type diabetes mellitus treated by metformin and 17 controls. The results were compared within groups. RBP4 in the group with IR and in controls was correlated with insulin.
Results
The highest level of RBP4 (561.6 ± 209 ng/ml) was found in obese individuals with IR (IRHOMA 3.9) and the lowest level in patients with 2 DM treated by metformin (391.1 ± 133.5 ng/ml, $P < 0.01$). The controls had significantly lower level of RBP4 in comparison to obese individuals with IR (452.8 ± 104.6 ng/ml $P < 0.05$), however, RBP4 was not significantly higher in comparison to obese individuals with 2 DM treated by metformin (391.1 ± 133.5 ng/ml). RBP4 correlated with insulin ($r = 0.46$, $P < 0.03$).
Conclusions
The increase of RBP4 in obese individuals through a back regulation GLUT4 in adipocytes contributes to the development and worsening of IR. Thus, metformin by influencing the expression of RBP4 in adipocytes can improve the overall insulin sensitivity in obese individuals (also with MS) and slower the manifestation of 2 DM. RBP4 could be considered as a marker of the worsening tolerance of glocose in obese individuals.

Reproductive Endocrinology 2 – OC11

OC11.1

Hypogonadotrophic hypogonadism in mice lacking a functional Kiss-1 gene

Xavier d'Anglemont de Tassigny[1], Mark Carlton[2] & William Colledge[1]
[1]PDN, University of Cambridge, Cambridge, United Kingdom, [2]Paradigm Therapeutics Ltd, Cambridge, United Kingdom.

Activation of the G-protein coupled receptor GPR54 (AXOR12, OT7T175) by peptide ligands (kisspeptins) encoded by the *Kiss-1*gene is central to acquisition of reproductive competency in mammals. Administration of exogenous kisspeptins stimulates GnRH release from hypothalamic neurons in several species including humans. To confirm that kisspeptins are the natural agonist of GPR54 *in vivo* and to determine if these ligands have additional physiological functions, we have generated mice with a targetted disruption of the *Kiss-1*gene. *Kiss-1* null mice are viable but fail to undergo sexual maturation at puberty. Mutant female mice do not progress through the oestrus cycle, have thread-like uteri, small ovaries and do not produce mature Graffian follicles. Mutant males have small testes and spermatogenesis arrests mainly at the early haploid spermatid stage. Both sexes have low circulating gonadotrophin (LH and FSH) and sex steroid (β-estradiol or testosterone) hormone levels. Migration of GnRH neurons into the hypothalamus appears normal with appropriate axonal connections to the median eminence and total GnRH content. The hypothalamic-pituitary axis is functional in these mice as shown by robust LH secretion after peripheral administration of kisspeptin-10. These data provide the first direct proof that kisspeptins are the true physiological ligand for the GPR54 receptor *in vivo* and that loss of *Kiss1* cannot be overcome by compensatory mechanisms.

OC11.2

Leukemia inhibitory factor promotes the chemomigration of immature GnRH neurons

Elena Dozio[1], Massimiliano Ruscica[1], Anna Cariboni[1], Hajime Watanobe[2], Marcella Motta[1], Roberto Maggi[1] & Paolo Magni[1]
[1]University of Milan, Milano, Italy; [2]University of Health and Welfare, Otawara, Japan.

Leukemia inhibitory factor (LIF), a pleiotropic cytokine of the interleukine-6 superfamily, is involved in several functions including the control of reproduction at the embrionic-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 β (LIFRβ) subunit and the gp130 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 LIFRβ and gp130 genes and proteins. Exposure to 100 ng/mL LIF activated the Janus kinases (Jak)-signal transducer and activator of transcription 3 (STAT3), the mitogen-activated protein kinase (MAPK)-extracellular regulated kinase 1/2 (ERK1/2) and the 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 Jak involved in LIF signalling. LIF stimulated chemotaxis at a concentration-dependent manner, with a plateau at 100 ng/mL after both 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 may modulate the development of the reproductive axis by directly influencing the migration of GnRH neurons to the hypothalamus.

OC11.3

Sex steroid and leptin regulation of KISS1/GPR54 system, a new regulator of the neuroendocrine reproductive axis, in human fetal GnRH-secreting neurons

Annamaria Morelli[1], Mirca Marini[2], Rosa Mancina[1], Linda Vignozzi[1], Anna Pezzatini[3], Michaela Luconi[3], Sara Mungai[1], Benedetta Fibbi[1], Sandra Filippi[4], Gabriella Barbara Vannelli[3], Gianni Forti[3] & Mario Maggi[1]
[1]University of Florence, Dept. Clinical Physiopathology, Andrology Unit, Florence, Italy; [2]University of Florence, Anatomy, Histology and Forensic Medicine, Florence, Italy; [3]University of Florence, Dept. Clinical Physiopathology, Endocrinology Unit, Florence, Italy; [4]University of Florence, 2Interdep. Lab of Functional and Cellular Pharmacology of Reproductio, Florence, Italy.

The molecular mechanisms underlying the reawakening of hypothalamic GnRH neurons at puberty remain to be elucidated. Recently, the G protein-coupled receptor 54 (GPR54) and its endogenous ligand kisspeptin, encoded by the KISS1 gene, have been involved. In fact, GPR54 mutations cause idiopathic hypogonadotropic hypogonadism in human and mice. We used the previously characterized primary culture of human fetal olfactory GnRH-secreting neurons, FNC-B4, to study *in vitro* the KISS-1/GPR54 regulation. Kisspeptin and GPR54 were immunolocalized in fetal olfactory mucosa, and in FNC-B4. Using confocal microscopy, co-expression of GnRH and GPR54 or GnRH and kisspeptin was found in fetal olfactory mucosa and FNC-B4. The 24 h exposure to sex steroids regulated both gene (qRT-PCR) and protein (western blot and immunocitochemistry) expression of KISS1/GPR54 in FNC-B4. Increasing doses of 17beta-estradiol (0.01–1 nM) significantly and dose-dependently decreased KISS1/GPR54 mRNA. Conversely, androgens (DHT, 0.01–1 nM) significantly stimulated KISS1/GPR54 mRNA. Immunofluorescence with anti-kisspeptin confirmed that 1 nM 17beta-estradiol significantly reduced, whereas 1 nM DHT significantly increased, the % of kisspeptin-positive FNC-B4 cells. Testosterone treatment showed no effect, but, blocking its aromatization with letrozole, it mimicked DHT stimulatory activity. In addition, 24 h exposure to leptin (1 nM), an adypocyte-derived hormone acting on the hypothalamus to influence puberty, significantly increased KISS1/GPR54 gene and protein expression. Leptin treatment in FNC-B4 significantly increased also the androgen receptor (AR) mRNA, as well as the mRNA of its own receptor (LEPR), which resulted induced also by 1nM DHT. These data suggest a synergistic action between AR and LEPR to finally up-regulate KISS1/GPR54 system, which, in contrast, was inhibited by estrogen. In conclusion, our results revealed for the first time that sex steroids and leptin regulate KISS-1/GPR54 system in human GnRH neurons, providing new insights into the comprehension of those permissive signals for pulsatile GnRH secretion and puberty onset.

OC11.4

EGFR ligands mediate key events of female reproduction: reduced litter size due to impaired fertilization in a transgenic mouse model

Marlon R. Schneider[1], Ana A. Gratao[1], Maik Dahlhoff[1], Fred Sinowatz[2] & Eckhard Wolf[1]
[1]Institute of Molecular Animal Breeding and Biotechnology, University of Munich, Munich, Germany; [2]Institute of Veterinary Anatomy, University of Munich, Munich, Germany.

EGFR ligands, a family of seven related peptide growth factors, are emerging as key factors regulating different aspects of female reproduction including oocyte maturation and ovulation, and implantation. Betacellulin (BTC) is a rather neglected EGFR ligand whose biological activities have been mostly associated with the endocrine pancreas. During the routine breeding of recently established BTC transgenic mouse lines (Schneider et al., Endocrinology 146, 5237–5246, 2005), reduced female fertility became evident. Thus, a systematic study of different aspects of female reproduction was carried out. While puberty onset and estrous cyclicity were not affected in the transgenic animals, controlled matings revealed reduced litter size as the major reproductive deficit of BTC transgenic females (5.3 ± 0.7 *vs.* 9.9 ± 0.3 pups/litter in non-transgenic controls). Embryo implantation (visualized by injection of blue dye) was shown to be delayed. However, the number of embryos implanted or recovered from the uterus was already reduced by about 50% in the transgenic group, indicating that delayed implantation was not the cause of reduced litter size. Collection of oocytes from transgenic and control females mated to non-transgenic males revealed that the number of ovulated oocytes was not different between the groups (10.4 *vs* 10.7, respectively). However, the proportion of fertilized oocytes recovered from transgenic females was significantly reduced (54% *vs.* 81.7%). Next, *in vitro* maturation (IVM) and fertilization (IVF) were carried out to study these aspects more closely. While IVM rate was only slightly affected, the proportion of fertilized oocytes obtained from transgenic females was strongly reduced as compared to the rate observed in oocytes derived from the control group (57.5% *vs* 84.6% cleavage rate). Localization of strong transgene-derived BTC levels in the cumulus and granulosa cells of transgenic follicles supports this observation. In summary, excess of BTC perturbs oocyte maturation and fertilization. Implantation is delayed but appears to have no consequence for the overall reproductive performance of transgenic females.

OC11.5

Integration of the EGF network with early LH signal in preovulatory follicles

Sara Panigone[1], Minnie Hsieh[2], Luca Persani[1] & Marco Conti[2]
[1]Lab Experimental Endocrinology, IRCCS Istituto Auxologico Italiano, University of Milan, Cusano Milanino, MI, Italy; [2]Division of Reproductive Biology, Department of Obstetrics and Gynecology, Stanford, University School of Medicine, Stanford, CA, United States.

Recent studies demonstrate an essential role of the EGF network in propagating the LH signal within ovarian preovulatory follicles. However, the molecular bases for the integration are poorly characterized. Here, we propose that the early LH signal leading to ovulation is amplified through activation of the EGF network.

For this study, preovulatory follicles from euthanized gonadotropin-primed mice were isolated and cultured with or without recombinant LH (rLH) and/or specific inhibitors. Primary granulosa cells were used in additional experiments. Analysis of EGF receptor (EGFR) and MAPK activation was performed by immunoprecipitation, western blot and immunohistochemistry (IHC). An increase in EGFR phosphorylation was detected as early as 30 minutes after LH stimulation. This activation is most likely cAMP dependent and sensitive to AG1478, an EGFR kinase inhibitor, as well as to inhibitors of matrix-metalloproteases (GM6001 and TAPI-1), suggesting the involvement of shedding of EGF-like factors in LH-induced EGFR transactivation. A target of EGFR signaling is the MAPK pathway. In IHC assays, signal for phosphorylated MAPK was observed in mural granulosa cells of preovulatory follicles within 15–30 minutes of hCG stimulation, and in both granulosa and cumulus cells after 1 h. In cultured follicles, LH-induced MAPK activation is partially inhibited by AG1478 and GM6001, indicating that this pathway is regulated in part by the EGF network. Furthermore, treatment of granulosa cells with a combination of neutralizing antibodies against amphiregulin, epiregulin and betacellulin (EGF-like factors described as regulators of ovulation) significantly inhibits EGFR phosphorylation and MAPK activation, supporting a role for these ligands in the LH-induced EGFR signaling in mural granulosa cells.

In conclusion, we provide evidence of early activation of EGF network following LH stimulation, involving rapid shedding of EGF-like ligands and EGFR transactivation. This mechanism partecipates in the rapid amplification and propagation of the LH signal within preovulatory follicles.

OC11.6

Visceral fat amount as predictor for subclinical cardiovascular disease in women with polycystic ovary syndrome

Teresa Cascella[1], Stefano Palomba[2], Ilario De Sio[3], Francesco Manguso[4], Laura Vuolo[1], Francesco Giallauria[5], Alessandra Grieco[1], Gaetano Lombardi[1], Annamaria Colao[1], Domenico Tafuri[6] & Francesco Orio[7]
[1]Department of Molecular & Clinical Endocrinology and Oncology, "Federico II" University of Naples, Naples, Italy; [2]Department of Obstetrics and Gynecology, University "Magna Graecia" of Catanzaro, Catanzaro, Italy; [3]Department of Internal Medicine, Hepatology, and Gastroenterology, Second University of Naples, Naples, Italy; [4]Gastroenterology Unit, "Cardarelli" Hospital, Naples;, Naples, Italy, [5]Department of Clinical Medicine, Cardiovascular and Immunological Sciences, Cardiac Rehabilitation Unit, Institute of Internal Medicine and Metabolic Disease, "Federico II" University of Naples, Naples, Italy; [6]Teaching and Methods of Sportive Activity, "Parthenope" University of Naples, Naples, Italy; [7]Endocrinology, Faculty of Motor Science, "Parthenope" University of Naples and Department of Molecular & Clinical Endocrinology and Oncology, "Federico II" University of Naples, Naples, Italy.

Introduction and aim
Polycystic Ovary Syndrome (PCOS) is a common disorder associated with a wide range of endocrine and metabolic abnormalities. Obesity is present in about 45–50% of PCOS women. Increased cardiovascular risk factors and evidence of subclinical cardiovascular disease (CVD) have been reported in PCOS. The aim of the present study was to evaluate whether visceral fat amount may be considered as predictor for early CVD in PCOS women.
Patients and methods
The procedures used were in accordance with the guidelines of the Helsinki Declaration on human experimentation. The study was approved by the local Ethical Committee. Two-hundred PCOS women and 100 healthy age- and body mass index-matched women were enrolled in this prospective baseline-controlled clinical study. Non-invasive markers of early CVD [carotid intima-media thickness (IMT), brachial arterial flow-mediated dilation (FMD)] and visceral fat amount [using abdominal ultrasonography] were evaluated. Inflammatory biomarkers [C-reactive protein (CRP), fibrinogen, white blood cells (WBC) count, plasminogen activated inhibitor (PAI)-1], hormonal and metabolic parameters were also investigated.
Results
Subjects with PCOS had significantly ($P<0.001$) higher visceral fat compared to healthy women [$31.4+7.3$ *vs.* $28.0+6.1$, mm + SD), respectively] which were directly related to HOMA ($r=0.918$, $P<0.001$), AUC_{INS} ($r=0.879$, $P<0.001$) and WC ($r=0.358$; $P<0.001$). Stepwise linear regression model showed that visceral fat amount was an independent predictor of IMT, FMD and CRP.
Conclusions
The early impairment of endothelial structure and function, the increase of low-grade chronic inflammation and insulin resistance in women with PCOS are associated with increased central fat excess. Visceral fat amount could be an important predictor of subclinical CVD in PCOS.

OC11.7

The current definitions of the metabolic syndrome underestimate the prevalence of nascent metabolic abnormality in adolescents with PCOS

Didier Dewailly, Sophie Catteau-Jonard & Anne-Céline Reyss
Lille University Hospital, Lille, France.

Background
The prevalence of the metabolic syndrome (MS) is notably higher in patients with PCOS than in the general population. Presumably, this prevalence increases as a function of age but the subgroups of patients less than 20 yr. old studied so far are small.
Design
In order to further document this issue, we have selected for this study 498 patients with PCOS aged 12.5–38 yr (Rotterdam definition) and 188 control women aged 15.5–38.5 yr, that have been consecutively included in a database and in whom the required clinical, hormonal and ultrasound data were available. A metabolic score has been calculated according to the ATP-III classification and defined the MS when ≥ 3.
Results
The prevalence of the MS was significantly higher in the PCOS than in the control group (15.2% *vs* 4.8%, $P<0.0001$). It did not differ significantly ($P=0.063$) between the 3 subgroups of patients with PCOS according to age, i.e., 12.8% in patients aged $\leq$ 0 yr ($n=47$), 13.9% in patients aged 21–30 yr ($n=301$) and 18.7% in patients aged 31–40 yr ($n=150$). However, we observed that a metabolic score of 1 or 2 tended to be more frequent in the adolescent group than in the groups of older patients

(cumulated rate 1+2: 66.0% *vs* 51.8% and 48.7%, respectively, $P=0.09$). In the patients having a score=1, a HDL-Cholesterol (HDL-C) <0.5 g/L was found in 57.1% adolescents *vs* 26.3% and 27% patients aged 21–30 and 31–40 yr, respectively ($P=0.068$). In the same group, a waist circumference (WC) >80 cm was found in 35.7% adolescents *vs* 69.7% and 70.3% patients aged 21–30 and 31–40 yr, respectively ($P=0.047$). In patients with a score=2, a HDL-C <0.5 g/L and a WC>80 cm were found in 100% of cases in every subgroup.

Conclusions

By requiring some items that reflect relatively late complications of insulin resistance (hypertriglyceridemia, hypertension, glucose intolerance or diabetes), the current definitions of the MS in adults underestimate the prevalence of nascent metabolic abnormality in adolescents. Our data indicate that a HDL-C <0.5 g/L is the most sensitive marker of such abnormality, while a WC >80 cm seems to be less sensitive than in adults.

Diabetes – OC12

OC12.1

Hypoglycemia and cerebral ATP synthesis in Type 1 Diabetes

Martina Mandl[1], Stefan Zbyn[1], Vladimir Mlynarik[2], Miriam Promintzer[1], Anton Luger[1], Michael Krebs[1], Christian Anderwald[1] & Martin G. Bischof[1]
[1]Division of Endocrinology and Metabolism, Department of Internal Medicine III, Medical University of Vienna, Vienna, Austria; [2]MR Center of Excellence, Medical University of Vienna, Vienna, Austria.

The mechanisms responsible for the progressive failure of hypoglycemia counter-regulation in type-1 diabetes (T1DM) are poorly understood. Alterations of brain energy metabolism could influence glucose sensing by the brain and, thus contribute to hypoglycemia associated autonomic failure. Thus, we measured intraneuronal kinetics of total ATP-synthesis from PCr (k_{for}) in T1DM patients and effects of hypo/hyperglycemia on this brain energy metabolism. Healthy nondiabetic humans (CON; 5 m/1f, BMI=23.5±1.0 kg/m^2, age=25±1 yr, HbA1c=5.1±0.1%), T1DM patients with good ($T1DM_{good}$; 5 m/1f, BMI=25.5±0.4 kg/m^2, age=24±2 yr, HbA1c=6.8±0,1%) and poor ($T1DM_{poor}$; 5 m/1f, BMI=24.9±1.6 kg/m^2, age=25±2 yr, HbA1c=8.9±0.3%) glycemic control were examined before, during and after hyperinsulinemic-(1.5 mU·kg^{-1}·min^{-1})-hypoglycemic-(~50 mg/dl) or –hyperglycemic-(~250 mg/dl)-clamp tests. k_{for} in the occipital lobe was measured by ^{31}P-nuclear-magnetic-resoance spectroscopy (3T) using saturation transfer, and calculated with *McConnell* equations. In $T1DM_{poor}$, k_{for} was increased during hypoglycemia (0.58±0.07 s^{-1}), when compared to CON (0.36±0.03 s^{-1}; $P=0.006$), $T1DM_{good}$ (0.41±0.02 s^{-1}; $P=0.03$), and baseline (0.43±0.05 s^{-1}; $P=0.03$). During post-hypoglycemic recovery, $T1DM_{poor}$ showed higher k_{for} (0.57±0.07 s^{-1}), when compared to CON (0.40±0.05 s^{-1}, $P<0.05$), and $T1DM_{good}$ (0.37±0.01 s^{-1}, $P=0.03$). HbA1c-levels were positively correlated with k_{for} during hypoglycemia ($r=0.47$, $P=0.02$), but not at baseline ($r=0.20$, $P=0.37$) or during recovery ($r=0.39$, $P=0.07$).

Conclusion

^{31}P NMRS with saturation transfer can be used for non-invasively measurement of cerebral ATP-synthesis during hypoglycemia *in vivo*. The positive correlation of HbA1c levels and k_{for} during hypoglycemia hints at an involvement of the CK system in the pathogenesis of hypoglycemia associated autonomic failure.

OC12.2

Uncoupling protein 2 mutations – a new explanation for congenital hyperinsulinism?

Henrik Thybo Christesen[1], Khalid Hussain[3], Lone Svargo[2], Bendt Brock Jacobsen[1] & Klaus Brusgaard[2]
[1]HC Andersen Children's Hospital, Odense, Denmark; [2]Dept. of Clinical Genetics, Odense University Hospital, Odense, Denmark; [3]London Center for Paediatric Endocrinology and Metabolism, Great Ormond Street Hospital, London, United Kingdom.

Background

Congenital Hyperinsulinism (CHI) is genetically unexplained today in up to 50% of the patients with persistent or recurrent disease. The uncoupling protein 2 (UCP2) gene is a candidate gene for medical-responsive CHI, since knock out studies have shown that UCP2 deficiency leads to increased glucose-stimulated insulin secretion.

Patients and methods

In a large series of 142 patients with transient, persistent or recurrent CHI, we examined for mutations using DHPLC and direct sequencing, or cutting with restriction enzyme for specific variations, in the known disease-causing genes *ABCC8* ($n=141$), *KCNJ11* ($n=140$), *Gck* ($n=21$), *GLUD1* ($n=27$), *SCHAD* ($n=10$), and *UCP2* ($n=46$), (number of investigated patients in brackets).

Results

In 53 of all patients (37%), a genetic explanation was found, while 90 patients had no mutations detected. Of these, 46 had persistent or recurrent medical-responsive hyperinsulinaemic hypoglycaemia and available DNA for *UCP2* analysis. No mutations were found in *UCP2*. The well-known polymorphism A55V was seen in 29 patients.

Conclusion

UCP2 mutations are rarely – if ever – found in CHI patients with persistent or recurrent CHI. Other genetic explanations should be considered.

OC12.3

Adhesion molecules two years after gestational diabetes

Thomas Prikoszovich[1], Giovanni Pacini[2], Christine Winzer[1], Anton Luger[1], Oswald Wagner[3] & Alexandra Kautzky-Willer[1]
[1]Medical University Vienna, Department of Internal Medicine III, Division of Endocrinology and Metabolism, Vienna, Austria; [2]Institute of Biomedical Engineering, CNR, Padova, Italy; [3]Medical University Vienna, Department of Medical and Chemical Laboratory Diagnostics, Vienna, Austria.

Objectives

We investigated in women with prior GD (pGD) at risk of diabetes and premature atherosclerosis in comparison to women with normal glucose tolerance during and after pregnancy (C) parameters of inflammation, endothelial dysfunction and glucose tolerance in a follow-up study.

Methods

119 pGD and 41 C underwent an oral glucose tolerance test 3 months, 1 and 2 years after delivery with measurements of plasma concentrations of circulating adhesion molecules (cAMs: VCAM, ICAM-1, ELAM), endothelin, leptin, sCRP, IL-6, fibrinogen, PAI-1 and ADMA. Intima-media-thickness (IMT) of the common carotid artery was measured by ultrasound and insulin sensitivity (S_I) was calculated from insulin-modified FSIGTs at baseline.

Results

At baseline ICAM ($P<0.0001$), VCAM ($P<0.005$), ADMA ($P=0.0005$), sCRP ($P=0.04$) and PAI-1 ($P=0.01$) were higher and S_I ($P=0.001$) was lower in pGD than in C. S_I inversely related to all cAMs ($r=-0.20$; $P<0.02$), sCRP ($r=-0.52$; $P<0.0001$), IL-6 ($r=-0.25$; $P=0.01$), and fibrinogen ($r=-0.22$; $P=0.006$). All cAMs also related to leptin ($r=0.17$; $P<0.04$) and BMI ($r=0.18$; $P<0.03$). IMT was associated with S_I ($r=-0.32$; $P=0.03$), BMI ($r=0.31$; $P=0.02$) and PAI-1($r=0.30$; $P=0.03$). After two years ELAM ($P<0.02$), ADMA ($P<0.0007$), PAI-1 ($P<0.001$), vWF ($P<0.04$), blood pressure ($P<0.001$) decreased, while ICAM-1, VCAM and BMI remained unchanged. Leptin ($P=0.01$), TNFα ($P<0.001$) and endothelin ($P<0.04$) increased compared to baseline. Higher age ($P<0.05$) and BMI ($P<0.0001$), increased levels of ELAM ($P<0.003$), Leptin ($P<0.0005$) and a lower insulin sensitivity (OGIS;$P=0.01$) at baseline characterised those pGD with deterioration of their initial normal glucose tolerance ($n=15$) in comparison to those who retained normal glucose tolerance ($n=65$) within 2 years. Logistic regression revealed BMI (OR[CI]: 1.313[1.03-1.67]) and ELAM (OR[CI]: 1.064[1.01-1.12]) as independent predictors of a deterioration of glucose tolerance.

Conclusion

Women with pGD are characterised by higher plasma ICAM and VCAM relating to insulin-resistance and inflammatory parameters. Moreover the degree of obesity and ELAM at baseline predicted deterioration of glucose tolerance within 2 years after delivery.

OC12.4

Polymorphisms of PSMA6 gene and its adjacent genomic sites and their association with type II diabetes mellitus in the Latvian population

Ilva Poudziunas, Tatjana Sjakste, Martins Kalis, Maris Lagzdins, Valdis Pirags, Nikolajs Sjakste & Jelizaveta Sokolovska
University of Latvia, Riga, Latvia.

Introduction

A possible involvement of proteasomes in the pathogenesis of type II diabetes mellitus has been recently reported. Therefore, association of polymorphism of proteosomal genes with type II diabetes mellitus is of particular interest. In this study, molecular markers of the proteasomal alpha subunit 6 gene PSMA6 and its adjacent genomic sites have been analyzed.

The goal of this study was to characterize polymorphisms of the HSMS801, HSMS702, HSMS701 and HSMS602 HSMS006 HSMS602 microsatellite

repeats and SNPs at positions –110 and –8 from the translation start of PSMA6 gene and to investigate their eventual association with type II diabetes mellitus.
Methods
In this study, 250 DNA samples of type II diabetes and healthy controls were used. Genotyping was performed using allele-specific PCR and restriction fragment analysis.
Results
For the HSMS006 marker, the 193 bp allele was more common the group of cases rather than controls (0.154 and 0.085 respectively, $P=4.64\%$). HSMS801 allele of 155 bp was found more often in the control group, as the HSMS602 marker allele of 169 bp. HSMS801 genotype of 148 bp/152 bp was more frequent in the control group (0.000 and 0.041 respectively, $P=4.22\%$). Significant differences were observed between cases and controls in all ten haplotype distributions created by combinations of all the microsatellites by two. In these combinations linkage disequilibrium was revealed, indicating the non-random association of alleles in two or more loci on a chromosome. Genotype –8CG was significantly more frequent in type 2 diabetes patients, and haplotype C^{-110}/G^{-8}, compared to $C^{-110}/C^{-}8$ was associated with a higher risk of type II diabetes.
Conclusion
These results show association between microsatellite and SNP alleles of PSMA6 gene and its adjacent genomic sites with type II diabetes mellitus.

OC12.5

The influence of concomitant diabetes mellitus on mortality in Addison's disease

Ragnhildur Bergthorsdottir[1], Maria Leonsson-Zachrisson[1], Anders Odén[2] & Gudmundur Johannsson[1]
[1]Department of Endocrinology, Sahlgrenska University Hospital, Gothenburg, Sweden; [2]Department of Mathematical Sciences, Chalmers University of Technology, Gothenburg, Sweden.

Background
The prevalence of type 1 diabetes mellitus (DM) among patients with primary adrenal insufficiency (Addison's disease) is 3-4 times higher than the expected prevalence in the population. The mortality rate due to DM is more than 3-fold the mortality rate in the background population. The impact of DM on mortality rate in patients with Addison's disease is not known.
Objectives
To study the frequency of DM and its impact on mortality rate in patients with Addison's disease.
Study design
In a population-based retrospective observational study between the years 1987 and 2001 using the Swedish Hospital Register we followed patients from the first registered hospitalisation where the diagnosis of Addison's disease appeared until end of follow-up or death. We looked for the concomitant presence of DM at the time of detection.
Results
We identified 1675 patients, 995 women and 680 men, diagnosed with primary adrenal insufficiency. Concomitant DM was observed in 199 (12%) of the identified patients. DM had a significant influence on total mortality with the relative risk (RR) for death 1.82 (CI 1.29–2.06) for men and 1.52 (CI 1.11–2.07) for women with Addison's disease and DM compared with those patients with Addison's disease without DM.
The impact of DM on the excess mortality in the whole group of Addison's patients was limited since excluding patients with concomitant DM only decreased the RR for death by 7% in both men (2.19 *vs* 2.04) and women (2.86 *vs*. 2.68).
Conclusions
Having DM and Addison's disease significantly increased the risk of death when compared with having Addison's disease alone. However, the overall impact of concomitant DM on the total mortality in all patients with Addison's disease was minor.

OC12.6

Short-term effects of atorvastatin on endothelial functions and oxidized LDL levels in type 2 diabetic patients

Aysen Akalin[1], Gokhan Temiz[2], Nevbahar Akcar[3] & Banu Sensoy[3]
[1]Osmangazi University Medical Faculty Department of Endocrinology, Eskisehir, Turkey; [2]Osmangazi University Medical Faculty Department of Internal Medicine, Eskisehir, Turkey; [3]Osmangazi University Medical Faculty Department of Radiology, Eskisehir, Turkey.

Objective
We aimed to investigate the short term effects of atorvastatin on endothelial function and oxidized LDL (oxLDL) levels and to evaluate the association of endothelial dysfunction to oxLDL levels and inflammatory markers in type 2 diabetic patients.
Material and methods
After ethical committee approval thirty type 2 diabetic and 11 healthy subjects with LDL levels between 100–160 mg/dl. Without a history of cardiovascular event were included in the study.Both groups were matched with respect to age, gender, body mass indices, body composition and lipid levels. Flow-mediated dilatation (endothelium-dependent, FMD) and nitroglycerine-induced dilatation (endothelium- independent, NID) were measured in the brachial artery using high-resolution ultrasound in all participants. Carotid artery intima media thickness (IMT) was also evaluated. OxLDL levels, lipid parameters, blood glucose, C-peptide, HbA1c and inflammatory markers including C-reactive protein(CRP), fibrinogen, erithrocyte sedimentation rate (ESR) were studied. Type2 diabetic patients recived 10 mg. Atorvastatin for 6 weeks and FMD, NID, IMT reevaluated and ox-LDL levels and inflammatory markers were measured.
Results
Basal FMD, NID, IMT and ox-LDL levels besides inflammatory markers were not significantly different between patients and controls. No correlation was found between inflammatory markers and FMD and NID. Only IMT correlated with the NID and fibrinogen levels obtained before treatment. In nondiabetics, IMT also correlated with oxLDL levels (*P*:0.013). FMD and NID significantly improved after atorvastatin therapy (7.62 ± 7.6 *vs*. 12.65 ± 7.8, $P<0.001$ and 18.22 ± 9.57 *vs*. 21.43 ± 9.6, $P=0.007$, respectively). Atorvastatin significantly reduced ox-LDL levels (57.85 ± 10.33 *vs*. 44.36 ± 6.34, $P<0.001$) and IMT (0.627 ± 0.17 *vs*. 0.597 ± 0.16, *P* 0.02) in diabetics.
Conclusions
Atorvastatin improves endothelial functions and reduces oxLDL levels in type 2 diabetics with average lipid levels in the short term and may have beneficial effects in the prevention of early atherosclerotic changes.

OC12.7

A propensity-based comparison of haemodialysis and peritoneal dialysis among diabetic patients with end-stage renal disease in the United States

LA O'Shea & AG Stack
Department of Renal Medicine, LGH, Co. Donegal, Ireland.

Renal transplantation is the optimal treatment strategy for patients with end-stage renal disease (ESRD); few are afforded the opportunity due to limited organ supply. Of the alternatives, peritoneal dialysis (PD) and hemodialysis (HD), it is unclear which confers the greater survival advantage, as prior comparisons have demonstrated conflicting results due to lack of case-mix adjustment, limited follow-up, and failure to consider switches in modality over time.

We compared all-cause and cause-specific mortality between PD and HD in national cohort of 263,556 new ESRD patients in the U.S. who began treatment between 5/1995 and12/2000, and followed until 12/2001. A propensity analysis, predicting the probability of assignment to PD, was used to control for baseline differences through regression adjustment and matching based on 23 demographic and comorbid indicators. The C-statistic for this model was 0.75, indicating excellent discrimination between treatments. Time-dependent Cox regression, stratified by age and diabetes, compared PD and HD using an intent-to-treat and as-treated approach and patients were censored at transplantation, loss to follow-up or end of study.

There were 122,672 deaths (46.5%), 24,596 renal transplants (9.3%) and 17,432 (6.6%) patients lost to follow-up within the 6-yr period. The adjusted relative PD/HD hazards ratios [RR] with 95% Confidence Intervals for all-cause and cause-specific mortality are shown (intent-to-treat analysis).

Mortality risks were significantly greater for PD compared with HD among diabetic patients and were principally confined to older patients. The excess mortality could be accounted for, in decreasing order, by increased death risk from infection, cardiac, stroke and the other causes of death category.

In conclusion, haemodialysis should be preferentially considered over PD among older (>50 yrs) diabetics with ESRD in order to improve patient survival.

Poster Presentations

Comparative Endocrinology – presented on Sunday

P1

Human adrenal NCI-H295R cells produce more C19 steroids than NCI-H295A cells – a possible model to study regulation of androgen biosynthesis?

Elika Samandari, Petra Kempna, Gaby Hoffer, Primus E Mullia & Christa E Fluck
University Hospital, Bern, Switzerland.

The human adrenal cortex consists of three layers in which specific steroid hormones are produced. Human adrenal NCI-H295A (A) and NCI-H295R (R) cells, originate from the same adrenocortical tumor and express all genes essential for steroidogenesis. Therefore they often serve as a suitable model to study human steroidogenesis. No data are available comparing steroidogenesis of A vs. R cells. Assuming no difference, research data from these two cell lines are directly compared. To characterize A and R cells, we investigated steroidogenesis of both cell lines. We found differences in the steroid profile of A and R cells. A cells converted [^{3}H]-pregnenolone predominantly to aldosterone and cortisol while only traces of androgens were produced. R cells converted [^{3}H]-pregnenolone to aldosterone, cortisol and androgens. The observed differences may be either due to differences in gene expression and /or posttranslational modifications which may lead to different activities of specific enzymes. Having found a profound difference in androgen synthesis, we compared HSD3B2 and CYP17 gene expression performing RT and real time PCR. We observed higher HSD3B2 expression in A cells compared to R cells while no difference in the expression of CYP17 was found. Functional studies were performed for P450c17 and 3betaHSDII enzymes. To study the activities of P450c17 (17alpha-hydroxylase and 17, 20 lyase), cells were treated with trilostane (3betaHSD inhibitor) prior to [^{3}H]-pregnenolone or [^{3}H]-17alpha-hydroxypregnenolone incubations. R cells showed higher 17, 20 lyase activity. To study 3betaHSDII activity, cells were incubated with [^{3}H]-DHEA. Interestingly, lower 3betaHSDII activity was detected in R cells. In summary, we show that A and R cells differ in their steroid profile. R cells produce significantly more androgens. Further comparative studies of A vs. R cells may help to understand mechanism/s regulating human androgen production in health and disease.

P2

Effects of ethanol and blokade of synthesis of nitric oxide on level of ACTH in female rats

Dragoslava Djikic, Sanja Vranjes-Djuric, Mirela Budec & Andjelka Spasic
University of Belgrade; Institute for Medical Research, Belgrade, United States, Institute for Nuclear Sciences "Vinca", Belgrade, United States; University of Belgrade, School of Medicine, Institute for Forensic Medicine, Belgrade, United States.

We showed previously that a single dose of ethanol acts as a stressor in female rats (Milovanovic *et al.*, 2003). In order to extend this observation, we investigated whether the effect of ethanol on ACTH is dose-related and possible interactions between nitric oxide (NO) and alcohol on the level of ACTH. To this end, adult female Wistar rats showing diestrus day 1 were treated with: (a) ethanol (2 or 4 g/kg, i.p.), (b) Nω-nitro-L-arginine-methyl ester (L-NAME), which blocks the activity of all isoforms of nitric oxide synthase, (30 mg or 50 mg/kg, s.c.) followed by ethanol (2 or 4 g/kg, i.p.) 3 h later and (c) L-NAME (30 mg or 50 mg/kg, s.c.) followed by saline 3 h later. Untreated rats were used as controls. The animals were sacrificed 0.5 h after ethanol administration. Blood ethanol levels were measured using gas chromatography. Plasma concentrations of ACTH were determined by radioimmunoassay. Obtained results showed that acute ethanol treatment significantly, dose-relatedly, enhanced the level of ACTH ($P<0.01$). The same phenomenon was observed in the groups treated with different doses of L-NAME followed by ethanol ($P<0.05$). Elevated concentration of ACTH was also found in the groups injected with L-NAME followed by saline ($P<0.05$).

Our results suggest that acute ethanol treatment increases the level of ACTH in dose-dependent manner. Although endogenous NO exerts negative influence on ACTH, it seems that it is not involved in the observed effect of ethanol under these experimental conditions.

Milovanovic T. *et al.* J Stud Alcohol 64:662–668, 2003.

(Supported by grant 145087 from the Ministry of Science of Serbia)

P3

Study of the hypothalamic-pituitary-adrenal axis in patients with the antiphospholipid syndrome

Renato Carignola[1], Valeria Data[1], Rosetta Vitetta[1], Adriana Severino[1], Tiziana Bertero[2], Mario Bazzan[3], Giuseppe Reimondo[1], Fulvia Daffara[1], Alberto Angeli[1] & Massimo Terzolo[1]
[1]Dipartimento di Scienze Cliniche e Biologiche – Medicina Interna I, ASO San Luigi, Orbassano, Italy; [2]UONA Immunologia e Allergologia Clinica, Ospedale Mauriziano, Torino, Italy; [3]UOA Ematologia e Malattie Trombotiche, Humanitas Cellini, Torino, Italy.

Objective
The Antiphospholipid Syndrome (APS) is a thrombophilic disorder characterised by recurrent venous and/or arterial thromboses and increased pregnancy morbidity. There is growing evidence supporting a functional interplay between the neuroendocrine and immune system; the hypothalamic-pituitary-adreal (HPA) axis plays a pivotal role in this network. Previous studies have described normal cortisol levels in APS patients while occurrence of acute adrenal failure was reported as a manifestation of this syndrome. However, it is still unknown whether subtle alterations of the HPA axis do exist in APS patients without overt hypoadrenalism.
Method
In the present study, we performed either a low-dose (1 μg) short Synacthen test (LDSST) or a 250 μg Synacthen test (SST) in 15 subjects of both sexes with primitive APS (diagnosed according to the Sapporo Criteria) and in 11 age and sex-matched healthy subjects. In addition, the patients underwent 1 mg dexamethasone suppression test (DST). None of the evaluated subjects were receiving any drug known to affect the HPA axis. The local Ethical Committee approval has been obtained.
Results
The patients with APS showed significantly higher cortisol levels than controls either at baseline (31.2 ± 15.6 vs. 18.3 ± 9.0 μg/dl, $P<0.01$) or at $+30$ min following 250 μg ACTH (57.3 ± 14.2 vs. 39.6 ± 12.8 μg/dl, $P<0.01$). Cortisol levels after 1 μg ACTH were also significantly increased in the subjects with APS compared to controls ($P<0.01$). Moreover, in only 2 patients we observed cortisol levels lower than 1.8 μg/dl after 1 mg DST (mean, 3.4 μg/dl; range 1.4–9.2) and two patients had cortisol values above 5.0 μg/dl after suppression.
Conclusions
In conclusion, although APS may cause adrenal insufficiency in selected cases, the present data seem to suggest that the HPA axis is not suppressed in APS patients. A possible explanation might be the state of chronic stress that usually accompanies long-standing autoimmune diseases.

P4

Survey of thyroid function of Hungarian Vizsla population in Hungary

Julianna Thuroczy[1], Aniko Tibold[1] & Lajos Balogh[2]
[1]SzIU, Faculty of Veterinary Science, Budapest, Hungary; [2]FJNCH, National "FJC" Research Institute for Radiobiology and Radiohygiene, Budapest, Hungary.

The prevalence of hypothyroidism in women of childbearing age is relatively high. The incidence of hypothyroidism during pregnancy has been calculated as between 0.3% and 0.7%. Overt abnormalities in thyroid function are common endocrine disorders affecting more than 19.2% of pregnant women in certain geographic areas of Hungary. 80% of Hungarian inhabitants are living in an iodine deficient area. The aim of this study was to investigate the prevalence of thyroid dysfunction in Hungarian Vizsla, a traditional breeding dog population.

A screening study was done on 95 Hungarian Vizsla, females and males. Serum total thyroxin, free thyroxin, triiodotyronine, total cholesterol and triglyceride concentrations were measured. The owners were asked to fill in a questionnaire concerning feeding and reproductive problems. TT4, freeT4 and T3 concentrations were determined by ELISA validated for use in canine serum.

The means and standard errors of the data were calculated and subjected to ANOVA and Student's *t*-test where appropriate. Significance was set at $P\leq0.05$.

Total T4 concentration of 36 dogs was lower (15.72 ± 2.62 (mean $\pm$ S.D.)) than the reference range (20.0–45.0 nmol/l). Total T4 level of 56 dogs was in reference range 26.83 ± 4.68 and of five was higher, 92.97 ± 64.86, than range. Total T4, free T4 and K values were different in the three groups at level of significance. T3 concentrations of suspected hypothyroid dogs (0.66 ± 0.24), dogs with normal thyroid function (0.77 ± 0.45) and dogs with suspected hyperthyroidism (0.67 ± 0.06) were not different at level of significance. TT4 concentrations of 25 (26.3%) dogs with familiar relations were out of reference range.

Our approach of a clinical investigation-based screening was rather efficient in suspicion of overt thyroid dysfunction but not for detecting many cases with

subclinical dysfunction. The high incidence of TT4 values out of range indicates a suspicion that the effect of iodine deficiency on thyroid function of dogs is similar to that in human subjects.

P5

Pancreatic polypeptide (PP) radioimmunoassay in acute phase and regression of cerulein induced pancreatitis

Grazyna Orlicz-Szczesna & Jolanta Zdanowska-Filipczak
Department of Internal Diseases University School of Medicine, Lublin, Poland.

Acute pancreatitis is a real medical problem with high patients mortality. Pathogenic interdependence between pancreas follicles function and islet endocrine secretion is under research. PP cells are pancreatic polypeptide (PP) producing cells, they determine about 1% islets, but their function is not completely known yet. Vagus nerve and peptidergic stimulation regulates PP secretion.

The aim of study was to estimate cerulein induced pancreatitis effects on rat serum PP concentration and pancreas morphology characteristics.

The study was conducted on male Wistar rats. They were anaesthetized with ketamine. We measured serum PP concentration during experimental cerulein-induced acute pancreatitis and different inflammatory process regression stages. Acute pancreatitis was developed through i.v. cerulein infusion 5 μg/kg per hour. Rats were divided into several groups in dependence on infusion time – 3,6,9,12 hours. Then rats had free access to standard nourishment and water. Blood samples from rat group with 12 hours cerulein infusion were taken after 3,6,9 and 12 days of observation. Control groups received i.v. 0.9% NaCl infusion. Pancreas histological changes were analyzed. Serum amylase and PP concentrations were assessed with DRG International Inc. (USA) kit. Both rabbit serum with antibodies against PP and goat's anti-rabbit gamma-globulin in buffer were used.

After 12 hours lasting cerulein infusion we obtained full biochemical and morphological acute pancreatitis picture. These changes start to regress after cerulein infusion withdrawal. Serum PP concentration was decreased after 3 hours of cerulein infusion, still decreased until the end of infusion (0.99 pg/ml). After cerulein cessation, progressive PP increase was observed, attained control PP concentration after 9 days (2.4 pg/ml) and exceed it after 12 days (3.5 pg/ml).

Cerulein significantly influence on serum PP concentrations - decreases it during pancreatitis induction and increases in regression stage. PP determines exocrine function stimulation, correlates with tissue destruction degree and pancreas enzymes disturbances.

P6

Evaluation of neuroendocrine disfunction in hypothalamo-pituitary-adrenal axis in diagnosis of depressive and non-depressive alcohol-dependent persons

Martina Matovinovic, Darko Katalinic, Andreja Maric, Miljenko Solter, Josip Ivica, Josip Resetic, Jure Murgic & Milan Vrkljan
Sestre Milosrdnice University Hospital, Zagreb, Croatia.

Acute and chronic alcohol intake and alcohol withdrawal induce dysfunction of neuroendocrine and other regulatory systems. The aims of this study were to assess a possible hypothalamo-pituitary-adrenal (HPA) axis dysfunction in population of alcoholics, using dexamethasone suppression test (DST). The study was approved by local Ethical Committee. The serum and urinary cortisol were compared between the groups of 89 male patients (64.5% depressive and 35.5% nondepressive alcoholics) (Hamilton test), before and after DST. In nondepressive patients, 50% was nonsupressive in DST. In depressive patients 46% was suppressive in DST test (serum cortisol). Twenty-four hours urinary excretion in group of nondepressive patients was supressed in 78% of cases; depressive patients showed 50.9% nonsupressors. Basal serum cortisol secretion was significantly lower in group of nondepressive than depressive patients. Also, serum concentration at 16 hours were significantly higher in group of the depressive nonsupressive patients. Basal urinary cortisol excretion was in normal range in all patients, but after dividing the patients into suppressible and nonsuppressible groups, significantly higher ($P<0.002$) basal urinary cortisol concentrations were found in latter. We concluded on the basis of DST test, as well basal cortisol measurement, that the neuroendocrine dysfunction of alcoholic patients could be present even if the depression is pronounced.

P7

Effects of melatonin on glutathione peroxidase activity after adriamycin in normal and pinealectomized rats

Katarzyna Dabrowska[1], Michal Stuss[1], Jolanta Gromadzinska[2], Wojciech Wasowicz[2] & Ewa Sewerynek[1]
[1]Department of Bone Metabolism; Medical University of Lodz, Lodz, Poland; [2]The Nofer Institute of Occupational Medicine, Lodz, Poland.

Adriamycin (ADR) is a potent chemotherapeutic agent, effective in treatment of leukemias, lymphomas and of many solid tumours. However, its clinical usage is often limited by cardiotoxicity, induced by oxygen radical damage of membrane lipids.

Melatonin (MEL), is a well-known antioxidant. It has been shown that melatonin can scavenge free radicals, both directly or indirectly, stimulating the activity of antioxidative enzyme, e.g., glutathione peroxidase (GSH-Px).

The aim of the study was to examine the effect of Mel on the GSH-Px activity in serum, erythrocytes and the heart after adriamycin.

Materials and Methods

Wistar rats were divided into the 3 groups: pinealectomized (PX), sham-operated (Sham-PX) and control animals (Intact). Each of the groups was divided into 4 subgroups, injected with: 1 – saline, 2 – MEL, 3 – ADR and 4 – MEL + ADR. ADR was administered 2 months after PX as a single dose (15 mg/kg, i.p.), 1 hour after the fourth melatonin injection. Melatonin (5 mg/kg, i.p.) was administered for 4 days before and 4 days after ADR. After 8 days of treatment the rats were killed by decapitation. Their hearts and blood were collected for measurements.

Results

The activity of GSH-Px in the heart increased significantly in all the examined groups after ADR injections. On the contrary, in serum, GSH-Px activity decreased in all the groups after ADR. In erythrocytes, GSH-Px decreased after ADR in Px-animals. Mel did not change GSH-Px activity after ADR.

Conclusion

MEL did not influence the activity of GSH-Px, either in normal or in pinealectomized rats after ADR.

Grant No 502-11-293 of the Medical University of Łódź.

Cytokines and growth factors – presented on Sunday

P8

Is there any role for anti-inflammatory cytokine Interleukin-10 in advanced congestive heart failure?

Azza Farrag, Nouran El Gahndour & Mohab Halim
Cardiology Department, Cairo University, Cairo, Egypt; Internal Medicine Department, Cairo University, Cairo, Egypt; Biochemistry Department, Cairo, Egypt.

Background

CHF manifestations can be explained by the biologic effects of tumor necrosis factor-alpha (TNF-alpha). Interleukin-10 (IL-10) has potent deactivating properties and is considered a potent anti-inflammatory cytokine that inhibits TNF-alpha production. This study was designed to examine the role of IL-10 in patients with CHF and to test its correlation with pro-inflammatory markers.

Methodology

Fifty patients with CHF were studied. Patients were classified according to NYHA functional class into 29 (NYHA II), 11 (NYHA III) and 9 patients (NYHA IV). Serum samples for TNF-alpha, IL-10, soluble TNF receptors (sTNF-R1 and sTNF-R2), transforming growth factor-beta (TGF-beta) as well as high sensitivity C-reactive protein (hs-CRP) were taken from all patients and also from healthy, age and sex matched 50 controls.

Results

CHF patients had a significantly lower level of IL-10 compared to controls (2.28 ± 1.1 vs 5.39 ± 1.4 pg/ml, $P<0.0001$). Patients with NYHA class IV had the lowest serum levels of IL-10 and TGF-alpha which were statistically significant when compared to patients with NYHA class III (0.67 ± 0.4 vs 1.9 ± 0.5 pg/ml, $P<0.001$) and (1348 ± 92 vs 1653 ± 111 pg/ml, $P<0.05$) respectively, but they had the highest serum level of TNF-alpha, sTNF-R1, sTNF-R2 and hs-CRP when compared to the same group (8.6 ± 1.9 vs 7.1 ± 0.8 pg/ml, $P<0.01$), (2380 ± 141 vs 1831 ± 185 pg/ml, $P<0.01$), (3410 ± 174 vs 2841 ± 191 pg/ml, $P<0.01$) and (26.4 ± 2.7 vs 14.4 ± 3.9 mg/L, $P<0.01$) respectively.

Conclusion

Patients with CHF had a significant decrease in their serum level of IL-10 and increase in TNF-alpha, sTNF-R1, sTNF-R2 and hs-CRP when compared to normal subjects and these levels change significantly with advanced NYHA class.

P9

Regulation of GAGEC1, a cancer-testis associated antigen family member, by sex steroid hormones and TGF-beta: implications for prostatic disease

Natalie Sampson[1], Gerlinde Schuhfried[1], Eugen Plas[2] & Peter Berger[1]
[1]Institute of Biomedical Aging Research, Austrian Academy of Sciences, Innsbruck, Austria; [2]Ludwig Boltzmann Institute for Andrology and Urology, Hospital Lainz, Vienna, Austria.

Prostate homeostasis and function are regulated by complex interactions between the fibromuscular stroma and secretory epithelium via locally-derived and systemic paracrine- and autocrine-acting growth factors and sex steroid hormones. Stromal tissue remodelling due to alterations in transforming growth factor beta (TGF-β) and sex steroid hormone signalling are associated with benign prostatic hyperplasia (BPH) and prostate cancer (PCa), two of the most common proliferative disorders affecting elderly men.

We previously demonstrated that GAGEC1, a member of cancer-testis associated antigens, is up-regulated in response to TGF-β in *in vitro* models of age-associated prostatic stromal remodelling. GAGEC1 expression is restricted to male and female reproductive tissues and is up-regulated in the prostates of patients with symptomatic BPH and PCa. Consistent with its restricted expression profile to classical steroidogenic tissues, *GAGEC1* is induced by sex steroid hormones, particularly oestradiol and dihydrotestosterone. Transiently expressed recombinant GAGEC1 undergoes constant shuttling between cytoplasmic and nuclear cell compartments, a process that may be regulated via post-translational phosphorylation.

Our data suggest that age/disease-associated changes in TGF-β1 and sex steroid hormones may account for the reported increase in *GAGEC1* expression in BPH and PCa. Functional analyses indicate that the biological activity of GAGEC1 is regulated via phosphorylation-dependent nucleo-cytoplasmic trafficking raising the possibility that GAGEC1 is involved in signal transduction mechanisms. Given that its expression is restricted in males to the prostate and testis, *GAGEC1* represents a promising target for therapeutic intervention of BPH and PCa.

P10

Normalization of serum testosterone level alters local GnRH-II and IL-2R mRNA expression in peripheral lymphocytes in patients with idiopathic hypogonadotrophic hypogonadism (IHH)

Fatih Tanriverdi[1], Hilal Akalin[2], Okay Caglayan[2], Umit Demirkoparan[1], Yusuf Ozkul[2], Munis Dundar[2], Fahri Bayram[1] & Fahrettin Kelestimur[1]
[1]Erciyes University Medical School Department of Endocrinology, Kayseri, Turkey; [2]Erciyes University Medical School Department of Genetics, Kayseri, Turkey.

Although the existence of the interaction between sex steroids and immune system is well known, the mechanisms of this interaction are still unclear. Recently a second form of GnRH (GnRH-II) has been described in human, which is significantly expressed in immune tissues suggesting a potential function. In a recent *in-vitro* study it has been demonstrated that GnRH-II decreases local expression of IL-2R in peripheral lymphocytes (1). However *in-vivo* interactions of testosterone, IL-2R and GnRH-II expression at lymphocyte level have not been investigated yet. Therefore in the present study we investigated the effects of conventional gonadotrophin therapy on local GnRH-II and IL-2R expression in peripheral lymphocytes in patients with IHH.

Fourteen males with IHH (24.5 ± 6.3) and 15 age-sex matched controls were investigated. Patients were treated with hCG and hMG for 12 months. Quantitative Real-Time RT-PCR (2 independent repeats) was used to determine the expression of GnRH-II (target gene), IL-2R (target gene) and beta-actin (reference gene) in peripheral lymphocytes derived from patients before and after treatment, and the controls.

Serum testosterone level before treatment in patient group was significantly low when compared to controls. After gonadotrophin treatment testosterone level significantly increased. Baseline GnRH-II and IL-2R mRNA levels (% of the control) were % 1451 ± 300 and % 285 ± 46 in the patient group, respectively. Significant decrease in GnRH-II and IL-2R mRNA levels were found after treatment**.**

In-vivo interactions between testosterone, IL-2R and GnRH-II at lymphocyte level were shown first time in the literature. Present findings clearly suggest that some immune effects of the sex steroids may occur via regulating the local GnRH-II and IL-2R expression.

P11

Blocking undesired leptin action *in vivo* with leptin antagonists prepared by site-directed mutagenesis of human, ovine, rat and mouse leptin site III

Leonora Niv-Spector, Dana Gonen - Berger, Gili Salomon, Isabelle Callebaut, Jean Djiane & Arieh Gertler
Institute of Biochemistry, Food Science and Nutrition, Faculty of Agricultural, Food and Environmental Quality Sciences, The Hebrew University of Jerusalem, Rehovot 76100, Israel.

Recent reports have revealed that leptin's effects are not restricted to central control of body weight, demonstrating instead that leptin is a pleiotropic hormone with a wide variety of different biological actions. Leptin exhibits undesirable effects in autoimmune diseases, in atherosclerosis, and possibly in several types of cancer, and increases the risk of cardiovascular disease in obese people. Therefore, preparation of reagents capable of abolishing leptin's action is both valid and timely.

As no structural information on the 3D structure of leptin receptor (LEPR) is available, the model of interleukin 6 (IL6) was applied. We identified leptin's putative binding site III, which does not affect binding but is necessary for receptor activation, by modeling LEPR on the basis of its alignment with gp130, and fitting leptin on IL6 in the IL6/gp130 complex.

Six muteins of human, ovine, rat, and mouse leptins, mutated to Ala in amino acids 39–41 or 39–42, were prepared by site-directed mutagenesis of the putative site III, and purified to homogeneity. All muteins had typical cytokine secondary structure, acted as true antagonists—namely, they interacted with LEPR with an affinity similar to that of the wild-type hormone (as evidenced by SPR and RRA), were devoid of biological activity in several leptin-response bioassays, and specifically inhibited leptin action *in vitro* and *in vivo.* These muteins can be prepared in gram amounts and thus serve as a novel tool for studying leptin function *in vitro* and *in vivo.* To prolong their lives in circulation, some muteins were pegylated using 40-, 30- and 20-kDa polyethylene glycol. Although pegylation decreased their *in-vitro* activity, increasing circulation half-life can compensate for this deficit *in vivo.*

Antagonizing leptin has been suggested as a possible therapy in autoimmune diseases and heart failure. Thus, leptin antagonists not only offer a novel tool to elucidate the role of leptin in mammalian physiology but have a potential role as a therapeutic drug.

P12

Role of soluble Fas-antigen (sFas), interleukin-6 (IL-6) and vascular endothelial growth factor (VEGF) in adrenocortical carcinoma patients

Nikolai Kushlinsky, Timur Britvin, Irina Kazantseva & Arian Kalinin
Russian N.N.Blokhin Cancer Research Center of RAMS, Moscow, Russia; M.F.Vladimirsky Moscow Regional Clinical and Research Institute, Moscow, Russia.

The numerous growth factors and cytokines take part in mechanisms of tumor growth and metastasing.

The aim of this study was determination of sFas, IL-6 and VEGF serum levels in 19 patients with adrenal cortical carcinoma (11 women and 8 men aged 21–72 years). The control group comprised 40 practically healthy donors (22 women and 18 men aged 19–70 years). sFas, IL-6 and VEGF before adrenalectomy and in the control were measured by ELISA. Mean IL-6 (4.6 ng/ml) and VEGF (438.7 pg/ml) levels in adrenocortical carcinoma patients were significantly ($P = 0.004$) higher than in the control (IL-6 – 1.3 ng/ml, VEGF – 126.5 pg/ml). There was no difference in serum sFas between patients (2.0 ng/ml) and the control (0.8 ng/ml). sFas, IL-6 and VEGF were markedly elevated in patients with advanced (III-IV) stages of the disease as compared to early (I-II) stages. In patients with nonfunctional adrenal cortical carcinoma, serum level of VEGF (571.9 pg/ml) was significantly ($P = 0.046$) higher than that in patients with Cushing's syndrome (460.1 pg/ml). No differences in serum sFas and IL-6 levels were revealed between patients with nonfunctional and hormonally-active tumors. Direct correlation was found between VEGF and IL-6 ($P = 0.56$; $r = 0.009$). 5-year overall survival (100%) of patients with serum VEGF less then 300 pg/ml was significantly ($P = 0.049$) higher compared to patients with serum VEGF exceeding 300 pg/ml (34.3%). 5-year overall survival didn't depend on the pretreatment serum sFas and IL-6 levels.

We suggest that VEGF serum level in adrenal cortical carcinoma patients may be used as a factor of clinical behaviour and prognosis.

P13

Rosiglitazone interferes with the inflammatory response induced in human endothelial cells by TNFalpha and IFNgamma through a new mechanism involving extracellular signal-regulated kinases (ERKs)

Adriana Lombardi[1], Elisabetta Piscitelli[1], Michela Francalanci[1], Tommaso Mello[1], Veronica Santarlasci[2], Stefania Gelmini[1], Giulia Cantini[1], Andrea Galli[1], Lorenzo Cosmi[2], Francesco Annunziato[2], Gianni Forti[1], Mario Serio[1] & Michaela Luconi[1]
[1]Dept. Clinical Physiopathology, University of Florence, Florence, Italy; [2]Dept. Internal Medicine, University of Florence, Florence, Italy.

Microvascular endothelium is one of the main character and target involved in the inflammatory response. Upon specific activation, endothelial cells massively recruit Th1 IFNgamma secreting lymphocytes at the inflammatory site. In the present study, we investigate the intracellular signalling mediating TNFalpha (T) and IFNgamma (I) inflammatory response in a human endothelial cell line (HMEC-1) *in vitro* and the interfering effects of rosiglitazone (RGZ), a peroxisome proliferator-activated receptor (PPARgamma) agonist currently used in clinical treatment of diabetes mellitus. We show that T and I alone stimulate interferon gamma-inducible protein-10 (IP-10) secretion by HMEC-1, effect which is dramatically increased when the two cytokines are used in combination. IP-10 secretion in response to T, I and RGZ is accompanied by a re-modulation of surface expression of cell adhesion molecules (CAM), such as VCAM-1 and ICAM-1. Although these stimulatory effects of T and I are mediated by a similar rapid increase in phosphorylation/activation of ERK1/2, as demonstrated by the use of ERK inhibitors, confocal microscopy analysis suggests that the synergistic action of T and I is partly mediated by a different subcellular localization of the activated ERKs. Concomitant treatment with RGZ reverts both activation of ERKs and interferes with IP-10 secretion and CAM expression elicited by T and I through a novel rapid mechanism not involving transcriptional activity of PPARgamma, as further confirmed by the inability of BADGE, an inhibitor of such a transactivational action, to revert RGZ effects. Our findings shed new light on the molecular mechanisms underlying the inflammatory response in the endothelium and on the possible therapeutic use of RGZ in such a process.

This study has been supported by project TRESOR of Region of Tuscany

P14

Role of growth hormone/insulin like growth factor 1 system in the remodelling process of the right ventricle in top levels rowers

Giovanni Vitale[1], Maurizio Galderisi[2], Annamaria Colao[3], Pasquale Innelli[2], Germano Guerra[4], Ermelinda Guerra[3], Frank Lloyd Dini[5], Antonio Soscia[4], Oreste de Divitiis[2] & Gaetano Lombardi[3]
[1]Chair of Endocrinology, Faculty of Medicine – University of Milan, Istituto Auxologico Italiano, Milan, Italy; [2]Cardioangiology and CCU Division, Department of Clinical and Experimental Medicine, University of Naples Federico II, Naples, Italy; [3]Department of Molecular and Clinical Endocrinology & Oncology, University of Naples Federico II, Naples, Italy; [4]Department of Biomorphological and Functional Sciences, University of Naples Federico II, Naples, Italy; [5]Cardiovascular Diseases Unit 2, Ospedale Santa Chiara, Pisa, Italy.

The intensive physical activity is often associated with cardiac changes, particularly involving the right ventricular (RV) chamber. However, the molecular mechanisms involved in the RV physiologic adaptation to long-term training are not completely understood. In the present study we investigated the role of the growth hormone/insulin like growth factor 1 (GH/IGF-1) axis in the RV remodeling of athletes.

Nineteen male top levels rowers and 19 age-matched healthy sedentary male controls underwent blood determination of fasting serum GH, IGF-1, IGF binding protein 3 (IGFBP-3) and acid-labile subunit levels and standard Doppler echocardiography combined with pulsed Tissue Doppler of RV tricuspid annulus. Myocardial pre-systolic (PS_m), systolic (S_m), early diastolic (E_m) and atrial (A_m) velocities as well as myocardial time intervals adjusted for heart rate were calculated.

Rowers had serum IGF-1 levels ($P<0.05$), RV internal chamber size ($P<0.05$) and RV wall thickness ($P<0.0001$) significantly higher than controls. Additionally, rowers had improved RV systolic (higher tricuspid annular systolic excursion, higher PS_m and S_m velocities; lower myocardial pre-contraction time) and diastolic function (lower A velocity, shorter deceleration time, isovolumic relaxation time and myocardial relaxation time; higher E/A ratio, E_m and E_m/A_m ratio) compared to controls. In the rowers, IGF-1 was associated with PS_m velocity ($r=0.55$, $P=0.01$) and myocardial pre-contraction time ($r=-0.57$, $P=0.01$), GH with pre-ejection period ($r=-0.50$, $P<0.05$) and E_m ($r=0.47$, $P<0.05$). These associations remained significant after adjusting for age, heart rate and body surface area.

In conclusion, this study shows for the first time that the GH/IGF-1 axis is responsible for the RV functional remodeling in high-top rowers, improving mainly the systolic activity. This effect seems to be primarily modulated by the IGF-1 overproduction, as a physiological adaptation to prolonged training.

P15

Clinical significance of simultaneously determined serum interleukin-6, dehydroepiandrosterone and its sulphate levels in melanoma

Mariann Boldizsár, Borbála Vincze, Katalin Gilde, Teodora Bánfalvi, Zsuzsanna Fejos, Kinga Borbola, Bence Kapuvári, Gabriella Liszkay & Szabolcs Otto
National Institute of Oncology, Budapest, Hungary.

Among well-known circulating melanoma markers 5-S-cysteinyldopa (5-SCD), a precursor of pheomelanin biosynthesis and S-100 beta (S-100B) are extensively investigated. Our earlier observations confirmed that serum concentration of 5-SCD and S-100B correlates well with the stages and progression of the disease. Interleukin-6 (IL-6) is a multifunctional inflammatory cytokine implicated in advanced stage of various diseases and tumour recurrence. Malignant melanoma cells are known to secrete IL-6. According to the recent reports skin could produce DHEA and DHEA-S due to the presence of key enzymes. This study was aimed to establish the significance and the possible relationship among different serum parameters. In 124 melanoma patients with ($n=63$) or without ($n=61$) metastasis, concentrations of IL-6, DHEA, DHEA-S were simultaneously measured in comparison with the metastatic markers of 5-SCD and S-100B. The presence of metastasis was verified by conventional imaging techniques. Serum 5-SCD concentration was determined by high pressure liquid chromatography with electrochemical detection. Serum levels of IL-6, DHEA, DHEA-S and S-100B were measured by RIA/IRMA and ILMA methods. For statistical analysis MedCalc Software was used. In patients with metastases compared to the metastasis-free cases significant increase in 5-SCD, S-100B and IL-6 serum levels were observed. On the contrary, significant decrease in DHEAS and DHEA concentrations was found. Correlations between serum concentrations of 5-SCD and IL-6 ($P<0.0001$), as well as DHEA and DHEA-S ($P<0.0001$) were significant and Spearman's coefficient of rank correlation (rho) was 0.69 and 0.71, respectively. Using multiple regression analysis a negative correlation between IL-6 and DHEA or DHEAS levels was found. These results suggest that simultaneous determination of IL-6, DHEA and DHEA-S together with 5-SCD and S-100B measured in melanoma patients could be predictive factors of the disease.

This research was supported by the Hungarian Research Fund (OTKA No. T 049814).

P16

Changes in growth hormone messenger RNA (GHmRNA) expression in rats anterior pituitary after single Interferon (IFN)alpha administration.

Wojciech Romanowski, Ryszard Braczkowski, Aleksander Danikiewicz, Ewa Nowakowska-Zajdel, Malgorzata Muc-Wierzgon, Zuzanna Muryn & Barbara Zubelewicz-Szkodzinska
Dept. of Internal Medicine Bytom, Silesian Medical University, Katowice, Poland.

Introduction
Interferon alpha (IFN alpha) is a cytokine with pleiotropic effects and via different pathways influences secretion of certain cytokines and hormones. Growth Hormone (GH) secreted from the pituitary has its physiological effects on various target tissues. The question is how IFN alpha administered in various type of diseases influences GH secretion. This study investigated acute effect of IFN-alpha on GH mRNA expression in rat anterior pituitary
Objective
The aim of the study was to measure the cellular expression of GH mRNA by *in situ*hybridisation in anterior pituitary after IFN alpha single administration
Material and methods
Rats were administered intraperitoneal injection of IFN alpha or saline. Rat pituitaries were taken 2 and 4 hours after IFN/saline administration and kept frozen until *in situ*hybridisation histochemistry. 31–base^{35}S-labelled oligonucleotide probe complementary to part of the exonic mRNA sequences coding for GH mRNA was used. All control and experimental sections were hybridised in the same hybridisation reaction

Results
Interferon α acute administration increases GH mRNA expression in the anterior pituitary in 4 hours group in comparison to the control group, and there was no difference between control group and 2-hours rats.
Conclusion
The influence of single IFN alpha administration on anterior pituitary GH mRNA expression has been found. These observations may pave the way for presenting a new possible IFN alpha action.

P17

Does stress test influence Interleukin (IL)-2 and IL-8 concentration in serum patients with stable ischaemic heart disease?

Wojciech Romanowski[1], Janusz Szkodzinski[2], Andrzej Kaszuba[1], Adam Blazelonis[1], Zuzanna Muryn[1], Pietka-Rzycka Anna[1] & Barbara Zubelewicz-Szkodzinska[1]
[1]Dept. of Internal Medicine Bytom, Silesian Medical University, Katowice, Poland; [2]Silesian Center for Heart Diseases, Zabrze, Poland.

Background
There is growing evidence that adhesion molecules, proinflammatory cells and cytokines play an important role in a variety of cardiac pathophysiological conditions; Cytokines are responsible for the modulation of immune and inflammatory processes. It has been suggested that cytokines such as IL-1, IL-2, IL-6, IL-10 and TNF alpha are important modulators of atherosclerotic effects with IL-2 and Interferon γ having a proinflammatory atherogenic effect and IL-8 and IL-10 having an anti-inflammatory protective role. Atherosclerotic lesions in the coronary vessels are heavily infiltrated by cellular components associated with inflammation (macrophages/monocytes, t-lymphocytes, eosynophils and NK-cells). These cells are also a source of cytokines and that is why the **Objective** of the present study was to measure IL-2 and IL_8 concentration in serum patients with stable ishaemic heart disease (i.h.d.).
Patients and method
26 patients (11 women, 15 men) age 47–65 years with diagnosed stable ischaemic heart disease were included into study. The control group consists of 20 patients matched with age and sex. All patients from examined group fulfilled the inclusion criteria (stable i.h.d. with standard treatment, rest ECG without ischaemic changes and with coronary sufficiency belongs to I or II class of CCS-(Canadian Cardiovascular Society scale).The exclusion criteria were typical for the study concerns cytokines.
In all patients we have measured the concentration of IL-2 and IL-8 concentration in serum by ELISA using R&D System kits
Additionally, patients with diagnosed i.h.d. had IL-2 and IL-8 concentration measured after the stress test done to assume the cardiac sufficiency in that group.
Results
Concentration of IL-2 and IL-8 in patients with i.h.d. is significantly higher then in the control group ($P<0.05$). After stress test in i.h.d. patients there were no significant changes of IL -2 concentration ($P=0.054$) and increase of IL-8 ($P<0.001$) concentration observed.

P18

Concentration of inflammatory factors such as CRP (acute phase response protein) and Interleukin (IL)-6 in serum patients with stable ischemic heart disease during trimetazidine treatment

Janusz Szkodzinski[1], Marek Szewczyk[2], Wojciech Romanowski[2], Aleksander Danikiewicz[2], Malgorzata Muc-Wierzgon[2] & Barbara Zubelewicz-Szkodzinska[2]
[1]Dept. of Internal Medicine Bytom, Silesian Center for Heart Diseases, Zabrze, Poland; [2]Silesian Medical University, Katowice, Poland.

Background
The patomechanism of developing ischemic heart disease (i.h.d.) is stenosis of coronary blood vessels with plaque placed on vascular endothelium built with monocytes/macrophages, foam cells, oxidized LDL, leukocytes, platelets and collagen. Atherosclerotic lesions are heavily infiltrated by cellular components associated with inflammation and influenced by other inflammatory factors. Trimetazidine, a clinically effective antianginal agent acts by optimizing cardiac energy metabolism through inhibition of free fatty acid oxidation.

Up to now there have been no study associating trimetazidine possible anti-inflammatory effect which could be a result of trimetazidine influence on granulocytes in-flow to ischemic region and atherosclerotic plaque and in consequence influence on granulocyte products such as cytokines and other inflammatory predictors.
Objective
The aim of the study was to determine if trimetazidine treatment in stable ischemic heart disease altered the concentration of certain inflammatory factors such as CRP (acute phase response protein) and Interleukin (IL)-6.
Patients and method
26 patients (11 women, 15 men) age 47–65 years with diagnosed stable ischemic heart disease were included into study. All of them fulfilled the inclusion criteria (stable i.h.d. with standard treatment, rest ECG without ischemic changes and with cardiac sufficiency belongs to I or II class of CCS-(Canadian Cardiovascular Society scale).
All patients have measured the concentration of Il-6 and CRP at the onset of trimetazidine treatment and 3 months after. Il-6 concentration has been measured by ELISA using R&D System kits and CRP concentration by immunoturbidometric method.
Results
3-months trimetazidine treatment caused significant decrease of CRP concentration in serum of patients with stable i.h.d. ($P<0.001$) and significant increase of IL-6 concentration ($P<0.05$).
Conclusion
Decrease of CRP concentration in serum after 3 months of trimetazidine treatment could be due to trimetazidine hepatoprotective properties. An increase of Il-6 concentration after 3 months of treatment with trimetazidine is possibly a result of different mechanism of its action.

P19

Insulin decreases IGF-I bioactivity in patients with impaired glucose tolerance and in healthy subjects

Ayman M Arafat[1], Jan Frystyk[2], Martin O Weickert[1], Joachim Spranger[1], Christof Schöfl[1] & Mathias Möhlig[1]
[1]Department of Endocrinology, Diabetes and Nutrition, Charité-University Medicine Berlin, Campus Benjamin Franklin and the German Institute of Human Nutrition Potsdam-Rehbruecke, Berlin, Germany; [2]Medical Research Laboratories and Medical Department M, Aarhus University Hospital, DK-8000 Aarhus C, Denmark.

Objectives
Insulin resistance (IR) is a very common metabolic abnormality in obesity, which is often associated with reduced growth hormone (GH) secretion. GH deficiency is associated with increased in intra-abdominal fat and several parameters of the metabolic syndrome. IGF-I improves IR but the IGF-binding proteins are supposed to regulate its bioactivity although only little information exists. We postulated that the elevated insulin levels due to IR do not only suppress GH but also IGF-I bioactivity, and therefore we tested the effect of insulin on serum levels of bioactive IGF-I.
Methods
24 healthy subjects (12 men; age 21–72 years; BMI 25.9±0.9 kg/m2) and 19 patients with impaired glucose tolerance (IGT; 8 men; age 26–71; BMI 28.9±1.2) were studied using an OGTT and a hyperinsulinemic euglycemic clamp. IR was estimated by calculating the homeostatic model assessment (HOMA-IR) index and the glucose infusion rate (GIR). IGF-I bioactivity was estimated using a novel IGF-I kinase receptor activation assay (KIRA) under fasting conditions and during the steady state of the clamp. Ethical Committee approval was obtained.
Results
Insulin significantly decreased IGF-I bioactivity in IGT patients (1.8±0.2 vs. 1.5±0.2 μg/l, $P=0.004$) and in healthy controls (1.8±0.2 vs 1.6±0.2 μg/l, $P=0.001$). Age, BMI and fasting IGF-I bioactivity did not significantly differ between groups. However, patients with IGT showed a higher HOMA-IR and a lower GIR (2.3±0.4 vs. 1.3±0.2 and 2.5±0.3 vs. 4.7±0.3 mg/kg min, $P<0.05$, respectively). Moreover, inverse correlations were seen between bioactive IGF-I levels and age ($r=-0.38$, $P=0.01$), BMI ($r=-0.46$, $P=0.002$) and waist to hip ratio ($r=-0.51$, $P=0.01$).
Conclusion
Our data indicate that insulin infusion acutely decreased serum IGF-I bioactivity in humans. Hyperinsulinemia as seen in IR may per se be responsible for this reduction. Estimation of IGF-I bioavailability using the KIRA method may, therefore, have a predictive value in the diagnosis of the metabolic syndrome.

P20

Plasma free fatty acids and adipocytokines concentration in relation to insulin sensitivity in patients with anorexia nervosa.

Monika Karczewska-Kupczewska, Irina Kowalska, Marek Straczkowski, Agnieszka Adamska, Agnieszka Nikolajuk, Malgorzata Karolczuk-Zarachowicz, Elzbieta Otziomek & Maria Gorska
Department of Endocrinology, Diabetology and Internal Medicine, Medical University of Bialystok, Bialystok, Poland.

Anorexia nervosa (AN) is an eating disorder, resulting in sustained low weight. In AN, similarly to syndromes of lipodystrophy, one observes the significant loss of the adipose tissue. In lipodystrophies, despite the lack of subcutaneous adipose tissue, insulin resistance is observed. Adipose tissue is known as a source of a variety of bioactive peptides, known as adipocytokines. The aim of the present study was to examine the plasma concentration of adipocytokines in relation to insulin sensitivity in women with AN.

The study group consisted of 16 women with AN, 16 women with obesity and 18 healthy normal weight female controls. The oral glucose tolerance test and euglycemic hyperinsulinemic clamp were performed in all the patients. The plasma concentrations of adiponectin, TNF-α, soluble TNF-α receptors (sTNFR1, sTNFR2) and IL-6, soluble form of IL-6 receptor (sIL-6R) were estimated.

Insulin sensitivity index (M) was not different in AN and healthy controls, but was significantly increased in AN in comparison to obese women ($P=$ 0.002). Adiponectin plasma levels were significantly higher in AN than control subjects and obese women ($P=0.01$, $P=0.003$, respectively). There were no differences in plasma concentrations of TNF-α, sTNFR1, sTNFR2, IL-6, sIL-6R among groups, however plasma free fatty acids (FFA) were significantly lower in AN than control subjects and obese women ($P=$ 0.00003, $P=0.00001$, respectively). Adiponectin levels were negatively correlated with BMI ($r=-0.40$, $P=0{,}005$) and waist girth ($r=-0.44$, $P=$ 0.002). Fasting FFA concentrations were related negatively to insulin sensitivity ($r=-0.55$, $P=0.00007$) and to adiponectin concentrations ($r=-0.34$, $P=0.026$).

Our data show that lack of adipose tissue observed in anorectic patients has no influence on insulin sensitivity, probably due to low plasma FFA concentration. It points out that in AN the adipocytes are still capable of functioning at the level that is sufficient to prevent the metabolic consequences.

P21

Improved glucose metabolism and altered pancreatic structure in transgenic mice overexpressing betacellulin

Maik Dahlhoff[1], Nadja Herbach[2], Rüdiger Wanke[2], Eckhard Wolf[1] & Marlon R. Schneider[1]
[1]Institute of Molecular Animal Breeding and Biotechnology, University of Munich, Munich, Germany; [2]Institute of Veterinary Pathology, University of Munich, Munich, Germany.

Betacellulin, one of several peptides activating the EGFR (ErbB1) and related receptors, is a multipotent growth factor known to posses the unique ability to promote growth and differentiation of pancreatic β-cells.

We investigated the effects of betacellulin overexpression in a recently established transgenic mouse model (Schneider *et al.*, Endocrinology 146, 5237–5246, 2005). In transgenic animals, overall glucose metabolism was improved as demonstrated by reduced blood glucose levels in fasted animals and a better response after a glucose tolerance test (associated with increased serum insulin levels). Unexpectedly, the absolute and relative (proportional to body weight) pancreas weights were significantly reduced in transgenic mice. Histomorphometrical analyses revealed a reduction in the volume of the exocrine pancreas while the islet and β-cell volume remained unchanged. This resulted in an increase in the relative volume of the latter compartments. Interestingly, the proportion of β-cells within the islets remained unchanged in betacellulin transgenic mice. While betacellulin is normally expressed in the islets, immunohistochemistry revealed that the growth factor is, in addition, strongly expressed in the exocrine pancreas in transgenic mice. This uncovers a hitherto unknown negative effect of betacellulin in the exocrine compartment. Finally, we identified, by immunohistochemistry, an opposite expression pattern of ErbB1 and ErbB4, the primary receptors for betacellulin, in the pancreas. In this organ, ErbB1 is expressed predominantly in the islets, while ErbB4 expression is mostly restricted to the exocrine compartment. Thus, this particular receptor distribution may provide an explanation for the opposing effects exerted by betacellulin in the different pancreatic compartments.

Current experiments involve the study of cell proliferation and apoptosis as well as functional studies on isolated islets including insulin content and release.

P22

Screening of 120 adipokines in subcutaneous adipose tissue of patients with growth hormone deficiency reveals changed protein levels

Daniela Gasperikova[1], Jozef Ukropec[1], Adela Penesova[1], Henrike Sell[2], Martina Skopkova[1], Miroslav Vlcek[1], Zofia Radikova[1], Juraj Koska[1], Vitazoslav Belan[3], Mikulas Pura[4], Peter Vanuga[4], Juraj Payer[5], Jurgen Eckel[2] & Iwar Klimes[1]
[1]Institute of Experimental Endocrinology, Bratislava, Slovakia; [2]German Diabetes Center, Dusseldorf, Germany; [3]Department of Radiology, Faculty Hospital, Bratislava, Slovakia; [4]National Institute for Endocrinology and Diabetes, Lubochna, Slovakia; [5]V. Clinic of Internal Medicine, Faculty Hospital, Bratislava, Slovakia.

The role of adipokines and inflammatory cytokines of adipose tissue for development of the growth hormone deficiency (GHD)-related metabolic derangements has not yet been completely understood. Therefore, we screened the protein level of 120 adipokines in subcutaneous adipose tissue (ScAT) of patients with GHD in adulthood.

Subjects and methods

Sixteen GHD (10M/6F) with BMI 27 ± 1.0 kg/m^2, age 30 ± 2 yrs and sixteen controls matched for BMI, sex and age were included into the study. ScAT biopsies were performed after an overnight fast. Protein expression of adipokines was determined in tissue lysates using the RayBio®Human Cytokine Antibody Array C Series 1000.

Results

GHD subjects had higher waist circumference, circulating hsCRP levels and impaired glucose tolerance (as assessed by oGTT) ($P<0.05$). From 120 proteins, one showed to have higher (IGFBP-1) and three (BDNF, NT-3, SDF-1) lower levels in ScAT of the GDH subjects in comparison with controls ($P<0.05$). Majority of the observed changes were related to waist circumference, as became evident when we had separated individuals of both groups according to the IDF criteria (men ≥ 94 cm and female ≥ 80 cm). Interestingly, CNTF, EGF, GDNF, IL-1α, MIP3A, TGFβ1 and GCP2 were elevated, and GM-CSF lowered in parallel with increasing waist circumference selectively in the GHD individuals. On the other hand, HGF and TIMP2 were elevated while IL-7, MIP-3A, GITR, IGF1 SR, IL-17, IL-2Rα, MIP1β and Oncostatin M lowered with increasing waist circumference only in the controls.

Conclusions

Our data provide the first information on specific changes in the ScAT adipokine protein levels in GHD adults. Moreover, they implicate a different regulation of cytokine ScAT levels in a comparable inflammatory setting, i.e. in equally obese subjects who differ in their metabolic status.

Supported by APVV-51-0406/02 and Slovak Diabetes Association. The study was approved by the local Ethics Committee and conforms to the ethical guidelines of the Helsinki Declaration.

P23

Human somatotrophic (GH) adenoma cells – interleukin (IL)-1β induces production of il-6 and il-8.

Signe Diness[1], Åse Krogh Rasmussen[1], Klaus Bendtzen[2], Michael Kosteljanetz[3] & Ulla Feldt-Rasmussen[1]
[1]Department Of Medical Endocrinology, Copenhagen University Hospital, Copenhagen, Denmark; [2]Institute Of Inflammatory Research, Copenhagen University Hospital, Copenhagen, Denmark; [3]Department Of Neurosurgery, Copenhagen University Hospital, Copenhagen, Denmark.

Aim

to establish a human *in vitro* system for the study of pituitary cells in culture and subsequently to study the influence of the pro-inflammatory cytokines interleukin (IL)-1β and tumour necrosis factor (TNF)-α on the function of the somatotrophic cells inclusive the ability of the cells to produce IL-6 and IL-8.

Methods

Pituitary adenomas were obtained from hypophysectomies of patients with acromegaly. The tissue was enzymatically digested and cultured in 24-chamber polystyrene plates in medium supplemented with nutritional factors and

antibiotics and with 10^5 cells per well. GH and cytokines were measured in the harvested supernatants.
Results
GHRH (GH releasing hormone) (30,000 ng/ml) stimulated significantly 72 h GH production from the somatotrophic cells (25% (10–50), median (range), $n=12$ chambers, $P<0.05$) compared to controls (3525 mU/l (49-17450)), while somatostatin (0.1–10,000 ng/ml) inhibited the 72 h GH production from the cells compared to controls ($P<0.05$, $n=12$–18). The GH production was significantly lower in cells cultured more than 15 days compared to younger cell cultures (<15 days). IL-1β (1000 and 100 pg/ml) stimulated modestly the 72 h GH production from the cells compared to controls (20% (10–50), $n=18$) and (15% (10–60), $n=18$), while TNF-α had no influence the function of the cells. The effect of IL-1β was reversible. IL-1β (10,000, 100, 10 pg/ml) also stimulated 72 h IL-6 and IL-8 production from the cells. IL-1β (10,000 pg/ml) induced a mean 12.3 and 8.2-fold increase in IL-6 and IL-8, respectively compared to control (mean 1472 pg/ml and 1948 pg/ml, respectively) in 4 different cultures.
Conclusion
We have established a robust *in vitro* system for studying the function of GH producing pituitary cells; GH production from the cells exhibited the expected responses to GHRH and somatostatin. IL-1β further stimulated the release of IL-6 and IL-8 from the cells, an effect that has been established also in other endocrine cells such as e.g. thyrocytes. The physiological and/or pathophysiological roles of these findings remain to be shown.

Diabetes and cardiovascular – presented on Sunday

P24

Serum ferritin concentrations in an impaired fasting glucose population and their normal control group

Faranak Sharifi, Yahya Jaberi & Nuraddin Mousavinasab
Zanjan University of Medical Sciences, Zanjan, Iran.

Background
Some recent studies have revealed the relationship among excess ferritin, coronary heart disease, and insulin resistance. To assess the association between serum ferritin concentration and Impaired Fasting Glucose, a prediabetes situation with insulin resistance, this study was designed in Zanjan, Iran.
Materials & Methods
187 people including 91 impaired fasting glucose (IFG) subjects and 96 normal glucose subjects who had been recognized in a large epidemiological study in Zanjan in 2001 were enrolled. The cohorts were well matched for age, sex and BMI. Body mass index and blood pressure of the participants were measured and serum cholesterol, triglyceride and ferritin were evaluated. All the data were analyzed by t-test, x^2 test and analysis of variance.
Results
Serum ferritin was higher in the IFG cohort (85.5±6.6 μg/l vs. 49.4±3.7 μg/l, *P*: 0.001). A positive correlation was found between fasting plasma glucose and serum ferritin in this study (*r*: 0.29, *P*: 0.001). Using multiple regression analysis, we found an association between serum ferritin and BMI (0.06, *P*:0.4), blood pressure (0.15, *P*:0.01), FPG (0.29, *P*:0.001), triglyceride(0.08, *P*:0.01) and cholesterol(0.07, *P*:0.03). The odd's ratio for the association of IFG in male subjects with the high serum ferritin level was 8.3 (C.I 95%:1.2–11.9, *P*:0.01) and for females was 3.06 (C.I 95%:0.58–15, *P*:0.1).
Conclusion:
Our study, implying that hyperferritinemia occurs before elevation of plasma glucose concentration more than 126 mg/dl. If prospective and interventional studies Confirm an etiologic role of iron overload in the pathogenesis of insulin resistance and type 2 diabetes, reduced dietary iron intake, especially in men with additional risk factors for type 2 diabetes, would appear to be a logical consequence.

P25

Implications of serum resistin in overweight diabetic patients with ischemic heart disease

Azza Farrag, Sameh Salama & Amal Rizk
Cardiology Department, Cairo University, Cairo, Egypt; Cardiology Department, Cairo University, Cairo, Egypt; Critical Care Medicine, Cairo University, Cairo, Egypt.

Background
Resistin is a recently discovered adipocyte-secreted hormone that links obesity with insulin resistance and/or metabolic and cardiovascular risk. This study was designed to investigate whether serum resistin concentrations constitute a significant coronary risk factor, with a particular focus on diabetes and one of its microvascular complications; nephropathy.
Methodology
Serum resistin was measured in 86 overweight patients with acute coronary syndrome (ACS) and 16 overweight healthy controls. Patients were divided into two groups according to presence or absence of diabetes: IHD with diabetes ($n=46$), and IHD without diabetes ($n=40$). In addition, patients with diabetes were subdivided into two groups: diabetics with microalbuminuria ($n=26$) and without ($n=20$).
Results
Non-diabetic IHD patients had a significantly higher level of serum resistin when compared to control participants (15.3±13 vs 6.3±2.7 ng/ml, $P=0.008$). IHD patients with diabetes had a significantly higher level of serum cholesterol, LDL and resistin compared to IHD patients without (204±43 vs 181±31 mg/dl, $P=0.048$), (129±36 vs 111±23 mg/dl, $P=0.048$) and (41±33 vs 15.3±13 ng/ml, $P=0.002$) respectively. Working on diabetic patients, the only significant difference between patients with microalbuminuria and those without is serum resistin concentration (55±37 vs 23±14 ng/ml, $P=0.011$). Pearson correlations including all subjects showed that serum resistin concentration had a significant positive correlation with both total serum cholesterol ($r=270$, $P=0.05$) and serum LDL ($r=313$, $P=0.026$).
Conclusion
This study showed that serum resistin concentration is associated independently with coronary atherosclerosis in overweight patients. Serum resistin is increased in patients with diabetes mellitus particularly those with microalbuminuria.

P26

The protective effect of tribulus terrestris in diabetes

Amr Amin, Mohamed Lotfy & Ernest Adeghate
UAE Universiy, Al-Ain, United Arab Emirates.

Tribulus terrestris (*TT*) is used in the Arabic folk medicine to Q1 treat various diseases. The aim of this study was to investigate the protective effects of *TT* in diabetes mellitus (DM). Diabetes is known to increase reactive oxygen species (ROS) level that subsequently contributes to the pathogenesis of diabetes. Rats were divided into six groups and treated with either saline, glibenclamide (Glib), or *TT* for 30 days. Rats in group 1 were given saline after the onset of streptozotocin (STZ)-induced diabetes; the second diabetic group was administered Glib (10 mg/kg body weight). The third diabetic group was treated with the *TT* extract (2 g/kg body weight), while the first, second, and third nondiabetic groups were treated with saline solution, Glib, and *TT* extract, respectively. At the end of the experiment, serum and liver samples were collected for biochemical and morphological analysis. Levels of serum alanine aminotransferase (ALT) and creatinine were estimated. In addition, levels of malonyldialdehyde (MDA) and reduced glutathione (GSH) were assayed in the liver. The tested *TT* extract significantly decreased the levels of ALT and creatinine in the serum ($P<0.05$) in diabetic groups and lowered the MDA level in liver ($P<0.05$) in diabetic and ($P<0.01$) nondiabetic groups. On the other hand, levels of reduced GSH in liver were significantly increased ($P<0.01$) in diabetic rats treated with *TT*. Histopathological examination revealed significant recovery of liver in herb-treated rats. This investigation suggests that the protective effect of *TT* for STZ-induced diabetic rats may be mediated by inhibiting oxidative stress.

P27

Prevalence of I27L polymorphism of hepatic nuclear factor-1[alpha] in diabetic patients younger than 35 years old.

Norma Amador, Manuel López, Jose Sánchez, Juan Guízar, Alejandra Zapién & Silvia Martínez
Mexican Institute Of Social Security, Leon/Guanajuato, Mexico.

Objective
In last decades prevalence of type 2 diabetes mellitus (DM) in children and young people worldwide has been reported increase. It is necessary to know according to biochemical and genetic characteristics the frequency of DM no corresponding to type 1 in our population. The objective of this study was to determine the prevalence of mutations on hepatic nuclear factor 1 [alpha], and 4 [alpha] in

diabetic patients younger than 35 years old with features of clinical autosomal dominant inheritance.
Material and Methods
The study included 140 diabetic patients (85 children and 55 young adults). It was approved by the local Ethical Committee. Glucose, C peptide, and β-cell autoantibodies measurements were performed. Polymorphisms of HNF [1 alpha] (I27L, G319S), and HNF [4 alpha] (T130I) were determined in all patients, when one of the polymorphisms was identified in a patient, all his/her family was studied by genetic evaluation.
Results
More than 50% patients showed overweight or obesity. The presence of DM in the father, overweight, and C peptide levels were higher in adults, while obesity, hypercholesterolemia, and β-cell autoantibodies were more frequent in those patients younger than 18 years old. Forty one (29.2%) patients showed the I27L polymorphism (24-Ile^{27}Leu and 17-Leu^{27}Leu). These patients were older, had higher BMI and C peptide levels than Ile^{27}Ile patients, and only 3 of them showed β-cell autoantibodies. In 5 patients we identified Thr^{130}Ile, and in one Gly^{319}Ser polymorphisms. I27L mutation was present in 30 families and T130I in one family. Patients in these families were older and showed higher BMI and C peptide levels, but lower glucose levels.
Conclusion
I27L polymorphism was present in almost a third part of diabetic patients with clinical autosomal dominant inheritance of the disease. These patients showed clinical and biochemical characteristics of DM no corresponding to Type 1 DM.

P28

Novel mechanism of chronic exposure of oleic acid-induced insulin release impairment in rat pancreatic β-cells

Takanori Kudo[1], Yoshiji Ogawa[1], Sechiko Suga[2], Ken Tomotsune[1], Noriyuki Hasegawa[1], Toshihiro Suda[1] & Makoto Wakui[3]
[1]Third Department of Internal Medicine, Hirosaki University School of Medicine, Hirosaki, Aomori, Japan; [2]Center for Education and Research of Lifelong Learning, Hirosaki University, Hirosaki, Aomori, Japan; [3]Division of Clinical Research, Hirosaki National Hospital, Hirosaki, Aomori, Japan.

A sustained, high circulating level of free fatty acids (FFAs) is an important risk factor for the development of insulin resistance, islet beta-cell dysfunction, and pathogenesis of type 2 diabetes. Here, we report a novel mechanism of chronic exposure of oleic acid (OA)-induced rat insulin release impairment. Following a 4-day exposure to 0.1 mM OA, there was no significant difference in basal insulin release when comparing OA-treated and untreated islets in the presence of 2.8 mM glucose, whereas 16.7 mM glucose-stimulated insulin release increased 2-fold in control, but not in OA-treated, islets. Perforated patch-clamp recordings showed that untreated beta-cells exhibited a resting potential of $-62.1 +/-0.9$ mV and were electrically silent, whereas OA-treated beta-cells showed more positive resting potentials and spontaneous action potential firing. Cell-attached single-channel recordings revealed spontaneous opening of ATP-sensitive potassium (K(ATP)) channels in control, but not in OA-treated, beta-cells. Inside-out excised patch recordings showed similar activity in both OA-treated and untreated beta-cells in the absence of ATP on the inside of the cellular membrane, whereas in the presence of ATP, K(ATP) channel activity was significantly reduced in OA-treated beta-cells. Electron microscopy demonstrated that chronic exposure to OA resulted in the accumulation of triglycerides in beta-cell cytoplasm and reduced both the number of insulin-containing granules and insulin content. Collectively, chronic exposure to OA closed K(ATP) channels by increasing the sensitivity of K(ATP) channels to ATP, which in turn led to the continuous excitation of beta-cells, depletion of insulin storage, and impairment of glucose-stimulated insulin release.

P29

Quality of care in a diabetic outpatient clinic

Ema Lacerda Nobre, Paula Chambel, Andreia Domingues, Marlene Paes da Silva, Zulmira Jorge, Valentim Santos, Joaquim Raimundo & João Jácome de Castro
Hospital Militar Principal, Lisboa, Portugal.

Background and aims
Type 2 Diabetes Mellitus affects a growing number of people allover the world. It is associated with serious complications. Several studies have shown that it is possible to prevent and minimize type 2 diabetes complications if it is treated appropriately over time. In our Hospital there is, since 1998, an outpatient clinics of diabetes. This study aimed to determine the quality of care provided to diabetic patients in our institution.
Subjects and methods
We reviewed the medical records of 776 diabetic patients, receiving care at our outpatient clinics since 1998.
Results
A total of 588 patients were included in the study, 58% were men with a mean age of 66.8 ± 27.2. HbA1c levels averaged 7.2 ± 1.65. 25.3% met the target blood pressure of 130/80 mmHg; 48% met the goal LDL cholesterol level <100 and 80% <130 mg/dl. 6.8% of patients met the combined ADA goal for BP, LDL and HbA1c. Concerning therapeutic regimens: 71.5% used oral hypoglycaemic agents (OAD) alone (52.1% of these were using 2 or more agents); 28.5% were treated with insulin (16.2% in combination with OAD).
Conclusions
HbA1c values reflects a good metabolic control. We emphasise the importance of combined therapy in the achievement of optimal glycaemic levels.The percentage of patients treated to the recommended BP of 130/80 mmHg is consistent with the results of other studies. LDL cholesterol levels compares favourable to the NHANES III study and is comparable with other published data. Despite the proved benefits of CV risk factors control in diabetic patients, international recommendations are difficult to achieve in clinical practice.

P30

Cardiovascular risk factors (CVRF) as predictors of microalbuminuria (MA) in type 2 diabetes mellitus (T2DM) patients

Francisco Javier del Cañizo-Gomez[1] & Maria Natividad Moreira-Andres[2]
[1]Hospital Virgen de la Torre, Madrid, Spain; [2]Hospital Universitario 12 de Octubre, Madrid, Spain.

MA is a marker of greatly increased cardiovascular morbidity and mortality in T2DM patients.
Objective
To perform a prospective study of normoalbuminuric T2DM patients, analysing the association between CVRF at baseline and the development of MA at follow-up.
Materials and methods
The prospective observational study was performed at Montes de Barbanza public health center, a specialized secondary referral center, which provides services to the 31 urban district of Madrid, Spain, and consisted in 348 T2DM patients. The inclusion criterion at baseline in 2002 was normoalbuminuria (urine albumin <30 mg/24 h.), and the exclusion criteria were previously diagnosed micro or macroalbuminuria or nephropathy. The clinical end-point was MA (urine albumin 30–300 mg/24 h.) at follow-up in 2005. The variables at baseline in 2002 were age, gender, onset-age of T2DM, HbA_{1C}, systolic (SBP) and diastolic blood pressure (DBP), total cholesterol (TCh), HDL-Ch, LDL-Ch, triglycerides (TGs), BMI and smoking; and were obtained from our records. Diagnosis of MA was made by two consecutive quantitative test of urine collected over 24 h. Comparison of mean levels were performed with the Student's "*t*" test for unpaired samples, and proportions with the chi-square test. Logistic regression analyses were performed with MA as a dependent variable, and age, gender, diabetes duration, and other CVRF as independent variables. An odds ratio (OR) >1.0 signifying a positive association, and $P<0.05$ was considered significant (SPSS, v. 13.0).
Results
Compared to those who still had normoalbuminuria at follow-up, the ones progressing to MA were males ($P=0.000$), and more likely to have a higher SBP ($P=0.001$) and TGs ($P=0.005$), and a lower HDL-Ch ($P=0.002$). The principal independent CVRF at baseline for the development of MA at follow-up were male gender (OR:3.36; $P=0.000$), elevated TGs (OR:2.17; $P=0.005$) and increased SBP levels (OR:1.03; $P=0.001$).
Conclusions
Male gender, elevated TGs and increased SBP, were independent CVRF for the development of MA in T2DM patients of the population studied. Other CVRF, as decreased HDL-Ch, was associated to MA in T2DM patients

P31

The prevalence of metabolic syndrome and its relation to metabolic control in patients with diagnosed type 2 diabetes

Florian Toti[1], Gazmend Bejtja[2], Kliti Hoti[1], Eduart Shota[1] & Erion Thengjilli[2]
[1]UHC "Mother Theresa" Service of Endocrinology & Metabolic Diseases, Tirana, Albania; [2]Institute of Public Health, Tirana, Albania.

Background and Aims
The identification of metabolic syndrome (MS) is important so that components of this syndrome can be managed appropriately to prevent or delay progression of associated cardiovascular risk factors. The aim of our study was to determine the prevalence of the Metabolic Syndrome as the NCEP/ATP III criteria in a selected population of type 2 diabetes from the Tirana Register of Diabetes.
Materials and Methods
In Tirana district we randomly selected 300 patients from the Tirana Register of Diabetes. 220/300 (73.3%) of the patients responded. All the patients had completed anthropometric measures and lipid profile after an 8-hour fast. All the patients having three or more of the criteria were defined as having Metabolic Syndrome (MS).
Results
The prevalence of the MS was 64.5%, in men 56.8% and 75.7% for women. The prevalence increased with age, from 16% before 40 years of age to 78% after 70 years. Diabetes duration was not different in patients with MS than those without it (M: 6.7 ± 3.4 vs 6.9 ± 3.7; F: 7.2 ± 3.8 vs 6.8 ± 3.6 yrs). The number of components of the MS was related to the age (ANOVA $P<0.05$) but not to diabetes duration. Central obesity was present to 36% of men and 85.4% of women, HTA 49.6 and 60.2%, low HDL 52 and 90%, high triglycerides 70.9 and 66.7% respectively. HbA1c was higher in persons with MS (9.6 ± 2.2 vs $8.7\pm1.4\%$, $P<0.01$).
Conclusion
The results show that MS is two-fold more prevalent in type 2 diabetes, compared with the general albanian population (64.5 vs 32%). The levels of cardiovascular risk factors are increased in type 2 diabetics and urged immediate efforts directed at controlling the components (mainly obesity, physical inactivity and lipid control) of MS especially in type 2 diabetes.

P32

Effects of rosiglitazone (RGZ) and pioglitazone (PGZ) on serum androgens and urinary steroid profile in patients with type 2 diabetes: A prospective, randomised cross-over study

Bernard Chappuis, Monika Braun, Christoph Stettler, Peter Diem & Emanuel Christ
Department of Endocrinology; Diabetology and Clinical Nutrition, University Hospital of Bern, Inselspital, Bern, Switzerland.

Background
Glitazones (GZ) influence androgen biosynthesis in PCO syndrome. At present it is unknown whether a) steroid hormone metabolism is influenced by GZ in patients with type 2 diabetes b) there is a differential effect of RGZ and PGZ on steroid hormone metabolism c) this effect is sex-specific and d) this effect is mediated by changes in insulin sensitivity. Therefore, urinary steroid profiles and serum total testosterone and DHEA levels were analysed before and after therapy with RGZ and PGZ in patients with type 2 diabetes.
Methods
17 patients with type 2 diabetes (7 women, 10 men, age: 60.8 ± 9.6, years, mean $\pm$ SD; BMI: 29.2 ± 4.7, kg/m^2; $HbA1_c$: $7.6\pm0.6\%$) were included in the study and assigned to RGZ or PGZ in a randomised cross-over study design for 12 weeks with an eight-week wash-out period in-between. Identical investigations (24-h-urinary steroid profile, plasma glucose (FPG), insulin (FI), $HbA1_C$, serum total testosterone and DHEA concentrations) were performed before and after each treatment period.
Results
RGZ and PGZ therapy resulted in a similar decrease in $HbA1_C$, FPG and FI concentrations without sex-specific differences. In *men*, RGZ resulted in a significant increase in serum testosterone levels compared to PGZ (RGZ: $+2.5\pm2.1$; nmol/L; mean $\pm$ SD; PGZ: $+0.5\pm3.3$; $P<0.04$), whereas DHEA concentrations remained unchanged. In *men* changes of urinary androstentriol, an androgen precursor, were significantly different after RGZ compared to PGZ (RGZ: $+45.7\pm158.1$; mcg/24 h; PGZ: -119 ± 161.1; $P<0.05$). In *women*, RGZ therapy resulted in a significant decrease in serum testosterone concentrations after RGZ compared to PGZ (RGZ: -0.3 ± 0.3; nmol/L; PGZ: $+0.3\pm0.4$; $P<0.05$). Serum DHEA levels were unaffected by PGZ and RGZ. In *women*, there were similar effects of PGZ and RGZ on urinary androgen metabolites.
Conclusion
These data suggest that 1. GZ impact on steroid hormone synthesis, 2. there is a differential effect of RGZ and PGZ 3. this effect is sex-specific and 4. this effect is not mediated by a differential effect of RGZ

P33

Abnormal glucose challenge test reflects mild gestational diabetes

Ayse Kafkasli[2], Ayse Sertkaya Cikim[1], Engin Burak Selcuk[3], Kezban Dogan[2], Feza Burak[5] & Saim Yologlu[4]
[1]Inonu University Faculty of Medicine Department of Internal Medicine Division of Endocrinology and Metabolism, Malatya, Turkey; [2]Inonu University Faculty of Medicine Department of Obstetrics and Gynecology, Malatya, Turkey; [3]Inonu University Faculty of Medicine Department of Family Medicine, Malatya, Turkey; [4]Inonu University Faculty of Medicine Departmant of Biomedical Statistics, Malatya, Turkey; [5]Ozel Dogu Hastanesi, Malatya, Turkey.

Objective
The status of carbohydrate metabolism of pregnant women with positive glucose challenge test (GCT), but normal oral glucose tolerance test (OGTT); and their neonates have not defined clearly.
Methods
Pregnant women with normal GCT (*n*: 120), with abnormal glucose challenge test (AGCT) but normal OGTT (*n*: 67) and those with gestational diabetes (GDM) (*n*: 67) were included into the study. Local ethical committee approval was obtained. Insulin sensitivity was evaluated by fasting insulin level, homeostasis model assessment of insulin resistance index (HOMA-IR); quantitative insulin check index (QUICKI) and IS_{OGTT}. Serum insulin and glucose values during OGTT were documented. The patients with both AGCT and GDM were treated either with diet or if needed with insulin until achieving the goals for defined glucose values. Perinatal outcome and delivery modalities were also compared between these three groups.
Results
Both GDM (31.6 ± 5.9 yrs) and AGCT groups (29.0 ± 4.0 yrs) were older than control subjects (28.1 ± 4.9 yrs). Body mass index (BMI) was found to increase with a correlation to the severity of carbohydrate intolerance as the predominant factor affecting both AGCT and GDM groups (odds ratios were 3.78 and 5.97 respectively). Despite there was no significance between insulin indices; serum glucose and insulin values were similarly different than controls in both AGCT and GDM groups. Macrosomic infant and caesarean section rates were higher than control group in both GDM and AGCT groups in favor of gestational diabetics (6.6% vs. 18.9%; $P=0.0001$ and 20% vs. 27.7% $P=0.0001$ respectively).
Conclusion
Pregnant woman with abnormal glucose challenge test have impaired carbohydrate metabolism as in gestational diabetics with a lesser severe degree.

P34

Ambulatory blood pressure reduction after rosiglitazone treatment in normotensive type 2 diabetic patients

Yasemin Kemal[1], Neslihan Tutuncu[1], Nilgun Guvener[1], Asli Unal[1], Ilyas Atar[2] & Haldun Muderrisoglu[2]
[1]Baskent University Faculty of Medicine Department of Endocrinology, Ankara, Turkey; [2]Baskent University Faculty of Medicine Deoartment of Cardiology, Ankara, Turkey.

Objective
The thiazolidinediones are new and potentially useful developments in the treatment of type 2 diabetes and impaired glucose tolerance. We tested the effects of the thiazolidinedione, rosiglitazone on blood pressure in normotensive type 2 diabetes.
Methods
After receiving approval from the local ethics committee, 25 normotensive diabetic patients were were enrolled to the study. Before the rosiglitazone treatment we measured plasma glucose, HbA1c, Hb, lipid profile and BMI. Also each subject underwent ambulatory blood pressure recording. Subjects were then placed on rosiglitazone treatment (8 mg per day) for twelve weeks, and baseline tests were repeated.

Results
At the end of twelve weeks there were significant decreases in total average diastolic blood pressure (67.02±8.06 vs 62.58±5.90, $P<.009$) and daytime average diastolic blood pressure (68.64±8.51 vs 65.12±6.34, $P<.01$). In addition, there were also significant decreases in fasting plasma glucose ($P=.007$), postprandial plasma glucose ($P=.01$), HbA1c ($P=.010$), and Hb levels ($P=.005$). Correlation analysis revealed that changes in diastolic blood pressures were not correlated with the decrease in both Hb, HbA1c. Also there was no significant correlation between the improvement in fasting and postprandial blood glucose and the decline in blood pressure.
Conclusion
Our study demonstrated a significant and sustained reduction in diastolic blood pressure with rosiglitazone therapy for 12 weeks, which was independent from the blood-glucose–lowering effect of the drug. Long-term studies are needed to determine the TZD-associated effects on blood pressure and other cardiovascular risk factors.

P35

Time dependent effects of rosiglitazone on heart and fluid dynamics: a 6-month follow up study

Yasemin Kemal[1], Nilgun Demirag[1], Neslihan Tutuncu[1], Aylin Yildirir[2], Asli Atar[2] & Asli Unal[1]
[1]Baskent University Faculty of Medicine Department of Endocrinology, Ankara, Turkey; [2]Baskent University Faculty of Medicine Department of Cardiology, Ankara, Turkey.

Objective
Thiazolidinediones (TZDs) have become a powerful tool for lowering insulin resistance. The problem of cardiovascular adverse events including fluid retention and risk of heart failure, although of a low incidence, should be well known and recognized. We aimed to evaluate the effects of rosiglitazone treatment on cardiac function and show whether these effects are reversible when we continued this treatment.
Methods
Fourty-six type 2 diabetic patients -without any symptoms and findings of heart failure-were randomized to treatment with rosiglitazone, metformin and control group after receiving approval from the local Ethical Committee. There were no significant differences between the groups in the duration of diabetes, HbA1c and plasma brain natriuretic pepetide (BNP) levels, body mass index (BMI) and myocardial performance indexes (MPI) before the treatment. After three months and after six months all these parameters were repeated.
Results
After three months period with rosiglitazone treatment, plasma BNP levels increased rapidly. Except one subject we did not see any clinical adverse effect including excessive weight gain, edema, and dyspnea so we continued rosiglitazone treatment. At the end of the six months period, this rapid increase didn't continue. Similarly, lateral wall MPIs worsened after three months- although statistically nonsignificant- and then improved significantly after six months in rosiglitazone group ($P=0.001$). Also the changes in hemoglobin values were highly correlated with other results that provide evidence of these reversible findings.
Conclusion
Our study showed the stability and reversibility of the adverse effects of TZDs on cardiovascular function and fluid dynamics in type 2 diabetics.

P36

Peculiarities of heart rate control in patients with non-insulin dependent diabetes mellitus and hypertension

Andris Vitols, Daina Voita & Mara Vitola
Institute of Cardiology, University of Latvia, Paul Stradins Clinical Hospital, Riga, Latvia.

Objective
To assess the sensitivity of exercise induced heart rate (HR) and baroreceptor reflex (BR) chronotrope reaction and HR variability for an early detection of autonomic nervous system impairment in non-insulin dependent diabetes mellitus (NIDDM) patients with arterial hypertension.
Design and Methods
On 25 NIDDM pts (group A, 63±1.8 yrs. aged men, HbA_{1C} 10.2±0.9%), 17 essential hypertension (EH) pts without glucose metabolism disturbances (group B, gender and age matched) and 20 controls (C) at rest and during handgrip (with force 50% of maximal for 60 s), beat-to-beat HR and finger mean arterial pressure (MAP) were monitored and bradycardic reaction to BR activation (by neck suction −60 mmHg) was analysed. HR variability by time and frequency domain analysis of ECG 512 R-R interval files was performed in supine and upright postures.
Results
Group A comparing to B and C was characterised by increased HR (81±2 vs.72±3 vs.70±3 bpm; $P<0.05$) and decreased bradycardic reaction to BR activation (1.95±0.3 vs. 4.9±0.9 vs.10±0.6 bpm; $P<0.05$). At 60th sec of handgrip MAP increase was similar in all groups but HR increase was reduced in group A vs. B vs.C (12±2 vs. 24±2 vs.18±2 bpm; $P<0.05$), but reaction to BR activation disappeared in group A and B, whereas in C remained in 32±11% of resting value. R-R interval variability in group A and B was diminished ($P<0.01$), but its decrease in upright position was less in group A than in C (108±12 vs. 254±21 ms; $P<0.05$), whereas the difference of increase in low-high frequency band ratio (LF:HF) was not significant in group A and B.
Conclusion
In patients with non-insulin dependent diabetes mellitus and hypertension, HR reaction to exercise and BR activation has an advantage over HR variability analysis to ascertain an early impairment of autonomic control of sinus node.

P37

One injection of Detemir insulin administered before the lunch improves the metabolic control in type 1 diabetic patients

Juan Carlos Ferrer-García[1], Juan Francisco Merino-Torres[2], Raquel Segovia-Portolés[2], Vicente Campos-Alborg[2], Agustín Herrera Ballester[1], Francisco Piñón Sellés[2] & Carlos Sánchez Juan[1]
[1]University General Hospital, Valencia, Spain; [2]University Hospital La Fe, Valencia, Spain.

Objective
To compare 2 modalities of bolus-basal insulin therapy with aspart-detemir, according to the moment of administration of detemir (DET), before the lunch or bed-time, in type 1 diabetic patients with poor metabolic control.
Methods
We conducted a prospective study of 40 type 1 diabetic patients, with poor metabolic control (HbAc 7–12%), randomized to receive treatment with 1 injection of DET before the lunch or bed-time and followed-up during 24 weeks. Physician decided the addition of one second dose, administering DET every 12 hours (DET-12 h) if the objectives in glycemic control were not obtained. Insulin analog aspart was used for the post-prandial control. Weight, insulin units/Kg/day, HbA1c, score in a test of quality of life (ITQ7) and hypoglycemias were determined.
Results
19 patients in DET pre-lunch group and 16 in DET bed-time group completed the study. 10 patients of group DET pre-lunch and 12 of DET bed-time needed DET-12 h. After 24 weeks of bolus-basal insulin therapy, a reduction of HbA1c was demonstrated, and the group DET pre-lunch showed a major reduction of HbA1c. By groups of treatment: DET pre-lunch 8.5 vs 7.1% ($P<0,05$); DET bed-time 9.0 vs 7.6% ($P<0.05$.) and DET-12 h 8.8 vs 8.1% ($P<0.05$.). The ITQ7 demonstrated an improvement without differences between the groups (score baseline visit 74.5±17.3 versus 62.0±19.2; $P<0.01$). There were no differences in weight and number of non-serious hypoglycemia. Serious hypoglycemia was presented in one patient of DET bed-time group. An increase in the insulin requirements was demonstrated in the 3 groups of treatment (average: 0.78±0.2 u/kg/day in baseline visit versus 0.86±0.2; $P<0.05$).
Conclusion
after this study, we recommend to begin detemir insulin treatment with one injection administered before the lunch. However, a strict monitoring is necessary because some patients will require two injections of detemir.

P38

Oral antibodies to insulin receptor are found effective in the treatment of streptozotocin-induced diabetes in rats

Julia Dugina[1], Andrey Martyushev-Poklad[1], Irina Kheyfets[1], Alexander Spasov[2], Maria Samokhina[2], Oleg Epstein[1] & Svetlana Sergeeva[1]
[1]'Materia Medica Holding' Company, Moscow, Russia; [2]Volgograd State Medica University, Volgograd, Russia.

An experimental study was designed to test a drug candidate for the treatment of diabetes mellitus in rats with streptozotocin (STZ) diabetes.

Diabetes was induced in outbred male rats (280–300 g) by single iv injection of streptozotocin 50 mg/kg. The animals showing hyperglycemia (12–15 mM) 72 hours after injection were randomized to receive daily intragastric doses of distilled water, glibenclamid 8 mg/kg, or polyclonal antibodies to C-terminal fragment of insulin receptor, beta subunit (ultra-low doses, anti-InsR); the last group received insulin subcutaneously (12 U/kg). For 7 weeks, the animals were monitored for fasting glycemia, glycosuria, and glucose tolerance.

STZ caused a sustained hyperglycemia (12–21 mM versus 2.3–3.2 in intact rats, maximum at day 42) and glycosuria (2.7–3.7 mM versus 0.8–1.8 mM in intact rats). Glucose tolerance reduced 3.5–5.5-fold (calculated by AUC in glucose load test). The rats featured polydipsia (an 2.7–3.2-fold increase in water consumption), body weight reduced by 50%. Due to diabetes and its complications, survival rate reduced to 12.5% (from 100% in intact rats).

Glycemia reduced by 30–50% in insulin group, and by 10–42% glibenclamid group, though remained abnormal. STZ-induced glycosuria remained unaffected in both groups. Survival rate increased up to 20%. Peroral anti-InsR was much more effective in reduction of glycemia (to normal values, 5.0–3.0 mM) and glycosuria (below 0.8 mM). Anti-InsR enhanced survival to 30%. The increase in glucose tolerance was most considerable in insulin and anti-InsR groups, less marked in glibenclamid group.

The peroral anti-InsR agent is regarded as a promising candidate therapeutic for the treatment of diabetes mellitus.

P39

1 year endurance training at the level of the ventilatory threshold in type-2 diabetics reduces by 50% healh costs: a controlled randomized trial

Brun Jean-Frédéric, Jaussent Audrey, Picot Marie-Louise, Mercier Jacques & Oréfaut Christian
CERAMM Service Central de physiologie Clinique, Hôpital Lapeyronie, Montpellier, France.

This trial was undertaken in order to evaluate the effects of endurance training on health cost in type 2 diabetes. 35 diabetic patients were randomly assigned to 2 groups: After 10 drop-outs, 15 followed a training program (8 sessions followed by training at home at the level of the ventilatory threshold V_T) while 10 had only routine treatment. Both groups were followed over 1 year with evaluation at 30, 120, 240 and 365 days for health costs, blood pressure, and a standard maximal exercise test, glycemic and lipid equilibrium, 6-min walking test, and exercise (Voorips) and quality of life questionnaires. The effectiveness of training was confirmed in the trained group by an increase in the Voorips score (5.25 ± 3.3 $P < .001$) and a lack of decrease in VO_{2max} and P_{max} while in the untrained group VO_{2max} decreased slightly (-2.16 ± 2.5 $P = 0.014$). Thus trained subjects at the end of the study reached a higher percentage of the theoretical maximal power ($P = 0.041$). The 6-min walking distance (472.2 ± 98.9 vs 547.6 ± 56.7 $P = 0.020$) was also higher than in the control group. Blood pressure, lipid profile and glycemic control did not significantly improve during this period in either groups, due decreasing doses in treatments prescribed by their physicians. In the trained group there was no hospitalization, in contrast ($P = 0.047$) with controls in whom there was 1.27 ± 2.20 (ie, 0 to 5 days) of hospitalization. The total health cost over this period is lowered by 50% in the trained group ($P = 0.018$). In conclusion, endurance training at the level of the V_T significantly prevents the progressive decline in aerobic working capacity evidenced in untrained diabetics over this period of observation. It results in a marked reduction in health cost due to a decrease in treatment and fewer hospitalizations.

P40

PED levels are increased in peripheral blood leucocytes from euglycaemic subjects at-risk of type 2 diabetes

Rossella Valentino[1], Gelsy Arianna Lupoli[1], Pietro Formisano[1], Iolanda Esposito[1], Anna Teresa Alberobello[1], Anna Perfetti[1], Michele Cirillo[2], Nicola Gennarelli[2], Giovanna Gargiulo[2], Roberto Lobello[2] & Francesco Beguinot[1]
[1]Dpt. Cellular and Molecular Biology and Pathology, Naples, Italy; [2]Dpt. Endocrinology/General and Digestive Surgery, Naples, Italy.

Background
Phosphoprotein enriched in diabetes (PED) is a scaffold protein widely produced in different tissues; it is involved in multiple cellular functions, including insulin-regulated glucose transport. Previous findings showed that in individuals with type 2 diabetes (T2D) the PED gene is overexpressed in skeletal muscle (SM) and adipose tissue (AT), both target tissues for insulin activity. Our group has recently evidenced that PED protein is also expressed in peripheral blood leucocytes (PBLs) and overexpressed in about 30% of diabetics.
Aim
To investigate the presence of any correlation in PED expression between PBLs and insulin-sensitive tissues, in order to validate this method as a possible screening in at-risk subjects for T2D.
Subjects and methods
21 subjects were recruited: 14 euglycaemic (7 T2D first degree relatives (FDR) and 7 without T2D family history) and 7 T2D patients. We evaluated PED protein expression analysing lysates from AT and SM, and PBLs by immunoblotting with specific PED antibodies.
Results
A two-fold increase in PED levels in AT and SM was found both in T2D patients and in FDR, compared with euglycaemic controls. On the whole, PED levels were 30% higher in PBLs than in SM and AT ($P < 0.001$) from the same subjects. Moreover, in all subjects there were significant correlations between PED levels in the PBLs and those in AT and in SM ($P < 0.001$).
Conclusions
PED expression can be detected in PBLs and its expression is correlated with that in insulin-sensitive tissues. Therefore, this method could become a valid aid to identify at-risk individuals for diabetes in large scale studies.

P41

Effectiveness of α-lipoic acid in prevention of peripheral diabetic neuropathy

Nino Jikurauli, Ellen Giorgadze, Nino Svani, Vasil Chachibaia, Felix Bestavashvili, Tea Lominadze, Nia Chanturia & Anna Malazonia
N4 clinical hospital, Tbilisi, Georgia.

Background
The aim of our study was to assess the effectiveness of α-lipoic acid for the reducing the risk of developing peripheral diabetic neuropathy.
Subjects and methods
We have studied 38 patients with type I and II diabetes, from age 28 to 35. The mean duration of disease was 9 years. Patients were divided in two groups. In group I were included 15 patients which got α-lipoic acid in order to prevent peripheral diabetic neuropathy in dosage 600 mg-50 ml a day i/v infusion during 3 weeks, then 600 mg a day per-os, during 2 months. The treatment was provided twice a year during the 2 year period. In group II were included 23 patients, who did not got α-lipoic acid. In both groups we studied HbAIC, fasting glucose, lipid profile, sensation screening tests; knee-jerk and tendon reflexes, subjective complaints were estimated by TSS scale.
Results
In I group of the patients the mean value of HbAIC was 7.0%, fasting glucose 133 mg/dl (±20); total Chol. 208 (±30), Trig 198 (±15), HDL 76 (±10); LDL 112 (±12), vibration sensation was decreased in 4 and a temperature sensation in 2 patients. The tactile and pain sensation were preserved. Knee-jerk and tendon reflexes were not changed, subjective symptoms by TSS were – 1.00;
In group II HbAIC was 7.2%, fasting glucose 138 mg/dl (±25); total Chol. 234 (±33); Trig 265 mg/dl; HDL 71 mg/dl; (±10); LDL 116 mg/dl, vibration sensation was decreased in 10, tactile sensation - in 2 and a temperature sensation in - 6 patients. The tactile and pain sensation were preserved. Knee-jerk and tendon reflexes were not changed, subjective symptoms by TSS was – 1.33;
Conclusion
Administration of α-lipoic acid for the lowering the risk of developing of peripheral diabetic neuropathy is required.

P42

Adhesion molecules s-VCAM-1 and s-ICAM-1 in members of families with familial combined hyperlipidemia

David Karásek, Helena Vaverková, Milan Halenka, Zdenek Fryšák, Dalibor Novotný & Marie Budíková
University Hospital, Olomouc, Czech Republic.

Objective
Familial combined hyperlipidemia (FCH) is the most common familial hyperlipidemia with a high risk of the early atherosclerosis. The aim of this study was to compare levels of s-ICAM-1 and s-VCAM-1 in asymptomatic members of FCH families with healthy controls and to find out relation between s-ICAM-1, respective s-VCAM-1, and risk factors accompanying FCH. We also investigate association between adhesion molecules and intima-media thickness of common carotid artery (IMT) in FCH families.
Methods
82 members of 29 FCH families were divided into the 2 groups: HL (probands and hyperlipidemic first-degree relatives, $n=47$) and NL (normolipidemic first-degree relatives, $n=35$). The control groups – HL-C ($n=20$) and NL-C ($n=20$) – consisted of sex- and age–matched healthy individuals.
Results
Hyperlipidemic members had significantly higher concentration of s-ICAM-1 (633.7 ± 169.6 ng/ml vs 546.2 ± 155.9 ng/ml, $P<0.05$). The elevation of s-VCAM-1 was not significant (880.8 ± 202.9 ng/ml vs 826.5 ± 174.6 ng/ml, N.S.). Levels of s-ICAM-1, respectively of s-VCAM-1 in normolipidemic relatives were not significantly different compared to the control group (530.8 ± 113.9 ng/ml vs 530.0 ± 101.0 ng/ml, respectively 860.2 ± 265.7 ng/ml vs 822.1 ± 197.0 ng/ml). There was significant correlation between s-ICAM-1 and apoB ($r=0.42$; $P<0.01$) in hyperlipidemic subjects and between s-ICAM-1 and proinsulin ($r=0.54$; $P<0.01$) in normolipidemic subjects. S-ICAM-1 correlated with IMT ($r=0.32$; $P<0.05$) in all members of FCH families.
Conclusions
The increase of s-ICAM-1 in asymptomatic hyperlipidemic members of FCH families reflects their high cardiovascular risk. The positive association between s-ICAM-1 and IMT could indicate s-ICAM-1 as a potential predictor of atherosclerosis manifestation.
This work was supported by grant IGA MZCR NR/ 9068-3.

P43

Body fat distribution, lipid and adipokine levels in South African type 2 diabetic patients of African and Indian origin

Rita Waisberg, Janice Paiker, Jaya George & Nigel Crowther
NHLS, Johannesburg, Gauteng, South Africa, WITS; Johannesburg, Gauteng, South Africa.

Introduction
The increased insulin resistance seen in Type 2 diabetic patients has been shown to be associated with abdominal fat accumulation, hypertension, and dyslipidaemia. The dyslipidaemia is characterized by raised serum triglycerides and decreased high density lipoprotein cholesterol (HDL) levels. The aim of the present study was to investigate the relationship between abdominal fat, lipid and adipokine secretion in South African type 2 diabetic patients of Indian and African origin.
Methods
Plasma and serum samples were collected from 20 African and 20 Indian diabetic females. Adipokines were measured using ELISA kits. Fasting plasma glucose, serum cholesterol, HDL-cholesterol, and triglycerides were assayed on the ROCHE MODULAR System. Insulin resistance was calculated using HOMA. CT-scans were performed to measure abdominal visceral and subcutaneous fat areas.
Results
Data presented as mean values $\pm$ SEM. The results for diabetic African (DA) and diabetic Indians (DI), respectively were as follows: Leptin (ng/ml) 40.6 ± 2.49 and 43.6 ± 2.1, soluble leptin receptor (U/ml) 21.0 ± 1.71 and 20.5 ± 1.7, IL-6 (pg/ml) 3.15 ± 0.58 and 3.87 ± 1.08, TNF-alpha (pg/ml) 7.06 ± 1.38 and 2.26 ± 0.42 ($P=0.003$), CRP (mg/l) 11.4 ± 3.09 and 8.97 ± 1.58, cholesterol (mmol/l) 4.78 ± 0.22 and 5.24 ± 5.24, HDL (mmol/l) 1.17 ± 0.05 and 1.76 ± 0.4, and triglyceride (mmol/l) 1.41 ± 0.11 and 2.11 ± 0.36, respectively. HOMA results for DI were 7.54 ± 0.74 and for DA 6.56 ± 1.26 ($P=0.507$). The visceral fat area was higher in diabetic Indian 117.47 ± 9.94 compared to African diabetic patients 93.85 ± 6.22 ($P=0.044$). No difference in BMI was noted between the groups.
Conclusions
Although visceral fat area is higher in diabetic Indian than diabetic African patients this seems to have no influence on adipokine levels. However, it may influence triglyceride metabolism.

P44

Low dose cyclosporin and methotrexate administration induces remission of Type 1 diabetes mellitus

Douglas Sobel, Val Abbassi & Annette Henzke
Georgetown University, Washington, DC, United States.

Although, high doses of cyclosporine (cyclo)has been demonstrated to inhibit the development of type 1diabetes mellitus (T1D), its usefulness was limited by its toxicity. Since methotrexate (Mtx) and cyclo have been shown to synergistically act in other disease processes, we determined if low dose cyclo and Mtx therapy could inhibit the development of diabetes and reduce or eliminate the need for insulin therapy in a pilot study.
Methods
Insulin dose and glycemic control were compared in 7 children (mean age 13.7 year) with new onset T1D who were administered cyclo at 7.5 mg/kg/day for 6 weeks and then 4 mg/kg/day and Mtx 5 mg/kg/day for one year and in 10 newly diagnosed diabetic control children (mean age 12.5 year). After 6 weeks, cyclo doses were adjusted to maintain blood cyclo levels 100–200 ng/ml. All children were treated with two daily doses of NPH and fast acting insulin. Clinical and biochemical toxicity of drug therapy was assessed. The study was approved by the Institutional Review Board.
Results
There were two episodes of mild mucositis which required transient lowering of the Mtx dose and one case of transient mild elevation of bilirubin. There were no abnormalities in other liver function tests, creatinine, BUN, or CBC. Mean HbA1c levels were similar in the experimental and control groups at baseline (12.6% vs 11.5%) and at 3, 6, 9, and 12 months. Daily Insulin requirements of the groups were similar at baseline. However the mean insulin dose (u/kg) at 3, 6, 9, and 12 months were significantly ($P<0.001$) lower in the experimental group (0.14 vs 0.56 at 3 months, 0.12 vs 0.61 at 6 months, 0.16 vs 0.55 at 9 months, and 0.22 vs 0.71 at 12 months). No control subjects became non-insulin requiring. However 4 of 7 experimental drug treated subjects were entirely off insulin therapy for 2.5, 4.5, 7 and 12 months. While off insulin therapy, the HbA1c levels of 3 of the 4 subjects were normal. The other subject's HbA1c was only mildly elevated at 6.7%. In conclusion. low dose cyclosporine and MTX treatment of subjects with new onset T1D can safely induce remission of disease and decrease the amount of required insulin.

P45

Progression of diabetic retinopathy in pregestational diabetes mellitus

R Guerrero[1], MA Martinez Brocca[1], MA Pomares[1], S Palma[1], I Gonzalez[1], D Acosta[1], E Moreno[2] & F Villamil[1]
[1]Departament of Endocrinology and Nutrition, Hospital Universitario Virgen del Rocío, Sevilla, Spain; [2]Departament of Obstetrics and Gynecology, Hospital Universitario Virgen del Rocío, Seviila, Spain.

Background
Pregnancy may adversely affect the progression of diabetic retinopathy and can have serious implications for the pregnant women.
Aim
To asses the impact of pregnancy on the progression of diabetic retinopathy in women with type 1 and type 2 diabetes mellitus and to identify risk factors for the progression of retinopathy during pregnancy.
Methods
306 diabetic women, 229 (75.4%) with type 1 and 77 (23.9%) with type 2 diabetes, referred to the Diabetes and Pregnancy Unit of the Hospital Virgen del Rocio from January 1995 through February 2004 were studied retrospectively. Dilated fundal examination was performed at booking, second and third trimester. At early postpartum was performed fluorescein angiographies.
Results
Retinopathy at booking was seen in 54 (17.6%). Any women without retinopathy at booking developed retinopathy during pregnancy or in early postpartum. Progression to proliferative retinopathy was seen in one patient (0.32%), while progression to moderate or severe non proliferative retinopathy was found in eight (2.6%). One women developed during pregnancy macular edema (0.32%). Progression of retinopathy was significantly increased in women with duration of diabetes >10 years (6.9% vs 0%, $P<0.05$). Laser therapy was needed in four (1.3%). Although glycaemic haemoglobin A1C (HbA1c) at booking was higher (7.95 ± 1.81 vs 7.02 ± 1.27) and the fall in HbA1c between booking and 16 weeks was greater (1.66 ± 1.33 vs 1.34 ± 1.08) in those women showing progression of retinopathy, these changes were not significant.
Conclusions
Progression of retinopathy in pregnancy was uncommon, but significantly more frequent in women with duration of diabetes more than 10 years. Laser therapy

was necessary in one percent of pregnancies, which is much lower than reported in earlier studies.

P46

Comparison of insulin monotherapy and combination therapy with insulin and metformin or insulin and rosiglitazone or insulin and acorbose in type 2 diabetes

Hamiyet Yilmaz, Alptekin Gursoy, Mustafa Sahin, Neslihan Bascil Tutuncu & Nilgun Guvener Demirag
Baskent University Faculty of Medicine, Department of Endocrinology and Metabolic Diseases, Ankara, Turkey.

The aim of this study was to compare the efficacy of treatment with insulin alone, insulin plus acarbose, insulin plus metformin or insulin plus rosiglitazone in type 2 diabetic subjects who were previously on insulin monotherapy, and to evaluate the effects of these treatments on cardiovascular risk factors including lipid profile, C-reactive protein (CRP) and fibrinogen. Sixty-six poorly controlled type 2 diabetic patients on insulin monotherapy were involved. They were randomized to insulin alone, insulin plus acarbose, insulin plus metformin or insulin plus rosiglitazone groups for six months period. Mean fasting and postprandial glucose values as well as HbA1c levels significantly decreased in all groups except for insulin plus acarbose group. The greatest improvement in HbA1c was observed in insulin plus rosiglitazone (2.4%) and insulin plus metformin (2%) groups. Daily total insulin dose increased 12.7 units/day in insulin alone group, decreased 4.7 units/day in insulin plus rosiglitazone group, 4.2 units/day in insulin plus metformin group, and 2.7 units/day in insulin plus acarbose group. Least weight gain occurred in insulin plus metformin group (1.4 kg) and greatest weight gain occurred in insulin plus rosiglitazone group (4.6 kg). Except for the improvement of total cholesterol levels in insulin plus rosiglitazone group, no significant change in lipid levels was observed in any groups. CRP levels decreased significantly both in insulin plus metformin and insulin plus rosiglitazone groups. Fibrinogen levels decreased in insulin alone, insulin plus metformin, and insulin plus rosiglitazone groups. All groups were comparable in hypoglycemic episodes. No serious adverse event was noted in any group.

P47

Competition between catecholamines and glucose for binding sites on proteins of erythrocytes

Michael Lepschy, Erich Moestl & Rupert Palme
Veterinary Medicine Vienna, Vienna, Vienna, Austria.

Glucose is slowly linked to haemoglobin in a non-enzymatic reaction and the determination of a glycated protein (HbA1c) is used for long term monitoring blood glucose concentrations. Radiometabolism studies in sheep showed that a haemoglobin-adduct formation also takes place with epinephrine or norepinephrine.

The aim of our study was to elucidate if there is a competition between catecholamines and glucose for binding sites on proteins of the erythrocytes.

Heparinised canine blood was obtained and centrifuged at 1500 g and the cells were washed three times with isotonic NaCl-solution. Afterwards, 5 portions of erythrocytes (0.7 ml each) were re-suspended in 7 ml TCM 'Eagle' and incubated with epinephrine and norepinephrine (1 and 10 ng/ml) for 3 days at 38.6 °C. One portion served as control.

Afterwards the erythrocyte portions were split into sub-samples of 0.2 ml each. One half was incubated with ^{3}H-norepinephrine for 1 h (unhaemolysed), the second half with ^{14}C-glucose for 10 days after haemolysis by freezing. To determine the uptake of ^{3}H-norepinephrine, samples were centrifuged and the radioactivity of the supernatant was measured. In total 410 ± 7 Bq of the added 574 ± 16 Bq were measured in the control samples, whereas all groups preincubated with non-radioactive catecholamines showed significantly ($P < 0.05$) higher radioactivity. To determine the binding of ^{14}C-glucose, proteins were precipitated. After centrifugation, 567 ± 24 Bq of the added 1916 ± 80 Bq were measured in the supernatant of the control samples. As in the experiment using ^{3}H-norepinephrine, significant ($P < 0.05$) higher values were measured in the supernatant of the preincubated erythrocytes, indicating a lower binding of ^{14}C-glucose to the proteins.

Therefore we conclude that catecholamines are blocking binding sites on proteins of erythrocytes for additional adduct formation with ^{3}H-norepinephrine or ^{14}C-glucose.

P48

Body composition, emotional state and quality of life in patients with diabetes mellitus type 2

Lina Lasaite & Jurate Lasiene
Institute of Endocrinology, Kaunas University of Edicine, Kaunas, Lithuania.

Diabetes mellitus type 2 (DM2) is affecting physical and psychological health.
Objective
Compare anthropometric data, body composition, lipids levels, emotional state, quality of life (QoL) of DM2 patients and that of healthy persons of the same age. 39 persons (58.1 ± 9.6 years) with DM2 (18 male, 21 female) and 41 healthy persons (54.3 ± 9.9 years) (22 male, 19 female). Profile of Mood State (POMS) used for emotional state evaluation, WHO Brief Quality of Life Questionnair – for QoL.
Results
In male weight (107.6 ± 30.1 vs 86.7 ± 23.1 kg, $P = 0.008$), body mass index (35.0 ± 9.9 vs 28.1 ± 5.4 kg/m^2, $P = 0.013$), fat mass (37.7 ± 21.0 vs 25.1 ± 12.3 kg, $P = 0.041$), lean mass (69.7 ± 10.6 vs 61.6 ± 11.7 kg, $P = 0.022$), water mass (52.1 ± 9.1 vs 45.1 ± 7.6 kg, $P = 0.007$, waist-to-hip ratio (0.97 ± 0.06 vs 0.91 ± 0.05, $P = 0.018$) were significantly higher in DM2 patients than in controls. In female weight (90.5 ± 14.6 vs 74.5 ± 18.7 kg, $P = 0.002$), body mass index (34.9 ± 6.2 vs 28.0 ± 5.7 kg/m^2, $P = 0.013$), fat mass (42.0 ± 10.4 vs 30.6 ± 12.2 kg, $P = 0.003$), lean mass (48.5 ± 6.4 vs 43.5 ± 7.8 kg, $P = 0.05$), water mass (38.1 ± 4.8 vs 33.5 ± 4.9 kg, $P = 0.004$), waist-to-hip ratio (0.90 ± 0.4 vs 0.83 ± 0.1, $P = 0.002$) were significantly higher in DM2 patients than in healthy female. In male and female no significant differences between research and control groups in high and low density cholesterol were found. In male, but not female QoL (79.3 ± 8.6 vs 85.3 ± 8.7, $P = 0.032$), POMS vigor (-11.8 ± 3.8 vs -15.8 ± 4.8, $P = 0.009$) were significantly lower in DM2 than in control group. Significant correlations were found in male between vigor and waist-to-hip ratio ($r = 0.347$, $P = 0.041$), in female between vigor and water mass ($r = 0.313$, $P = 0.049$), POMS total and waist-to-hip ratio ($r = 0.362$, $P = 0.046$), depression and low density cholesterol ($r = 0.430$, $P = 0.028$), vigor and lean mass ($r = 0.385$, $P = 0.014$).
In conclusion
Weight, body mass index, fat mass, lean mass, water mass, waist-to-hip ratio were significantly higher in male and female; quality of life and vigor were significantly lower in DM2 male than in healthy persons of the same age.

P49

Deleterious effects of beta-blockers on arterial stiffness and central pulse pressure in menopausal women: baseline findings from the Cashmere trial

S Laurent[2], S Christin Maitre[1], A Gompel[3], P Boutouyrie[2], P Jaillon[1], Ch Thuilliez[4], F Zannad[5], B Pornel[7], B Pannier[8], H Struijker-Boudier[9], L Van Bortel[10], Ch Kuntz[11], A Keanry-Schwartz[5], I Pithois-Merli[6], D Simonneau[6] & T Simon[1]
[1]Saint Antoine-UMPC-ParisVI university Hospital, Paris, France; [2]HEGP-Paris V-University Hospital, Paris, France; [3]Hotel Dieu-Paris V-University Hospital, Paris, France; [4]CHU Rouen, Rouen, France; [5]CHU Nancy, Nancy, France; [6]Pfizer, Paris, France; [7]Centre de menopause, Bruxelles, Belgium; [8]Department of cardiology, fleury-merogis, France; [9]Department of cardiology, Maastricht, Netherlands; [10]Department of cardiology, Ghent, Belgium; [11]CHU strasbourg, Strasbourg, France.

Beta-blockers (BB) may be less effective than other antihypertensive drugs for stroke prevention in patients with primary hypertension (ASCOT and LIFE studies). Our study compares arterial stiffness and central PP between users (BB+) and non users of BB (BB-), among menopausal women with hypercholesterolemia and no history of CV disease.
Methods and Results
We used the baseline data of 664 menopausal women, screened for the Cashmere study, a12-month double-blind randomized trial comparing the effects of atorvastatin (80 mg/day), vs placebo, ±with hormone therapy, on the progression of CCA-IMT and arterial stiffness. Aortic stiffness was measured by carotid-femoral pulse wave velocity (PWV); central PP and augmentation index (AI, wave reflection) were determined by applanation tonometry; carotid stiffness was calculated from relative stroke change in diameter (echotracking system) and carotid PP. BB were used in 104 women for treating headache, tachycardia, arrhythmia, and hypertension. 97% BB used were devoid of vasodilating properties. Age (60 ± 6 vs 58 ± 5 years, $P < 0.0001$) and mean BP (MBP: 91 ± 12 vs 88 ± 11 mmHg, $P < 0.0001$) were slightly but significantly higher in BB+ than in BB- ($n = 560$). After

adjustment to age and MBP, BB+ had 10% higher central PP ($P<0.0001$), 6% higher AI ($P<0.001$), 4% higher PWV ($P=0.04$), and 5% higher carotid stiffness ($P<0.01$) than BB-. BB+ had 4% higher central SBP ($P<0.0001$) than BB-, despite a non significantly higher brachial SBP only (1%, $P=$NS). To rule out an influence of hypertension on arterial parameters, we compared users of anti-hypertensive drugs ($n=110$) to non users ($n=554$). No significant difference was observed concerning the above parameters, excluding or not BB- users.
Conclusions
In menopausal women with hypercholesterolemia and no CV disease, the use of non-vasodilating BB was associated with higher aortic and carotid stiffness. These data are consistent with the results of the CAFÉ trial. Whether the deleterious effects of BB on large arteries increase the risk of CV events in women remains to be determined.

P50

Prevalence of GADA and IAA in elderly patients with type 2 diabetes

Maria Kurowska[1], Jerzy S Tarach[1], Helena Jankowska[2], Mariusz Kowalczyk[1], Joanna Malicka[1] & Andrzej Nowakowski[1]
[1]Dpt Endocrinology, Skubiszewski Medical University, Lublin, Poland; [2]Dpt Nuclear Medicine, Skubiszewski Medical University, Lublin, Poland.

Little is known about the prevalence and significance of islet cell immunity in elderly patients with type 2 diabetes. The low antibody titers against islet-cell antigens in LADA elderly patients may be a sign of a less aggressive autoimmune diabetes.
The objective
To establish the changing frequency and titers of GADA and IAA in elderly diabetics.
Material
83(56F;27M)diabetic patients (60–91 y) divided in age related groups. Group 1: 55 (36F;19M) patients (60–69 y). Group 2: 14 (9F;5M) pts (70–79). Group 3: 14 (11F;3M) pts (80–91). Mean duration of diabetes 5.6 ± 6.4 y.
Method
GADA and IAA determined by RIA (ANTI-INSULIN RIA and GAD-AB kits), (CIS). IAA estimated in patients not treated with insulin. The positive GADA and IAA titers were over 1 U/ml and 5.5%B/T.
Results
Group 1: Positive GADA were found in 13(27%) assays, 5(10.2%) patients with the level 7.1–64.5 U/ml and 8(16.5%) subjects 1.02–2.1 U/ml. In 11(22.9%) patients GADA titers 0.38–0.98 U/ml were found (method sensitivity >0.3 U/ml). The positive IAA were in 20(40.8%) assays (5.6–13.2%B/T). Group 2: In 3(21.5%) patients, the GADA were >1 U/ml (1.63; 38.5;68.5 U/ml). 4(28.6%) patients had GADA 0.93–0.99 U/ml. The positive IAA were obtained in 4(28.6%) patients (9.1–19.7%B/T). Group 3: There were positive GADA in 4(30.8%)assays (1.3–12.1 U/ml). In 8(61.5%%) patients GADA ranged 0.61–1.42 U/ml. In 6(42.9%) subjects the positive IAA was obtained (1 patient 36.1%B/T and the rest 5.6–6.95%B/T).
Summary
The percentage of patients with high GADA titer didn't significantly change with the age. In the older patients the frequency of GADA low titers (close to 1 U/ml) clearly increased. The IAA frequency and titer didn't significantly change with the age.
Conclusion
Eldery diabetic patients are characterized by increasing frequency of GADA sublimited titers as they aged. The autoantibodies low level may signify a less aggressive beta-cell autoimmunity as well as instability of the immunological system related to aging or both.

P51

Comparison of plasma homocysteine concentrations (HYC) in patients with acute coronary syndrome (ACS) and newly or previously diagnosed type 2 diabetes

Maria Kurowska[1], Jerzy S Tarach[1], Wojciech Gernand[2], Janusz Kudlicki[3], Adam Tarkowski[3], Andrzej Nowakowski[1], Teresa Czekajska[3] & Janusz Solski[2]
[1]Dpt Endocrinology, Skubiszewski Medical University, Lublin, Poland; [2]Dpt Laboratory Medicine, Skubiszewski Medical University, Lublin, Poland; [3]Dpt Cardiology, Skubiszewski Medical University, Lublin, Poland.

Introduction
The patients with ACS and scheduled for an elective coronary angiography have high frequency of both newly and previously diagnosed diabetes. The diabetic patients with acute myocardial infarction have an increased risk of death. Elevated blood HYC is strongly related to an increased risk for atherosclerosis and cardiovascular disease. This association is particularly evident in patients with diabetes.
Aim of the study
An attempt to evaluate whether cardiovascular risk expressed by serum HYC in ACS patients differs between groups of patients with newly or previously diagnosed type 2 diabetes.
Group of patients
95 cases (30F and 65M) of which 71 pts (18F; 53M) without previously diagnosed disorders of carbohydrate metabolism and 24 patients (12F and 12M) with previously diagnosed type 2 diabetes. Patients aged 41–90 years.
Methods
In all patients the following parameters have been measured: 1 The blood glucose level in the course of acute coronary disorders (admission glucose); 2 Fasting blood glucose in the next day; 3 Serum HYC applying chemiluminescence method (IMMULITE, DTC reagents). Diagnosis of type 2 diabetes has been established according WHO criteria.
Results
Patients with recent diagnosed t. 2 diabetes constituted 13% of group without previously known symptoms of carbohydrate disorders. The mean admission glucose level in the group with newly diagnosed diabetes was 151.8 ± 26.9 mg/dl; in the group with previously known diabetes was 218.8 ± 127.1 mg/dl. Mean HYC in the former group was 18.4 ± 7.3 μmol/l (F-20.2 ± 9.9; M- 17.5 ± 6.6 μmol/l) and 15.3 ± 5.2 μmol/l (F-15.3 ± 4.9; M-15.4 ± 5.6 μmol/l) in the latter, respectively. In the group with normoglycemia the mean serum HYC were 15.02 ± 5.2 μmol/l (M-15.5 ± 5.5 μmol/l, F-13.6 ± 4.6 μmol/l).
Conclusions
The cardiovascular risk estimated according to serum HYC is higher in ACS patients with newly diagnosed type 2 diabetes.

P52

The association between carotid artery intima-media thickness and cardiovascular mortality and morbidity in Type 2 diabetes: a retrospective study

Hayriye Esra Ataoglu, Mustafa Yenigun, Tayyibe Saler, Zehra Yigit, Levent Temiz, Zuhal Saglam, Faik Çetin, Baki Kumbasar, Fuat Sar & Suleyman Ahbap
Haseki Training and Research Hospital 4th Clinic of Internal Medicine, Istanbul, Turkey.

Carotid artery intima-media thickness (CCA-IMT) highly correlates with cardiovascular events in type 2 diabetes (T2DM). We aimed to determine the cardiovascular mortality and morbidity incidence regarding CCA-IMT and Framingham Score compared with preceding results of T2DM individuals. Our aim was to determine whether ultrasonographic evaluation of carotid arteries may predict cardiovascular mortality, morbidity and diabetic complications in T2DM patients.
Method
Demographic and clinical data of 102 T2DM individuals were registered including blood pressure, HbA_{1c}, lipid parameters, albumin excretion rate (AER), ECG and ultrasonographic evaluation of carotid IMT and reevaluated seven years later (2004). Primary end point was defined as cardiovascular mortality and morbidity. Student-t test, regression analysis and $[chi]^2$ tests were used. $P<0.05$ was significant.
Results
The percentage of patients reaching primary end point was 45.10%. Age ($P=0.043$), diastolic blood pressure (DBP) ($P<0.0001$), systolic blood pressure (SBP) ($P=0.004$), A_{1c}% ($P=0.042$), (AER) ($P=0.017$), triglyceride levels ($P=0.038$), IMT/CCA ($P=0.001$) and percentage of coronary risk assessment by Framingham Score were significantly high($P=0.001$) in patients presenting with any of the primary end points. Reevaluation at the end of 7 years revealed that measuring DBP, SBP and IMT/CCA was statistically important at assessing the risk of presenting with any primary end points in T2DM patients (Constant:$P<0.0001$).
Conclusion
Although Framingham Score predicts 10-year risk for cardiovascular mortality and morbidity in diabetic patients, we suggest that DBP, hypertriglyceridemia and microalbuminuria should also be included in risk scoring as well as the measurement of carotid IMT.

P53

Comparison of the effects of gliclazide and glibenclamide on insulin resistance and metabolic parameters

Rabia Gokturk, Hayriye Esra Ataoglu, Mustafa Yenigun, Suleyman Ahbap, Zuhal Saglam, Levent Temiz, Faik Çetin, Fuat Sar & Baki Kumbasar
Haseki Training and Research Hospital 4th Clinic of Internal Medicine, Istanbul, Turkey.

Introduction
It has been shown with many studies that sulphonylureas may have a negative effect on parameters of insulin resistance while improving glucose regulation. However not all of the sulphonylureas have the same effect. This analysis assessed the different effects of sulphonylureas on some metabolic parameters of insulin resistance.
Method
Newly diagnosed 25 T2DM individuals who were naive of oral antidiabetic therapy were recruited and randomized to either long lasting gliclazide (30–90 mg/day;$n=13$) or glibenclamide (1–3 mg/day;$n=12$) group. Body-mass index (BMI), waist-hip ratio and blood pressure as well as biologic parameters like blood glucose, $A1_c$(%), BUN, creatinine, uric acid, lipid parameters, microalbuminuria, CRP, insulin, c-peptide, glucagon, proinsulin and IGF1 levels were recorded at baseline and at the end of the third month. The ratios of glucose/insulin, proinsulin/insulin, HOMA-IR were assessed for each patient. Comparisons between groups were performed by Students t test. $[Chi]^2$ test was used for categorical variables. All analyses were two sided with a significance level of [alpha]=0.05.
Results
By the end of three months, gliclazide caused a decrease in c-peptide and insulin levels whereas glibenclamide resulted with a significant increase. Although insulin resistance was decreased in both groups it was evident in glibenclamide group. Creatinine levels were elevated in both groups which was significant with glibenclamide group. Uric acid levels were decreased in gliclazide group contrary to glibenclamide group in which uric acid levels were elevated.
Conclusion
Sulphonylureas have different effects on metabolic parameters of insulin resistance. These data suggest that gliclazide has a lowering effect on hyperinsulinemia. Yet this study is an observation based on small number of patients, studies with bigger numbers and longer duration are required for confirmation.

P54

24-hour ambulatory blood pressure and aortic dimensions in women with Turner syndrome

Britta Hjerrild[1], Keld Sorensen[2], Erik Morre Pedersen[3], Erik Lundorf[3], Arne Horlyck[4], Jens Sandahl Christiansen[1] & Claus Hojbjerg Gravholt[1]
[1]Medical Department M (Endocrinology and Diabetes) and Medical Research Laboratories, Aarhus University Hospital, Aarhus, Denmark; [2]Department of Cardiology, Skejby Hospital, Aarhus University Hospital, Aarhus, Denmark; [3]The MR Centre, Skejby Hospital, Aarhus University Hospital, Aarhus, Denmark; [4]Department of Radiology, Skejby Hospital, Aarhus University Hospital, Aarhus, Denmark.

Study objective
To study blood pressure (BP) levels and aortic dimensions in women with Turner syndrome (TS).
Materials and methods
102 women with TS (mean age 37.7 years; 18–62 years). 24 hour ambulatory BP measurement and echocardiography was performed on participants.
Results
Mean BP systolic (sys) and diastolic (dia) values were ($\pm$SD): sysBPday 128.0$\pm$15.3; diaBPday 81.6$\pm$11.8; sysBPnight 110.4$\pm$14.0 and diaBPnight 68.1$\pm$11.5. Heart rate (HR): 77.5$\pm$9.7.
Hypertension was found in a large proportion of the women: sysBPday. 36/97 (37%); diaBPday 44/97 (45%); sysBPnight 27/96 (28%) and diaBPnight 49/96 (51%). 34/97 (35%) did not have elevated BP levels, 22/97 (23%) had elevated levels in all 4 measures. 19 women already received antihypertensive treatment, and sys BP was significantly higher in this group. Aortic diameters (cm):

	Mean	SD	% above cutoff
Aortic Annulus	1.83	0.18	0
Sinus	2.80	0.41	15
Sinotubular level	2.63	0.42	11
Brachial trunk	2.08	0.40	0

17 individuals had aortic diameters above expected levels. A positive correlation was found between systolic BP ($r=0.36$; $P=0.001$) and age, but not weight or BMI. HR correlated negatively to VO_2max ($r=0.22$; $P=0.038$). We found no correlation between BP and aortic diameters or age and aortic diameters. There was however a significant increase in aortic diameters in TS with karyotype 45,X compared to others ($P<0.02$) and in TS with bicuspid aortic valves ($P<0.02$).
Conclusion
Hypertension is common in TS, affecting more than 50% of the study group, and subjects on antihypertensive treatment were insufficiently treated. Aortic dimensions are larger in TS (17%), especially with the karyotype 45, X. In this study we found no correlation between BP and aortic dimensions.

P55

Plasma marker of lipid peroxidation and type 2 diabetes in subject with coronary artery disease in Iranian subjects

Fariborz Haghparast & Jaffar Nourooz-Zadeh
Islamic Azad University-Larestan Branch, Larestan, Iran.

Objective
Abnormal lipid profile is an important risk factor in the development of macrovascular atherosclerotic complications in patients with type 2 diabetes mellitus (T2D). The aim of this study was to investigate the relationship between lipid profile and lipid peroxidation in type 2 diabetics with and without coronary artery disease (CAD).
Materials and methods
We studied 80 patients with T2D, 40 with CAD and 40 without CAD. We also studied 50 non-diabetics, 30 with CAD, and 20 without CAD. Lipid profile was estimated by the total, HDL, LDL cholesterol and triglyceride (TG). To evaluate the oxidative status we measured circulating malondialdehyde (MDA), plasma levels of superoxide dismutase (SOD), glutathione (GSH), as well as vitamin E and C.
Results
No significant difference was found in the lipid profile in patients with T2D and CAD patients. There was significantly difference in the level of MDA between the groups. In diabetics, MDA positively correlated with the total cholesterol, LDL -C, total lipid, and the relations between LDL/HDL and TG/HDL ($P<0.001$). In non-diabetic with CAD group, MDA positively correlated with total cholesterol, ($P<0.05$). There was significant difference in the SOD, glutathione, vitamin E / total lipid and vitamin C between the groups of diabetics and were lower in the diabetes group with CAD ($P<0.05$). There were significant negative correlations between MDA and vitamin E and C in groups with T2D, but it was statistically significant in the non-diabetic with CAD ($P<0.05$).
Conclusion
Type 2 diabetes is associated with excess risk of CAD and primary therapy should be directed first at lowering lipid peroxidation. CAD and T2D alone and combined carry similar atherosclerotic burden concerning lipid profile, enzymatic and nonenzymatic antioxidative status and lipid peroxidation.

P56

Abstract unavailable

P57

Effect of testosterone replacement therapy on adipocytokines in hypogonadal men with Type 2 diabetes

D Kapoor[1], S Clarke[3], R Stanworth[1], KS Channer[2] & TH Jones[3]
[1]Centre for Diabetes and Endocrinology, Barnsley NHS Foundation Trust Hospital, Barnsley, United Kingdom; [2]Department of Cardiology, Royal Hallamshire Hospital, Sheffield, United Kingdom; [3]Academic Unit of Diabetes, Endocrinology and Metabolism, University of Sheffield, Sheffield, United Kingdom.

Serum testosterone level is known to inversely correlate with insulin sensitivity and obesity in men. Furthermore, there is evidence to suggest that testosterone replacement therapy reduces insulin resistance and visceral adiposity in Type 2 diabetic men. Adipocytokines are hormones secreted by adipose tissue and contribute to insulin resistance. We report a double-blind placebo controlled crossover study in 20 hypogonadal Type 2 diabetic men examining the effect of

testosterone replacement therapy on adipocytokines and CRP. Patients were treated with testosterone (Sustanon 200 mg) IM every 2 weeks or placebo for 3 months in random order followed by a wash-out period of 1 month before the alternate treatment phase. At baseline, leptin levels significantly correlated with BMI ($r=0.71$; $P<0.001$) and waist circumference ($r=0.78$; $P<0.001$). There was also a significant inverse correlation between IL-6 levels and total testosterone ($r=-0.68$; $P=0.002$) and bioavailable testosterone levels ($r=-0.73$; $P=0.007$). CRP levels also correlated significantly with total testosterone levels ($r=-0.59$; $P=0.01$). Testosterone treatment reduced leptin (-7141.9 ± 1461.8 pg/ml; $P=0.0001$) and adiponectin levels (-2075.8 ± 852.3 ng/ml; $P=0.02$). There was a significant reduction in waist circumference (-2.1 ± 0.81 cm; $P=0.02$). No significant effects of testosterone therapy on resistin, TNF alpha, IL-6 or CRP levels were observed.

In conclusion, testosterone replacement treatment decreases leptin and adiponectin levels in Type 2 diabetic men. Moreover, low levels of testosterone in men are associated with inflammation, though testosterone treatment over 3 months had no effect on inflammatory markers.

P58

A role of the liver in the infringements of lipid metabolism of patients with diabetes mellitus type 2 and metabolic syndrome

Vadim Korpachev[1], Alla Kovalchuk[1], Natalija Kushnareva[1] & Volodymyr Kovalchuk[2]
[1]V.P. Komissarenko Institute of Endocrinology and Metabolism, Kyiv, Ukraine; [2]O.O. Bogomolets National Medical University, Kyiv, Ukraine.

Different interdependences with symptoms of insulin resistance give us the possibility to consider steatosis as a disorder of the liver with metabolic syndrome (MS).

The aim of study is to assess the role cholesterol-HDL in rise of diabetic steatohepatosis.

40 patients with Diabetes Mellitus type 2 (DM) and signs of MS were examined to determine spreading of steatohepatosis as one of the factors of insulin resistance. Only 8 of them didn't have diabetic hepatopathy, while 32 patients had adipose infiltration of the liver (according to the results of the ultrasonic examination).

Actual difference between the two groups was revealed in the rate of HDL decrease. So, if the patients with DM type 2 and symptoms of MS with steatohepatosis have the rate of cholesterol-HDL decrease which is $34.36\pm4.2\%$ from the low norm measure, the patients with the same symptoms, but without steatohepatosis, had $6.8\pm0.2\%$ ($P<0.05$). We distinguished a group of patients who had prevalent fasting hyperglycemia. Those patients who had prelevant postprandian hyperglycemia formed the group of comparison. Analyzing the findings, it is necessary to mention that the group of patients with prevalent fasting hyperglycemia were effected by more serious disorders with lipid metabolism, they had a lower level of cholesterol-HDL than those who had rather high postprandian hyperglycemia (0.89 ± 0.03 vs 1.027 ± 0.05 mmol/l, $P<0.05$) and rather high percentage of a waste circle growing that indicates of a greater aggressiveness of MS factors.

Thus, it was determined that prevalent fasting hyperglycemia which effects patients with DM type 2 and diabetic hepatopathy in condition of adipose infiltration confirmed by echographic results is a proof of a major role of the liver in the infringement of lipid metabolism that contributes to increasing of insulin resistance due to, so called, 'lipid toxity'.

P59

Radionuclide study of hepatobiliary system function in patients with type 2 diabetes and metabolic syndrome

Valentin Slavnov, Alla Kovalchuk, Vadim Korpachev & Natalija Kushnareva
V.P. Institute of Endocrinology and Metabolism, Kyiv, Ukraine.

The aim of the research is to study the functional state of liver parenchyma in patients with type 2 diabetes and to analyze hepatobiliary system disorders depending on marker of metabolic syndrome (MS).

The study involved 22 patients with type 2 diabetes and MS and 8 healthy persons. Dynamic hepatoholecystoscintigraphy was performed using RKC 301T gamma camera after Tc-99m mesida administration and bile-expelling meal.

We established a reliable increase of maximum accumulation time of radiopharmaceutical in parenchymatous cells of the liver (22.0 ± 3.76 vs 14.6 ± 0.83, $P<0.01$) comparing to healthy. Also these patients have infringements of secretory functions that are confirmed by meaningful increase of radionuclide half-deduction time (T1/2) from the liver (60 ± 4.16 vs 45.2 ± 3.49, $P<0.03$). Also a reliable T1/2 delay occurs in patients with type 2 diabetes and metabolic syndrome comparing to healthy. But in patients with smaller body mass index was found significant lowering of time of radiopharmaceutical occurrence in intestine that testifies the hypotonia of Oddi's sphincter. In patients with decompensation stage of carbohydrate metabolism comparing to subcompensation occurs meaningful increase of liver T1/2 that points on excretory function delay. Nevertheless we have not found any significant relations between delay of liver excretory function and HOMA, level of C-peptide, triglycerides, cholesterol, HDL-cholesterol, LDL-cholesterol, WHR, arterial hypertension in patients with type 2 diabetes and metabolic syndrome. Different impacts of per oral hypoglycemic drugs displayed significant lowering of excretory function in patients taking metformin comparing to those who were taking sulfonylurea due to T1/2 elongation of liver (61.75 ± 5.54 vs 39.75 ± 6.62, $P<0.05$). The obtained findings suggest that absorbing and excretory functions of liver slow down at increase of BMI and decompensation stage in patients with type 2 diabetes and metabolic syndrome. But other markers of metabolic syndrome are not defining in early disturbances of liver excretory function in mentioned patients.

P60

Omega-3 polyunsaturated fatty acids in the treatment of cardiovascular autonomic neuropathy in Type 2 diabetes mellitus patients

Victoria Serhiyenko, Alexander Serhiyenko & Ludmila Serhiyenko
Medical University, Lviv, Ukraine.

Background and aims
The aim of this study was to assess the effects of docosahexaenoic (DHA) and eicosapentaenoic acid (EPA) on the heart rate variability (HRV), some biochemical parameters in patients (pts) with Type 2 diabetes mellitus and cardiovascular aunonomic neuropathy (CAN).
Materials and methods
39 pts with CAN (54 ± 5 yrs) were allocated in two groups: A ($n=26$) were receiving capsules of fish oil every day (2.0 g EPA, 2.0 g DHA and 0.1% α-tocopherol acetate), B ($n=13$) - placebo capsules of olive oil. We investigated the activities of protein-kinase C (PK-C), Na^+, K^+-ATPase, Ca^{2+}, Mg^{2+}- ATPase in the membranes of RBC's, levels of the ^{125}I-6-ketoprostaglandin F1alpha (6--ketoPGF1alpha), ^{125}I-thromboxane B_2 (TXB_2) in the blood plasma.
Statitics
ANOVA.
Results
The manifestation of the CAN is accompanied by decrease of the Na^+, K^+-ATPase, Ca^{2+}, Mg^{2+}-ATPase activities ($P<0.001$), 6-ketoPGF1alpha, EPA level ($P<0.001$) with increase of TXB_2, PK-C activity, stage of platelet's aggregation, QTc interval. After four months of treatment there were a decrease of TXB_2 level (141.2 ± 15.4 pg/ml, $P<0.001$), activity of PK-C (14.46 ± 4.52 pmol ^{32}P/mg protein per 1 min, $P<0.001$), degree and speed of an aggregate of thrombocytes with simultaneous increase activities of Na^+, K^+-ATPase (0.1 ± 0.004 mMol P_i/mg protein per 1 hour, $P<0.001$), Ca^{2+}, Mg^{2+}-ATPase and the level of the 6-ketoPGF1alpha in the group A marked. Also, we observed significant improvement of HRV parameters, decrease of QTc interval ($P<0.01$).
Conclusion
DHA and EPA at moderate doses may exert antithrombotic effects and may be used for prophylaxis and treatment of patients with Type 2 diabetes mellitus and cardiovascular aunonomic neuropathy.

P61

Nicotinamide and alpha-lipoic acid in the treatment of cardiovascular autonomic neuropathy in Type 2 diabetic patients

Ludmila Serhiyenko, Alexander Serhiyenko & Victoria Serhiyenko
Medical University, Lviv, Ukraine.

Background and aims
The present study has examined the effect of α-lipoic acid (ALA) and nicotinamide (NA) on the heart rate variability (HRV), superoxide dismutase (SOD), gluthation peroxidase (GPO), catalase activities, reduced gluthatione (GSH), malondialdegide (MDA) contents in the RBCs' in Type 2 diabetic patients (T2DM) with cardiovascular autonomic neuropathy (CAN).
Materials and methods
59 patients with T2DM and CAN (59.3 ± 7.9 years) were allocated to three treatment group: (1) daily per os dose of ALA 600 mg ($n=29$); (2) NA 700 mg ($n=18$); (3) ALA 600 mg and NA 700 mg ($n=12$) during 2 months.

Statistics
ANOVA.
Results
The progress of CAN is accompanied by decrease of the activities of SOD (7.38 ± 0.29, $P < 0.001$), GPO and catalase ($P < 0001$), the content of GSH and increase of the MDA ($P < 0.001$) in RBC's. After 2 months of a treatment course with ALA it the increasing spectral power in the low- and high frequency ($P < 0.01$), coefficient of variation ($P < 0.05$). Simultaneously, activity of SOD, GPO ($P < 0.001$) and GSH concentration were authentically augmented, and the contents of MDA, QTc interval parameters (0.52 ± 0.057, $P < 0.05$) was reduced ($P < 0.01$). Simultaneously introduction of NA and ALA is conducted with more significant increasing SOD - 9.14 ± 1.25 IU/ml Rbc's, $P < 0.001$; GPO - 298.14 ± 19.45 mcmol GSH/min Hb, $P < 0.001$) and GSH concentration (1.97 ± 0.04 mcM/g Hb, $P < 0.001$), TRAC ($P < 0.001$), HRV, decreasing of MDA concentration ($P < 0.001$) and QTc interval parameters.
Conclusion
Usage of ALA and NA is accompanied by improvement of HRV, QTc interval, antioxidant defence parameters and may be used for the treatment of CAN.

P62

Plasma measures of oxidative stress and antioxidant status in type 2 diabetes mellitus

Fariborz Haghparast & Jaffar Nourooz-Zadeh
Islamic Azad University-Larestan Branch, Larestan-Fars, Iran.

Objective
The aim of this study was to test the hypothesis that type 2 diabetes mellitus is associated with increased oxidative stress in Iranian subjects.
Materials and methods
The study population consisted of Fifty-nine patients with type 2 diabetes (mean age 62.5 ± 8.7 years). Type 2 diabetes was diagnosed according to the American Diabetes Association criteria. 36 patients had diabetes complications and 23 patients had no complications. For the normal control subjects, fifty-five age- and sex- matched healthy control subjects (mean age 63 ± 5.7 years) were included. Plasma α-tocopherol (α-ToH) was analyzed with HPLC. Malondialdehyde (MDA), plasma glutathione (GSH), vitaminC and superoxide dismutase (SOD) were spectrophotometrically measured. Total cholesterol, triacylglycerol, LDL-cholesterol, HDL-cholesterol, HbA1c, uric acid, blood urea nitrogen (BUN) and creatinine (Cr) were studied.
Results
Plasma α–TOH-to-lipid ratio, glutathione and vitamin C levels were significantly decreased in type 2 diabetes compared with controls (all $P < 0.05$). Plasma vitamin C and glutathione levels in diabetic patients with complications were significantly lower than in those without complications (51.86 ± 2.6 vs. 62.31 ± 2.7 μmol/L, $P < 0.001$, 64.02 ± 7.6 vs. 125.33 ± 25.6 nmol/L, $P < 0.05$, respectively). MDA concentration was significantly higher in patients compared with controls ($P < 0.005$) as well as diabetes with complication compared to without complications ($P < 0.05$). Plasma levels of α–TOH/ total lipid was similar in diabetic patients with or without complications. Plasma concentration of uric acid and SOD were significantly lower in patients with diabetes than in control subjects.
Conclusions
Our results support the oxidative stress hypothesis for type 2 diabetes mellitus. We therefore suggest that oxidative stress is an early stage in the disease pathology, which may contribute to the development of complications.

P63

Does screening of primary hyperaldosteronism only lead to diagnosis of more adrenal hyperplasia?

Helga Sigurjonsdottir[1], Mikael Gronowitz[2], Ove Anderson[3], Robert Eggertsen[4], Hans Herlitz[5] & Gudmundur Johannsson[1]
[1]Dept. of Endocrinology and Metabolism, Sahlgrenska University Hospital, Gothenburg, Sweden; [2]Nödinge Primary Care Center, Nödinge, Sweden; [3]Hypertension outpatient clinic, Sahlgrenska University Hospital, Gothenburg, Sweden; [4]Mölnlycke outpatient clinic, Mölnlycke, Sweden; Nephrology outpatient clinic, Sahlgrenska University Hospital, Gothenburg, Sweden.

Background
The prevalence of primary hyperaldosteronism (PA) has recently been reported as high as 7.5% in patients with hypertension from unselected populations (USP) and up to 22% in selected populations (SP). Whether increased screening and diagnosis of PA will lead to more findings of curable PA (aldosterone producing adenoma, APA) is unclear.
Methods
Three-hundred fifty-three consecutive patients with hypertension, age 20 to 88 years were included from two primary care centers (230 USP) and specialized university hypertension outpatient clinics (123 SP) in the same catch-up area. Plasma renin activity and serum aldosterone levels were sampled. Patients with criteria for PA did positional and salt-loading test for confirmation. Further investigation was with CT-adrenals, ^{131}I-chol-scintigraphy and adrenal vein sampling (AVS), with few exceptions. The local ethical committee approved the study.
Results
Forty-six patients, 28 SP (22.8%) and 18 USP (7.8%), had criteria for PA. Six USP and 22 SP were available for further investigation. Confirmation tests found 8 patients to be normal. Nine of eighteen (53%) CT-adrenals had positive findings. Three of 17 (18%) ^{131}I-chol-scintigraphies had positive findings. Ten of 14 AVS (71%) had positive findings. Four AVS were unsuccessful. Supplementary information from ^{131}I-chol-scintigraphy and CT-adrenals lead to clear diagnosis in 3 of them, 1 AH and 2 APA. Further investigation after screening lead to clear diagnosis in 27 of 28 (96%) patients, 8 AH, 11 APA.
Conclusion
The study confirms high prevalence of PA found in recent studies, higher in SP than USP as expected. Our results indicate that screening for PA finds more patients with curable cause of PA. All APA were from the SP group. Our findings indicate that ^{131}I-chol-scintigraphy is less accurate for diagnosing APA than CT-adrenals and that the AVS is superior to both of them, further comparison of the methods are needed.

P64

Liquorice in moderate doses decreases serum levels of vitamin B12 but does not affect the serum lipid levels

Helga Sigurjonsdottir[1], Margret Arnadottir[2] & Sven Wallerstedt[3]
[1]Dept. of Endocrinology and Metabolism, Sahlgrenska University Hospital, Gothenburg, Sweden; [2]Dept. of Nephrology, Landspitali University Hospital, Reykjavik, Iceland; [3]Dept. of Internal Medicine, Sahglrenska University Hospital, Gothenburg, Sweden.

Background
Liquorice in moderate doses increases blood pressure (BP) in healthy individuals (NT) as well as patients with hypertension (HT) due to increased cortisol effect. Glycyrrhetinic acid, the active substance in liquorice, inhibits 11beta-hydroxysteroid-dehydrogenase type 2 (11betaHSD2) which converts the active hormone cortisol to the inactive hormone cortisone. Recently it has been reported that treatment with glucocorticoids decreases serum levels of cobalamin (B12), it is also known that increased cortisol levels negatively affect different metabolic risk-factors as serum lipids. Hence, it is possible that liquorice due to its increased cortisol effect can decrease serum levels of B12 and affect the lipid levels negatively.
Methods
Thirty-six individuals, 25 NT (13 men and 12 women) and 11 HT (8 men and 3 women), 22–44 years old, consumed 100 g of liquorice (150 mg GA) daily for 4 weeks. Blood tests were taken, 24-hour-urin collected and BP measured before and after the liquorice consumption. The study was approved by the local ethical committee.
Results
Serum-B12 decreased from 299 ± 78 pmol/L to 284 ± 78 pmol/L in the whole group ($n = 36$, $P = 0.005$), from 322 ± 77 pmol/L to 303 ± 74 pmol/L ($P = 0.005$) in the NT group and from 288 ± 82 pmol/L to 270 ± 77 pmol/L ($P = 0.007$) in men. Serum levels for total-cholesterol, LDL-cholesterol, HDL-cholesterol, triglycerides, apolipoprotein A1 and lipoprotein(a) did not change after liquorice consumption. Serum levels for apolipoprotein B (ApoB) decreased from 0.83 ± 0.22 g/L to 0.81 ± 0.22 g/L ($P = 0.04$) in the whole group ($n = 36$). The ratio for urinary free cortisol/cortisone (Q, an indicator of 11betaHSD2-activity) increased significantly in all groups ($P < 0.001$ in all groups). No statistical difference was found between the genders or between the NT-and HT-groups.
Conclusion
The glucocorticoid-effect induced by liquorice consumption in moderate doses for 4 weeks is sufficient to significantly decrease the serum concentration of B12, which is a novel finding. Even if the decrease is not substantial it can be of clinical importance. This moderate dose of liquorice does not affect the serum lipid levels.

P65

Decreased insulin sensitivity in young lean hypertensive men is not associated with increased visceral fat and changes in plasma adipocytokines

Adela Penesova[1], Zofia Radikova[1], Eva Cizmarova[2], Vitazoslav Belan[3], Milan Vigas[1] & Juraj Koska[1]
[1]Institute of Experimental Endocrinology SAS, Bratislava, Slovakia; [2]Outpatients Clinic of Pediatric Cardiology, Karlova Ves, Bratislava, Slovakia; [3]Radiological Clinic of Faculty Hospital, Bratislava, Slovakia.

Objective
Increased abdominal visceral adipose tissue (VAT) deposition is associated with insulin resistance in obese and/or hypertensive patients. We investigated the association of insulin sensitivity with the amount of VAT in young, lean, non-treated males with recently established high normal blood pressure or hypertension grade 1 (HT).
Subjects and methods
Twenty-one subjects with HT (age 20.3±0.6 years, BMI 22.4±0.5 kg/m^2, systolic BP 141±2, diastolic BP 73±2 mmHg, mean ±SE) and 19 normotensive controls (NT; age 23.1±1.0, BMI 22.1±1.4 kg/m^2, systolic BP 117±3, diastolic BP 67±2) underwent a 75-g oral glucose tolerance test (OGTT) and magnetic resonance imaging for measurement of abdominal adipose tissue distribution. Fasting concentrations of leptin and adiponectin, and fasting and post load concentrations of glucose and insulin were measured in plasma. Indices of insulin sensitivity Cederholm (ISI_{CED}), Matsuda (ISI_{MAT}) and insulin resistance (IR HOMA) were also estimated. Abdominal VAT and subcutaneous adipose tissue depots (SAT) were measured from single transverse MRI scan in the space between L4 and L5. The study was approved by the Ethics Committee of the IEE.
Results
All subjects had normal fasting glucose levels and normal glucose tolerance. HT patients had higher IR HOMA (2.4±0.4 vs. 1.2±0.1, $P=0.007$) and lower ISI_{CED} and ISI_{MAT} (58±3 vs. 77±4, $P=0.0001$ and 5.1±0.6 vs. 9.0±0.8, $P=0.001$, respectively) than NT subjects. The two study groups did not differed in amount of VAT and SAT (31.80±8.63 vs. 47.35±6.78; 93.58±15.66 vs. 111.05±10.80 cm^2, NS), and in plasma levels of leptin and adiponectin (3.82±0.52 vs. 3.45±0.49 ng/ml; 1.71±0.40 vs. 1.40±0.21 μg/ml NS).
Conclusions
These results demonstrate that even lean subjects with recently established higher blood pressure and with normal fasting and post-load glucose levels display signs of insulin resistance. These changes were however not related to abdominal adipose tissue distribution or circulatory levels of leptin and adiponectin.

P66

Comparison of twice daily NPH insulin versus once daily glargine insulin in the frequency of nocturnal hypoglycemia in Type2 diabetic patients with congestive heart failure

Neslihan Kurtulmus[1], Fatma Demirdogen[2] & Tufan Tukek[2]
[1]Vakif Gureba Educational Hospital, Department of Endocrinology, Istanbul, Turkey, [2]Vakif Gureba Educational Hospital, Department of Internal Medicine, Istanbul, Turkey.

Aim
We had the aim to determine the insulin treatment strategy that could prevent or decrease the occurrence of hypoglycaemia while providing better regulation of blood glucose in Tip 2 diabetic patients with cardiac failure.
Method
The patients demonstrating similar characteristics with respect to the age, body mass index, the duration of diabetes and heart failure were randomized into two groups as insulin glargine (*n*: 19) and NPH (*n*: 11). The subjects have been prospectively followed up for 12 weeks.
Results
Basal blood glucose level was detected as 197.21±69.01 in insulin glargine group(group1), it was 175.45±52.26 in NPH insulin group(group2) ($P=0.339$). Basal postprandial blood glucose in group1 was found to be 191.42±63.42, it was 186.18±81.82 In group2 ($P=0.857$). The nocturnal(3.00 am) blood glucose was 191.42±63.42 in group1, it was 186.18±81.82 in group2 ($P=0.857$). In group1, basal HbA1c value was 8.11±1.98, which was found to be 7.88±1.49 in group2 ($P=0.728$). At week 12 of insulin therapy, HbA_{1c} value was 6.86±1.59% in group1, markedly decreased compared to initial HbA_{1c} value ($P<0.001$). In NPH group, HbA_{1c} was found to be 7.31±1.36% at week 12, which was also lower than that at the beginning of the treatment, however this result was not statistically significant ($P=0.417$). The frequency of nocturnal hypoglycaemia in group1 was detected to be 10.5%, compared to 9.1% in group2. In two groups did not show any statistical difference related to the frequency of nocturnal hypoglycaemia.
Conclusion
In our study, while the use of insulin glargine provided a better metabolic control compared to NPH insulin,but it failed to decrease the frequency of nocturnal hypoglycaemia in diabetic subgroup with cardiac failure.

P67

Intravenous constant ghrelin infusion in healthy young men: sustained cardiovascular effects of supraphysiological ghrelin levels

Esben Thyssen Vestergaard, Niels Holmark Andersen, Troels Krarup Hansen, Lars Melholt Rasmussen, Niels Moller, Keld Sorensen, Erik Sloth, Jens Sandahl Christiansen & Jens Otto Lunde Jorgensen
Aarhus University Hospital, Aarhus, Denmark.

Objective
The short-term cardiovascular effects of continuous ghrelin infusion in healthy humans remain to be studied.
Methods
Fifteen healthy, young and normal-weight men volunteered to participate in a randomized double-blind, placebo-controlled cross-over study. The local ethics committee approved the study. We used a constant infusion of human ghrelin at a rate of 5 pmol/kg body weight per minute for 180 minutes and measured peak left ventricular myocardial systolic velocity V_{max}, tissue tracking *TT* (GE Vivid Seven with a 2.5 MHz transducer) and endothelium-dependent flow-mediated vasodilatation of the radial artery (Acuson Sequoia C256, 8 MHz linear array vascular ultrasound transducer).
Results
Ghrelin infusion increased serum ghrelin levels ~6-fold (5.2 to 6.5) ($P<0.001$) , V_{max} increased ~9% ($P=0.002$), *TT* increased ~10% ($P=0.004$), while endothelium-dependent flow-mediated vasodilation did not change ($P=0.10$). Concomitantly, growth hormone peaked after 60 minutes of infusion (36.8±4.7 ng/ml, $P<0.001$), glucose levels increased 0.5±0.1 mmol/l ($P<0.001$), free fatty levels increased 1.7-fold ($P=0.002$), cortisol levels increased 1.4-fold (P 0.002), while insulin levels were constant.
Conclusion
Supraphysiological levels of ghrelin persistently improve left ventricular function in healthy young normal-weight men without changing endothelium-dependent flow-mediated vasodilation. It remains to be studied whether ghrelin exerts direct myocardial effects or indirect effects through the concomitant changes in glucose, growth hormone, free fatty acids and cortisol levels.

P68

Circulating retinol binding protein 4 and protein C inhibitor are not related to insulin resistance

Miriam Promintzer[1], Michael Krebs[1], Anton Luger[1], Martin Georg Bischof[1], Peter Nowotny[1], Christoph Binder[2], Harald Esterbauer[2] & Christian Anderwald[1]
[1]Division of Endocrinology and Metabolism, Department of Internal Medicine III, Medical University of Vienna, Vienna, Austria; [2]Clinical Institute of Medical and Chemical Laboratory Diagnostics, Medical University Vienna, Vienna, Austria.

Recent data suggest that circulating retinol binding protein-4 (RBP4) is involved in the pathogenesis of insulin resistance in rodents and humans. Moreover, protein C inhibitor (PCI) which specifically binds retinoic acid was found to be increased in myocardial infarction survivors who are also insulin-resistant.

Therefore, we investigated the association of insulin resistance with plasma retinol binding factors (RBP4 and PCI active antigen) in nondiabetic humans with high (IS; $n=20$, f/m=14/6, age: 47.2±1.9 years, BMI: 26±1 kg/m2) and low (IR; $n=20$, f/m=14/6, age:45.5±1.7 years, BMI:28±1 kg/m2) insulin-stimulated glucose-disposal (M), measured by 2-h hyperinsulinemic-(40 mU•min-1•m-2)-isoglycemic clamp-tests.

M (80–120 min) was higher in IS (10.9±0.6 mg•min-1•kg-1) than in IR (4.0±0.2; $P<10$–12). Fasting plasma RBP4 concentrations were comparable in IS (4.4±0.3 mg/dl) and IR (4.6±0.3). Fasting plasma PCI active antigen was similar in both groups (IS: 106.6±15.6%; IR: 95.3±4.0%). Plasma RBP4 and PCI were not significantly related to M.

In conclusion, our data demonstrate that healthy, nondiabetic, insulin-resistant humans do not show altered plasma retinol binding factors, such as RBP4 and PCI. Both do not significantly correlate with insulin sensitivity. Thus, our findings do not support the hypothesis of insulin sensitivity modulation by proteins involved in retinol transport.

P69

KCNJ11 and ABCC8 promotor variants in congenital hyperinsulinism

Klaus Brusgaard[1], Khalid Hussain[2], Lone Svargo[1], Bendt Brock-Jacobsen[1] & Henrik Christesen[1]
[1]Odense University Hospital, Odense, Denmark; [2]Great Ormond Street Hospital, London, United Kingdom.

Congenital hypoglycemic hyperinsulinemia (CHI) is a clinical and genetic heterogeneous entity. Clinical manifestations can vary from serious life threatening to milder difficultly identifiable cases. Children who don't react adequate to medical treatment are subject to pancreatic recession. The molecular ethiology are from recessive mutations of the *ABCC8* (SUR1) and *KCNJ11* (Kir6.2) to dominant mutations of the *GCK* or *GDH* genes. Focal dysplasia characterised by loos of maternal Chromosome 11 and hereby *ABCC8* and *KCNJ11* is a common cause of CHI. In some studies mutations in the *ABCC8* promotor have been shown to cause CHI. In approximately 50% of the incidences the disease is still genetically unexplained necessitating the search for other genetic factors.

The purpose of the present study was to identify new genetic causes of CHI in patients with a hitherto unexplained manifestation.

46 children and there parents was tested for mutations in the *ABCC8* and *KCNJ11* promotors by D-HPLC and sequencing. Samples with deviating chromatographic patterns were sequenced.

The a region covering 1063 bp including the minimal *KCNJ11* promoter and a region covering 930 pb including the *ABCC8* minimal promoter was analysed. In 13 samples a c.-507 del T mutation was found in the *KCNJ11* gene. This variant has not previously been described. Using SIGSCAN and TRANSFAC software possible transcription factor binding sites was predicted in this region site. No other variants were found in either of the two genes. If the c.-507 delT variant is a common cause of CHI in Denmark has to be further investigated.

P70

Effect of L- carnitine supplementation on glycemic profile in patients with type 2 diabetes mellitus

Rahebeh Sakerhosseini, Alireza Rahbar, Avid Saadat, Amir hamzeh Pordal, Feroug azam Taleban & Banefsheh Golestan
National Nutrition and Food Technology Research Institute, Tehran, Iran.

Background
It has been thought that L-carnitine is effective in improving insulin-mediated glucose disposal either in healthy subjects or in type 2 diabetic patients,and carnitine plays an important role in diabetes mellitus omplications (cardiovascular disease).
Objective
We designed this study to investigate the effects of oral L-carnitin administration on fasting plasma glucose (FPG) and glycosylated hemoglob (HbA1c), in patients with diabetes mellitus type II.
Materials and methods
The effect of L-carnitine on FPG and lipid parameters was investigated in 22 male and 14 female type II diabetic patients, mean age $\pm$ SD was 51.3 $\pm$ 3.7 years. The patients were randomly divided into 2 groups (i.e. test and control groups). One gram of L-carnitine or placebo was given orally three times a day to the test and control groups respectively for a period of 12 weeks.
Results
Fasting plasma glucose in the test group decreased significantly from 143 $\pm$ 35 mg/dl to 130 $\pm$ 35 mg/dl ($P = 0.03$. There were no significant changes in HbA1c, between the two groups.
Conclusion
L-carnitine significantly lowers fasting plasma glucose in type II diabetic patients

P71

Raised serum, adipocyte and adipose tissue retinol binding protein 4 (RBP4) in women with polycystic ovary syndrome

Bee Kang Tan[1], Jing Chen[1], Sheryl Pua[1], C Richard Kennedy[2] & Harpal S Randeva[1]
[1]Warwick Medical School, University of Warwick, Coventry, West Midlands, United Kingdom; [2]University Hospitals Coventry & Warwickshire NHS Trust, Coventry, West Midlands, United Kingdom.

Context
Polycystic ovary syndrome (PCOS) is associated with insulin resistance (IR) and obesity, both predisposing factors to type 2 diabetes. A very recently described adipokine, retinol-binding protein 4 (RBP-4), has been shown to modulate insulin signalling and possibly lead to IR. At present, there is no data that depict the relative expression of RBP-4 in either serum or adipose tissue of PCOS women.
Objectives
In women with PCOS compared to matched control women, we studied the mRNA expression of RBP-4 from subcutaneous (sc) and omental (om) adipose tissue and sc adipocytes. Furthermore, RBP4 protein levels were assessed in adipose tissue; serum RBP4 was also determined.
Methods
Real-time RT-PCR and western blotting were used to assess the relative mRNA and protein expression of RBP4. Biochemical measurements were also conducted. The Local Research Ethics Committee approved the study and all patients involved gave their informed consent, in accordance with the guidelines in The Declaration of Helsinki 2000.
Results
There was significant upregulation of RBP4 mRNA in both sc ($P < 0.05$) and om ($P < 0.01$) adipose tissue of PCOS women, when compared to normal controls; these findings were also reflected in isolated sc adipocytes (PCOS > controls; $P < 0.01$). In addition to elevated serum RBP4 levels in women with PCOS ($P < 0.05$), when compared to normal controls, RBP4 protein levels were significantly greater in both sc and om adipose tissue of PCOS women ($P < 0.05$ and $P < 0.05$, respectively).
Conclusions
RBP4, a new adipokine, is elevated in PCOS women. Our findings potentially introduce a novel concept into the aetiopathogenesis of insulin resistance in these women.

P72

Polymorphisms of von Willebrand factor gene promoter modulate the corticosteroid-mediated increase of VWF levels in Cushing's syndrome.

Viviana Daidone[1], Francesca Sartorello[2], Nora Albiger[1], Franco Mantero[1], Antonio Pagnan[2], Alessandra Casonato[2] & Carla Scaroni[1]
[1]Division of Endocrinology, Department of Medical and Surgical Sciences, University of Padova, Padova, Italy; [2]Division of Intenal Medicine, Department of Medical and Surgical Sciences, University of Padova, Padova, Italy.

Cushing's syndrome (CS) is associated with hypercoagulable state, mainly dependent on corticosteroid-induced increase of von Willebrand factor (VWF) levels, even though this does not affects all patients. In normals plasma VWF levels are genetically determined by ABO blood groups and polymorphisms G/C −1793, C/T −1234, A/G −1185, G/A −1050 of VWF promoter. These SNPs segregate as haplotype 1 (G/C/A/G) and haplotype 2 (C/T/G/A) with genotype 1/1 (GG/CC/AA/GG) associated with higher VWF:Ag levels than genotype 2/2 (CC/TT/GG/AA), and intermediate VWF values in heterozygote subjects (genotype 1/2). In this study we aim to investigate the relationship between SNPs of VWF promoter and VWF levels in CS patients, in order to evaluate whether glucocorticoid effects may be influenced by VWF promoter genotypes.

50 patients with Cushing's syndrome and 200 normal subjects were analyzed.

Patients were divided by ABO blood group into groups A (increased VWF) and B (normal VWF). While a significant difference in VWF levels was observed between the two groups ($P < 0.001$), cortisol values were similar ($P = 0.44$). A direct correlation between cortisol and plasma VWF levels was observed in group A ($P < 0.001$), while no correlation was found in group B ($P > 0.1$).Genotype distribution differed significantly between the two groups being 25.8% genotype 1/1, 22.6% type 2/2 and 38.7% type 1/2 in group A, as opposed to 0% type 1/1, 57.9% type 2/2 and 31.6% type 1/2 in group B ($P = 0.03$) and their genotypes also differed from the controls ($P = 0.003$ for group A, $P = 0.03$ for group B). Our findings suggest that corticosteroid-mediated increases of VWF, and its associated prothrombotic state, are dependent on peculiar haplotypes of VWF gene promoter. CS patients presenting genotype 1/1 have a higher risk of developing thrombosis than patients with genotype 2/2.

P73

Circulating pro- and anti-inflammatory cytokines in women with gestational diabetes

Mariusz Kuzmicki[1], Beata Telejko[2], Anna Zonenberg[2], Jacek Szamatowicz[3], Adam Kretowski[2], Agnieszka Nikolajuk[2] & Maria Gorska[2]
[1]Department of Pathophysiology of Pregnancy, Medical University, Bialystok, Poland; [2]Department of Endocrinology, Diabetology and Internal Medicine, Medical University, Bialystok, Poland; [3]Department of Gynecology, Medical University, Bialystok, Poland.

Gestational diabetes mellitus (GDM) identifies a population of women at high risk of subsequent type 2 diabetes mellitus, representing an early stage in the natural history of the disease. Systemic inflammation is associated with the development of type 2 diabetes but the data concerning pro- and anti-inflammatory cytokines in patients with GDM are limited. The aim of our study was to investigate serum concentrations of interleukin-8 (IL-8), IL-18 and IL-10 in pregnant women with various degree of glucose intolerance. The group studied consisted of 58 patients with GDM, 31 pregnant women with normal glucose tolerance (NGT) and 32 women with an abnormal result of a 50 g glucose challenge test (GCT) but a normal result of 75 g oral glucose tolerance test (OGTT). Serum IL-8, IL-10, IL-18 and CRP concentrations were measured by immunoenzymatic assays. Patients with GDM had markedly higher IL-8 and IL-18 levels than women with NGT (3.86 ± 5.44 vs 0.8 ± 0.57 pg/ml, $P=0.00001$ and 264.4 ± 111.98 vs 203.57 ± 108.14 pg/ml, $P=0.0005$, respectively), as well as significantly lower IL-10 concentrations (1.37 ± 2.04 vs 2.86 ± 1.53 pg/ml, $P=0.00001$). There were no significant differences in interleukin levels between patients with NGT and abnormal GCT. There were significant correlations between IL-8 concentration and prepregnancy BMI ($R=0.2093$, $P=0.031$), insulin ($R=0.42075$, $P=0.00004$), HOMA-IR ($R=0.45857$, $P=0.000001$), and glucose ($R=0.2030$, $P=0.03$), as well as between IL-18 level and insulin ($R=0.20055$, $P=0.0301$) and HOMA-IR ($R=0.20385$, $P=0.028$). IL-10 correlated inversely with insulin ($R=-0.26822$, $P=0.0036$) and HOMA-IR ($R=-0.29127$ $P=0.0016$). CRP correlated with insulin ($R=0.28875$ $P=0.0017$) and HOMA-IR ($R=0.28836$, $P=0.0019$). Our results suggest that GDM is associated with elevated concentrations of pro-inflammatory cytokines IL-8 and IL-18, as well as with low level of anti-inflammatory IL-10. This association seems to be mediated in part by the indices of insulin resistance.

P74

Abstract unavailable

P75

Metabolic improvement in diabetic patients with glucose continuous monitoritation in real time

Cristobal Morales Portillo, Isabel Serrano Olmedo, Silvia Maraver, Amaya Fernandez Arguelles, Juan Manuel Garcia-Quiros Muñoz, Guillermo Martinez De Pinillos, Carmen De La Cuesta Mayor & Angel Sendon Perez
Virgen Macarena Hospital, Seville, Spain.

Objective
To study if the use of continuous glucose monitoritation systems in real time (GUARDIAN REAL TIME®) in the diabetic patients could improve the metabolic control.
Material and method
15 type 1diabetic patients studied (age: 33.8 ± 13.40, years of evolution: 17.06 ± 11.32) in intensive treatment (5 multidoses, 10 insulin pump) which were made one first blind 4 days long monitoritation to them, PERIOD 1 (P. BLIND) with sensor CGMS Gold®. Next it was made another monitoritation with a real time system with glycemia and alarms of hyperglycemia and hypoglycemia, GUARDIAN RT®, PERIOD 2 (P. Real time). These 2 monitoritations were consecutive in each patient, establishing in the second period the levels of alarm of hypoglycemia in 50 mg/dl and hyperglycemia in 200 mg/dl. We studied in both periods: Average glycemia Glycemia Variability, Percentage of time in hiperglycemia (>180), normoglycemia and hipoglycemia (<70). A non-parametric test for matched up data was made (Wilcoxon)
Results

	PERIOD 1 (BLIND)	PERIOD 2 (R.T)	P
Average glucose	**157.17 ± 34.30** (110–224)	**137.49 ± 21.99** (115–203)	**0.053**
Variability	**73.72 ± 19.60** (38–103)	**48.23 ± 13.20** (25–75)	**<0.005**
% High	**32.56 ± 19.25** (7.0–75.0)	**19.90 ± 13.87** (1.23–56.03)	**<0.05**
% Euglucemia	**55.49 ± 17.79** (25.0–86.0)	**74.98 ± 14.22** (43.97–98.77)	**<0.005**
% Low	**11.94 ± 8.04** (0.00–29.00)	**5.10 ± 5.16** (0–17.00)	**<0.005**

Conclusions
In our patients we observed during the monitoritation in real time: – longer time in normoglycemia with decrease of the frecuency in hipoglycemia and hiperglycemia. – smaller glycemia variability.The monitoritation in real time could be a useful tool at the time of assuring a better metabolic control and to diminish the exhibition to hipoglycemias.

P76

The role of stress related aldosterone secretion in essential hypertension

Athina Markou[1], Ioannis Androulakis[1], Aggeliki Gouli[1], Kostantina Dimitriou[1], Achilleas Zacharoulis[2], Kostantinos Papadopoulos[2], Theodoros Barlogiannis[2], Evangelos Chariatis[2], Antony Halapas[2], Eleni Kourlaba[2], Despoina Ragkou[1], Georgia Kondyli[1] & George Piaditis[1]
[1]General Hospital of Athens "G. Gennimatas", Department of Endocrinology & Diabetes Center, Athens, Greece; [2]General Hospital of Athens "G. Gennimatas", Cardiology Department, Athens, Greece.

Introduction
Approximately 90% of the hypertensive population is characterized as having essential hypertension. Apart from renin and K^+, ACTH plays an important role in aldosterone secretion, being a potent stimulant under situations of stress. Up to date, the association between stress and aldosterone-related hypertension has not been studied and that is the aim of our study.
Methods
36 hypertensive patients and 14 matched for age and sex controls (BP < 140/90 mmHg), had baseline biochemical profile, TSH, cortisol, ACTH, aldosterone, active renin and 24 hr urine Na^+/K^+ measurements, followed by a Bruce protocol exercise test aiming at the 80% of maximal effort according to Froelicher normograms and repeated the hormonal profile at peak exercise. 17 hypertensines and 7 controls had a 0.03 mcg ACTH stimulation test. Hypertensive patients on treatment were switched to a calcium channel blocker for at least 3 weeks before. Exclusion criteria were any cause of secondary hypertension, renal, hepatic or heart failure, ischemic heart disease and diabetes mellitus. CT scan of the adrenals was performed in both groups.
Results
Exercise test: baseline ACTH and aldosterone to renin ratio (ARR) did not differ but at peak exercise hypertensives had statistically higher ACTH and ARR levels compared to controls [35.97 ± 5.59 (mean $\pm$ S.E.M.) vs 23.24 ± 4.25 pg/ml, $P=0.046$ and 138.83 ± 34.22 vs 55.22 ± 34.45 pmols/L/pg/ml, $P=0.015$].
0.03 mcg ACTH test: there was a trend towards higher values in ARR at peak in hypertensives that did not reach statistical significance probably due to the low number of patients.
Conclusions
Using an exercise test at sub maximal effort in order to mimic every day's life physical stress, we observed a higher response of aldosterone to stress in patients with hypertension. Therefore, stress related aldosterone hyper secretion may play a causative role in essential hypertension with major implications in its treatment.

P77

Dialysis therapy and its complications

Evgeniya Bublik, Gagik Galstyan, Galina Melnichenko, Victor Safonov, Euginy Shutov, Pavel Filipcev & Oleg Udovichenko
[1]National Research Centre of Endocrinology, Moscow, Russia; [2]Moscow Sechenov Medical Academy, Moscow, Russia.

Aim
To study prevalence of foot complications in patients on dialysis therapy and evaluate the role the haemodynamic changes during dialysis procedure in development of foot problems.

Methods
109 dialysis patients, mean age 49 years. 60 of them had diabetes mellitus (DM): 29 on haemodialysis (HD), 31 on peritoneal dialysis (PD). Non-diabetic patients (NDM): 24 HD, 25 PD.
Vascular status: doppler, photoplethysmography. Polyneuropathy: NDS. Monitoring of BP: during and after dialysis (follow-up period 14 months).
Results
Peripheral vascular disease (PVD) was associated with DM (16 DM vs. 1 NDM). Polyneuropathy: 51 DM, and in 7 NDM.
Ulcers were diagnosed only in DM patients (prevalence 25%). The number of patients with diabetic ulcers was increased after starting dialysis therapy (from 4 to 12) and it was due to increasing neuroischemic ulcers (from 0 to 8, $P=0{,}016$). Transient intra- and postdialysis hypotension (TH) was determined in 15 HD, 5 PD. This group had significant fall of pressure in toe arteries during TH. Neuroischemic ulcers were frequently diagnosed in the group with PVD and HD, than with PVD and without HD; $P=0{,}001$.
Conclusions
Diabetic patients on dialysis therapy have high risk of neuroischemic ulcers. TH can intensify PVD and provoke neuroischemic ulcers.

P78

The effect of interferon treatment on glucose metabolism in patients with chronic hepatitis

Atilla Alev[1], Ayse Kubat Uzum[2], Ali Murat Tatli[1], Fatih Oner Kaya[1], Iskender Dik[1] & Burhan Bedir[1]
[1]Istanbul Training and Research Hospital, Istanbul, Turkey; [2]Istanbul University, Istanbul Faculty of Medicine, Department of Internal Medicine, Division of Endocrinology and Metabolism, Istanbul, Turkey.

Introduction
In recent years, interferon (IFN) is used in treatment of chronic hepatitis and the studies about the side effects of IFN therapy are increasing.
Objective
We aimed to investigate the effects of IFN therapy on glucose metabolism.
Materials and methods
Study group was consisted with 30 patients who were diagnosed as chronic hepatitis. Sixteen of 30 were chronic hepatitis B and 14 were chronic hepatitis C. Diagnose was confirmed by serology and liver biopsy. Patients with chronic hepatitis B were prescribed alpha-IFN, 9–10 MU/three times/week and chronic hepatitis C were given alpha-IFN, 3 MU/three times/week, subcutaneously. All patients were evaluated by fasting plasma glucose concentrations (FPG) and oral glucose tolerance test (OGTT) at the beginning and and at the 4th week of IFN treatment. Diagnose of impaired fasting glucose (IFG), impaired glucose tolerance (IGT) and diabetes mellitus (DM) was approved by American Diabetes Association (ADA) criteria.
Results
The study group was consisted of 16 (53.3%) female and 14 (46.7%) male patients. Mean age was 42 ± 13.67 years. Twenty eight patients had normal FPG concentrations, whereas two had IFG. No patient had DM. Mean FPG concentrations of chronic hepatitis B and C was 91.13 ± 8.25 and 96.5 ± 97.07 mg/dl, respectively. At the 4th week of the therapy, we reevaluated the patients for glucose metabolism. Difference between FPG levels before and after treatment were not statistically significant (93.63 ± 10.54 and 94.33 ± 16.01 mg/dl; $P>0.05$). However OGTT results were affected by the therapy. Nineteen patients (63.3%) had normal, six had IGT anf 5 had DM. Mean glucose concentrations during initial and second OGTT were 106 ± 26.53 and 132 ± 17 mg/dl respectively ($P<0.001$).
Conclusion
IFN treatment alteres glucose metabolism. Therefore, patients who had chronic hepatitis and treated with IFN should be followed-up closely for diabetes mellitus during and after the therapy.

P79

Unacylated ghrelin (UAG) enhances the early insulin response to meal, improves glucose metabolism and decreases free fatty acids levels in healthy volunteers

Flavia Prodam[1], Fabrizio Riganti[1], Elena Gramaglia[1], Andrea Benso[1], Barbara Lucatello[1], Fabio Broglio[1], Thierry Abribat[2], Aart Jan van der Lely[3] & Ezio Ghigo[1]
[1]Dept. Internal Medicine – University of Turin, Turin, Italy; [2]Alizé Pharma, Lyon, France; [3]Dept. Internal Medicine – Erasmus University, Rotterdam, Netherlands.

Ghrelin circulates in two different forms. Acylated ghrelin (AG), a natural ligand of the GH Secretagogue receptor (GHS-R) type 1a, exerts several biologic central and peripheral actions including stimulation of GH secretion, but also modulation of insulin secretion, glucose and lipid metabolism. Unacylated ghrelin (UAG), despite unable to bind the GHS-R1a, is biologically active showing some influence *in vitro* and *in vivo* on glucose and lipid metabolism likely mediated by still unknown receptors. Based on these data, the aim of our study was to investigate the endocrine and metabolic effects of prolonged UAG administration in humans in physiological conditions. To this goal, the effects of UAG (1.0 mcg/kg/h infused iv over 16 hours from 21.00 to 13.00 h) or saline were studied in 8 normal subjects who had isocaloric balanced standardized meals at h21.20 and h09.00. Blood samples were collected every 20 min. Compared to saline, UAG infusion significantly modified the profile of all parameters, except glucagon. Compared to saline, UAG decreased glucose ($P<0.01$) and FFA AUCs ($P<0.01$). The glucose decrease during UAG was particularly relevant at fasting during nighttime ($P<0.01$) while FFA profile was reduced both post-prandially and at fasting($P<0.01$). UAG did not modify total insulin AUC; however, the early insulin response to both dinner ($P<0.01$) and breakfast ($P<0.05$) was enhanced by UAG infusion that was associated to decrease in the nighttime HOMA index ($P<0.01$). During UAG, cortisol ($P<0.01$) and GH ($P<0.05$) AUCs were lower than those during saline, but cortisol levels remained within physiological values. Thus, the intravenous infusion of UAG in normal subjects enhances the early insulin response to meals, improves glucose metabolism and insulin sensitivity, and inhibits lipolysis. Thus, UAG displays a remarkable metabolic impact suggesting a promising anti-diabetogenic action through an original mechanism of action.

P80

Weight-related concentrations of steroid hormones in patients of both sexes with preserved gonadal function suffering from coronary artery disease

Zbigniew Sablik[1], Anna Samborska-Sablik[2], Jan Henryk Goch[1] & Krzysztof Kula[3]
[1]Department of Cardiology and Cardiac Surgery, Medical University of Lodz, Lodz, Poland; [2]Department of Emergency Medicine and Disaster Medicine, Medical University of Lodz, Lodz, Poland; [3]Department of Andrology and Endocrynology of Fertility, Medical University of Lodz, Lodz, Poland.

Aim
To define concentrations (conc) of steroid hormones in patients (pts) with preserved gonadal function, suffering from coronary artery disease (CAD), and to assess their relations to patients' weight.
Material
Pts with coronarographically proved CAD: C-W group (gr)-52 women (W) in the age 43 ± 3 years, with stable menstrual rhythm, C-M gr-46 men (M) in the age 52 ± 6 years. Healthy volunteers: H-W gr-15 W (H-W) in the age 41 ± 4 years, H-M gr-13 M in the age 51 ± 6 years.
Methods
In all pts occurrences of common risk factors of CAD including values of body mass index (BMI) and waist-hip-ratio (WHR) were defined. To assess concentrations of hormones in pts of all grs blood samples from cubital vein were taken at 8.00 a.m., in W in 4-7 day of sexual cycle. Using immunological methods conc of estradiol (E2), testosterone (T), dehydroepiandrosterone sulphate (DHEAS), follicle-stimulating hormone (FSH), luteinizing hormone (LH), progesterone (P) and cortisol (Cort) were measured.
Results
Only conc of T was significantly higher in C-W than in H-W (3.5 ± 1.7 vs 2.4 ± 1.0 nmol/l, $P<0.02$). In C-W a negative correlation between BMI or WHR and conc of T and DHEAS was found.
In C-M, comparing to H-M, conc of P and conc of Cort were higher (3.5 ± 1.6 vs 1.4 ± 1.0, $P<0.001$, and 345 ± 97 vs 246 ± 96 nmol/l, $P<0.01$, respectively) and a there was a trend towards lower conc of T (10.3 ± 3.8 vs 12.2 ± 3.3 nmol/l, $P<0.1$). In C-M we found a negative correlation of BMI or WHR with conc of P and DHEAS and positive correlation with conc of E2. Because in C-M a positive correlation between conc of P and T, and conc of P and Cort was present, there was an indirect negative relationship between BMI or WHR and conc of T and Cort.
Conclusions
T is involved in pathogenesis of CAD and plays proatherogenic role in young women and probably antiatherogenic role in men. In both sexes excessive weight is a potent risk factor of CAD, because it influences conc of steroid hormones of gonadal and adrenal origin including changing conc of T in unfavourable manner.

P81

Diabetic patient's evaluation of continuous glucose monitoring sensors versus capillary glucose measurements

Guilermo Martínez De Pinillos Gordillo, Amaya Fernández-Argüelles & Juan Manuel Garcí-Quirós Jm
Hospital Virgen Macarena, Sevilla, Spain.

Objective
To evaluate the monitoritation systems acceptance: capillary glucose measurements and continuous glucose sensors (CGSM and GUARDIAN).
Research design and methods
15 diabetics patients were monitoritatied in two diferents periods of time. (Period 1: Guardian, 86 hours long. Period 2: CGSM 72 hours long). Later, they had to fill a satisfaction questionnaire concerning several aspects which were valued from 0–6.

Table 1

Results	Capillary	*P*	Guard	*P*	CGMS
System satisfaction	4.4	Ns	4.2	Ns	3.8
Information given	4.8	Ns	4.9	Ns	5.1
Recomend-able system	4.9	Ns	5.1	$P<0.05$	4.1
Wish to continue	4.4	Ns	3.8	Ns	3.1
Uncomfort-ability	2.1	Ns	3.2	Ns	4.1
Anxiety	1.0	Ns	1.8	Ns	1.9
Interference with: work	1.8	Ns	1.3	$P<0.05$	2.5
social life	1.5	Ns	1.1	$P<0.05$	2.1
physical activity	1.1	$P<0.05$	2.7	Ns	3.1
hygiene	0.5	$P<0.005$	2.3	$P<0.005$	4.2
sexual life	0.5	$P<0.05$	2.5	$P<0.05$	3.5
dream quality	0.3	$P< 0.05$	1.5	Ns	2.3
clothing	0.6	Ns	2.1	Ns	3.0

Conclusions
The information given both by capillary mesearuments and continous clucose sensors was valued positively by our patients without significative differences between them but with a bigger acceptance with the Guardian. Real time monitorization did not generate greater anxiety than the blind registry. Glucemia sensors interfere in the daily life of the patients in most of the studied aspects but less with the Guardian than the CGSM sensor.

P82

Dehydroepiandrosterone therapy in men with verified coronary heart disease: the effects on fibrinogen, plasminogen activator inhibitor-1 (PAI-1) and tissue plasminogen activator (tPA)

Michal Rabijewski, Lucyna Papierska & Wojciech Zgliczynski
Department of Endocrinology; Medical Center for Postgraduate Education, Warsaw, Poland.

Objectives
The aim of this study was to analyze the influence of DHEA therapy on fibrinogen, plasminogen activator inhibitor-1 (PAI-1) and tissue plasminogen activator (tPA) plasma concentrations in men with decreased serum DHEAS levels and angiographically verified coronary heart disease (CHD).
Material and methods
The study included thirty men aged 41–60 years (mean age 52 ± 0.90 yr) with serum DHEAS concentration <2000 μg/l, who were randomized into a double-blind, placebo-controlled, cross-over trial. Subjects completed the 80 days study of 40 days of 150 mg oral DHEA daily or placebo, and next groups were changed after 30 days of wash-out. Fasting early morning blood samples were obtained at baseline and after each treatment to determine serum hormones levels (testosterone, DHEA-S, LH, FSH and estradiol) and also fibrinogen, plasminogen activator inhibitor-1 (PAI-1) and tissue plasminogen activator (tPA) plasma concentrations.
Results
Administration of DHEA was associated with 4.5-fold increase in DHEA-S levels. Estrogen levels significantly increased after DHEA from 22.1 ± 0.7 pg/mL to 27.4 ± 1.6 pg/L (mean $\pm$ S.E.M.; $P<0.05$), while testosterone levels did not changed. Fibrynogen concentrations significantly decreased in DHEA group from 4.5 ± 0.3 g/L to 3.83 ± 0.2 g/L ($P<0.05$ vs placebo). Changes of tissue plasminogen activator (tPA) and plasminogen activator inhibitor-1 (PAI-1) were not statistical significant (respectively: 8.37 ± 0.4 ng/mL vs 8.93 ± 0.5 ng/mL and 82.3 ± 6.3 ng/mL vs 92.7 ± 9.1 ng/mL (mean $\pm$ SEM; NS vs placebo). Mean testosterone levels did change. Tolerance of the treatment was good and no adverse effects were observed.
Conclusions
DHEA therapy in dose of 150 mg daily during 40 days in men with DHEAS levels <2000 μg/l and angiographically verified coronary heart disease (CHD) was connected with significant decreasing of fibrinogen concentration and increasing of estradiol levels, and did not influence on plasminogen activator inhibitor-1 (PAI-1) and tissue plasminogen activator (tPA) plasma concentrations.

P83

Insulin resistance and insulin secretion in non-diabetic acromegalic patients

Dan Niculescu[1], Mariana Purice[2], Radu Lichiardopol[1] & Mihail Coculescu[1]
[1]Carol Davila University, Bucharest, Romania; [2]C. I. Parhon Institutute of Endocrinology, Bucharest, Romania.

Aim
To set up, in acromegaly without diabetes mellitus, a correlation between the disease activity in GH-secreting adenoma (AA) - assessed by minimum GH serum level during an oral glucose tolerance test (OGTT) - and severity of insulin resistance (IR), assessed by HOMA-IR index.
Methods
75 out of 88 consecutive patients with acromegaly hospitalized in our department were included in this study. 13 patients proved to have diabetes mellitus and were excluded. Serum glucose, GH and insulin levels were measured by immunor-adiometricassay basal and at 30, 60 and 120 minutes after a 75 g OGTT in 88 patients with active or cured acromegaly. IR was assessed using HOMA-IR index. A value over 2.5 was considered indicating IR. An Ethical Committee approval has been obtained for this study.
Results
Out of 75 patients without diabetes mellitus, 36 subjects (48%) were presenting with IR (34 with active disease, 2 cured). We found a significant positive correlation ($r=0.56$, $P<0.001$) between AA and HOMA-IR. The GH minimal level corresponding to the intersection of the exponential regression curve with the HOMA-IR level of 2.5 was 8.8 ng/mL, a cut-off point indicating IR with 82% specificity and 78% sensitivity. The odds ratio for developing IR becomes significant at a minimum GH level during OGTT of 2 ng/mL (odds ratio 7.6, 95% confidence interval 2–29).
Conclusions
The severity of IR revealed by acromegaly correlates with GH production. A GH serum level higher than 2 ng/mL during OGTT indicates an increased risk for developing IR. This cut-off level of GH can be used as one of criteria of cured disease, regarding the lack of metabolic effects.

P84

Response to metformin treatment in adolescent siblings with familial partial lipodystrophy of the dunnigan variety (FPLD) due to the R482W LMNA gene mutation

James Ryan[1], Patrick Kiely[2], Vivion Crowley[3], Michael Maher[4], Rosemary O'Connor[2] & Domhnaill O'Halloran[1]
[1]Department of Endocrinology, Cork University Hospital, Cork, Ireland; [2]Department of Biochemistry, University College Cork, Cork, Ireland; [3]Department of Clinical Chemistry, Trinity College, Dublin, Ireland; [4]Deparment of Radiology, Cork University Hospital, Cork, Ireland.

FPLD is a rare monogenic cause of insulin resistance. We document responses to treatment with metformin in 2 adolescent sisters with FPLD due to heterozygosity for R482W *LMNA* gene mutation.

The probands, aged 14 and 16 years, presented with secondary amenorrhoea, hirsutism and progressive acanthosis nigricans. Phenotypically they showed central obesity, nuchal enlargement, and thin muscular arms. These changes occurred post-pubertally. Anthrpometric and metabolic parameters of the probands, their R482W

mutation positive father and three R482W negative sisters are shown in Table 1. Proband B had impaired glucose tolerance at diagnosis. Limb MRI of the probands showed almost complete absence of subcutaneous fat; neck MRI showed lipohypertrophy. Liver ultrasound of the probands and father showed diffuse fatty infiltrate. Both probands had cystic ovaries. A therapeutic trial with metformin in both probands showed a modest improvement in insulin resistance scores(Table 1). Proband A had regression of acanthosis nigricans, Proband B regained normal glucose tolerance. Both regained menses.

Table 1

	Proband A	Proband B	Father C	Sibling D	Sibling E	Sibling F
Fasting Insulin(mIU/L)/ C-peptide(μg/L)	71.9/6.52	44.92/6.29	32.64/6.02	9.62/1.75	0.37/2.2	0.81/0.98
Baseline HOMA-IR	13.74	9.78	7.25	1.84	0.067	0.14
Baseline OGIS (ml/min/m2)	159	195	285	426	449	543
HOMA – IR @6/12	10	8.37				
@6/12 OGIS (ml/min/m2) @6/12	185	184				

This kindred demonstrate the classical phenotype associated with FPLD, including marked insulin resistance. While FPLD may be rare, it is nonetheless vital to recognise this condition, as it is associated with significant morbidity and mortality. Furthermore, while lamin mutations are associated with different diseases this particular mutation is not well studied. We document a modest decrease in insulin resistance and regression of secondary amenorrhoea in response to metformin. Further longitudinal studies are required to fully evaluate metformin as a treatment modality for FPLD.

P85

Insulin sensitivity and lipid levels in pateints with primary hyperparathyroidism

Goran Cvijovic, Dragan Micic, Aleksandra Kendereski, Svetlana Zoric, Mirjana Sumarac-Dumanovic, Danica Pejkovic & Maja Georgiev
Institute of endocrinology, diabetes and diseases of metabolism, Belgrade, United States.

Patients with primary hyperparathyroidism (PHPT) are insulin resistant. The effect of PHPT on lipid levels and low-grade inflammation leves is unknown. The aim of our study was to estimate the cardiovascular risk profile in patients with PHPT. Methods: In patients with PHPT ($N=19$; age: 58.15 ± 8.38 years; PTH 180.83 ± 104.15 ng/l, calcium 2.97 ± 0.19 mmol/l) insulin sensitivity (measured using euglycemic hyperinsulinemic clamp - M value), lipids (total cholesterol, HDL-C, LDL-C, triglycerides, ApoA1 and ApoB) and CRP levels were measured. Results: There were low-normal level of insulin sensitivity (M value: 4.29 ± 0.52), slightly elevated levels of total cholesterol (6.07 ± 1.39 mmol/l) and LDL-C (3.72 ± 1.04 mmol/l) and normal levels of HDL (1.28 ± 0.08 mmol/l), triglycerides (1.80 ± 0.19 mmol/l), ApoA1 (1.54 ± 0.09 g/l), ApoB (1.19 ± 0.09 g/l) and CRP (1.58 ± 0.52 mg/dl) levels. There were negative correlations between M index and total cholesterol ($r=-0.56$, $P<0.05$) and Apo B ($r=-0.77$, $P<0.05$) levels, while there was positive correlation between PTH and CRP levels ($r=0.55$, $P<0.05$). In conclusion, low-normal insulin sensitivity and elevated levels of total holesterol and LDL-C were observed in our group of patients with PHPT. Further evaluation of low-grade inflammation is necessery in this group of patients.

P86

The effect of surgical treatment on insulin sensitivity in pateints with primary hyperparathyroidism

Aleksandra Kendereski, Dragan Micic, Goran Cvijovic, Svetlana Zoric, Mirjana Sumarac-Dumanovic, Danica Pejkovic & Maja Georgiev
Institute of endocrinology, diabetes and diseases of metabolism, Belgrade, United States.

It was reported that patients with primary hyperparathyroidism (PHPT) are insulin resistant, and that surgical treatment may improve insulin sensitivity in this group of patients. The aim of our study was to evaluate the effect of surgical treatment on insulin sensitivity in patients with PHPT. Methods: In patients with PHPT ($N=19$; age: 58.15 ± 8.38 years) insulin sensitivity was estimated using euglycemic hyperinsulinemic clamp (M value) before and 3 months after surgical treatment. Results: There was significant reduction of PTH (180.83 ± 104.15 vs 46.11 ± 19.45, $P<0.05$) and calcium serum levels (2.96 ± 0.19 vs 2.29 ± 0.17 mmol/l, $P<0.05$) after surgical treatment. We have observed low-normal insulin sensitivity before operation with significant improvement after surgical treatment (M value: 4.29 ± 0.52 vs 8.21 ± 1.44, $P<0.01$). There was no change in BMI (25.72 ± 3.70 vs 24.93 ± 3.33 kg/m2, $P>0.05$) and waist/hip ratio (0.82 ± 0.11 vs 0.85 ± 0.13, $P>0.05$) before and after operation (when the tests were performed). There were no correlations between chages (%Δ) of M index and PTH ($r=0.32$, $P>0.05$) and calcium ($r=0.05$, $P>0.05$) levels. In conclusion, surgical treatment improves insulin sensitivity in patients with PHPT. The mechanism of insulin resistance and its improvement after surgical treatment remains unclear in patients with PHPT.

P87

Insulin-sensitivity and glycaemic control improve on rosuvastatin (RSV) treatment in hypertriglyceridaemic type-2 diabetes (T2DM)

Giorgio Silvani, Andreas Tartaglia, Silvia Acquati, Anna Laura Vacirca, Antonella Bondi, Lorenza Gagliardi & Maurizio Nizzoli
Unit of Endocrinology, GB Morgagni-L Pierantoni Hospital, Forlì, Italy.

Aims
Studies on statins and insulin-sensitivity in T2DM are highly controversial. We aimed to evaluate the effect of RSV in type-2 diabetic people and whether its action may be phenotype-dependent, i.e. triglyceride (TG)-related (study approved by the local Ethical Committee).
Methods
48 type-2 diabetic pts (22 M:26 F), in a poor glycaemic control with oral agents, insulin or a combination therapy (unchanged over the study), were given RSV 10 mg for 12 weeks and stratified in 2 groups (23:25) by fasting TG-levels (<150 and 150–400 mg/dl), matched for age (59.7 ± 9.8 vs 60.0 ± 10.2 years), BMI (29.8 ± 4.9 vs 29.9 ± 5.5 kg/mq), waist (103.9 ± 10.8 vs 105.7 ± 11.1 cm), HbA1c (8.3 ± 1.0 vs $8.5\pm1.2\%$), total cholesterol (TC) (245.8 ± 33.6 vs 264.7 ± 26.1 mg/dl), LDL-C (167.2 ± 31.2 vs 176.6 ± 35.0), HDL-C (45.7 ± 11.6 vs 44.0 ± 14.4), and Apo-B (152.4 ± 34.8 vs 170.2 ± 28.1). Baseline- and 12-wk samples were taken for TC, LDL-C, HDL-C, TG, Apo-B, HbA1c, fasting plasma glucose and insulin. Homeostasis Model Assessment for Insulin-Resistance (HOMA-IR) was calculated, baseline score being higher in the 2nd group (4.68 ± 1.0 vs 6.32 ± 1.5 $P<0.05$).
Results
In both groups RSV lowered LDL-C (-47.2 vs -45.8%) and Apo-B (-40.7 vs -39.6%) significantly and to a similar extent. HDL-C was significantly increased ($+5.3$ vs $+4.4\%$) irrespective of changes in TG levels, mostly affected by RSV in the 2nd group: 133.5 ± 47.9 (-17.2%) vs 250.5 ± 60.1 (-25.9%) $P<0.001$. HOMA-IR correlated with TG ($r=0.21$) and was significantly decreased by RSV-treatment in hyper-TG-group (3.35 ± 0.9 vs 6.32 ± 1.5 $P<0.001$), as far as HbA1c showed a slight but significant improvement (-0.7% $P<0.05$), while no change was detected in HOMA-score or in HbA1c level in normo-TG-one, BMI and waist being not modified in both.
Conclusions
Perturbations in large-VLDL- and TG-metabolism generate an atherogenic lipid profile in T2DM and are closely linked with insulin-resistance. So in our data RSV improves HOMA-IR and HbA1c in hyper-TG type-2 diabetic pts by lowering TG-levels and seems to have both phenotype-independent and –dependent (TG-related) actions.

P88

Gestational diabetes mellitus and adiponectin levels

Cavit Culha, Suheyla Gorar, Rustu Serter, Yavuz Demir, Pinar Karakaya & Yalcin Aral
Ankara Education and Research Hospital, Department of Endocrinology, Ankara, Turkey.

Aim
Hypoadiponectinemia is known to be associated with insulin resistance, diabetes and obesity. Since gestational diabetes is together with increased body mass index (BMI) and decreased insulin sensitivity, the evaluation of adiponectin levels in these patients is interesting. We investigated the relationship between adiponectin and glucose tolerance during pregnancy and after delivery.

Materials and methods
We evaluated plasma adiponectin levels, insulin resistance and glucose tolerance in women with gestational diabetes mellitus (GDM, $n=16$) and in normal pregnancies (controls, $n=18$). Measurements were performed two times as in 28–31 weeks of pregnancy and at 3 months after delivery. Insulin resistance was calculated by the homeostasis model assessment (HOMA-IR).
Results
Four of the GDM patients remained as impaired glucose tolerance after delivery. Adiponectin levels during pregnancy were significantly lower in women with GDM compared to normal pregnancies (7.68 ± 6.26 μg/ml vs 12.72 ± 3.72 μg/ml; $P<0.01$). Adiponectin levels increased significantly after delivery both in GDM and control groups. Despite the increment after delivery, adiponectin remained significantly lower in women with GDM compared to controls (11.75 ± 6.11 μg/ml vs 16.55 ± 3.05 μg/ml; $P<0.01$). In HOMA-IR, the differences between two groups before and after delivery, and also the changes with delivery within the groups, were not found statistically significant. Adiponectin was correlated negatively with HOMA-IR ($r=-0.39$, $P<0.05$), third-trimester BMI ($r=-0.37$, $P<0.05$) and one-hour plasma glucose ($r=-0.33$, $P<0.05$); and positively with HDL-cholesterol ($r=0.34$, $P<0.05$) in women with GDM. These correlations including the adiponectin-HOMA-IR one disappeared following the delivery.
Conclusion
Decreased adiponectin levels in GDM do not normalise instantaneously following the delivery. The difference is more apparent in adiponectin levels than in HOMA-IR. There is a moderate correlation between adiponectin and one-hour plasma glucose in GDM.

P89

Lipoprotein Lp(a) in patients with systemic lupus erythematosus. Relationship with disease activity and anticardiolipin antibodies

Panagiotis Athanassiou[1], Ifigenia Kostoglou-Athanassiou[2], Despina Papadopoulou[1], Chrysoula Galanaki[1], Thomais Kalogirou[3], Athanasios Kordalis[3], Christodoulos Antoniadis[1] & Philippos Kaldrymidis[2]
[1]Department of Rheumatology, Asclepeion Hospital, Athens, Greece; [2]Department of Endocrinology, Metaxa Hospital, Piraeus, Greece; [3]1st Department of Internal Medicine, Asclepeion Hospital, Athens, Greece.

Systemic lupus erythematosus (SLE) is a multisystem multifactorial autoimmune disorder. The survival of SLE patients has been improved by the administration of immunomodulatory therapy. Patients, however, are affected by late onset complications of the disease such as atherosclerosis. Lipoprotein Lp(a) is a known risk factor for the development of atherosclerosis.

The aim was to study Lp(a) levels and their relationship with disease activity in SLE patients.

Patients with SLE, $n=74$, aged 21–64 years, and normal controls, $n=74$, of the same age and sex were studied. In patients and controls the levels of hematocrit, hemoglobin, erythrocyte sedimentation rate, C-reactive protein, antinuclear antibodies, complement, anti-dsDNA antibodies, cholesterol, triglycerides, HDL, LDL and lipoprotein Lp(a) were measured.

Lp(a) levels (normal values <30 mg/dl) were found increased in 23 of 74 (31.1%) patients with SLE and in 9 of 74 (12.2%) controls. Within the group of 23 SLE patients with increased Lp(a) levels 17 (73.9%) had active disease. In 11 of 23 (47.8%) SLE patients with increased Lp(a) levels anticardiolipin antibodies were detected, while anticardiolipin antibodies were found in 12 of 51 (23.5%) patients with Lp(a) levels within the normal range. All patients with active disease and increased Lp(a) levels had renal and/or central nervous system involvement. A strong relationship was observed between Lp(a) levels and anti-dsDNA antibodies.

Lp(a) levels were higher in SLE patients. Increased Lp(a) levels were found to be related to disease activity in SLE, specifically with renal and central nervous system involvement and anticardiolipin antibodies. Increased Lp(a) levels may contribute to the development of atherosclerosis and cardiovascular disease in SLE patients.

P90

Lipoprotein Lp(a) in patients with rheumatoid arthritis and its relationship with disease activity

Panagiotis Athanassiou[1], Ifigenia Kostoglou-Athanassiou[2], Despina Papadopoulou[1], Chrysoula Galanaki[1], Thomais Kalogirou[3], Athanasios Kordalis[3], Christodoulos Antoniadis[1] & Philippos Kaldrymidis[2]
[1]Department of Rheumatology, Asclepeion Hospital, Athens, Greece; [2]Department of Endocrinology, Metaxa Hospital, Piraeus, Greece; [3]1st Department of Internal Medicine, Asclepeion Hospital, Athens, Greece.

Epidemiological studies indicate that rheumatoid arthritis (RA) patients have increased mortality. Cardiovascular disease seems to be one of the major causes of death in patients with RA. Lipoprotein disorders are observed in patients with systemic autoimmune disorders as well as in patients with RA. Lipoprotein Lp(a) is an independent risk factor for the development of cardiovascular disease.

The aim of the study was estimate lipoprotein Lp(a) levels and their relationship with disease activity in RA patients.

Patients with RA, $n=92$, aged 22–71 years and normal controls, $n=92$, of the same age and sex were studied. All the patients fulfilled the American College of Rheumatology criteria for the diagnosis of RA. In patients and controls the levels of hematocrit, hemoglobin, erythrocyte sedimentation rate, C-reactive protein, cholesterol, triglycerides, HDL, LDL and lipoprotein Lp(a) were measured. DAS28 disease activity index was calculated in all RA patients.

Lipoprotein Lp(a) levels (normal values <30 mg/dl) were found increased in 24 of 92 RA patients (26.1%) and in 11 of 92 controls (12%). Within the group of 24 RA patients with increased Lp(a) levels 18 (75%) had increased inflammation markers and increased DAS28. A strong relationship was observed between Lp(a) levels, erythrocyte sedimentation rate ($P<0.01$) and C-reactive protein ($P<0.01$).

Lipoprotein Lp(a) levels were found increased in RA patients. RA patients are at an increased risk for the development of atherosclerosis. The increase in Lp(a) levels seems to be observed specifically in patients with active RA. Inflammation may be the factor responsible for the increase in Lp(a) levels in RA patients.

P91

Impaired proinsulin secretion before and during oral glucose stimulation in HIV-infected patients, who display fat redistribution

Steen B Haugaard[2], Ove Andersen[2], Ian Halsall[4], Johan Iversen[1], Charles Nicholas Hales[4] & Sten Madsbad[3]
[1]Dept. Infectious Diseases, Hvidovre University Hospital, Copenhagen, Denmark; [2]Clinical Research Unit, Hvidovre University Hospital, Copenhagen, Denmark; [3]Dept. of Endocrinology, Hvidovre University Hospital, Copenhagen, Denmark; [4]Dept. of Clinical Biochemistry, University of Cambridge, Cambridge, United Kingdom.

Beta-cell function of HIV-infected patients on highly active antiretroviral therapy (HAART), who display lipodystrophy, may be impaired. An early defect in beta-cell function may be characterized by an increased secretion of 32–33 split proinsulin (SP) and intact proinsulin (IP).

To address this issue the secretion pattern of SP and IP of 16 HIV-infected men with lipodystrophy (LIPO) and 15 HIV-infected men without lipodystrophy (NONLIPO) were studied during an oral glucose tolerance test (OGTT). All patients received HAART. Insulin secretion rates were determined by deconvolution of plasma C-peptide concentrations.

More LIPO than NONLIPO patients displayed diabetes mellitus and impaired glucose tolerance than a normal glucose tolerance (LIPO 2/8/6 vs NONLIPO 1/2/12, $P=0.05$). LIPO had increased fasting SP, IP, ratio of SP/IP, area under the curve (AUC) of SP and IP during early phase of the OGTT (0, 10, 20 minutes), and AUC-SP and AUC-IP during the late phase of the OGTT (45, 75, 105 minutes), respectively, compared to NONLIPO (Ps <0.05). LIPO exhibited significantly increased fasting SP/IP ratio, fasting SP/insulin ratio and ratios of total proinsulin to C-peptide during the OGTT. LIPO displayed increased incremental secretion of IP during the first 10 minutes of the OGTT ($P<0.05$), despite the fact that the incremental insulin secretion during this period did not differ between LIPO and NONLIPO.

These data suggest that HIV-infected patients with lipodystrophy display major perturbations of proinsulin secretion in the fasting state and during an OGTT, which is compatible with the notion of a beta-cell dysfunction of such patients.

P92

Concentration of vasopressin and of N- terminated naturetic propeptyde type B – potent predictors of survival of patients after cardiac arrest

Anna Samborska-Sablik[1], Zbigniew Sablik[2], Wojciech Gaszynski[3], Jan Henryk Goch[2] & Krzysztof Kula[4]
[1]Department of Emergency Medicine and Disaster Medicine, Medical University of Lodz, Lodz, Poland; [2]Department of Cardiology and Cardiac Surgery, Medical University of Lodz, lodz, Poland; [3]Department of Anaesthesiology and Intensive Therapy, Medical University of Lodz, Lodz, Poland; [4]Department of Andrology and Endocrynology of Fertility, Medical University of Lodz, Lodz, Poland.

Aim
To assess concentration (conc) of arginine vasopressin (AVP) and conc of N-terminated naturetic propeptyde type B (NTpBNP) in patients (pts) after cardiac arrest (CA) and their role for clinical state of pts after CA.
Material
52 pts after CA, 36 men and 16 women, in the age 62±13 years. CA was caused by ventricular fibrillation in 31 cases, by asystolia in 15 and by pulseless electrical activity in 6. 28 pts died after CA (P-CA-D), 24 survived and were discharged from hospital (P-CA-S).
Methods
Clinical state of pts after CA was assessed by common scales used in critical care: Glasgow Coma Scale (GCS) and Acute Physiology and Chronic Health Evaluation II (APACHE II). Venous blood samples to measure conc of AVP and NTpBNP were taken from each patient just after admission to hospital and in 2 consecutive days at 8.00.
Results
Just after CA mean conc of AVP was higher in P-CA-D than in P-CA-S (87±58 vs 60±46 pmol/l, but $P<0.1$). Mean conc of NTpBNP was higher in P-CA-D in 3 consecutive days, significantly in 1 day after CA (11,4000±112000 vs 45000±58,000 pmol/l, $P<0.027$).
In logistic regression analysis as well in Kaplan-Meyer survival analysis an important relation between conc of AVP just after CA and survival after CA, and between levels of NTpBNP just after CA and in first day after CA and survival was revealed.
We proved negative correlations among blood conc of AVP, conc of NTpBNP and values of GCS and positive correlations among levels of them and values of APACHE II.
Conclusions
1-Mechanisms involving biological function of AVP and of brain naturetic peptide play an important role for covalescence in early stage after CA.
2- Conc of AVP and NTpBNP are important predictors of survival after CA.

P93

Changes in plasma adiponectin during the treatment of diabetic ketoacidosis

A Emre Yildirim[1], Yavuz Selim Demir[2], Abidin Ozturk[1], A Ozden Barazi[3], Hacer Cetiner[1], Gokcen Kilic[1], Tugrul Ozcan[1], Yasar Acar[1], Berrin Demirbas[1] & Gul Gursoy[1]
[1]Ankara Education and Training Hospital Department of Internal Medicine, Ankara, Turkey; [2]Ankara Education and Training Hospital Department of Endocrinology and Metabolism, Ankara, Turkey; [3]Ankara Numune Education and Training Hospital Department of Biochemistry, Ankara, Turkey.

Background
Low plasma adiponectin concentrations are associated with diabetes mellitus. Resuts from animal studies suggest that adiponectin plays an important role in regulating insulin action. Leptin levels found to be low in patients with diabetic ketoacidosis (DKA). The recent studies showed that insulin replacement during DKA increased leptin concentrations. In our study, we aimed to determine the effect of insulin replacement on serum leptin and adiponectin concentrations in patients with DKA.
Methods
Our study included 31 patients (F/M) with DKA. 18 of 31 patients have been treated with oral anti diabetic agents and 13 of 31 patients have been treated with insulin treatment before admission. Leptin and adiponectin concentrations are analyzed in samples which are collected on admission and at resolution of ketoacidosis in 24–48 hours.
Results
Mean age of the the patients was 46.7±17.3 years, mean period of diabetes was 7.1±7.3 years, and body mass index was 26.5±4.3 kg/m^2. There was a signifcant negative correlation between plasma adiponectin levels and blood ketone concentrations on admission ($r=-0.66$). Significant positive corelation between age and body masss index was also determined ($r=0.477$). While plasma adiponectin levels didn't change after DKA treatment ($P=0.095$), leptin levels increased significantly ($P<0.001$). In patients using oral antidiabetic agents adiponectin levels didn't change ($P=0.103$) but leptin levels increased significiantly ($P=0.002$) at the end of treatment. In the patients using insulin therapy, adiponectin levels didn't change ($P=0.56$), however leptin levels increased significantly ($P<0.001$).
Conclusions
In our study leptin levels are increased after the treatment of DKA. This result can be described with the increase of calori intake and the regulation of glucose utilization in adipocytes caused by insulin. Adiponectin levels found to be still low in DKA after the treatment. These results could be explained by early period of treatment.

P94

Power spectral analysis (PSA) of heart rate variability (HRV) in the detection of cardiac autonomic neuropathy (CAN) in subjects with diabetes mellitus

Amjed Khamis, SR Rizvi, Shu Hoashi, Marie Byrne, RG Firth & Brendan Kinsley
Diabetes and Endocrine department, Mater Misercorea University Hospital, Dublin, Ireland.

Standard Autonomic Function tests AFT may not detect subclinical CAN. Modalities exist (Frequency Domain) using PSA of HRV, which may detect subclinical CAN.

We performed standard AFTs (HR response to deep breathing, Valsalva, Tilt at 1 min and 6 min) and PSA of HRV on these tests in 46 subjects (29 DM and 17 Controls) matched for age and sex. We sought to establish if those DM subjects considered normal by AFT would exhibit abnormalities in PSA of HRV. We measured mean spectrum RR interval and LF/HF ratio (measures of sympathovagal tone).

Diabetic Subjects were divided into group A (normal AFT), group B (any abnormal AFT), and to subgroup B1–3 by number of abnormal AFTs.

We then sought to identify if subjects in Group A would demonstrate differences in results of PSA of HRV tests compared to controls, which might suggest subclinical CAN.
Results
In standard AFT, HRV in deep breathing was 22±10 beats/min in Group A and 20±6 beats/min in Control, $P=$NS. In contrast results of PSA of HRV show that LF/HF ratio in deep breathing was 7.1±3.3 (Control), 3.3±1.6 (Group A) and 1.8±1.5 (Group B), 1.2±0.6 (GroupB2), 2.3±1.6 (Group B3), $P<0.01$ Control vs Group A and Group B. $P<0.001$ Control vs Groups B1, B2, B3.
Summary
LF/HF ratio to deep breathing is a useful parameter to detect presence of subclinical CAN and to stratify severity of CAN. It may be useful as a screening test for the presence of subclinical CAN in subjects with diabetes mellitus.

P95

Glicoregulation in obese diabetics treated with glargin insulin in combination with metformin and with glargin in combination with glimepirid

Zelija Velija-Asimi
University Clinical Centre of Sarajevo, Sarajevo, Bosnia and Herzegovina.

Aims
The aim of study was to evaluate glucose control in obese diabetics during six months of treatment with glargin insulin in combination with metformin and glimepirid.
Methods
In beginning of study excluded patients who had coronary heart and kidneys disease before. In study included 43 obese diabetics with type 2 diabetes [23 male and 20 female, BMI=29.82±1.91 kg/m^2, aged 42–65 yr], who had previously been treated with different orally antidiabetics. Previous treatment was without results, because all treated patients had bed glicoregulation. Patients divided in two groups. Both groups were without significant difference in BMI, age and sex. Twenty five patients (14 male and 11 female) received glargin s.c. once a day and metformin orally at the dose of 3×850 mg/d. Eighteen diabetics (10 male and 8 female) received glargin s.c. once a day and glimepirid orally at the dose of 2–4 mg/d. Glicoregulation evaluated by measuring fast blood glucose (FBG), postprandial blood glucose (PBG) and HbA1c. Duration of study was six months. Percentile, average and correlation analysis have been utilized in statistical analysis.
Results
The results of study, after six months treatment with glargin and metformin, show statistical significantly decreasing of FBG (6,7+/−1.4 mmol/l, vs 9.9+/−2.9 mmol/l, $P<0.05$), PBG and HbA1c (7.0+/−1.3% vs 9.1+/−1.3%, $P<0.05$). BMI decreased for 10% (27.1+/−0.9 kg/m^2 vs 29.82±1.91 kg/m^2). In group treated with glargin and glimepirid FBG, PBG and HbA1c (7.7+/−1.2% vs 9.3+/−1.1%, $P<0.05$) as well decreased but no more then group treated with glargin and metformin.
Conclusion
Glargin in combination with metformin is more effective in treatment of obese diabetics then glargin in combination with glimepirid.

P96

High-sensitivity C-reactive protein in diabetes mellitus type II according to micral test findings

Zarida Hambali
University Putra, Malaysia.

Cardiac complications account for three quarter of deaths among diabetic patients. Many studies have shown that high-sensitivity C-reactive protein (hs-CRP) correlated with the inflammatory process of atherosclerosis in the coronary artery. This study is designed to determine the levels of plasma hs-CRP in Type II diabetic patients with microalbuminuria and its association with other biochemical markers used for diabetic monitoring. All biochemical parameters were analyzed using HITACHI 919 Analyzer. Microalbuminuria levels were assessed using Micral Test in 120 diabetics and 100 normal subjects (control). hs-CRP is significantly higher among diabetics ($P<0.05$) as compared to the control group. The concentrations of hs-CRP increases significantly with increasing levels of microalbuminuria which are classified into 0 mg/dL, 20 mg/dL and more than 50 mg/dL ($P<0.01$). Among diabetics, hs-CRP is significantly higher in those with miroalbuminuria compared to those without microalbuminuria ($P<0.001$). In contrast, hs-CRP is not significantly correlated with fasting blood glucose, LDL-chol, total cholesterol and triglyceride ($P>0.05$). This case-control study confirms the findings of higher concentration of hs-CRP among diabetic patients and may suggest the ongoing inflammation associated with atherosclerosis. This study suggests that by measuring the concentration of plasma hs-CRP in addition to other biochemical parameters as recommended by the Malaysian Clinical Practice Guideline, a proper planning to monitor complications of coronary artherosclerosis among diabetic patients with or without microalbuminuria can be done.

Endocrine tumors and neoplasia – presented on Sunday

P97

Localization of an ectopic adrenocorticotropin-secreting tumour using ^{18}F- Dopa PET/CT

Séverine Dubois[1], Olivier Morel[2], Patrice Rodien[1], Christian Jeanguillaume[2], Bernard Enon[3], Frédéric Illouz[1], Jean-Paul Saint-André[4], Sylvie Girault[2] & Vincent Rohmer[1]
[1]Pôle d'Endocrinologie, Diabètes et Nutrition, Centre Hospitalier et Universitaire, Angers, France; [2]Pôle de Médecine Nucléaire et Cancérologie, Centre Hospitalier et Universitaire, Angers, France; [3]Pôle de chirurgie thoracique et cardiaque, Centre Hospitalier et Universitaire, Angers, France; [4]Département d'anatomo-pathologie et de cytologie, Centre Hospitalier et Universitaire, Angers, France.

Ectopic adrenocorticotropin secretion (EAS) accounts for 10–15% of cases of Cushing's syndrome and comprises a spectrum of lesions from highly malignant tumours to a variety of less aggressive neuroendocrine tumours. Selective removal of the primary lesion is the optimal management. It is therefore mandatory to localize the source of ectopic ACTH.

As no single test is accurate enough to distinguish the ectopic from the pituitary sources of ACTH, no single imaging technique can itself identify every tumour responsible for EAS.

We report on the use of Photon Emission Tomography (PET) scanning using ^{18}F-fluoro-Dopa in the localization of an occult ACTH-secreting carcinoid tumour.

An 18-yr-old man was referred for evaluation of EAS. Evidence for EAS included: plasma ACTH and ß LPH levels above the normal reference range, no serum cortisol suppression after high-dose dexamethasone suppression test, normal pituitary MRI and lack of central to peripheral gradient on bilateral inferior petrosal sinus sampling. The patient had a history of post-infectious bronchiectasis since 6 years. The chest computed tomographic (CT) scan showed a widespread lobar disease already known and compatible with bronchiectasis. In-111 pentreotide scintigraphy was interpreted as normal. A low-intensity uptake was seen on ^{18}FDG PET scanning located in the middle right pulmonary lobe. As the patient suffered from a respiratory infection, interpretation of this image was difficult. An ^{18}F-fluoro-dopa PET scanning revealed a pathologic uptake localized in the right lung middle lobe.

The pulmonary lesion was surgically treated after adrenolytic medication. Histology revealed a bronchial carcinoid tumor. Hypercortisolism was replaced by prolonged corticotropic insufficiency. Until now, hypercortisolims did not relapse.

In conclusion, no imaging technique should be neglected in the localization of an occult EAS.

P98

Adrenocortical carcinosarcoma: first european case report

Frédéric Somda[1], Julie Leger[1], Laurent Guy[2], Olivier Norha[3], Françoise Desbiez[1], Jean-Paul Boiteux[2], Jean-Louis Kemeny[3], Philippe Thieblot[1] & Igor Tauveron[1]
[1]CHU Gabriel Montpied, Endocrinology, Clermont-Ferrand, France; [2]CHU Gabriel Montpied, Urology, Clermont-Ferrand, France; [3]CHU Gabriel Montpied, Pathology, Clermont-Ferrand, France.

Adrenocortical sarcoma is an extremly rare tumour associated with a quite pejorative evolution.We report the case of a fifty-eight years old woman presenting a chronic asthenia and acute flank pain. She had no hypertension, no clinical sign of virilization or hypercorticism. Abdominal ultrasonography revealed an 8 centimeters mass above the right kidney. CT scan aspect evoked an adrenal carcinoma embolizing vena cava. Hormonal assays did not reveale any inappropriate secretion (17 alpha hydroxyprogesterone, 11 desoxy-cortisol, cortisol, dehydroepiandrosterone-sulfate, delta-4 androstenedione, testosterone, aldosterone, renin, 24-hour urine metanephrine and normetanephrine). A radical adrenalectomy associated with a nephrectomy was performed. Tumour measured $13\times7.5\times5$ centimeters, weighed 760 grams. Histological study confirmed the diagnosis of adrenal carcinoma, but described a sarcomatous component occupying nearly twenty percent of the total mass. Immunohistochimical labelling was positive for anti-vimentin, anti-desmin and anti-actin antibodies. In addition to surgical resection, the patient received mitotane as adjuvant treatment (6 g per day, mitotanaemia: 20.6 mg/l). After a 16 month evolution, physical exmination, CT scan, PET scan and hormonal monitoring don't show any evidence of local reccurence or metastasis. In the last twenty years, only four cases of adrenocortical carcinosarcoma have been reported in literature. One was a non secreting tumor, the three others were revealed by aldosterone, androgen or catecholamine secretion. Considering pathology, one had an osteogenic and chondroid differenciation, the two others a rhabdomyosarcomatous differenciation. To our knowledge, this is the first observation of an adrenal carcinosarcoma expressing a smooth muscle phenotype. The strikingly good evolution in our patient is also particularly unusual. Indeed adrenocortical sarcoma is a cancer with a very poor prognosis since in all other cases, life expectancy after diagnosis has never exceeded 8 months.

P99

The genetic association of medullary thyroid carcinoma with Hirschsprung's disease

Sarka Dvorakova[1], Eliska Vaclavikova[1], Richard Skaba[2], Petr Vlcek[3] & Bela Bendlova[1]
[1]Dept. of Molecular Endocrinology, Institute of Endocrinology, Prague, Czech Republic; [2]Dept. of Pediatric Surgery of the 2nd Faculty of Medicine, Charles University and Hospital, Prague, Czech Republic; [3]Dept. of Nuclear Medicine and Endocrinology of the 2nd Faculty of Medicine, Charles University and Hospital, Prague, Czech Republic.

Medullary Thyroid Carcinoma (MTC) can be associated with Hirschsprung's disease (HSCR). Mutations in exon 10 of the RET proto-oncogene were found in patients with co-occurrence of HSCR and MTC. The aim of the study was to screen the MTC risk exons in patients with HSCR. The genetic analysis comprised 73 HSCR patients (53 males, 20 females) who were operated on and followed-up during 2001-2006. The cohort consisted of 48 patients with classical HSCR, 11 with long colonic aganglionosis and 14 with total colonic aganglionosis (TCA). DNAs were isolated from blood after signing informed consent approved by ethical committee. HSCR patients and 10 available family members were tested for RET mutations in exons 10,11,13,14,15 and 16. Direct sequencing revealed RET mutations in 7 (9.6%) HSCR patients. Three groups of mutations were detected. Typical MTC risk mutations were found in 2 HSCR patients with TCA: Cys609Tyr and Cys620Arg (both exon 10). Atypical mutation Tyr791Phe (exon 13) was detected in 2 classical HSCR patients. This mutation is causative for MTC only and has not been associated with HSCR till now. Novel mutations with unknown function for HSCR and MTC were found in 3 patients – del603(A) (exon 10), Gly798Ser (exon 13) and Ser649Leu (exon 11). Two of these patients had TCA and the third one had classical HSCR. MTC developed in 2 patients and 2 family members with typical mutations for HSCR-MTC. These mutation carriers underwent total thyroidectomy (TTE), the other RET positive patients are screened for calcitonin level and they are without TTE till now. Results showed the benefit of systematic RET mutation screening in HSCR families in order to identify the risk of MTC. We recommend to investigate not only exon 10 but also other MTC risk exons in all HSCR patients. This work was supported by grant GACR 301/06/P425.

P100

Inhibition of $C_{17,20}$-lyase activity by new 17β-exo-heterocyclic androsterone derivatives

Mihály Szécsi[1], István Tóth[1], János Wölfling[2], Gyula Schneider[2] & János Julesz[1]
[1]Endocrine Unit and Research Laboratory, University of Szeged, Szeged, Hungary; [2]Department of Organic Chemistry, University of Szeged, Szeged, Hungary.

17α-Hydroxylase-$C_{17,20}$-lyase ($P450_{17\alpha}$) is a key regulator enzyme of the steroid hormone biosynthesis in both the adrenals and the testes. Inhibition of this enzyme can block androgen synthesis in an early step, and may thereby be useful in the treatment of prostatic carcinoma, which is androgen-dependent in the majority of cases. Abiraterone and its analogues have been found strong inhibitors of $P450_{17\alpha}$ suggesting that steroid derivatives with heterocyclic substituent on the C-17 position may bear such potential.

We investigated inhibitory effect on $C_{17,20}$-lyase exhibited by newly synthesised androsterone derivatives with heterocyclic 17β substituents. $C_{17,20}$-lyase inhibition was tested via conversion of 17α-hydroxy-progesterone to androst-4-en-3,17-dione in the homogenate of rat testis *in vitro*. Incubation was carried out with ^{14}C labeled substrate at 37 °C for 20 min. Following an extraction procedure and isolation by thin layer chromatography, the enzyme product and the residual substrate were quantified by their radioactivities. Ketokonazole, a $P450_{17\alpha}$ inhibitor applied in medical practice was used as a reference compound. Among test compounds the non-substituted tetrahydrooxazolone and tetrahydrooxazinone derivatives were found to be the best $C_{17,20}$-lyase inhibitors; IC_{50} values were 4.2 and 6.0 μM, respectively. The N-phenyl-tetrahydrooxazinones did not show substantial inhibition ($IC_{50} > 50$ μM).

The 17β-exo-heterocyclic androsterone derivatives which proved to be potential $C_{17,20}$-lyase inhibitors in the present study, also exhibited marked inhibition against prostatic 5α-reductase activity in our previous investigations. This dual effect might be particularly beneficial in the therapy of prostate cancer.

P101

Cigarette smoking increases high calcitonin levels

Michele d'Herbomez[1], Catherine Bauters[2], Laurence Leclerc[2], Emmanuelle Leteurtre[3], Bruno Carnaille[4], Jean-Louis Schlienger[5], Philippe Caron[6] & Jean-Louis Wémeau[2]
[1]Nuclear Medicine, Lille, France; [2]Endocrinology, Lille, France; [3]Pathology, Lille, France; [4]Endocrine Surgery, Lille, France; [5]Internal Medicine, Strasbourg, France; [6]Endocrinology, Toulouse, France.

Increased basal or pentagastrin-stimulated calcitonin level is the cornerstone for the biological diagnosis of medullary thyroid carcinoma, but is also observed in patients with C-cell hyperplasia (CCH) of the thyroid. In a prospective multicenter study we re-evaluated the reference ranges of basal calcitonin (bCT) in 287 euthyroid controls without thyroid disease (142 men-45 smokers, 3 deprived, 145 women-27 smokers). The CT levels were measured using 2 different assays (Cis-Bio International, Advantage Nichols). After exclusion of the main causes of increased CT levels, 11 (8%) male controls had bCT concentrations > 10 pg/mL within the two assays. All, except one, were active or deprived smokers. Then, we evaluated preoperative bCT and pentagastrin-stimulated CT levels in patients with CCH of the thyroid (more than 50 C cells per field at 3 low-power magnification microscopic fields). In 27 smokers or deprived patients (23 men and 4 women, median age 53 years) total thyroidectomy was performed for nodular pathology. CCH was diffuse and bilateral ($n=17$), diffuse and unilateral ($n=4$), nodular ($n=1$) or diffuse and nodular ($n=5$). Preoperative bCT was normal (< 10 pg/mL), between 10 and 20 pg/mL or > 20 pg/mL in 8, 13, and 6 patients, respectively. Pentagastrin- stimulated CT level was normal (< 50 pg/mL), between 50 and 100 pg/mL, and > 100 pg/mL in 2, 3, and 15 patients respectively.

In conclusion, there are evidences that cigarette smoking induces: 1) diffuse and bilateral CCH of thyroid, 2) increased bCT level, 3) abnormal pentagastrin-simulated test, particularly in men.

P102

Cortisol excess, inflammatory markers and echocardiographic alterations in adrenal incidentalomas

Federica Ermetici[1], Alexis Malavazos[1], Calin Coman[2], Lelio Morricone[1], Massimiliano Corsi[3] & Bruno Ambrosi[1]
[1]Endocrinology Unit, Department of Medical and Surgical Sciences, IRCCS Policlinico San Donato, University of Milan, San Donato Milanese, Italy; [2]Echocardiographic Unit, IRCCS Policlinico San Donato, San Donato Milanese, Italy; [3]Institute of General Pathology, Laboratory of Clinical Pathology, University of Milan, Milan, Italy.

An increased cardiovascular risk has been described in patients (pts) with adrenal incidentaloma (AI), similarly to pts with overt Cushing's syndrome (CS). Some echocardiographic abnormalities and alterations in adipokine secretion involved in insulin resistance, inflammation and atherosclerosis have been reported in pts with CS. In this study the possible correlation between echocardiographic parameters and adipokine levels in pts with AI was evaluated.

Subjects and methods

Morphological and functional echocardiographic characteristics and plasma IL-6, TNF-α, MCP-1 and resistin levels (ELISA methods) were studied in 7 pts (60.0 ± 2.5 yrs, BMI 31.1 ± 2.1) with AI and subclinical Cushing's syndrome (SCS) and in 17 pts (58.8 ± 2.3 yrs, BMI 29.5 ± 1.2) with non functioning masses. All adrenal masses were identified as cortical adenoma. In all pts plasma ACTH, serum cortisol and urinary free cortisol (UFC) were measured.

Results

In pts with SCS the interventricular (IV) septum thickness was significantly greater than in pts with non functioning masses (13.2 ± 0.1 vs 10.7 ± 0.03 mm, $P<0.05$) and in 8 obese normotensive subjects (10.5 ± 0.5 mm, $P<0.001$). Plasma IL-6, TNF-α, MCP-1, and resistin levels were higher in pts than in 20 normal subjects (60.3 ± 2.5 vs 5.5 ± 0.6 pg/ml, 27.2 ± 1.3 vs 22.1 ± 1.4 pg/ml, 164.3 ± 17.0 vs 104.3 ± 19.4 pg/ml, 12.9 ± 2.4 vs 5.1 ± 0.2 ng/ml, respectively, $P<0.05$). The other echocardiographic parameters and adipokine values were not different in pts with SCS and with non functioning AI. In all patients, UFC excretion positively correlated with left ventricular (LV) diameter end-systole ($r=0.549$, $P=0.01$) and with LV mass ($r=0.479$, $P<0.05$). Significant correlations were found between early wave diastolic filling velocity and IL-6 and TNF-α levels ($r=-0.633$, $P=0.01$ and $r=-0.547$, $P<0.05$, respectively), and between late wave diastolic filling velocity and TNF-α levels ($r=-0.520$, $P<0.05$), in all pts.

Conclusions

In AI a long-lasting exposure to an even slight cortisol excess and inflammatory stimuli might be responsible for a gradual impairment of both diastolic function and cardiac morphology.

P103

Prognostic value of anti-thyroperoxidase antibodies in high malignancy degree breast cancer

Emilio Fiore[1], Claudio Giani[1], Elisa Giustarini[1], Ilaria Muller[1], Claudia Mammoli[1], Daniela Campani[3], Manuela Roncella[2], Loredana Fustaino[2] & Aldo Pinchera[1]
[1]Department of Endocrinology and Metabolism, Pisa, Italy; [2]Department of Surgery, Unit of Senology, Pisa, Italy; [3]Department Pathology, Pisa, Italy.

A high incidence of serum anti thyro-peroxidase antibodies (TPOAb) has been found in breast cancer (BC). Aim of this study was to evaluate the predictive value of TPOAb in BC. The study group included 47 women submitted to mastectomy for high malignancy degree BC (tumor size > 5 cm and/or n° lymph-nodes > 3), followed for 5 years. No patient had distant metastases. All were evaluated for thyroid disorders after breast surgery and before any anti-tumoral adjuvant therapy. Thirty-one out of 47 (65.9%) patients were alive 5 years after BC diagnosis (survivors group: SG), 16/47 (34.1%) were dead during follow-up (deaths group: DG), (mean age 53.1 ± 10.9 yrs and 53.3 ± 8.5 yrs, respectively) (p NS).

Estrogen Receptor (ER) was measured in 43/47 (91.5%) BC speciments. ER was detected (ER+) in 19/30 (63.0%) patients in SG and 3/13 (23.1%) in DG ($P=0.01$, x^2 5.9). Five year mortality in ER- BC was 10/21 (47.6%), and in ER+BC was 3/22 (13.6%) ($P=0.008$). The overall prevalence of TPOAb was 15/47 (31.9%); 14/31 (45.1%) patients in SG and 1/16 (6.2%) in DG were TPOAb+ ($P=0.008$). Five years mortality was 15/32 (46.9%) in TPOAb- and 1/15 (6.7%) in TPOAb + ($P=0.01$). TPOAb were detected in 8/21 (38.1%) ER- patients and in 7/22 (31.8%) ER+; no relation was found between ER expression and TPOAb positivity (x^2 0.2; p 0.7). Age at diagnosis was not significantly related to five years survival (O.R. 0.98; 95%C.I. 0.92–1.04; $P=0.6$). Absence of ER expression (O.R. 6.54; 95%C.I. 1.70–25.21; $P=0.006$) and absence of TPOAb (O.R. 9.37; 95%C.I. 1.21–72.67; $P=0.03$) were related to a higher mortality rate. RE+ and TPOAb+ are positive prognostic parameters in BC and the absence of any relationship between them seems to propose an independent role on the prognosis of BC patients.

P104

Bone density in patients with non-functioning pituitary adenomas (NFA)

Stefan Fischli, Christoph Stettler, Rahel Sahli, Silvia Schwab, Bernard Chappuis, Stefan Jenni, Peter Diem & Emanuel Christ
Department of Endocrinology, Diabetology and Clinical Nutrition, University Hospital of Bern, Inselspital, Bern, Switzerland.

Background
Surgically treated patients with NFA often present with secondary hypogonadism. Hypogonadism is a well known risk factor for osteopenia or osteoporosis. The aim of this study was to assess (a) the frequency of osteopenia/osteoporosis in a single centre Swiss cohort of patients with operated NFA and (b) whether gender or hypogonadism impacts on bone density at follow up.
Methods
Data of patients with NFAs diagnosed between 1967 and 2005 were analysed. Clinical and endocrinological parameters were recorded before, immediately after surgery and at last follow-up. Bone densitometry (DEXA) was performed during follow up. Data were analyzed using Fisher's Exact Test for calculating relative risks (RR) and p-values.
Results
121 patients with NFA were included (71% male and 29% female). Mean age at diagnosis was 55.2 ± 14.7 years. 74% of male and 25% of female patients had secondary hypogonadism at follow up, 57% (20) of female were menopausal prior to surgery. DEXA was performed in 68% ($n=82$) of all patients. Overall, DEXA showed a normal bone density (T-score $\geqq -1$) in 26%, in 30% signs of osteopenia (T-score between -1 and -2.5) and in 12% signs of osteoporosis (T-score ≤ -2.5). The relative risk (RR) for osteopenia/osteoporosis in all patients with secondary hypogonadism at follow up compared to patients with normal gonadale function at follow up was 0.84 (95% CI 0.61–1.16; $P=0.36$) [men: 1.19 (0.59–2.40; 0.74), women: 1.50 (0.67–3.34;$P=0.37$)]. The RR for osteopenia/osteoporosis in female patients with hypogonadism (incl. menopausal females) compared to men with hypogonadism at follow up was 1.57 (95% CI 1.16–2.14; $P=0.013$).
Conclusions
(1) Osteopenia and Osteoporosis is a common problem in patients with NFA. (2) A diminished bone density is not only related to impaired gonadale axis in patients with NFA. (3) The influence of gender on bone density appears to be critical.

P105

Echo-enhanced ultrasound has a higher sensitivity than high-resolution CT in the detection of hepatic metastasis of adrenocortical carcinoma

Jürgen Bauditz[1], Wolfram Wermke[1], Dirk Beyersdorff[2], Herbert Lochs[1], Christian J. Strasburger[1] & Marcus Quinkler[1]
[1]Medicine, Center for Gastroenterology, Hepatology and Endocrinology, Charite Campus Mitte, Charite Universitaetsmedizin Berlin, Berlin, Germany, [2]Institute for Radiology, Charite Campus Mitte, Charite Universitaetsmedizin Berlin, Berlin, Germany.

Background
Adrenocortical carcinoma (ACC) is a rare and heterogeneous malignancy with incompletely understood pathogenesis and poor prognosis. Computerized tomography (CT) and magnetic resonance imaging (MRI) are routinely performed for imaging of the adrenal mass and for standard staging of chest and abdomen as lung and liver are the primary organs for metastatic spread in ACC. Contrast ultrasound is a non-invasive procedure which has been shown to have a high sensitivity and specifity for differentiation of hepatic and neuroendocrine tumours.
Methods
patients (7 women, 5 men; aged 24 to 77 years) with ACC were treated in our centre from 2004 to 2006. Patients received staging with HR-CT as well as with contrast ultrasound (Sonovue/Bracco, Acuson Sequoia/Siemens, CPS) of the liver.
Results
Contrast ultrasound demonstrated liver metastases in 8 of 12 patients (67%), HR-CT showed liver metastases in 6 of 12 patients (50%). In 2 of 8 patients (25%) HR-CT missed detection of liver metastases. Even retrospectively and with knowledge of the ultrasound results, the hepatic lesions were not recognized by HR-CT, but were detectable by HR-CT at a later time point. All hepatic lesions diagnosed by HR-CT were also seen by ultrasound. The detection of liver metastases by ultrasound resulted in a change of therapy in the 2 patients.
Conclusions
Contrast ultrasound has a higher sensitivity than HR-CT in detecting highly vascularized liver metastases of ACC and should be included in the staging algorithm of ACC.

P106

Characteristics of metabolic syndrome in patients with adrenal incidentaloma

Miomira Ivovic, Svetlana Vujovic, Milos Stojanovic, Milina Tancic-Gajic, Ljiljana Marina, Marija Barac & Milka Drezgic
Institute of Endocrinology, Belgrade, Serbia.

Several studies show that characteristics of metabolic syndrome are often seen in patients with adrenal incidentaloma. The aim of our study was to evaluate metabolic factors in these patients. 208 patients (148 female and 60 male, age 55.08 ± 11.02 y's and BMI 27.91 ± 4.6 kg/m^2) were admitted and biochemical, endocrine testing were performed. Lipid status: cholesterol 5.77 ± 1.26 mmol/L, triglyceride 1.92 ± 0.98 mmol/L. 113(55%) patients were hypertensive (mean systolic pressure was 150.3 ± 30.12 mmHg, diastolic 92.93 ± 16.48 mmHg). 34 (16.35%) patients had type 2 diabetes. According to OGTT (performed in 131 patients) more than 50% were diabetic or showed glucose intolerance. Insulin sensitivity was calculated by HOMA, QUIQI formula and 56,86% of patients had insulin resistance. After endocrine evaluation we divided them in two groups: first with subclinical hypercorticism and second without hypercorticism. First group: 46 patients (38 woman and 8 man mean age 56.6 ± 9.25 y's and BMI 27.83 ± 4.37 kg/m^2). Second group: 162 patients (110 women and 52 men, age 54.66 ± 11.45 years and BMI 27.93 ± 4.67 kg/m^2). No statistically significant difference was found for cholesterol (5.68 ± 1.24 vs. 6.09 ± 1.29 mmol/L; $P > 0.05$) and triglyceride (1.88 ± 0.9 vs. 2.08 ± 1.22 mmol/L; $P > 0.05$) between these subgroups. We also find no statistically significant difference in insulin resistance between groups (QUIQI: 0.34 ± 0.05 vs. 0.33 ± 0.03; $P > 0.05$). Mean systolic and diastolic blood pressure was not significantly higher in subgroup with sublinical hypercorticism (149.6 ± 29.92 vs. 152.7 ± 31.02 mmHg; $p > 0.05$ and 92.38 ± 16.42 vs. 94.89 ± 16.74 mmHg; $P > 0.05$).

Significant number of patients with adrenal incidentaloma had characteristics of metabolic syndrome even without proved endocrine hypersecretion. These patients are at high risk for cardiovascular events. Well-defined international study protocols should include screening for metabolic syndrome.

P107

The role of radio-guided surgery (RGS) with the use of ^{99m}Tc-EDDA/-HYNIC-octreotate in detection of unknown primary and secondary sites of neuroendocrine tumours of the gastrointestinal tract (GEP-NET) and improving the final outcome of patients

Alicja Hubalewska-Dydejczyk[1], Jan Kulig[2], Piotr Szybinski[2], Renata Mikolajczak[3], Anna Sowa-Staszczak[1], Katarzyna Fröss-Baron[1] & Bohdan Huszno[1]
[1]Department of Endocrinology, Medical College at Jagiellonian University, Cracow, Poland, [2]Gastrointestinal and General Surgery Department, Medical College at Jagiellonian University, Cracow, Poland, [3]Radioisotope Centre POLATOM, Otwock-Swierk, Poland.

Despite a wide spectrum of imaging diagnostics, GEP-NETs often stay undetectable until the time of dissemination. Removing of a primary tumour together with disseminated lymph nodes even with the presence of liver metastases is the most appropriate treatment to delay progression of the disease. SRS followed by RGS gives a possibility to detect occult GEP-NET intra-opratively. ^{99m}Tc-HYNIC/EDDA-octreotate, a somatostatin analogue with high affinity to sst2 was applied in the study. **The aim of the study** was to determine whether intra-operative radio-detection with the use of ^{99m}Tc-EDDA/HYNIC-octreotate, is able to reveal unknown primary tu and metastases of GEP-NET thereby improving surgical treatment and final prognosis.
Materials and methods
There were ten patients under examination with GEP-NET (with positive SRS and negative different pre-operative imaging tests). Insulinoma was suspected in 5 pts, non-functioning pancreatic NET - 1, and carcinoid in 5 cases. At surgery, suspected lesions were measured in vivo and ex vivo (Navigator-GPS) and the exact exploration of the abdominal cavity was performed.
Results
Amongst patients with pancreatic NET, ^{99m}Tc-EDDA/HYNIC-octreotate SRS followed by RGS detected 4 insulinomas, 1 glucagonoma and in one patient false

positive result appeared to be a cyst but nesidoblastosis was finally recognised. Three carcinoids with metastases were detected; in two cases the use of hand-held gamma probe extended the surgical procedure resulting in the successful excision of the metastatic lymph nodes. In one case the liver metastases were confirmed previously revealed by SRS only. Another false positive result was caused by ileitis.
Conclusion
In our study ^{99m}Tc-EDDA/HYNIC-octreotate SRS followed by RGS localized all primary GEP-NETs undetected with other imaging diagnostics. The main advantage of RGS in comparison to SRS is high sensitivity in detection of metastatic lymph nodes. The imaging properties of the ^{99m}Tc-EDDA/HYNIC-octreotate creates abilities for more common application of this tracer followed by RGS in oncology.

P108

Ascl1 is abundantly expressed in endocrine pancreatic tumors

Téresè Johansson[1], Margareta Halin Lejonklou[1], Sara Ekeblad[1], Peter Stålberg[2] & Britt Skogseid[1]
[1]Institution of Medical Sciences, Uppsala, Sweden, [2]Institution os Surgical Sciences, Uppsala, Sweden.

Background
Apart from inactivation of the *MEN1* gene, molecular events essential for tumorigenesis of the endocrine pancreas are poorly characterized. A potentially useful approach for understanding tumor progression is to study transcription factors operating in fetal pancreatic development. The Notch signaling cascade with expression of the transcription factors *Hes1*, *Hey1*, and *Ascl1* plays a vital role in sustaining the balance between cell proliferation, differentiation and apoptosis during the pancreatic development. They may play a similar role in the development of endocrine pancreatic tumors (EPT).
Aim
To study the expression of *Notch1*, *Hes1*, *Hey1* and *Ascl1* in EPT, by quantitative PCR (qPCR) and Immunohistochemistry (IHC)
Material and methods
Notch1, *Hes1*, *Hey1*, and *Ascl1* mRNA and protein expression were investigated in 26 EPT (ten were MEN1 associated). Immunohistochemistry was also performed on 11 normal pancreatic tissues adjacent to the tumor (five MEN1 and six sporadic). The immunoreactivity was graded (negative, weak, moderate or strong), and sublocalization of expression as nuclear and/or cytoplasmatic was determined.
Results
The statistical analysis of the qPCR data revealed a correlation between the *Notch1-Hes1* expressions in EPT. All tumors displayed *Ascl1* immunoreactivity, which was graded as strong in 85%. *Hes1* expression in EPT was graded as invariably weak, or completely absent (30%). In normal islets a weak nuclear *Hes1* staining was observed. *Hey1* and *Notch1* were expressed in the cytoplasm and nucleus of tumor cells and normal endocrine tissue.
Conclusions
Ascl1 is invariably and abundantly expressed in EPT. *Hes1* is either lacking or weakly expressed and confined to the cytoplasm of EPT. The lack of *Hes1* in tumor cell nuclei could contribute to the prominent *Ascl1* expression in EPT. These results show that *Notch1*, *Hes1*, *Hey1* and *Ascl1* are variable expressed in EPT and normal pancreatic tissues; and that they may be involved in endocrine pancreatic tumorigenesis.

P109

Histopathological and molecular studies in patients with goiter and hypercalcitoninemia: reactive or neoplastic C-cell hyperplasia?

Uberta Verga[1], Stefano Ferrero[3], Leonardo Vicentini[2], Tatiana Brambilla[3], Valentina Cirello[1], Marina Muzza[1], Paolo Beck-Peccoz[1] & Laura Fugazzola[1]
[1]Endocrine Unit, Dept. of Medical Sciences, University of Milan and Fondazione Policlinico IRCCS, Milan, Italy, [2]Endocrine Surgery Unit, Fondazione Policlinico IRCCS, Milan, Italy, [3]Pathology Unit, Dept. of Medicine, Surgery and Dentistry, University of Milan, Ospedale S. Paolo, Milan, Italy.

The cut-off values able to differentiate between reactive or neoplastic C cell hyperplasia (CCH) or to predict sporadic medullary thyroid cancer (MTC) are still debated both for basal and stimulated calcitonin (bCT and sCT). Aim of the present study was to define the prevalence and the histological patterns of CCH in 15 patients with multinodular goiter (MNG), bCT $>$ 10 pg/ml and sCT levels $>$ 50 pg/ml. These data were compared with those from 16 patients with MNG and bCT levels $<$ 10 pg/ml, and with those from 4 patients with familial medullary thyroid cancer (FMTC). For each case, 6 paraffin blocks were selected, CT immunoreactive cells were counted in sixty consecutive high power fields (400x) and classified as focal, diffuse, nodular or neoplastic. *RET* genetic analyses were performed at the germline and tissue level in MTC and, for the first time, in CCH cases. In patients with MNG, sCT levels $>$ 50 pg/ml were associated with CCH or with MTC, being the total number of C cells/60 fields significantly higher than that found in MNG with normal bCT ($P = 0.0008$), and comparable to that detected in FMTCs. Interestingly, in the group with sCT $>$ 50 pg/ml, the C cells displayed a neoplastic phenotype, concerning morphology, distribution and localization. No *RET* mutations were found neither at the germline nor at the somatic level.

In conclusion, sCT levels $>$ 50 pg/ml were associated with CCH in all cases and with MTC in 4 patients, without correlation between CT levels and the number of C cells or the final diagnosis. After serial blocking and high power field magnification, an elevated number of C cells were counted, often showing a morphology and a distribution pattern consistent with neoplastic CCH, thus strengthening the hypothesis that CCH might be the precursor also of sporadic MTC in the absence of *RET* mutations. Hence, sCT levels $>$ 50 pg/ml indicate the presence of CCH with a possible preneoplastic potential, suggesting the opportunity to perform a "prophylactic" surgical treatment.

P110

Thyroid cancer and pregnancy: clinical outcome and time of diagnosis in a series of 94 women

Perrino Michela[1], Vannucchi Guia[1], Vicentini Leonardo[2], Deborah Mannavola[1], Paolo Beck-Peccoz[1] & Laura Fugazzola[1]
[1]Endocrine Unit, Dept. of Medical Sciences, University of Milan and Fondazione Policlinico IRCCS, Milan, Italy, [2]Endocrine Surgery Unit, Fondazione Policlinico IRCCS, Milan, Italy.

Thyroid cancer represents the second more frequent tumor among those diagnosed during pregnancy. Indeed, during pregnancy thyroid volume increases by 20–30% and new nodules can appear, due to the effect of choriogonadotropin which stimulates thyroid growth. Hence, it has been proposed that thyroid cancer diagnosed during pregnancy could harbour a poorer prognosis. Aim of the present study was to compare the clinical outcome in the following 3 groups of patients affected with thyroid cancer: group 1 (Gr.1): 12 women with tumor diagnosed during pregnancy and submitted to thyroidectomy during the second trimester or in the first year after delivery; group 2 (Gr.2): 33 women with diagnosis of tumor at least 1 year after the delivery; group 3 (Gr.3): 49 women with diagnosis and treatment of the tumor before pregnancy or nulliparous. The 3 groups were matched for age, treatment, histology and follow-up. In particular, all patients of group 1 were treated with total thyroidectomy and radiometabolic treatment. Remission or persistence of disease were defined on the bases of basal thyroglobulin (Tg) levels before and after rhTSH, in the absence of anti-Tg antibodies, and of Total Body Scan. No significant differences in tumor size, capsular invasion and local/distant metastases were observed between the 3 groups. As far as the outcome is concerned, patients with the tumor diagnosed during pregnancy showed more frequently persistence or relapse of the disease with respect to the patients of the other groups (Gr. 1 *vs* Gr. 2: $P = 0.0035$; Gr.1 *vs* Gr. 3: $P = 0.0057$; Gr1 *vs* Gr. 2+3, $P = 0.018$; Gr.2 *vs* Gr.3: $P =$ NS). In particular, 9/12 patients of Group1 showed persistence of disease, with lymph-node metastases in 2 cases, distant metastases in 2 cases and elevated Tg levels in 5 cases.

In conclusion, the present data show that thyroid cancer diagnosed during pregnancy is associated with a poorer prognosis with respect to tumors developed in a non gravidic period, thus suggesting the need for an aggressive treatment by thyroidectomy during the second trimester and radiometabolic therapy soon after delivery.

P111

RET genotypes comprising specific haplotypes of polymorphic variants are associated with sporadic medullary thyroid cancer

Laura Fugazzola[1], Marina Muzza[1], Caterina Mian[3], Daniela Cordella[2], Susi Barollo[3], Valentina Cirello[1], Maria Elisa Girelli[3], Giuseppe Opocher[3], Paolo Beck-Peccoz[1] & Luca Persani[2]
[1]Endocrine Unit, Dept. of Medical Sciences, University of Milan and Fondazione Policlinico IRCCS, Milan, Italy, [2]Dept. of Medical Sciences, University of Milan and Istituto Auxologico Italiano IRCCS, Milan, Italy, [3]Endocrinology Unit, Dept. of Medical and Surgical Sciences, University of Padova, Padova, Italy.

Many single nucleotide polymorphisms (SNPs) of the *RET* gene have been described both in the general population and in patients with sporadic medullary thyroid cancer (sMTC), MEN2 or Hirschsprung disease. Some association studies reported a higher prevalence of these variants in the affected patients, suggesting a possible role in modifying the risk of occurrence of the disease. However, data from different cohorts of sMTC are discrepant and the aim of the present study was to determine if a variant *per se* or a combination of variants predispose to sMTC. Thus, a possible association of RET haplotype(s) and disease was looked for in 82 patients affected with sMTC and 49 age matched controls. Six *RET* SNPs were studied by PCR and direct sequencing. The most frequent SNPs were those in intron 1 (30 and 32% in sMTC and controls, respectively), exon 2 (22 and 24%) and exon 13 (24 and 26%). No significant differences were observed in the prevalence of single SNPs between patients and controls, including G691S, which is the only non-synonymous variant. Accordingly, functional analyses did not reveal an increased autophosphorylation for G691S. Twelve unique haplotypes, labelled A-N, were obtained. The distribution of haplotypes between cases and controls were significantly different ($P<0.05$). The study of the association of these different haplotypes in cases and controls lead to the identification of 30 different genotypes. Inspection of the genotypes in the two groups showed that the genotype distribution between cases and controls was different ($P<0.05$). In particular, there were 7 genotypes unique to controls, 13 unique to sMTC and 11 shared by the 2 groups. For example, AA, AC, AD and AH, all of which containing one allele without polymorphisms, are prominently or uniquely represented in sMTC. These data suggest that genotypes comprising specific pairs of RET haplotypes are associated with predisposition to sMTC. In this series, the absence on both alleles of the 6 SNPs analyzed was recorded only for MTC cases, indicating that the presence of RET variants could be protective against cancer development.

P112

Isolation of the Side population (SP) from murine adrenal glands renders cells with adrenocortical stem cell properties

Urs Lichtenauer[1], Igor Shapiro[1], Klaus Geiger[2], Anne-Marie Pulichino[3], Jaques Drouin[3] & Felix Beuschlein[1]
[1]Institute of Molecular Medicine and Cell Research, Albert-Ludwigs-University Freiburg, Freiburg, Germany, [2]Department of Internal Medicine I, Albert-Ludwigs-University Freiburg, Freiburg, Germany, [3]Laboratoire de Genetique Moleculaire, Institut de Recherches Cliniques de Montreal, Montral, Canada.

Radioactive and transgenic tracing experiments indicate that in the adult adrenal stem cells persist in the periphery of the cortex, which migrate centripetally and populate the inner cortical zones upon differentiation. However, investigation of these cells has been hampered by the lack of known marker genes. Vital Hoechst dye exclusion has been described as a method for isolating a side population (SP) from mouse bone marrow, which was enriched with stem cells. Utilizing this technique, we demonstrate the presence of SP cells in a variety of adrenal derived cell populations including normal mouse (0.71–0.83%) and human (0.01%) adrenals. After FACS sorting, isolation of SP and non SP (NSP) cells from murine adrenal glands revealed self-renewal and long-term culture capacities only for the SP fraction, which grew in a fibroblast-like manner, whereas the NSP cells did not proliferate. In addition, adrenal SP cells expressed adrenocortical markers such as MC2 receptor, StAR, and P450scc by means of RT-PCR and IHC. Interestingly, in a mouse model of ACTH deficiency (Tpit knock out animals, Tpit$-/-$), the proportion of SP cells was significant higher in comparison to heterozygous animals (Tpit$-/-$ $0.45\pm0.16\%$ *vs.* Tpit$+/-$ $0.13\pm0.04\%$;$P<0.004$). This higher SP cell proportion was associated with an increased width of the subcapsular cell compartment (Tpit$-/-$ $100\pm12.3\%$ *vs.* Tpit$+/-$ $259\pm10.7\%$; $P<0.0001$), which was characterized by the lack of expression of steroidogenic enzymes such as 3ßHSD. Short term ACTH treatment of Tpit$-/-$ animals resulted in a decrease of SP proportion (0.09%) and a shrinkage of the subcapsular zone similar to that of untreated Tpit$+/-$ controls ($130\pm10.2\%$;$P=0.33$). In summary, the adrenal Side Population displays certain stem cell properties. Moreover, we present indirect evidence that ACTH might be required for adrenocortical stem cell differentiation thus affecting adrenal zonation *in vivo*. Current studies including *in vitro* stimulation and *in vivo* transplantation experiments aim at the further characterization of this cell population.

P113

Selective intra-arterial calcium stimulation with hepatic venous sampling in investigation of hyperinsulinemic hypoglycemia

Mohd Shazli Yusof, Tomas Ahern, Thomas P Smith & Donal O'Shea
Department of Endocrinology and Diabetes Mellitus, Elm Park, Dublin 4, Ireland.

A retrospective analysis of the results of all intra-arterial calcium stimulations performed at St. Vincent's Hospital, Dublin, in the years 2001-2006. All patients with symptoms suggestive of hypoglycemia had 72 hour fasting test with evidence of inappropriately elevated insulin and c-peptide at the time of hypoglycemia. These patients were investigated further with pancreatic imaging and selective intra-arterial calcium stimulation with hepatic venous sampling (ASVS). Analysis of the results was performed using the Wilcoxon signed rank test. Results were available in 9 patients. The overall catherisation success rate was: minimum four arteries in 7/9, three arteries 1/9 and two arteries in 1/9. CT was positive in 2/7 patients, MRI 0/2, octreotide scan 0/2 and endoscopic ultrasound 0/2. Mean insulin increment was 11.91 fold (95% CI 6.51–17.30) in tumour area versus 1.61 fold (95% CI 1.21–2.01) $P=0.002$. ASVS was positive in 8 patients. 7 patients were found to have insulinoma and 2 patients were diagnosed with adult nesidioblastosis by means of histological diagnosis. One of nesidioblastosis patient had negative calcium stimulation test but had diffuse hyperinsulinemic picture on ASVS. Our results suggest that selective intra-arterial calcium stimulation with hepatic venous sampling remains a powerful tool for diagnosis of insulinoma. CT pancreas alone combined with ASVS should be the standard of investigation in biochemically proven insulinoma. Three fold insulin levels increment should be used as the cut-off point for positive test after calcium stimulation. We reported a case of failure ASVS. ASVS use should be restricted to units with expertise in this area.

P114

Somatostatin and dopamine receptor regulation and effects of a new somatostatin/dopamine chimeric compound on cell proliferation of the human androgen-dependent prostate cancer cell line LNCaP

Marica Arvigo[1], Massimiliano Ruscica[2], Elena Dozio[2], Michael D Culler[3], Francesco Minuto[1], Marcella Motta[2], Paolo Magni[2] & Diego Ferone[1]
[1]Department of Endocrinological & Metabolic Sciences and Center of Excellence for Biomedical Research, Genoa, Italy, [2]Center for Endocrinological Oncology, Istituto di Endocrinologia,, Milan, Italy, [3]Biomeasure Incorporate/IPSEN, Milford, United States.

The increasing use of somatostatin (SRIF) analogues prompted extensive investigations on SRIF receptor (SSR) in human tumours. Human prostate cancer (PCa) may differentially express SSRs from the normal tissue. Moreover, SSR and dopamine (D) receptors (DR) may interact to form homo- and heterodimers with enhanced functional activity. In the present study, using the human androgen-dependent PCa cell line LNCaP, we investigated: 1) SSR and DR subtype expression in different culture conditions (10% and 2% FBS); 2) the effects of SRIF and of a new SRIF/D chimeric molecules, BIM-23A760, able to bind with high affinity both sst_{2A} and D_2R on cell proliferation. LNCaP expressed sst_1, sst_{2A}, sst_3, sst_5, and D_1R and D_2R subtypes at gene (RT-PCR) and protein (Western blot) level. SSRs and D_2R expression was differentially regulated by the culture conditions: sst_{2A}, sst_5 and D_2R expression was not modified by serum concentration, whereas sst_1 and sst_3 were inversely correlated to serum concentration. Dose-response studies were performed at 24 and 48 h with a dose range from 10^{-11} to 10^{-7} M. SRIF inhibited cell proliferation ([^{3}H]thymidine incorporation) after 24 and 48 h at all doses. The clinically available analogue lanreotide inhibited cell proliferation after 24 and 48 h with a maximum effect at 10^{-11} M. However, the chimeric BIM-23A760 resulted more potent than lanreotide and significantly inhibited cell proliferation after 24 h at 10^{-9} M and after 48 h in a dose range from 10^{-7} to 10^{-11} M. These data indicate a heterogeneous expression of SSRs and DRs in PCa, depending on the culture conditions and show an enhanced potency of the chimeric BIM-23A760 in inhibiting cell proliferation, suggesting an important role of the dopaminergic pathway in PCa. Hence, LNCaP provides a model to study the interaction between membrane receptors and to further investigate chimeric SRIF/D compounds in human cancer.

P115

Somatostatin receptor regulation and effects of somatostatin and somatostatin analogues on cell proliferation of the human androgen-dependent prostate cancer cell line LNCaP

Massimiliano Ruscica[1], Marica Arvigo[2], Elena Dozio[1], Michael D Culler[3], Francesco Minuto[2], Marcella Motta[1], Diego Ferone[2] & Paolo Magni[1]
[1]Center for Endocrinological Oncology, Istituto di Endocrinologia, Università degli Studi di Milano, Milan, Italy, [2]Department of Endocrinological & Metabolic Sciences and Center of Excellence for Biomedical Research, University of Genoa, Genoa, Italy, [3]Biomeasure Incorporate/IPSEN, Milford, Massachusetts, United States.

Somatostatin (SRIF) has been demonstrated to inhibit *in vitro* proliferation of normal and transformed cells via SRIF receptors (SSRs). Moreover, like other neuroendocrine molecules, SRIF and SSRs may play a significant role in the progression and neuroendocrine differentiation of human prostate cancer (PCa). However, conflicting results have been reported in the literature on SSR heterogeneity and specific cell localization in PCa. In the present study, using the human androgen-dependent PCa cell line LNCaP, we investigated: 1) SSR subtypes expression in different culture conditions (10% and 2% FBS); 2) the effects of SRIF and of new agonists on cell proliferation. LNCaP expressed sst_1, sst_{2A}, sst_3, sst_5, at gene (RT-PCR) and protein (Western blot) level. SSR level of expression was differentially regulated by the culture conditions: sst_{2A} and sst_5 expression was not modified by serum concentration, whereas sst_1 and sst_3 expression was inversely correlated to serum concentration. Dose-response studies were performed at 24 and 48 h with a dose range from 10^{-11} to 10^{-7} M. SRIF inhibited cell proliferation ([^{3}H]thymidine incorporation) after 24 and 48 h at all doses. The sst_1 (BIM-23926), sst_2 (BIM-23120) and sst_5 (BIM-23206) preferential compounds did not affected cell proliferation. Conversely, lanreotide, inhibited cell proliferation after both 24 and 48 h with a maximum effect at 10^{-11} M, whereas, the bispecific sst_2/sst_5-preferential ligand BIM-23244 inhibited cell proliferation after 24 h at the dose of 10^{-9} M. The bi-specific sst_1/sst_2-preferential ligand BIM-23704 inhibited LNCaP proliferation after 48 h treatment, (dose range 10^{-10} M to 10^{-11} M). SSR subtype expression in PCa can be actively regulated by culture conditions, suggesting that receptor profile in PCa may depend from the tumor microenvironment. Finally, LNCaP represents a useful model for studying SSR regulation in PCa, intracellular subtype-linked signalling, and validate new analogues with different receptor affinities in PCa treatment.

P116

Conjugated and unconjugated serum steroid hormone concentrations in relation to tumour receptor status in postmenopausal breast cancer patients

Borbála Vincze, Ferenc Pommersheim, Nora Udvarhelyi, Zsolt Horváth, Bence Kapuvári, Mariann Boldizsár, István Köves, Zsolt Orosz & István Láng
National Institute of Oncology, Budapest, Hungary.

Human breast cancer tissue is able to concentrate estrogens. 17-beta-estradiol (E2) and estrone (E1) are produced locally through several mechanisms, e.g. from conjugated and unconjugated steroid hormones uptaken from the circulation. This study was aimed to investigate the correlation between endogenous serum sex steroid concentrations and tumour receptor status in postmenopausal breast cancer patients undergoing surgical intervention. The study involved 740 postmenopausal patients with primary breast cancer of Stage I-II prior to surgical intervention. None of them took hormone preparations and received chemo or radiotherapy. Serum levels of sexual hormones and precursors, sex hormone-binding globulin (SHBG), insulin-like growth factor-1 (IGF-1) and IGF binding protein-3 (IGFBP-3) were measured by fully automated equipment using RIA and IRMA methods. Estrogen (ER) and progesterone receptors (PR), HER2/neu expression in tumour tissues were determined using immunohistochemical methods (NCL-ER-6F, 11/2; NCL-L-PgR-312; CB11-RTU, Novocastra; Hercep Test, DAKO). In the ICH 2+/3+ cases HER2/neu gene amplification was confirmed by fluorescence in-situ hybridization. MedCalc Software was used for statistical analysis. Our investigation revealed significant correlations among steroid receptor status of tumour tissue and the serum E1 and androstenedione (AD) levels. Close relationship was observed among serum value of E1-sulfate, IGF-1, testosterone (TE), dehydroepiandrosterone sulphate (DHEA-S) and HER2/ER status of tumour tissue. Results demonstrate that the positivity of tumour tissue receptor status can be predicted on the basis of increased serum unconjugated (E1, DHEA, AD, TE) and conjugated (E1-S, DHEA-S) sexual hormone concentrations. It is suggested that circulating E1-S and DHEA-S might play a major role in the intratumoral estrogen synthesis. Our study supports the hypothesis that the serum E1, AD, E1-S, DHEA-S, TE and IGF-1 levels might also be useful for predicting the magnitude of response to postoperative chemotherapy.

This research was supported by the Hungarian Research Fund (OTKA No.T 049814).

P117

A novel activating germline mutation in the RET gene (Y606C) in a patient with medullary thyroid carcinoma

Michaela Luconi, Tonino Ercolino, Adriana Lombardi, Lucia Becherini, Elisabetta Piscitelli, Maria Sole Gaglianò, Mario Serio & Massimo Mannelli
University of Florence, Dept. Clinical Physiopathology, Florence, Italy.

Germline mutations in the RET gene cause MEN2, an inherited cancer syndrome associated with medullary thyroid carcinoma (MTC). We performed genetic analysis on DNA from whole blood of a 58 yr old female affected by a multifocal MTC. Exons 10, 11, 13, 15 and 16 of RET gene were amplified by PCR using specific primers and characterised by direct automatic sequencing. Here, we report a new RET point mutation: a heterozigous missense mutation Y606C, a G to A nucleotide substitution leading to a Tyrosine (Y) to Cysteine (C) amino acid change in exon 10. We approached the functional effects of such a mutation in an *in vitro* system by cloning the wild-type RET, the Y606C mutation as well as the C620Y mutation, previously described as less strong RET oncogene associated with MTC, in an expression vector and transiently transfecting NIH3T3 fibroblasts. All mutations were obtained by site-directed mutagenesis. We first demonstrated by western blot analysis using a specific antibody an increased tyrosine phosphorylation in the Y905 residue in the RET/Y606C, corresponding to receptor activation. Since RET activation results in an intracellular signalling cascade leading to extracellular signal regulated kinases (ERKs), we investigated ERK activity in our transfected cells. Results demonstrate a significant increase in ERK2 phosphorylation/activation in the RET/Y606C *versus* the wild type and RET/C620Y. We finally showed by gel electrophoresis of transfected cell lysates in non reducing conditions that the introduction of a C due to the Y606C mutation results in an increased dimerization of the receptor. All these findings suggest that the Y606C mutation confers constitutive activation of RET signalling.

P118

Novel germline VHL mutations associated to uncommon clinical presentations

Tonino Ercolino[1], Lucia Becherini[1], Lisa Simi[1], Maria Sole Gaglianò[1], Gabriella Nesi[2], Andrea Valeri[3] & Massimo Mannelli[1]
[1]University of Florence, Dept. Clinical Physiopathology, Florence, Italy, [2]University of Florence, Dept. Human Pathology, Florence, Italy, [3]AOUC, General and Vascular Surgical Unit, Florence, Italy.

The von Hippel-Lindau (VHL) syndrome is an inherited multi-tumor disorder characterized by clinical heterogeneity and high penetrance. Pheochromocytoma (Pheo) is present in 10-15% of cases. It can be isolated or associated with other lesions such as hemangioblastomas, kidney cysts or cancer, and pancreatic lesions. Pheos secrete norepinephrine and are generally located in the adrenals. While performing genetic testing in patients affected by apparently sporadic pheos or PGLs, we found two novel different VHL germline mutations in two patients presenting two uncommon clinical pictures (an adrenal incidentaloma and a neck tumor, respectively).

Coding regions and exon-intron boundaries of RET (exons 10, 11, 13, 14, 15), VHL, SDHD, SDHB and SDHC genes were amplified and sequenced. We identified two novel point mutations: a L198V missense mutation in a 32 yr old female affected by a right adrenal compound and mixed tumor constituted by an epinephrine secreting Pheo, a ganglioneuroma and an adrenocortical adenoma and a T152I missense mutation in a 24 yr old female affected by a left carotid body tumor. An extensive clinical, laboratory and radiological examination of the patients and the mutated relatives did not show any other lesion.

We also analyzed the three-dimensional structure of the wild-type and the mutated VHL protein showing that the mutations are located in functionally relevant sites.

These cases enlarge the list of VHL mutations and add new insights in the clinical variability of VHL disease, thus confirming the importance of genetic testing in patients affected by apparently sporadic Pheos or PGLs.

P119

The expression of alternatively spliced forms of type 1 deiodinase is changed in clear cell renal cell carcinoma

Agnieszka Piekielko-Witkowska[1], Adam Master[1], Tanski Zbigniew[2] & Nauman Alicja[1]
[1]Medical Center of Postgraduate Education, Warsaw, Poland, [2]Specialistic Hospital, Ostroleka, Poland.

Type 1 deiodinase (D1) catalyses deiodination of tyroxine (T4), which leads either to synthesis of triiodothyronine or reverse triiodothyronine (rT3). T3 can influence the process of neoplasia through its receptors which act as transcription factors and regulate the expression of many tumor suppressor genes and oncogenes. The aim of the study was to analyze the changes in expression of alternatively spliced variants of D1 mRNA in clear cell Renal Cell Carcinoma (ccRCC), which is the most common type of renal cancers (75% of primary renal

malignancies). Tissue samples were obtained with the permission of the local Ethical Committee of Human Studies. Using quantitive real-time PCR we have analyzed: 33 samples of ccRCC with their controls (the contralateral pole of the same kidney not infiltrated by cancer, assigned C) as well as control samples from patients suffering from other, nonneoplastic kidney abnormalities (6 samples, assigned N). The expression of the whole pool of D1 transcript variants was dramatically lowered in ccRCC tissues. The separately performed expression analysis of alternatively spliced D1 transcript variants, which differ in the presence or absence of subexon 1b, also exhibited about 90% decrease of mRNA in both transcript variants of cancer tissues. Simultaneously, the comparison of these alternatively spliced mRNA groups revealed that ratio: (whole pool of D1 transcripts)/(transcripts containing the1b exon) as well as relation: (whole pool of D1 transcripts)/(transcripts devoid of the1b exon) were increased several times in the ccRCC in comparison with controls. This observation suggests the existence of at least one another alternatively spliced variant, which extends the whole poll of D1 transcripts and possibly is overexpressed in ccRCC. Our results indicated that the alternative splicing process of deiodinase type I can be disturbed in ccRCC.

P120

Ret expression reduces estrogen-induced lactotrope hyperplasia

Carmen Cañibano, Noela Rodríguez, Sulay Tovar, María Jesús Vázquez, Montserrat Lavandeira, Carlos Díeguez & Clara Álvarez
Department of Physiology, School of Medicine, University of Santiago de Compostela, santiago de Compostela, Spain.

RET is a tyrosine kinase receptor activated by GDNF, NTN, ART and PSPN through GFRa1,2, 3 and 4 respectively. Activation of the receptor elicits intracellular pathways such us Ras/MAPK and PI3K/AKT leading to differentiation and proliferation. Our group has previously shown that RET is expressed specifically in the somatotroph cell population within the pituitary gland, both in rats and in humans. We have also shown that, in absence of its ligand GDNF, RET induces activation of caspase 3 PKCd/JNK/c/EBPa and CREB, causing apoptosis in cell cultures. Cell death is dependent on Pit-1 and p53 induction. This findings confirm previous hypothesis and strongly indicate that RET acts as a dependence receptor. Now we provide evidence that the same biological and biochemical mechanisms work *in vivo*.

For doing so, we have used a model of lactotroph hyperplasia induced by estrogen administration in rat. Hyperplasic pituitary glands were infected with purified high-titer retroviruses encoding RET or the corresponding empty virus as control. Viral delivery was achieved by estereotaxia, injecting the retrovirus directly into the pituitary of living anesthetized rats. Following treatment and infection rats were sacrificed and pituitary weights recorded. As expected, estrogen treatment induced a marked increase in pituitary size. Interestingly, viral-mediated RET expression caused a significant reduction compared to mock-infected pituitaries (26.6+/− 1.8 mg *vs* 18.0+/− 1.0 mg), restoring pituitary weight to values similar to pituitaries not treated with estrogens. We were able to detect RET expression in lactotrophs, suggesting that ectopic expression of the dependence receptor caused lactotroph cell death and hyperplasia reversal. Moreover, we show activation of the caspase 3-PKCd-JNK-c/EBPa-CREB apoptotic pathway, indicating that the same molecular events are elicited by RET in cell culture models and *in vivo*.

P121

Analysis of BRAF point mutation in papillary thyroid carcinoma

Vlasta Sykorova[1], Sarka Dvorakova[1], Jan Laco[2], Ales Ryska[2], Daniela Kodetova[3], Jaromir Astl[4], David Vesely[4] & Bela Bendlova[1]
[1]Institute of Endocrinology, Prague, Czech Republic, [2]Fingerland's Institute of Pathology, Hradec Kralove, Czech Republic, [3]Institute of Pathology and Institute for Pathological Anatomy and Molecular Medicine FN Motol, Prague, Czech Republic, [4]Department of ENT and Head and Neck Surgery FN Motol, Prague, Czech Republic.

BRAF point mutations are found in 29-69% of papillary thyroid carcinoma (PTC). BRAF is a serine-threonine kinase involved in the phoshorylation of MAPK signaling pathway. The mutation is located in the exon 15 of BRAF, resulting in the substitution of valine to glutamate at codon 600 (V600E). Mutation generates unregulated B-Raf activity that leads to increased cellular proliferation. The aim of this study was to determine the frequency of BRAF mutation in the Czech population and its changes in 1960-2006. We examined 145 of PTC: 92 paraffin-embedded formalin-fixed tissue samples, 44 fresh frozen tissues and 9 wash-out material from fine-needle aspiration biopsies (FNAB) after signing informed consent approved by ethical committee. For assessment of influence of Chernobyl nuclear accident we devided samples into 5 periods - one period before and four periods after the accident. DNAs from paraffin-embedded samples were extracted using the QIAamp DNA Blood Mini Kit and frozen samples using Trizol reagent. BRAF gene was screened using the single strand conformation polymorphism method (SSCP) and verified by direct sequencing. The V600E mutation was detected in 56 samples (38,6%). All BRAF mutations except one were heterozygous. Surprisingly, in the period before Chernobyl nuclear accident no BRAF mutation was found, in other periods 56 mutations were detected (41,2%). The female to male ratio was 3,7:1, mutation was found in 48,4% of male and in 36% of female patients. In our series difference between age at diagnosis in patients with and without mutation was not significant. Our study confirms a high rate of BRAF V600E mutation in Czech PTC patients. Results indicate that the mutation is the most frequent genetic alteration found in PTC and it could be used as a reliable genetic marker of PTC and applied for FNAB before surgery.

This study was supported by IGA MZ CR NR/7806-3.

P122

Effect of surgery on carotid vascular remodeling in patients with pheochromocytoma

Giampaolo Bernini, Fabio Galetta, Michele Bardini, Ferdinando Franzoni, Chiara Taurino, Angelica Moretti, Matteo Bernini & Antonio Salvetti
Department of Internal Medicine, Universiyt of Pisa, Pisa, Italy.

In vitro and *in vivo* studies suggest that catecholamines, in addition to their hemodynamic effect, exert a direct influence on the vascular wall, leading to eutrophic and hypertrophic remodeling. This finding is in agreement with that recently reported by our group on patients with pheochromocytoma (PHEO) who show carotid intima media thickness (IMT) and vascular fibrosis higher than essential hypertensives matched for classic cardiovascular risk factors, including blood pressure. To further confirm the direct vascular influence of catecholamines in humans, we compared carotid IMT, by ultrasound imaging, and vascular fibrosis, by imaging backscatter signal (IBS) analysis, in a group of patients with PHEO and high-normal blood pressure (n = 10; mean ± SD age 51 ± 13.2 yr, range 28–70 yr) before and after surgical cure (mean ± SD 20.5 ± 5.98 months, range 12–29 months). After removal of the tumor, no significant variation in systolic (126.5 ± 6.5 *vs* 138.3 ± 5.6 mmHg, mean ± SE) and diastolic (83.6 ± 3.1 *vs* 87.0 ± 4.1 mmHg) blood pressure and in total cholesterol (207.0 ± 9.6 *vs* 198.8 ± 12.6 mg/dl), HDL-cholesterol (62.8 ± 4.5 *vs* 61.3 ± 4 mg/dl), and LDL-cholesterol (118.3 ± 8.5 *vs* 117.9 ± 13.1 mg/dl) was observed, while a reduction in urinary metanephrines (normetanephrine: 480.0 ± 51.2 *vs* 2264.8 ± 681.4 μg/24 h, $P < 0.003$; metanephrine: 178.7 ± 23.5 *vs* 879.2 ± 290.8 μg/24 h, $P < 0.03$) and in catecholamines (plasma noradrenaline: 442.9 ± 54.7 *vs* 623.9 ± 115.0 pg/ml, N.S.; plasma adrenaline: 36.1 ± 7.2 *vs* 183.8 ± 99.3 pg/ml, $P < 0.02$; urinary noradrenaline: 49.4 ± 8 *vs* 86 ± 27.4 μg/24 h, N.S.; urinary adrenaline:8.6 ± 0.7 *vs* 18.0 ± 7.7 μg/24 h, NS) was shown. After surgery, IBS values significantly decreased (−22.82 ± 0.40 *vs* –21.17 ± 0.61 dB, $P < 0.005$) and a similar pattern was observed for carotid IMT (0.86 ± 0.06 *vs* 0.88 ± 0.06 mm, $P < 0.06$), though at not significant extent. A direct and significant correlation was found between the absolute reduction in IBS values and the absolute decrement in urinary normetanephrines levels ($r = 0.54$, $P < 0.03$). In conclusion, our results confirm that high catecholamine levels directly affect the vascular wall structure, indipendently of the hemodynamic discharge.

P123

A case report of ectopic Cushing's disease presented with trombocytopenia

Demet Ozgil Yetkin[1], Sayid S Zuhur[1], Ekrem Kara[1], Ayla Gurel Sayin[2], Buge Oz[3] & Ertugrul Tasan[1]
[1]Istanbul University Cerrahpasa Medical faculty Endocrinology and Metabolism Department, Istanbul, Turkey, [2]Istanbul University Cerrahpasa Medical faculty Cardiovascular Surgery Department, Istanbul, Turkey, [3]Istanbul University Cerrahpasa Medical faculty Pathology Department, Istanbul, Turkey.

PURPOSE
To report a case of Cushing's syndome caused by ectopic ACTH secretion related to a thymic carcinoid presented with trombositopenia.

CASE
years old male presented with fatigue, skin rash. At presentation, physical findings showed Cushingoid appearance, with moon face, hyperpigmentation, easy bruising and buffalo hump. His laboratory findings showed platelet: 90.000 (150.000–450.000), ACTH: 609 pg/ml (0–46 pg/ml), baseline cortisol level 60.8 μgr/dl (6.2–19 μgr/dl), potassium: 2.4 mEq/l (3.5–5 mEq/l), midnight cortisol level: 57.17 μgr/dl, urine cortisol level: >1000 μgr/24 hour. Serum cortisol levels failed to supress after low and high dose dexamethasone (DST) (1 mg: >60 μgr/dl, 8 mg: 42 μgr/dl), therefore confirming the diagnosis of ectopic ACTH production. Laboratory evaluation for trombositopenia showed, normal eritrocyte series, deficient trombocyte. PT, aPTT and FDP were normal, fibrinojen: 606 mg/dl (<350). Megacaryocyte level was elevated and platelet count was normal in bone marrow aspiration. His sella MRI was normal, thorax CT showed 2×1.5 cm lesion at anterior mediastinum, and surrenal hyperplasia on his abdomen CT. His octreoscan was normal. There was a hypermetabolic focus in anterior mediastinum and bilaterally adrenal gland on his 5FDG PET/CT. Under the diagnosis of ectopic ACTH production in anterior mediasten, he underwent mediastinotomy and thymectomy. Pathological examination showed ACTH, chromogranin and synaptophsin positive thymic benign carcinoid. After the operation his cortisol levels returned to normal (corisol:11 μgr/dl, ACTH: 54 pg/ml) and low dose DST was 1.6 μgr/dl. Three weeks after the operation his platelet count was 411.000, with exclusions of other causes of tromposytopenia and reversal of platelet counts to normal after the operation we concluded that his trombosytopenia was due to a paraneoplastic immune trombocytopenic purpura (ITP).
CONCLUSION
Thymic ACTH secreting carcinoid tumors are rare phenomenon of ectopic Cushing's syndome. To our knowledge this is the first case of ectopic Cushing's disease with paraneoplastic ITP.

P124

Influence of Lanreotide Autogel on insulin sensitivity among patients with acromegaly

Melek Eda Ertorer[1], Okan Bakiner[1], Inan Anaforoglu[1], Emre Bozkirli[1], Neslihan Bascil Tutuncu[2] & Nilgun Guvener Demirag[2]
[1]Baskent University Faculty of Medicine, Endocrinology and Metabolism, Adana, Turkey, [2]Baskent University Faculty of Medicine, Endocrinology and Metabolism, Ankara, Turkey.

There are limited data inquiring the relationship between somatostatin analogues and insulin sensitivity among acromegalic cases. This study was conducted to evaluate short-term effects of lanreotide autogel (LA), administered every 28 days by deep subcutaneous injection, on insulin sensitivity among acromegalic patients with pituitary tumors. Before and following six months of LA treatment, insulin resistance and beta-cell function were calculated by using homeostasis model assessment of insulin resistance (HOMA-IR) and beta-cell function (HOMA-beta) formula, and euglycemic hyperinsulinemic clamp test was performed for evaluating the whole insulin sensitivity. Naïve acromegalic patients (Case 1, Case 3) and cases who experienced any prior unsuccessful treatment modality and approved to consume LA (Case 2, Case 4, Case 5) were included. The study was approved by the local ethics committee. Euglycemic hyperinsulinemic clamp defined by De Fronzo was used and insulin sensitivity was derived from glucose disposal rate expressed as mg/kg/min and indicated as 'M' index. The characteristics of the cases regarding serum growth hormone (GH) levels and insulin sensitivity markers during follow-up are shown in Table. Although there were statistically insignificant difference between baseline and final GH, HOMA-IR, HOMA-beta% and M values ($P=0.150$, $P=0.447$, $P=0.158$, $P=0.151$, respectively), remarkable M value improvement was observed in Case 1, Case 2 and Case 3. This finding might be explained by the prominent decrease in their GH levels following LA treatment.

	Case 1	Case 2	Case 3	Case 4	Case 5
GH (ng/ml)*	34.20/15.30	4.25/0.74	5.0/0.66	1.2/1.0	5.8/3.2
HOMAI-IR*	2.32 / 2.25	2.31 / 0.41	4.29/ 5.59	3.23 / 2.59	4.36/3.45
HOMAI-beta (%)*	95/ 58.48	289.15 / 83.63	152.25/ 98.06	76.87 /67.14	228.91/218.05
M value*	1.03 / 8.22	2.98 / 4.70	5.09 / 13.09	5.72 /5.53	3.90/3.35

*Baseline/ following 6 months of treatment.

P125

A newly detected mutation of the RET proto-oncogene in exon 8 as a cause of multiple endocrine neoplasia Type 2A

S. Bethanis[1], Th. Palouka[1], Ch. Avgoustis[1], G. Koutsodontis[2], T. Bei[2], D. Yannoukakos[2] & S. Tsagarakis[1]
[1]Department of Endocrinology, Athens' Polyclinic, Athens, Greece, [2]BioGenomica, Centre for Genetic Research and Analysis, Athens, Greece.

Multiple endocrine neoplasia type2A (MEN 2A) is a syndrome of familial cancers characterized by medullary thyroid carcinoma (MTC), pheochromocytoma and hyperplasia of the parathyroid glands. RET protooncogene is the responsible gene for MEN 2A; in more than 96% of MEN 2A families mutations in RET exon 10 or exon 11 are identified. Herein we report a MEN 2A case affected by a mutation ($Gly^{533}Cys$) in exon 8. A 66-yr-old male patient was referred to our Department due to bilateral adrenal nodules, revealed incidentally on a computed tomography of the abdomen. Patient's family history was remarkable for pheochromocytoma in his mother. On physical examination there were no features of von Hippel-Lindau disease (VHL) or neurofibromatosis type 1(NF1). Biochemical evaluation (elevated normetanephrines and metanephrines excretion) and findings of the adrenals' magnetic resonance imaging (hyperintense adrenal nodules on T_2-weighted image) were compatible with the diagnosis of bilateral pheochromocytomas. The patient underwent laparoscopic bilateral adrenalectomy and histological examination confirmed the preoperative diagnosis of pheochromocytoma. Absence of phenotypic characteristics of VHL or NF1 and elevated basal and stimulated by pentagastrin serum calcitonin levels raised the possibility of MEN 2A syndrome. Total thyroidectomy was performed and histological examination showed the presence of MTC. Genetic testing for the presence of a RET mutation was also recommended. Direct sequencing of exon 8 from patient's genomic DNA revealed the mutation c.1597G–>T (Gly533Cys). So far, the above missense point mutation has been associated with familial MTC (FMTC) but, to the best of our knowledge, mutations in exon 8 have never been identified in a MEN 2A case. In conclusion, in patients with clinical suspicion of MEN 2A syndrome the analysis of RET exon 8 should be considered when routine evaluation of mutations in exons 10, 11 and 13 is negative.

P126

Clinical and biochemical effects of adjuvant mitotane treatment in patients with adrenocortical cancer (ACC)

Fulvia Daffara[1], Silvia De Francia[2], Giuseppe Reimondo[1], Barbara Zaggia[1], Paola Sperone[3], Francesco Di Carlo[2], Alberto Angeli[1], Alfredo Berruti[3] & Massimo Terzolo[1]
[1]Dipartimento di Scienze Cliniche e Biologiche - Medicina Interna I, ASO San Luigi, Orbassano, Italy, [2]Dipartimento di Scienze Cliniche e Biologiche - Laboratorio di Farmacologia, ASO San Luigi, Orbassano, Italy, [3]Dipartimento di Scienze Cliniche e Biologiche - Oncologia Medica, ASO San Luigi, Orbassano, Italy.

Objective
Seventeen patients (9 women, 8 men aged 36 years, 22–58) radically resected for ACC were treated with adjuvant mitotane and prospectically followed from 2000 to 2006.
Methods
Stage of ACC: was: 1 stage I,; 12 stage II, 4 stage III; Weiss score 6, 3–9; Ki67% 20, 4–67. Eleven patients had functional tumors. Median duration of treatment was 15 months (range:4–84) and 14 patients are currently on mitotane, 2 died, 1 discontinued treatment after 5 years. All patients were treated with a low-dose regimen (till to 3–4 g/die) and underwent monitoring of plasma mitotane level every 3 months. None of the patients discontinued mitotane definitively for side effects and 16/17 patients reached the therapeutic levels after a median time of 3 months. At the last follow up, 6/17 (35%) patients have relapsed, 15 patients are still alive.
Results
Hyperprolactinemia was observed in 50% of men and 40% of women, 62% of men become partially hypogonadic: reduction of free testosterone was greater than total testosterone. Central hypothyroidism developed in 9 patients who were treated, while 4 patients already on thyroxine required dose increment. Fifteen patients developed overt hypoadrenalism, while 1 patient showed normal cortisol and elevated ACTH, 11 patients developed hypoaldosteronism. Total cholesterol level were slightly enhanced with increase of HDL and reduction of LDL, triglicerides were normal. Reduction of folate level and consequent increase of homocysteine was also observed. Mitotane levels were inversely correlated with cortisol ($P=0.007$), aldosterone ($P=0.01$) and FT4 levels ($P=0.03$), while they were positively correlated with PRA ($P=0.004$) and HDL levels ($P=0.005$).
Conclusions
In conclusion, a low-dose regimen of adjuvant mitotane is well tolerated and able to reach the therapeutic interval. Adequate supplementation of adrenal and sex steroid and thyroid hormones is necessary. Some effects of mitotane may be ascribed to either adrenolytic or estrogen-like actions of the drug.

P127

Effectiveness of retinoic acid treatment for redifferentiation of thyroid cancer in relation to recovery of radioiodine uptake

Cristián A. Fernández Fernández[1], Manel Puig-Domingo[1], Francisco Lomeña[1], Montserrat Estorch[2], Angel L Bittini[3], Mónica Marazuela[5], Javier Santamaría[4], Tomás Martín[7], Purificación Martínez de Icaya[6], Immaculada Moraga[5], María Cuesta[7], Ana Mejías[8], Manel Porta[9], Dídac Mauricio[2] & Irene Halperin[1]
[1]Hospital Clinic i Provincial de Barcelona, Barcelona, Spain; [2]Hospital de Sant Pau, Barcelona, Spain; [3]Hospital Gregorio Marañon, Madrid, Spain; [4]Hospital de Cruces, Barakaldo, Spain; [5]Hospital de la Princesa, Madrid, Spain; [6]Hospital de Leganés, Madrid, Spain; [7]Hospital Virgen Macarena, Sevilla, Spain; [8]Hospital Juan XXIII, Tarragona, Spain; [9]Hospital de Vic, Vic, Spain.

Retinoic acid (RA) treatment has been used in the last decade for redifferentiation of metastatic thyroid cancer that have lost radioiodine uptake (RIup) with heterogeneous results.
Aim
To evaluate the improvement of RIup after a course of RA treatment.
Method
Retrospective analysis of 29 patients with radioiodine negative metastatic disease (17 men /12 women; 22 papillary, 4 follicular and 3 oncocytic tumours). RA was given at a dose of 0.66–1.5 mg/kg for 5–12 weeks, followed by a therapeutic ^{131}I dose (3700–7400 MBq). Thyroglobulin levels and CT imaging control after 3 months of RA were performed.
Results
In 44.8% of the patients (14 out of 29 cases, 11 papillary/3 follicular) a positive radioiodine scan was observed; in 7 additional cases (5 papillary, 2 oncocytic) a weak RIup was also apparent (total responders 21/29, 72.4%), and in the remaining 8 the RIup persisted negative (6 papillary, 1 follicular and one oncocytic). No correlation was observed between changes in thyroglobulin levels and recovery of RIup. In 11 RA positive treatments a stabilization of mestastasic growth was observed in 5, while in 6 tumoural mass increased at short term. No major side effects were detected.
Conclusion
A relatively high rate of reinduction of RIup after RA treatment may be possible in advanced stage papillary and follicular thyroid cancer patients, with uncertainty in relation to a potential modification of the natural course of the disease. Further studies, aiming to identify potential responders to RA treatment by a better characterization of the biological nature of these tumours, will be required for an improved indication of RA coadjuvant treatment of thyroid cancer in the future.

P128

Expression of the neuropeptide cortistatin in haematological malignancies

Virgil Dalm, Frank Staal, Leo Hofland, Aart-Jan van der Lely & Martin van Hagen
Erasmus MC, Rotterdam, Netherlands.

Introduction
Cortistatin (CST) is a 17 amino-acid neuropeptide involved in sleep regulation. Due to its structural resemblance to somatostatin (SS), CST binds with high affinity to the 5 known SS receptors. CST also binds to the putative MrgX2 receptor. Previously we demonstrated that various types of human immune cells and tissues as well as lymphoid cell lines express CST mRNA. We suggested that CST plays a regulatory role in immune cell function both in physiological and pathophysiological conditions.

In the present study we investigated CST expression in human haematological malignancies, in order to gain more insight in the potential significance of CST in these diseases.
Patients and methods
Bone marrow and peripheral blood samples of 38 patients with T-ALL and B-ALL were studied using micro-array technique (Affymetrix) and 5 lymph node biopsies from patients with non-Hodgkin's lymphoma (NHL) using Q-PCR. Expression of both SS and CST mRNA was investigated in all samples.
Results
In 11 out of 22 patients with B-ALL CST expression was found, whereas in only 1 patient SS expression could be detected. Moreover, in 14 out of 16 patients with T-ALL CST expression was detected, while SS expression was present in only 1 patient. In all 5 NHL biopsies low expression of CST mRNA was detected, while no SS mRNA was found.
Conclusion
In the present study we demonstrated that CST mRNA is widely expressed in samples of patients with leukemic disease and in malignant NHL. On the other hand, expression of SS is absent in most cases. These findings suggest that, in line with our findings in normal human immune cells, CST might play a regulatory role, potentially with respect to control of proliferation or cytokine secretion, in these diseases, rather than SS. Further studies will be necessary to evaluate the role of CST and the potential therapeutical implications of CST or CST-like peptides.

P129

A loss-of-function polymorphic mutation in the P2X7 receptor gene in patients with papillary thyroid cancer

Angela Dardano[1], Simonetta Falzoni[2], Antonio Polini[1], Alessia Bemi[1], Anna Solini[1], Nadia Caraccio[1], Francesco Di Virgilio[2] & Fabio Monzani[1]
[1]University of Pisa, Pisa, Italy; [2]University of Ferrara, Ferrara, Italy.

Extracellular nucleotides, via specific plasma membrane receptors (P2Rs) of the X and Y subtype, modulate several cell functions, including cell-to-cell cross talk. We have previously demonstrated the expression of several functional P2XRs subtypes, including $P2X_7$ in primary human thyrocytes. P2X7 is the main player in inflammation and immunomodulation; a strong expression of this receptor has been shown in several human solid tumors. Polymorphisms of the gene encoding for $P2X_7$ have been described; among these, 1513A>C induces loss-of-function while 489 C>T gain-of-function of the receptor.

We evaluated the presence of 1513A>C and 489C>T polymorphisms in patients with papillary thyroid carcinoma (PTC).

$P2X_7R$ genotypic analysis was performed in 83 patients with PTC (70 women; mean age 43±13 yrs; 29 with diameter <1 cm; 33 with follicular and 50 with classical variant) and 100 healthy subjects (Bone Marrow Bank donors, Ferrara). The single nucleotide polymorphisms were analyzed in genomic DNA samples by the TaqMan MGB probe technique. Results are summarized in the table.

Table 1

Polymorphism 1513A>C	Minor Allele Frequency	Genotype (%) A/A	A/C	C/C	p
Controls	0.2	62	36	2	
Patients	0.3	48	43	9	0.0004

Increased homozygous substitution 1513A>C was detected only in patients with the follicular variant (22%). A significant correlation with PTC dimension was also observed ($P=0.02$). No differences were detected in the allelic frequencies for 489C>T.

Overall, our data demonstrate an increased prevalence of 1513A>C polymorphism in patients with PTC. This loss-of-function polymorphism characterized the follicular variant and correlated with cancer dimension. Further studies are needed to evaluate the role of 1513A>C polymorphism as a novel clinical marker of differentiated thyroid carcinoma.

P130

Enhanced expression of functional $P2X_7$ receptor in human papillary thyroid cancer

Angela Dardano[1], Davide Ferrari[2], Sabina Cuccato[1], Eleonora Santini[1], Nadia Caraccio[1], Sara Gulinelli[2], Giulia Callegari[2], Pinuccia Faviana[1], Anna Solini[1], Francesco Di Virgilio[2] & Fabio Monzani[1]
[1]University of Pisa, Pisa, Italy; [2]University of Ferrara, Ferrara, Italy.

Extracellular ATP modulates several biological processes via activation of plasma membrane receptors (P2Rs) in normal human thyrocytes (NT). We characterized P2Rs expression and function in two thyroid cancer cell lines: FB1 (anaplastic cancer) and FB2 [papillary cancer (PTC)]. P2Rs expression was evaluated by RT-PCR and WB, intracellular Ca^{2+}changes by fluorimetric technique (Fura2-AM), IL-6 release by ELISA, intracellular [*i*(ATP)] and extracellular ATP [*e*(ATP)] concentration by luminometry.

FB1 and FB2 showed significantly higher *e*(ATP) and *i*(ATP) concentration than NT ($P<0.001$ for both). [Ca^{2+}] fluxes induced by *e*(ATP) (1 mM, in the presence of external Ca^{2+}) were higher in both FB1 and FB2 than NT cells, ($P<0.01$). Moreover, the addition of ATP (0.25 and 1 mM) induced a significantly higher IL-6 release respect to NT ($P<0.001$at both ATP concentrations) in both cell lines. The $P2X_7$ agonist BzATP, almost ineffective in NT, induced a huge IL-6 release in FB2 (from 6315 ± 328 to 11764 ± 1652 and to 25661 ± 2815 pg/ml/1.5x10^5 cells with BzATP 0.25 and 1 mM, respectively) and FB1, although at a lesser extent (from 7388 ± 170 to 8721 ± 1332 and to 10620 ± 2216, respectively). Moreover, IL-6 release was prevented either by oxidized-ATP or KN-62, selective blockers of human $P2X_7$. Accordingly, FB2 cells showed a strong expression of $P2X_7$, less evident in FB1 cells. These findings demonstrated an enhanced expression of functional $P2X_7$ receptors in thyroid cancer cell lines. Therefore, we checked $P2X_7$ expression in 33 human PTC histological samples, confirming an increased $P2X_7$ expression in cancer than in normal thyroid tissue both by RT-PCR ($P<0.0001$) and immunostaining (avidin-biotin method) ($72\pm15\%$ *Vs* $8\pm3\%$ of cells, respectively).

In conclusion, human thyroid cancer is characterized by an enhanced P2XRs function; specifically, PTC shows a strong $P2X_7$ expression in comparison to normal thyroid tissue. The increased $P2X_7$R function may play a role in the modulation of the inflammatory response to neoplasia.

P131

Results 90Y-DOTATATE therapy in patients with neuroendocrine tumours (NETs) - own experience

Jolanta Kunikowska[1], Alicja Hubalewska-Dydejczyk[2], Anna Sowa-Staszczak[2], Leszek Krolicki[1], Pawel Ochman[1], Renata Mikolajczak[3], Dariusz Pawlak[3], Malgorzata Kobylecka[1], Joanna Maczewska[1] & Bohdan Huszno[2]
[1]Nuclear Medicine Department, Medical Academy, Warsaw, Poland; [2]Department of Endocrinology, Medical College at Jagiellonian University, Cracow, Poland; [3]Radioisotope Centre POLATOM, Otwock-Swierk, Poland.

In the 1980 s the discovery of expression of somatostatin receptors on NET cells made the use of somatostatin analogues in diagnosis and therapy possible.
The aim
Of the study was to assess response of targeted radio-nuclide therapy with radio-labelled somatostatin analogue ^{90}Y-[DOTA^0,D-Phe^1,Tyr^3]-octreotate (DOTATATE) in treatment of disseminated NETs.
Material and methods
12 patients (aged 56.7+/−11.2): carcinoid-5 pts, insulinoma-1pt, gastrinoma-2 pts, pancreatic NET-2 pts, ca neauroendocrinale without primary tumor-1, stomach NET-1 pt) were enrolled in the study. Before the therapy, blood tests for hematology, kidney and liver function and CgA were performed. All patients underwent CT scans and ^{99m}Tc-HYNIC/EDDA-octreotate SRS. Treatment with ^{90}Y-DOTATATE) was repeated every 4–6 weeks up to the total of 200 mCi/m^2. Amino acids infusion was used for kidney protection.
Results
One year observation: regression of disease (PR -decrease of size and number of metastases, ↓CgA level, good clinical response) was observed in 6 pts, stable disease (SD-stable size and number of metastases, ↓CgA) in 3 pts. 3 patients died. No nephrotoxicity was observed. WBC and PLT levels were stable during therapy in 3 pts (without chemotherapy). In 1 pt with previous chemotherapy (last course a month before radiotherapy), PLT level decreased (220×10^3/mm^3 ® 47×10^3/mm^{3}after the first course); the patient died 2 months after the beginning of the therapy. In 8 pts leucopoenia was observed ($<4\times10^3$/mm^3) but serious neutropenia ($<2\times10^3$/mm^3) was found in 3 pts with previous chemotherapy. Thrombocytopenia (PLT $<100\times10^3$/mm^3) was observed in 2 patients with previous chemotherapy.
Two-year observation: prolonged PR – 4 pts; SD – 3 pts, progression of disease in 2 pts: with gastrinoma and stomach NET without hormonal activity (4 and 9 months after radiotherapy). Blood tests stable.
Conclusion
PR and SD were observed in 9/12 patients with disseminated NET. Severe haematologic toxicity was mainly observed in patients after prior chemotherapy –the question of optimising the time between chemotherapy and radiotherapy is still open.

P132

Results of treatment of patients with pituitary somatotroph adenomas

Andrey Grigoriev, Natalye Molitvoslovova, Galina Kolesnikova & Anatolij Kuzmin
Endocrinology Scientific Centre, Moscow, Russia.

In ESC for the period since October 2004 till October 2006 were operated 69 patients with acromegaly. Men were 22 (32%), women – 47 (68%). Age of patients changed from 24 till 68 (middle – 47).

All patients were separated into 2 groups: surgery (group 1) and combination treatment (group 2), which consist of surgery and somatostatin analogues therapy before and after surgery.

In most cases were macroadenomas, only 5 patients (7%) had microadenomas. Suprasellar invasion had 21 patients (30%), infrasellar – 28 (41%) and 32% patients had invasion to one or both cavernous sinuses.

50 patients operated by transnasal approach and 19 with endoscopic techniques. In 47 cases (69%) tumor was total removal, in 17 – subtotal (not less 90% tumor mass was removal), and in 5 cases (7%) – partial removal.
Results
Significant clinical improvement is seen in most patients – 66 (97%). Reduce diabetes mellitus we observed at 43% patients (6 from 14), visual improvement had 78% patients (14 from 18).
Nobody had CSF leak after operation. Diabetes insipidus had 6 patients (9%). Pulmonary embolus had 3 patients (1 patient died).
After 6–12 months were examination 14 patients from group 1 and group 2. GR was normalized in 79% of patients of each group. IGF-1 was normalized in 75% of each group. And postglucose GH level was normalized in 46% into group 1 and 58% into group 2.
Conclusion
Transsphenoidal surgery for acromegaly is safe and effective treatment with minimal mortality and morbidity.
Obvious distinctions in postoperative dynamics IGF-1 and postglucose GH in both groups it is not revealed. There is a tendency in greater efficiency of the combined treatment.

P133

Adrenal incidentaloma, an oncological or endocrinological enigma? Clinical analysis of 1300 cases observed at a single endocrinological centre

Anna Kasperlik-Zaluska[1], Elzbieta Roslonowska[1], Jadwiga Slowinska-Srzednicka[1], Wojciech Zgliczynski[1], Wojciech Jeske[1], Lucyna Papierska[1], Maciej Otto[2], Rafal Slapa[2], Andrzej Cichocki[3] & Jaroslaw Cwikla[4]
[1]Centre for Postgraduate Medical Education, Warsaw, Poland; [2]Warsaw University of Medicine, Warsaw, Poland; [3]Centre and Institute of Oncology, Warsaw, Poland; [4]Hospital of MSWiA, Warsaw, Poland.

Objective
Incidentally found adrenal tumour (adrenal incidentaloma = AI) is the most frequent adrenal disorder. Every patient with AI has to be evaluated carefully to choose the best method of management. We present our experience with a group of 1300 patients with AI, registered at our department.
Material and methods
Material consisted of 1300 patients (female/male ratio 2.6, age 10–87 years) with AI ranging in size from 0.8 to 23.0 cm. Methods: clinical examination, biochemical assays, hormonal determinations (cortisol, androgens, ACTH, aldosterone, metanephrines), imaging studies (ultrasound scans, CT, MRI), histological/immunocytochemical investigations in 420 patients treated by surgery.
Results
Basing on these examinations we diagnosed in our material 116 patients with adrenal cancer, 14 – with other primary malignant adrenal tumours, 48 – with metastatic tumours and 1122 with probably benign tumours. The most important criteria for surgery were imaging phenotype (mainly high density, over 20 HU in the I phase of CT), size (≧5 cm) rapid growth of the tumour and suspicion of a clinically silent chromaffin tumour (for fear of an unexpected metanephrines crisis). In some cases of adrenal cancer elevated levels of androgens have been noted. The most frequent form of subclinical hyperactivity has been pre-Cushing's syndrome (6.5%).
Conclusions
1/Malignant adrenal tumours were found in 178 patients (14%), in this number adrenal cancer in 9%. 2/ The oncological criteria for surgery were of primary importance in our material, with the elevated density in CT (I phase) as the main single indication.

P134

Frequency and type of adrenal tumors in our patients

Milica Medic-Stojanoska[1], Branka Kovacev-Zavisic[1], Tijana Radovanov[1], Ivana Bajkin[1], Jovanka Novakovic[1], Milena Mitrovic[1], Dusan Z Tomic[1], Nilkola Curic[2] & Ljiljana Todorovic-Djilas[1]
[1]Clinic for Endocrinology, Institute for Internal Medicine, Clinical Center of Vojvodina, Novi Sad, Vojvodina, Serbia; [2]Department for pathological physiology, Clinical Center of Vojvodina, Novi Sad, Vojvodina, Serbia.

In recent years, adrenal tumors (AT) are no rare disease. They may arise from all zones of adrenal cortex and medulla, benign or malignant, sometimes as metastases of distant malignances. Patient's present hormone excess or mass effect, but part of them is clinically silent. The aim of this study was to investigate the frequency, hormonal secretion and pathohistology of AT in our patients lasting years. All patients with AT which are hospitalized in the period from January 1st, 2000. to October 15th, 2006. in our Clinic are included in study. Data of clinical feature, hormonal secretion, imaging and pathohistology of AT are collected in our hospital register of admitted patients and medical records. Patients with AT are divided according to hormonal secretion and pathohistology per years. Linear trend are calculated.
Results
During this 7 years in our Clinic are admitted 102 patients with AT, 65 (63,72%) females and 35 (36,28%) males. It has been 2,38% of all hospitalized patients. Hormonally inactive are presented 64,71%. Patients with hormonally active AT be demonstrated as Cushing's syndrome (18,63%), Syndrome Conn (8,82%) and pheochromocytoma (3,92%). According to data of histology and immunohistology after surgery, 89,22% be presented as benign and 10,78% as malignant. Only 5,88% of malignant tumors has been metastases of distant tumors. Linear trend is pointed the increase of incidence patients with AT during period of observation.
Conclusion
The incidence of patients with AT have tendency to increase lasting years in our region. Benign and non-functionally AT are the most common.

P135

Papillary thyroid cancer – the possible role of death ligands in tumor immunology

Emese Mezosi[1], Ferenc Gyory[2], Agnes Borbely[2], Bernadett Ujhelyi[2], Gyorgyike Soos[2], Endre V. Nagy[2], Zsuzsa Varga[2] & Laszlo Bajnok[1]
[1]University of Pecs, School of Medicine, Pecs, Hungary; [2]University of Debrecen, Medical and Health Science Center, Debrecen, Hungary.

Papillary thyroid cancer (PTC) cells and immune cells can kill each other by death ligands. Death ligands induce apoptosis only in sensitive cells. The sensitivity to apoptosis is regulated in a complex and poorly understood manner. The aim of this study was to investigate the Fas ligand (FasL) and Tumor Necrosis Factor-Related Apoptosis Inducing Ligand (TRAIL) expression in PTC cells and tumor infiltrating immune cells. Twenty-six PTCs without and fifteen PTCs with cervical lymph node metastasis were examined by immunohistochemistry. Lymphocytic and macrophage infiltration, HLA-DR, FasL and TRAIL expressions were investigated. The intensity of positive staining was evaluated by a semiquantitative score system. Macrophages and lymphocytes infiltrated the majority of tumor samples. FasL expression of cancer cells was universal and did not show any correlation with the intensity of lymphocytic infiltration and lymph node metastasis. A small subgroup of lymphocytes in close proximity to tumor cells was strongly positive for FasL. Lymphocytes did not express TRAIL. TRAIL expression of tumor cells was increased in PTCs with lymph node metastasis ($P=0.01$). Macrophages were negative for death ligands. In summary, increased TRAIL expression of tumor cells may inhibit the anti-tumor immunity and promote the formation of lymph node metastasis. A subgroup of lymphocytes can use FasL for tumor cell killing.

This work was supported by a grant from the Hungarian Medical Research Council (ETT 186/2003).

P136

Leptin and adiponectin interact in regulating prostate cancer cell growth

Tina Mistry[1], Janet Digby[1], Ken Desai[2] & Harpal Randeva[1]
[1]University of Warwick Medical School, Coventry, United Kingdom; [2]University Hospital Coventry and Warwickshire, Coventry, United Kingdom.

Introduction
Leptin and adiponectin have opposing properties and are implicated as molecular mediators between obesity and (aggressive) prostate cancer. Adiponectin, circulates inversely proportional to visceral fat accumulation, and has demonstrated anti-proliferative effects in prostate cancer cells; circulating leptin levels increase with visceral fat accumulation and has shown mitogenic effects. We propose that adiponectin and leptin interact in prostate cancer cell growth regulation.
Materials and Methods
We studied the effect of full-length (fAd) and globular (gAd) adiponectin (0.01 nM–100 nM) $\pm$ 100 nM leptin on LNCaP and PC3 prostate cancer cell proliferation. *p53* tumour suppressor and *bcl-2* oncogene expression was measured using quantitative RT-PCR.
Results
LNCaP: co-incubation of fAd with leptin resulted in decreased cell proliferation; fAd alone had little effect. gAd alone slightly increased proliferation and had little effect when co-incubated with leptin. fAd alone increased *p53* mRNA expression and rescued leptin-induced inhibition of *p53* expression; both fAd and gAd alone increased *bcl-2* expression, but reduced expression to below basal when co-incubated with leptin. PC3: fAd decreased proliferation at 100nM, but reduced proliferation to half of basal when co-incubated with leptin; gAd alone increased proliferation but reduced proliferation to basal when co-incubated with leptin. Both fAd and gAd demonstrated significant dose-dependent increases in *p53* mRNA expression when co-incubated with leptin; both fAd and gAd reduced *bcl-2* expression to negligible levels despite the addition of leptin.
Conclusion
We show an interaction between adiponectin and leptin in the regulation of prostate cancer cell proliferation through modulation of *p53* and *bcl-2* expression; this is most marked in the advanced PC3 cell line. Concurrent hyperleptinaemia and hypoadiponectinaemia in obese patients may modulate prostate cancer progression, and serum leptin:adiponectin ratio could represent a new prognostic marker; increasing circulating fAd in these patients may be a novel treatment for this disease.

P137

A novel role for Visfatin/Pre-B cell colony-enhancing factor 1 (PBEF) /Nicotinamide phosphoribosyltransferase (NMPRTase) in prostate carcinogenesis

Tina Mistry[1], Janet Digby[1], Joe Cross[1], Ken Desai[2] & Harpal Randeva[1]
[1]University of Warwick Medical School, Coventry, United Kingdom; [2]University Hospital Coventry and Warwickshire, Coventry, United Kingdom.

Introduction
Visfatin/PBEF is a novel adipokine circulating inversely proportional to visceral fat mass and exerts insulin-mimetic effects; it is expressed in normal, inflamed and tumour tissues. Visfatin/PBEF has also been identified as NMPRTase, a key intracellular enzyme involved in NAD^+ metabolism, replenishing NAD^+ during cellular respiration. Inhibition of NMPRTase by the anti-cancer agent FK866 has been shown to induce apoptosis in tumours. Prostate cancer progression is associated with obesity and its metabolic sequelae, and we propose a role for visfatin/PBEF/NMPRTase in prostate carcinogenesis.
Materials and Methods
Visfatin expression was studied in normal and malignant prostate cancer tissue and LNCaP and PC3 human prostate cancer cell lines using RT-PCR, immunocytochemistry and confocal analysis. Regulation of visfatin expression by testosterone, 5-alpha dihydrotestosterone (DHT) (10^{-6}M) interleukin-6 (30 ng/ml) and insulin-like growth factor-1 (IGF-1) (10 ng/ml) was studied using quantitative RT-PCR and Western blotting. We also investigated the effect of visfatin $\pm$ IGF-1 on LNCaP and PC3 cell proliferation.
Results
Visfatin mRNA and protein were detected in LNCaP and PC3 cells and normal and malignant prostate cancer tissue; visfatin protein demonstrated cytoplasmic and nuclear distribution. Testosterone, DHT and IGF-1 increased visfatin mRNA and/or protein expression in both the androgen-sensitive LNCaP and androgen-insensitive PC3 cell line. Treatment of PC3 cells with visfatin resulted in a dose-dependent increase in PC3 cell proliferation which was enhanced in the presence of IGF-1; co-incubation of visfatin and IGF-1 showed a synergistic dose-dependent increase cell proliferation in LNCaP cells.
Conclusions
Our novel findings demonstrate a multifunctional (intra- and extra-cellular) role for visfatin in prostate carcinogenesis, and provide greater insight into the molecular association between obesity and prostate cancer. High visfatin expression in prostate cancer cells may indicate poor prognosis, and inhibition of visfatin may represent a novel therapeutic target for treatment of this disease.

P138

Initial presentation of patients with acromegaly - analysis of the German acromegaly register

Stephan Petersenn[1], Martin Reincke[2], Michael Buchfelder[3], Holger Franz[4] & Hans-Jürgen Quabbe[5]
[1]Division of Endocrinology, University of Duisburg-Essen, Essen, Germany; [2]Klinikum Innenstadt, University of Munich, Munich, Germany; [3]Department of Neurosurgery, University of Erlangen-Nuremberg, Erlangen, Germany; [4]Lohmann & Birkner Health Care Consulting GmbH, Berlin, Germany; [5]Prof. emer., Free University Berlin, Berlin, Germany.

Due to its rarity, initial endocrine abnormalities in acromegaly are difficult to investigate in a large cohort, especially with respect to cofounding variables. We searched the German Acromegaly Register for data on the first presentation of patients with acromegaly.

Up to November 2005, 1485 patients with acromegaly had been entered into the database. Male patients demonstrated significantly higher random GH (21.0 (0.2–620.0) ng/ml, median (range)) and IGF-1 (773.0 (118–2000) ng/ml) levels than females with 14.0 (0.06–556.0) ng/ml ($P<0.005$) and 679.0 (136–2103) ng/ml ($P<0.0001$). Furthermore, comparison of biochemical parameters for various age decades demonstrated a significant association between increasing age and decreasing random GH and IGF-1 levels. Gonadal insufficiency occurred in 18.8%, secondary adrenal insufficiency in 11.8%, TSH deficiency in 7.5%, and diabetes insipidus in 1.3% of subjects. Pituitary insufficiencies occurred with higher frequency in male patients (39.1% vs. 22.0%, $P<0.0001$), and in a significantly higher percentage of patients with macro- (31.6%) compared to microadenomas (18.1%, $P<0.005$). During initial biochemical analysis, 6.4%, 1.5%, and 3.7% of subjects revealed non-pathological results for random GH (<2.5 ng/ml), minimal GH during oGTT (<1 ng/ml), and IGF-1, respectively. None had normal, and 91,4% had pathological results for all three parameters. Whereas the combination of GH during oGTT and IGF-1 raised suspicion of acromegaly in all subjects, 0.5% and 1.1% of subjects demonstrated normal values with combinations of random GH and IGF-1, or random and glucose suppressed GH, respectively.

In conclusion, biochemical activity of acromegaly may depend on age and sex. Therefore, therapy may need to consider and being adapted according to these parameters. Patients with acromegaly may need to be evaluated for pituitary insufficiencies, even with microadenomas. The combination of glucose-suppressed GH and IGF-1 may be the best screening parameters for acromegaly.

Endocrine tumors and neoplasia – presented on Tuesday

P139

Survivin – a promising target for immunotherapy in patients with adrenocortical carcinoma

Martin Fassnacht[1], Sebastian Wortmann[1], Silviu Sbiera[1], Dorothee Kuehner[1], Marion Wobser[2], Patrick Adam[3], Juergen C. Becker[2] & Bruno Allolio[1]
[1]University of Wuerzburg, Dept. of Medicine I, Wuerzburg, Germany; [2]University of Wuerzburg, Dept. of Dermatology, Wuerzburg, Germany; [3]University of Wuerzburg, Dept. of Pathology, Wuerzburg, Germany.

Objectives
Adrenocortical carcinoma (ACC) is a rare tumor with poor prognosis and limited therapeutic options. Survivin is an anti-apoptotic molecule expressed by neoplastic and tumor-specific endothelial cells of various carcinomas, but rarely or only weakly in normal differentiated tissue. In melanoma and pancreatic cancer, preliminary results of a survivin vaccination trial (www.clinicaltrials.gov) indicated that an immunological response in patients is often paralleled by tumor control. Hence, we investigated, whether survivin may also be a reasonable target for an immunotherapy in ACC.
Methods
We performed survivin real-time-PCR in 14 ACCs and 13 normal adrenals. In addition, survivin protein was analysed by immunohistochemistry in 78 ACC samples and 5 normal adrenals using a tissue array (scoring of expression: 0–3). Finally, the presence of spontaneous survivin-recognizing T-cells in the peripheral blood of 7 ACC patients were investigated by indirect interferon-gamma-ELISPOT using HLA-A1, -A2 or -B35 restricted survivin peptides.
Results
Survivin RNA was detectable in 11/12 ACCs and 8/13 normal adrenals. However, the mean expression in ACC was an order of magnitude higher than in normal adrenals ($9071\pm5561\%$ vs. $100\pm25\%$, $P<0.001$). Immunohistochemistry confirmed survivin protein expression in 89% of ACCs. Moreover, in 38/78 of the ACCs but in none of the normal adrenals the expression was judged as moderate-to-high (score 2 or 3). Notably, in 1/7 ACC patients spontaneous HLA-A2-restricted survivin-specific T cells response was detected suggesting that the used epitope might be of immunotherapeutic value.
Conclusion
This is the first study addressing survivin expression in a large series of ACC patients. Since antiapoptotic survivin is overexpressed in many ACCs and exhibits immunogenic properties, it is an intriguing target for immunotherapy also in this rare disease. Especially in patients with refractory ACC having progressed after several cytotoxic therapies an experimental vaccination approach seems to be justified and promising.

P140

Thyroid cancer: with an unexpected location – in the pancreas and in an unexpected combination with Boeck's sarcoidosis

Zsuzsanna Valkusz[1], Éva Csajbók[1], János Gardi[1], Lilla Kardos[2], László Tiszlavicz[3] & László Pávics[4]
[1]Endocrin Unit, University of Szeged, Szeged, Hungary; [2]International Medical Center, Szeged, Hungary; [3]Dept. of Pathology, University of Szeged, Szeged, Hungary; [4]Dept. of Nuclear Medicine, University of Szeged, Szeged, Hungary.

The incidence of differentiated thyroid cancer gradually increased in the last few decades. Primery thyroid cancer is usually located in the thyroid gland and can be classified into well differentiated and poorly differentiated forms. Sooner or later, these cancers metastatize into local lymphnodes or distant organs.

We present the histories of two patients with anusual forms of thyroid cancer.

A woman of 64 was admitted in our department in 2004, due to an inoperable tumor in the pancreas. Histological sampling revealed a well differentiated ectopic follicular thyroid cancer. After total thyroidectomy (no malignancy in the thyroid), 131-I scintigraphy showed isotope accumulation int he pancreas.

Repeated high-dose 131-I therapy shrank the size of the pancreatic tumor and markedly decreased the thyroglobulin level in the serum. One year after these interventions, the patient feels well, has no further distant metastases and is treated for insulin-dependent diabetes mellitus; TSH is sctrictly suppressed by thyroxine medication.

A man 28 was admitted in our department for severe dyspnea in 2004. The computed tomography of the chest detected disseminated patches in the lung with enlarged lymphnodes both int he mediastinum and ont he neck. Total thyroid surgery plus modified cervical and mediastinal lymphnode dissection showed a papillary type thyroid cancer metastatizing into the lung and combined with Boeck's sarcoidosis. Postoperative thyroglobulin level was foud extremely high and 131-I scintigraphy showed pulmonary accumulation. Repeated radioiodine treatment resulted in decreasing thyreogobulin level and strongly improved picture of the chest by computed tomography. The patient is under TSH suppressing therapy.

P141

Thyroglobulin-antibodies in the "normal" range may decrease the diagnostic accuracy of thyroglobulin in the care of patients with differentiated thyroid cancer

Locsei Zoltán[1], Toldy Erzsébet[1], Szabolcs István[2] & Kovács L. Gábor[3]
[1]Markusovszky Hospital, szombathely, Hungary; [2]National Medical Centre, Budapest, Hungary; [3]University of Pécs, Pécs, Hungary.

Issue
The use of thyroglobulin (Tg) as tumor marker in differentiated thyroid cancer (DTC) is limited in the presence of thyroglobulin-antibodies (TgAb) but it is generally believed that this is true only for TgAb concentrations over the normal 'cut off' point.
The aim
Of this study was to investigate if TgAb-s in the normal range, considered to be physiological, may also influence the accuracy and clinical relevance of Tg measurement.
Methods
Recombinant human TgAb (Roche) was added stepwise to serum-samples ($n=45$) with TgAb concentrations near to the analytical sensitivity of the method (10 IU/ml), aiming to have TgAb concentrations of 50–100–150 and 200 IU/ml (ECLIA Elecsys 2010 Roche, normal 'cut off' <115 IU/ml). After this, Tg levels were measured at all TgAb concentrations by electrochemiluminescence immunoassay (ECLMA, Elecsys 2010, Roche). Additionally, 134 samples from 27 patients with DTC were measured for Tg, Tg-recovery (Tg%) and TgAb.
Results
In the *in vitro* experiment, TgAb and Tg concentrations showed strong correlation ($r=0.93$, $P<0.01$) both at normal and elevated TgAb levels, which could be described mathematically as: Loss of $Tg=-0.43\,Ln(TgAb\ IU/ml)+1{,}06$. Patients with non-detectable Tg had higher antibody levels than those with detectable Tg. There was a rather weak negative correlation ($r=-0{,}32$ $P<0.001$) of Tg% to TgAb and in 19% of the samples the results were clinically discordant. In 2/27 patients, on-T4 Tg levels of <2.0 ng/ml were corrected to be >2,0 ng/ml by using the above function. Subsequent off-T4 Tg levels appeared to be significantly elevated in both.
Conclusion
Physiological (normal) TgAb concentrations may also decrease serum Tg but their effect can be calculated from the actual Tg and TgAb concentrations by the

mathematical model described. The findings stress the importance of parallel Tg and TgAb measurements in patients with DTC expected to have undetectable or low Tg.

P142

Dopamine receptor expression and dopamine agonist effectiveness in post-surgical persistent medullary thyroid cancer

Rosario Pivonello[1], Piero Ferolla[2], Antongiulio Faggiano[1], Diego Ferone[3], Enrica Cerasola[3], Gabriella Angeletti[2], Fausto Santeusanio[2], Francesco Minuto[3], Gaetano Lombardi[1], Steven WJ Lamberts[4], Leo J Hofland[4] & Annamaria Colao[1]
[1]Department of Molecular and Clinical Endocrinology and Oncology, "Federico II" University, Naples, Italy; [2]Department of Internal Medicine and Endocrinological Sciences, University of Perugia, Perugia, Italy; [3]Department of Endocrinological and Metabolic Sciences, University of Genova, Genova, Italy; [4]Department of Internal Medicine, Erasmus Medical Center, Rotterdam, Netherlands.

Dopamine receptors was suggested to be expressed in medullary thyroid cancer (MTC). The aim of the current study was to evaluate the expression of D_2 dopamine receptor in MTC and the effectiveness of the dopamine agonist cabergoline in patients with MTC. Five paraffin-embedded cases of MTC obtained after thyroidectomy were used to evaluate D_2 receptor expression by immunohistochemistry. Fifteen patients (7 males, 8 females, 36–78 years) with post-surgical persistent and not operable MTC were treated with cabergoline for 4 months, in order to evaluate its effect on clinical syndrome, serum calcitonin (CT) and CEA levels, and metastasis number and size. Cabergoline was administered at the dose of 1 mg/week for the first month and 3.5 mg/week for the following 3 months. D_2 receptor was variably expressed in all 5 cases of MTC. Before treatment, all patients had progressively increasing serum CT and/or CEA levels. Lymph node metastasis were visible in 4, whereas liver and lung metastasis were identified in 1 and 2 patients, respectively. At the 4-month follow-up, a significant decrease of serum CT ($P=0.027$) but not CEA ($P=0.244$) levels was found. A $>50\%$ decrease in serum CT levels was found in 3 (20%), a 25–50% decrease was found in 10 (66.7%) and an increase in serum CT levels was found in 2 (13.3%) patients. A significant improvement in flushing ($P=0.039$) and fatigue ($P=0.023$) and a slight improvement in diarrhoea ($P=0.066$) score was also found. No significant change was found in body weight. No significant change was observed in metastasis number and size, although one patient experienced a disease progression. In conclusion, the results of this study demonstrated that D_2 receptor is expressed in MTC and that cabergoline treatment improve clinical syndrome and decrease serum CT levels in patients with post-surgical persistent MTC. Further studies on a larger number of patients and longer period of treatment are mandatory to draw definitive conclusions on the usefulness of cabergoline treatment in patients with MTC.

P143

Somatostatin analogues and the PI3K-AKT-MTOR-P70S6K pathway: how do they control the proliferation of neuroendocrine tumours?

Giulia Franchi, Simona Grozinsky-Glasberg, Antonio Ribeiro de Oliveira, Nabila Salahuddin, Márta Korbonits & Ashley B. Grossman
Department of Endocrinology, William Harvey Research Institute, Barts and the London, Queen Mary School of Medicine, University of London, EC1M 6BQ, UK, London, United Kingdom.

Background
Somatostatin analogues are very useful in the treatment of symptomatic neuroendocrine tumours, but effects on proliferation remain unclear. Over-expression of the proto-oncogene protein kinase Akt has been demonstrated in certain endocrine tumours, and activates downstream proteins including mTOR and p70S6K, which play a significant role in cell growth and proliferation. We have therefore explored the site of action of somatostatin in causing inhibition of proliferation in a neuroendocrine cell line.
Aims
To confirm the anti-proliferative effects of SS analogue treatment in a rat insulinoma cell line (INS-1), and to investigate whether the SS analogues act on the PI3K-Akt-p70S6K pathway.
Methods
RT-PCR was used to demonstrate SS receptors (SSTR) in the INS-1 cell lines. MTS and thymidine incorporation were used to determine the effects of the SS analogues octreotide (SSTR2 agonist) and pasireotide (SOM230, Novartis; activation of SSTR-1, 2, 3 and 5) on cell proliferation. Western blotting was used to characterise phosphorylated-Akt and p70S6K expression in the SS-treated cells.
Results
The INS-1 cells expressed SSTR 1, 2, 3 and 5. Treatment with octreotide and pasireotide caused significant dose-responsive inhibition of proliferation. No difference in phospho-Akt (either Ser473 or Ser308) expression was detected in the octreotide-treated INS-1 cell lysates. However, phospho-p70S6K (Thr389) expression was significantly reduced at 10 minutes-6 hours treatment with octreotide 10^{-9}M ($P=0.01$), while no effect on phospho-p70S6K (Thr229) expression was observed at 30 and 60 minutes. It is known that Thr229 site of phosphorylation is affected by PDK1 upstream of Akt. Treatment with IGF-1 (10nM) increased both phospho-p70S6K (Thr389) and phospho-Akt expression.
Conclusions
Octreotide and pasireotide treatment inhibited proliferation of INS-1 cells and, at a concentration achieved in clinical human use, octreotide attenuated p70S6K (Thr389) phosphorylation, but not Akt phosphorylation. We conclude that SS analogues acts downstream of Akt to inhibit the mTOR-p70S6K pathway.

P144

Angiotensin 4–8 and angiotensin 5–8 inhibit cell proliferation in GH3 rat pituitary lactosomatotroph tumor cell culture

Dorota Ptasinska-Wnuk, Jolanta Kunert-Radek, Hanna Lawnicka & Marek Pawlikowski
Medical University of Lodz, Lodz, Poland.

Introduction
In many tissues angiotensin peptides acts as the auto/paracrine growth factors. Their effects are dependent on activation of various intracellular signaling pathways, including mitogen-activated protein kinases (MAPK).

Angiotensin II (ang II) is the best known angiotensin peptide. The ang II derivatives, angiotensin III (ang III) and angiotensin IV (ang IV) posses biological activity as well. Both ang II and ang IV are known to promote the proliferation of rat prolactinoma cells *in vitro* and rat anterior pituitary cells *in vivo*. The role of ang IV degradation products, angiotensin 4–8 (ang 4–8) and angiotensin 5–8 (ang 5–8) in the regulation of cellular growth has not already been investigated.
Aim
In our study we examined the influence of ang 4–8 and ang 5–8 on the GH3 cells (rat pituitary lactosomatotroph tumor cells line) proliferation and the possible role of two MAPK pathways (p44/42 and p38) in ang 5–8 regulatory action.
Material and Methods
GH3 cells were cultured in F-10 medium and then plated at 96-multiwell plates (10×10^3 cells/well). After 12 hours of preincubation cells underwent to 72-hours treatment either with ang 4–8 or ang 5–8 alone or with the combination of ang 5–8 and p44/42 MAPK-kinase or p38 MAPK inhibitor (PD98059 or SB203580 respectively). Cell proliferation was evaluated using two colorimetric assays: based on the measurement of cell activation and on the BrdU incorporation during DNA synthesis.
Results
Ang 4–8 and ang 5–8 decreased both the cell activation and BrdU incorporation in GH3 cells culture. SB203580 prevented only the ang 5–8-induced inhibition of cells activation. Non of ang 5–8 effects was abolished by PD98059.
Conclution
Ang 4–8 and ang 5–8 inhibit GH3 cell proliferation. This mechanism is independent of both MAPK p44/42 and MAPK p38. They probably exert additional proapoptotic effect, mediated by MAPK p38.

P145

Epidermal growth factor receptor (EGFR) as a potential new target in the treatment of patients with adrenocortical carcinoma – results of pre-clinical studies

Martin Fassnacht[1], Stefanie Hahner[1], Barbara Heinrich[1], Patrick Adam[2], Sarah Johanssen[1], Marcus Quinkler[3], Ann-Cathrin Koschker[1], Philipp Stroebel[4], Alexander Marx[4] & Bruno Allolio[1]
[1]University of Wuerzburg, Dept. of Internal Medicine I, Wuerzburg, Germany; [2]University of Wuerzburg, Dept. of Pathology, Wuerzburg, Germany; [3]Charite University, Dept. of Medicine, Campus Mitte, Berlin, Germany; [4]University of Heidelberg, University Hospital Mannheim, Dept. of Pathology, Mannheim, Germany.

Objectives
Adrenocortical carcinoma (ACC) is a rare malignancy with incompletely understood pathogenesis and poor prognosis. Overexpression of epidermal growth factor receptor (EGFR) has been demonstrated in several tumors and is partly associated with a more aggressive phenotype and a worse prognosis. In addition, targeting the EGFR tyrosine kinase represents a successful new therapeutic strategy, e.g. in non-small cell lung cancer. Therefore, we investigated the role of EGFR in ACC as a potential therapeutic target.
Methods
EGFR expression was analyzed by immunohistochemistry in 95 ACCs and 5 normal adrenals using paraffin sections and tissue arrays (scoring of expression: 0–3). Utilizing the clinical data from the German ACC registry, Kaplan Meier survival analyses were performed. In 30 patients the tumor DNA was sequenced for mutations of the 'hot spot' exons 19–21 of the EGFR gene. In addition, cells of the ACC cell line NCI-h295 were incubated with the EGFR antibody cetuximab (1–100 μg/ml) and cell proliferation was measured by MTT tests.
Results
Immunohistochemistry revealed EGFR expression in 78% of ACCs. In 55/95 (58%) of the ACCs and 0/5 of the normal adrenals the expression level was judged as moderate-to-high (score 2 or 3). However, the expression level did not correlate with the clinical outcome in these patients. In addition, none of the sequenced tumor DNA samples showed a mutation in exon 19–21. Cetuximab exhibited a dose dependent antiproliferative effect in NCI-H295 cells (cell viability: 1 μg/ml: 95$\pm$2%; 10 μg/ml 90$\pm$3%*; 100 μg/ml 85$\pm$4%* vs untreated control cells: 100$\pm$3%; *=$P<0.01$).
Conclusion
EGFR is overexpressed in the majority of ACC. Moreover, *in vitro* experiments demonstrated that inhibition of EGFR signalling lead to moderate growth inhibition in ACC cells. Therefore, in patients with ACC refractory to established cytotoxic therapies the experimental use of EGFR inhibitors (combined with cytotoxic therapy) seems to be justified.

P146

Time necessary to achieve the maximum effect of goserelin, LH-RH agonist, in therapy of hormone dependent breast cancer

Jure Murgic, Martina Matovinovic, Lucija Fabijanic, Darko Katalinic & Milan Vrkljan
University hospital Sestre milosrdnice, Zagreb, Croatia.

Nearly one third of women diagnosed with invasive breast cancer are younger than 50 years with regular menstrual cycles. 60% of these tumors express estrogene and progesterone receptors. Common treatment procedure is surgery followed by chemotherapy, radiotherapy and hormone therapy. Often in younger patients chemotherapy causes permanent amenorrhoea. In case that menses afterwards occurs ovarial suppression is needed, mostly by goserelin, LH-RH agonist. The principle of therapy is to cause inhibition of LH and FSH pituitary secretion (medicamental ovariectomy). Sometimes in premenopausal women ovarian suppression is added to standard chemohormonal therapy. In this review two high-risk node positive premenopause breast cancer patients are presented, diagnosed at the age of 38 and 28. Both had hormone receptors positive tumor and underwent breast surgery followed by FEC regimen chemotherapy and radiotherapy. Chemotherapy caused them temporary amenorrhoea, but soon after radiotherapy and tamoxifen introduction regular menstrual cycle began. Due to high-risk node positive cancer combined therapy with tamoxifen 20 mg daily and goserelin 3,6 mg s.c. monthly was introduced. The first patient needed a three months goserelin application to obtain amenorrhoea but the other patient needed only one. After six months of goserelin plus tamoxifen therapy gynaecological and endocrinological evaluation was preformed. In both patients LH value was lower than 1.0 IJ/L but in the first one FSH, estradiole and progesterone values were within menopausal ranges with ultrasound proof of ovarial and endometrial inactivity. In the other patient FSH, estradiole and progesterone values were within fertile range, with present ovary follicles, although ammenorrhoic. This review refered that numerous individual factors influence the effect of adiuvant LH-RH agonist therapy in high-risk breast cancer patient and that different time period is needed to obtain its maximum effect.

P147

Acromegaly due to a lung carcinoid: a case report

Rezzoug malika & Chentli Farida
Bab El Oued Hospital, Algiers, Algeria.

Acromegaly secondary to a lung carcinoïd is a very rare entity. Secretion of GHRH (Growth hormone releasing hormone) or GHRH like by neuroendocrine tumor induces pituitary hyperplasia and a production of Growth hormone (GH) with or without others anterior pituitary hormones. Total resection of lung tumor induces normalisation of pituitary function as in our observation.

AD, 37 years, male, came to our unit for diabetes mellitus and acromegaly. His chest X ray showed a 7 cm right lung tumor. On hormonal exploration there was a very high GH =92 to 132 ng/ml ($N<5$), high prolactine (PRL)=120 ng/ml ($N<20$), elevated ACTH=70 pg/ml ($N=0$–46)) and cortisol =262 ng/ml ($N=50$–210) without clinical signs of Cushing's syndrome. Thyreotrop function was preserved but there was a gonadotrop deficit: testosterone =0,91 ng/ml ($N=3$–5). On MRI there was a huge pituitary process impading the third ventricle and a destroyed sella turcica.

GHRH and 5 HIA (5 hydroxyindolacetic acid) were not evaluated. Surgical exploration and pathology study showed typical picture of carcinoïd in the right lung. On post operative period there was a dramatic fall of GH (=1,2 ng/ml). PRL, ACTH and cortisol normalized and diabetes mellitus disappeared. Three month after surgery MRI showed a significant reduction of pituitary process with partial empty sella.
Conclusion
In this observation even if evaluation GHRH assay and immunohistochimy of the tumor was not available, clinical, biological and radiological evaluation confirmed that all endocrine abnormalities observed in our patient were due to lung carcinoïd.

P148

Intra- and supra-sellar immature teratoma mimicking pediatric craniopharyngioma

Chentli Farida
Bab El Oued Hospital, Algiers, Algeria.

Immature teratoma arising from the brain is very rare. The intra and supra sellar localization is very exceptional. Its clinical symptoms and radiological aspects on TDM are similar to those of craniopharyngiomas but on MRI the fat signal characterize teratomas, but only the histological exam gives the confirmation of this last lesion and makes differential diagnosis with mature tumor whose prognosis is better than the immature one. Our observation illustrates all these problems.
Observation
LM, 6 years, female, is referred to our unit for craniopharyngioma. She complaints of headaches, vomiting and a decrease in visual acuity. On clinical exam we noted a blindness, diabetes insipidus and a statural deficit with hypothyroidism wich are confirmed by hormonal results. On TDM there is huge (60$\times$32 mm) solid intra and supra sellar tumor with cysts and calcifications wich arrives to the third ventricule but on MRI there is a fat signal evocating a teratoma. histological exam of this very hemorrhage tumor argue for an immature teratoma.
Conclusion
This observation prouves that clinical and TDM aspects of craniopharyngiomas are similar with those of teratomas. Only the fat signal on the MRI argue for the teratoma. Histological exam is the only one wich makes the proof and the differential diagnosis between craniopharyngioma and mature or immature teratoma. The last one has the worst prognosis.

P149

Pituitary microprocess

Chentli Farida
–Bab El Oued Hospital, Algiers, Algeria.

Nowadays neuro-radiological explorations are so frequent that radiologists discover more and more pituitary lesions. In this work we would like to study pituitary microlesions (PML: inferior or equal to 10 mm) in order to analyze age and sex repartition, clinical symptoms leading to the diagnosis, position in the pituitary area and the apparent etiologies.

All our patients are examined and hormonal exploration is as complete as possible.

Results

Among 85 subjects with PML proved by TDM and or MRI, there are 79 women and 6 men (sex ratio = 13/1). Age at diagnosis = 30.8 years (14–73), most of them are between 21 and 30 years old. The complaints are: Gonadal dysfunction = 72%, galactorrhea = 10.5%, headaches = 5.8%, metabolic abnormalities = 6.7% and visual troubles = 4.3%. The diagnosis is really fortuitous in 2 subjects = 2.3%. For the apparent etiology there are 58 prolactinomas, 12 ACTH (19.2%), 10 non functioning (11.8%) and 5 somatotrop adenomas = 5.7%. The average size = 6.45 mm (3–10). 58% are in right pituitary area, 23%, in the left and 13% in the middle.

Conclusion

In our population the diagnosis of pituitary microlesions is rarely fortuitous. Gonadal abnormalities are the most complaints. This may be explained by the high frequency of female cases and secreting tumors. The diagnosis is relatively late (mean size = 6.5 mm). PML are frequently located in the right area. ACTH PML are the smallest and the GH one are the biggest

P150

Adrenal incidentalomas and insulin sensitivity – are there any differences between adenomas and hyperplasia?

Daniela Dudasova, Ivica Lazurova, Hedviga Wagnerova & Ingrid Dravecka
–Faculty of Medicine, P. J. Safarik University, Kosice, Slovakia.

It is well known that adrenal masses, particularly adenomas are frequently related to metabolic syndrome and insulin resistance. However, there are no reported data about the differences between adenomas and hyperplasia.

Authors examined the prevalence of symptoms of the metabolic syndrome and insulin resistance in 25 patients with adrenal incidentalomas (10 men, 15 women) of the mean age 57.9 + 15 years. 15 patients had adrenal adenoma determined by CT or MR scan and 10 had unilateral or bilateral hyperplasia. The prevalence of obesity was 72%, arterial hypertension 60%, diabetes mellitus or impaired glucose tolerance 28%, hyperlipidemia 56% and hyperuricemia 20%, respectively, which is more frequent occurrence than that in normal human population. Patients with adrenal adenomas had mildly but significantly higher body mass index (BMI, $P < 0.05$) and insulin resistance calculated as HOMA IR ($P < 0.05$) and FIRI ($P < 0.05$) and significantly higher values of serum ferritin ($P < 0.01$). Plasma cortisol values were slightly but not significantly higher in the group with adrenal adenomas.

Authors conclude that adrenal adenomas are probably more related to the metabolic syndrome than adrenal hyperplasia.

P151

Frequency of occurence of MEN1 syndrome in patients admitted with primary hyperthyroidism

Agata Baldys-Waligorska, Grzegorz Sokolowski, Malgorzata Trofimiuk, Filip Golkowski & Bohdan Huszno
Department of Endocrinology, Collegium Medicum of the Jagiellonian University, Krakow, Poland.

Background

Primary hyperparathyroidism (HPT) is the most common endocrinopathy in MEN1 and usually its first clinical manifestation. Yet MEN1 is a rare disease, representing only 2–4% of all cases of HTP. We studied the frequency of MEN1 syndrome in HPT patients admitted to our Department.

Methods

In a retrospective analysis of 84 suspected HPT patients hospitalized in 1999–2006, case reports of 11 patients with suspected MEN1 were analysed. MEN1 was stated if two of the three main MEN1-related endocrine tumours occurred.

Results

HPT diagnosis was confirmed in 69 patients: of mean age 55.4 ± 14.1 yrs. Median values of PTH and total calcium concentration were 57.4 pg/ml (min – 60.6, max – 1580) and 2.95 mmol/l (min – 2,2, max – 4,0), respectively. In parathyroid scintigraphy equivocal tracer accumulation was found in 72% of cases. MEN1 was diagnosed in 9 patients of mean age 51.3 ± 12,0 yrs, in 8 of whom (89.0%) HPT was confirmed. Pituitary adenoma was found in 7 patients: 3 prolactinomas, 1 acromegaly, 1 Cushing disease and 2 non-functioning tumours. In 2 patients pancreatic tumours were diagnosed: somatostatinoma and gastrinoma were confirmed by laboratory tests and immunohistochemistry. Four carcinoids: 3 gastric and one bronchial were found. Mean 5-HIAA (5-hydroxyindoloacetic acid) urine excretion in the carcinoid patients was 144.0 μmol/24hrs (norm: up to 40), mean serum concentration of CgA (chromogranin-A) 728.7 U/L (norm: up to 18.0). Moreover, in the patient with HPT and somatostatinoma concurrent von Recklinghausen's disease was diagnosed and in the HPT and prolactinoma patient, meningioma was found. Adrenal tumours were observed in two cases: one pheochromocytoma and one non-functioning tumour.

Conclusions

The frequency of MEN1 occurrence in our patients (13%) is much higher than that quoted in the literature (2–4%), clearly, due to referral of complicated cases to our Department. Patients with symptoms atypical for HPT should be screened towards MEN1.

P152

Evaluation of the efficacy of sandostatin LAR in the treatment of acromegaly

Agata Baldys-Waligorska, Anna Krzentowska, Filip Golkowski & Bohdan Huszno
Department of Endocrinology, Collegium Medicum of the Jagiellonian University, Krakow, Poland.

Background

Somatostatin analogues are used to treat acromegaly patients who, following surgery, have not fulfilled cure criteria (hGH < 2,5 ng/ml, IGF-1 below normal range for age and post-OGTT hGH < 1,0 ng/ml). We evaluated the efficacy of Sandostatin LAR in managing such patients.

Material and method

In our Clinic, 81 acromegaly patients (mean age 51.6 ± 14.4 yrs) were registered over the years 1983-2005. Based on CT i MRI, macroadenoma and microadenoma were stated in 63% and 37% of these patients, respectively. 70 patients (86.5%) underwent surgery, 6 (7.4%) refused surgery and 5 (6.1%) underwent radiotherapy., Independently of time after surgery, 60 patients underwent diagnostic tests to qualify them for Sandostatin LAR treatment. Treatment efficacy was based on measuring concentration of hGH i IGF-1 3, 6, 9 and 12 months, and performing control MRI 6 and 12 months after the beginning of Sandostatin LAR treatment (20 mg/month, increased to 30 mg/month if unsatisfactory).

Results

Criteria of post-surgery cure were not fulfilled by 40 patients (66.6% of the 60 evaluated). Due to poor tolerance, one patient was treated with Pegvisomant. 19 patients (31.6%) required no further treatment. After 6 months of treatment, hGH < 2.5 ng/ml was stated in 63%, and IGF-1 below normal ranges for age in 58.8% of patients, and after 12 months – in 68.4% and 36.8% of patients, respectively. In control MRI, recurrence, correlated with enhanced concentration of IGF-1, was stated in 7 patients (17,5%).

Conclusions

In terms of hGH and IGF-1 levels, satisfactory acromegaly control was obtained in about 40% of patients treated with Sandostatin LAR. This result may be biased by the high number of macroadenoma, and possible non-radical surgery in our patients. Due to evident disparity between 12-month normalization of hGH and of IGF-1 levels, measurements of IGF-1 concentration are of considerable diagnostic value in assessing the activity of acromegaly.

P153

The beta-HLH transcription factor neurogenin-2 is preferentially expressed by secreting pituitary adenomas

Amato Fratticci[1], Fabio Grieco[1], Cristina Spilioti[1], Felice Giangaspero[2], Vincenzo Esposito[2], Antonio Santoro[3], Luca Ventura[4], Edoardo Alesse[1] & Marie-Lise Jaffrain-Rea[1]
[1]Experimental Medicine, University of L'Aquila, L'Aquila, Italy; [2]Neuromed, IRCCS, Pozzili, Italy; [3]Neurosurgery, University "La Sapienza", Rome, Italy; [4]Pathology, S. Salvatore Hospital, L'Aquila, Italy.

Beta-HLH transcription factors are involved in the ontogenesis of neural/neuroendocrine cells, and may play a role in the pathogenesis of neuroendocrine tumours. Neurogenin 2 (Ngn2) is expressed by the developing mouse pituitary. After preliminary data indicating its expression in the normal human pituitary, we have studied its phenotypic expression in normal and adenomatous pituitary tissues.
Methods
Fifty-two pituitary adenomas (PA) – 23 clinically non-secreting (CNS) and 29 clinically secreting (CS) (13 GH-, 8 PRL-, 6 ACTH- and 2 TSH-secreting PA, respectively) - and 4 normal pituitaries (NP) were studied. Ngn2 transcripts were determined by realtime qRT-PCR and compared to beta-actin transcripts, using Taqman on-demand assays (Applied Biosystems). Immunohistochemistry was performed on 21 PA and 2 NP, using a rabbit polyclonal antibody (Chemicon). Mouse monoclonal antibodies for pituitary hormones (Dako) were used for co-localization experiments.
Results
Ngn2 transcripts were observed in all NP and 39/52 (75%) of PA, with a higher frequency in CS versus CNS PA (89.6% vs 56.5%, $\chi 2 = 7.51$, $P = 0.006$). Accordingly, Ngn2 levels were higher in CS than in CNS PA ($P = 0.006$, Mann-Whitney). Only a subset of PA ($11/52 = 21.1\%$) were found to moderately overexpress Ngn2 as compared to NP: 8 were CS and 3 were CNS, including 2 silent-secreting PA. Nuclear immunopositivity for Ngn2 was detected in scattered cells of the NP, co-localizing with most pituitary hormones, and in 17/21 PA (14/15 CNS and 3/6 CNS, respectively). No significant correlation was found between Ngn2 expression and tumour volume, invasiveness or Ki-67 labelling index.
Conclusions
Ngn2 is expressed by the NP and a significant subset of PA. Its preferential expression by CS PA, the lack of significant overexpression or correlation with tumour aggressiveness, suggest that Ngn2 may contribute to maintain a differentiated secreting phenotype in PA but plays no role in pituitary tumorigenesis itself.

P154

TGFß1 signalling in human insulinomas compared with human islets.

Anastasiya Nabokikh[1], Martin Bilban[2], Wolfgang Gartner[1], Greisa Vila[1], Bruno Niederle[3], Jens Nielsen[4], Christoph Zielinski[5], Anton Luger[1] & Ludwig Wagner[1]
[1]Medical University of Vienna, Department of Medicine III, Vienna, Austria; [2]Medical University of Vienna, Department of Medical and Chemical Laboratory diagnostics, Vienna, Austria; [3]Medical University of Vienna, Department of Endocrine Surgery, Vienna, Austria; [4]University of Copenhagen, Department of Medical Biochemistry and Genetics, Copenhagen, Denmark; [5]Medical University of Vienna, Department of Medicine I, Vienna, Austria.

Insulinomas are thought to be the result of reduced ß-cell death and hyperproliferation of this specific and highly differentiated cell type. Specific growth- factors are responsible for inducing ß- cell replication and might therefore be involved in insulinoma formation. Pluripotent islet progenitor cells are thought to be located at pancreatic ducts, which can give rise to novel islets as well as exocrine pancreas formation. TGFß1 signalling disruption has been shown to result in premalignant ductal lesions in mouse models as well as in humans.

The **specific objective** of this study was to evaluate the gene expression profile of human insulinoma tumors compared with human islets. The gene expression profile of three human insulinomas originating from different individuals was compared to one islet donor. The comparative Affimetrix gene chip analysis of 8000 spotted genes revealed 1102 upregulated ($>$ 1.5x) and 210 downregulated (>1.5x) genes. The results revealed significant differences in the expression of members of the TGFß signalling pathway. Insulinomas contained reduced TGFß1 and TGFß-induced proteins, but overexpressed TGFß receptors. These data were confirmed by quantitative real-time PCR expanding the numbers of insulinomas to 7 and islets-donors to 3. Our results suggest a novel important function of TGFß in development of human insulinomas and cell growth regulation at the islet of Langerhans. Furthermore they are in accordance with earlier data on the exocrine counterpart, where impairment of TGFß signalling is documented in ductal progenitor cells and premalignant ductal lesions leading to pancreatic adenocarcinomas. Apparently, in the presence of aberrant TGFß signalling, these unique pluripotent progenitor cells might be able to give rise to both endocrine and exocrine neoplasias.

P155

The effect of SOM230 on cell proliferation and cortisol secretion in the human adrenal carcinoma cell line H295R

Barbara Mariniello, Sara Cervato, Giuseppe Opocher, Nora Albiger, Gianluca Occhi, Carla Scaroni & Franco Mantero
Division of Endocrinology, Department of Medical and Surgical Sciences, University of Padova, Padova, Italy.

Adrenocortical carcinoma (AC) is a rare neoplasm with poor prognosis. Medical treatment of AC is actually based on the use of op'DDD (mitotane) with or without traditional chemotherapeutic agents. Only very few information are available about the effectiveness of somatostatin analogs in AC. In human adrenal gland the expression of all five somatostatin receptor (SSTR) subtypes was previously demonstrated. A differential expression was shown in adrenal adenomas and carcinomas.

SOM230 is a new somatostatin analog able to interact with SSTR type 5. The effect of SOM230 on cell proliferation and hormone secretion was demonstrated in corticotroph pituitary adenomas primary cultures, but no data are available on adrenal gland.

The aim of the present study was to evaluate the effect of SOM230 on H295R, a human cell line derived from adrenal carcinoma. Cell proliferation was assessed by MTT-assay, whereas cortisol secretion was determined, with and without forskolin stimulation, using a competitive chemiluminescence immunoassay. Moreover, SSTR expression profile study was performed by RT-PCR.

SSTR 3, 4 and 5 were expressed in H295R cells, whereas no expression of SSTR1 and 2 was shown instead. The effect of SOM230 on H295R was determined in a 5 days treatment. A slight decrease of cell proliferation (11.4%) was observed after 72 h of treatment with a high dose of SOM230 (10^{-5}M). At the same high dose (10^{-5}M) SOM230 significantly ($P < 0.05$) inhibits cortisol secretion already after 24 h. A lower concentration of the drug (10^{-8}M) is effective only after 72 h of treatment.

These preliminary data show that SOM230 seems to have an effect on adrenal cell proliferation only at high dose, while a significant dose dependent effect on suppression on cortisol release was observed at 72 h also at low doses. Further studies are required to determine if SOM230 might be used for treatment of patients with AC.

P156

MEN2B – Two simultaneous cases of a rare syndrome

Ágnes Sallai, Éva Hosszú[1], Péter Gergics[2], Zsolt Tulassay[2], Károly Rácz[2] & György Fekete[1]
[1]Semmelweis University 2nd Dep. of Paediatrics, Budapest, Hungary; [2]Semmelweis University 2nd Dep. of Medicine, Budapest, Hungary.

A 17-year-old boy was referred to our Department. In his medical history Crohn's disease had been supposed because of abdominal pain and distention. He had previously undergone minor surgery as having large tongue with neuromas and hypertrophic gums. Due to his marfanoid appearance, arachnodactyly, massive eyebrows and lips together with his medical history, multiple endocrine neoplasia type 2B (MEN2B) was suspected, which is a very uncommon hereditary disease. It consists of typical dysmorphia, mucosal neuromas, ganglioneuromatosis, medullary thyroid carcinoma (MTC) and phaeocromocytoma, and the prognosis depends on the presence of MTC.

Two weeks later a 10-year-old girl presented with a hard mass at her neck. She had massive lips, neuromas on the tongue and solitary thyroid nodule. Thyroid scan showed a cold nodule in the right lobe, and fine needle aspiration cytology suggested MTC.

Genetic analysis was carried out in both patients and revealed a point mutation at codon 918 (M918T) of the proto-oncogene RET. Adrenomedullary function tests showed normal levels of serum and urinary fractionated catecholamines, however, high levels of plasma calcitonin related to MTC. Imaging studies did not identify metastases. Both patients underwent total thyroidectomy and lymph node dissection. Histological examination verified MTC in the thyroids and in the lymph nodes, too. After the operation the plasma calcitonin level of the girl decreased, but it remained high in the boy, so PET-CT was performed to look for metastases. These were found at his cervical region, therefore a reoperation was made with a more extensive node dissection. Since the operations (2006) both patients have been doing well.

Our conclusion is that whenever the M918T mutation of proto-oncogene RET is found total thyroidectomy should be done right after the diagnosis, or if possible within the first 6 months of life.

P157

Prevalence of autonomous cortisol and aldosterone secretion in patients with a single benign cortical adrenal adenoma after modification of the diagnostic tests

George Piaditis[1], Gregory Kaltsas[1], Theodora Kounadi[1], Athina Markou[1], Nikos Mazarakis[1], Aggeliki Gouli[1], Polyzois Makras[1], Dimitrios Papadogias[1], Kostantina Dimitriou[1], Alexandra Stathopoulou[1], Kostantinos Kossyvakis[1], Ragkou Despoina[1], Vamvakidis Kyriakos[2], George Zografos[2], George Kontogiorgos[3] & George Chrousos[4]
[1]Department of Endocrinology & Diabetes Center, General Hospital of Athens G. Gennimatas, Athens, Greece; [2]3rd Department of General Surgery, General Hospital of Athens G. Gennimatas, Athens, Greece; [3]Department of Pathology, General Hospital of Athens G. Gennimatas, Athens, Greece; [4]First Department of Pediatrics, University of Athens Medical School, Agia Sophia Children's Hospital, Athens, Greece.

Background
The normal cut-offs of screening and diagnostic tests for autonomous aldosterone (AAS) and cortisol (ACS) secretion are poorly defined, mainly due to the presence of adrenal adenomas among those who have served as controls and the stimulating effect of ACTH on aldosterone secretion.
Methods
We investigated cortisol and aldosterone secretion in 151 patients with benign cortical adrenal adenomas (BCAA) and in 119 healthy controls with a normal CT of adrenals. Tests for AAS were performed before and after dexamethasone suppression to eliminate the ACTH effect on aldosterone secretion. Performed tests: 1. ACTH-test (250 μg ACTH 1-24, IV) for cortisol, plasma active renin (PRC), aldosterone (PAC) and PAC/PRC ratios measurements at 0, 30 and 60 min. 2. Classical saline infusion test (SIT, 2 liters NaCl 0.9%/4 h, IV) for PRC, PAC and PAC/PRC ratios measurements, 3. LDDST (0.5 mg DEX/6hX24 h) for ACTH and cortisol measurements. 4. A further saline infusion test (POST-DEX-SIT) 2 h after the LDDST.
Results
Using ROC analysis the POST-LDDST cortisol levels (26.90 nmol/L), as well as the POST-DEX-SIT PAC (53.45 pmols/L) and POST-DEX-PAC/PRC (6.18 pmols/L/mU/L) achieved a 100% sensitivity and specificity. Using these new cut-offs the estimated prevalence of ACS and AAS among the BCAA-patients was 61.58% and 33.74% respectively, whereas simultaneous AAS and ACS was observed in 15.68% of the patients. Both systolic and diastolic blood pressure were significantly correlated with POST-DEX-SIT PAC/PRC ratio ($^{P}<0.003$ and $^{P}<0.002$ respectively) and PAC/PRC ratio at 60 min of ACTH-test ($^{P}<0.0003$ and $^{P}<0.001$ respectively) but not with the basal measurements.
Conclusions
With the newly defined normal cut-offs even mild forms of ACS and AAS were identified. As a consequence the estimated prevalence of ACS and AAS in BCAAs was found much higher than the reported previously, whereas a high prevalence of simultaneous cortisol and aldosterone secretion was identified for first time.

P158

Immunohistochemical detection of angiotensin receptors AT1 and AT2 in adrenal tumors

Marek Pawlikowski, Beata led & Katarzyna Winczyk
Department of Neuroendocrinology, Chair of Endocrinology, Medical University of Lodz, Lodz, Poland.

Immunohistochemical detection of angiotensin receptors AT1 and AT2 in adrenal tumors

Angiotensin II is well known to affect the adrenal cell growth and function. Angiotensin receptors AT1 and AT2 were found to be present in the normal adrenal gland. However, the data on the expression of angiotensin receptors in the adrenal tumors is very scarce.

To overcome this gap, the paraffin sections of the adrenal cortical tumors and of pheochromocytomas from the archival material were immunostained with antibodies raised against AT1 (sc-1173) and AT2 (sc-9040) receptor proteins. In hyperplasia of the adrenal cortex and in benign adrenocortical adenomas, both functioning and non-functioning, the AT1 immunostaining was present mainly in the cell membranes. A positive immunoreaction was also found in a subpopulation of cell nuclei and within the cytoplasm. In the adrenal cancer, as well as in pheochromocytomas neither cell membranes nor cell nuclei were immunostained with anti-AT1 antibody. However, a weak AT1 immunostaining was present within cytoplasm of the tumoral cells. With anti-AT2 antibody, in all tumors investigated, the tumoral cells were immunonegative but moderate to strong AT2 immunostaining was observed in the walls of intratumoral blood vessels and in the interstitial tissue. Our data indicates that the expression of AT1 receptors is altered in adrenal cancer and in pheochromocytomas. The expression of AT2 receptors, in turn, may be connected with the process of tumoral neo-angiogenesis.

P159

Bilateral adrenal incidentalomas: exploration of aberrant responses and comparison with unilateral lesions

Eirini Vicha, Dimitra-Argyro Vassiliadi, Christos Avgoustis, Theodossia Palouka & Stylianos Tsagarakis
Department of Endocrinology, Athens' Polyclinic, Athens, Greece.

Aberrant hormone receptors have been demonstrated in macronodular adrenal hyperplasia or, rarely, unilateral adenomas causing Cushing's syndrome but their prevalence in adrenal incidentalomas (AI) remains uncertain. Therefore we evaluated patients with bilateral AI for evidence of abnormal response to physiological stimuli. We also compared their biochemical characteristics with those of patients with unilateral AI.

Assessment of adrenal function was performed in 93 patients (27 men, 66 women, mean age 59.2+/−12 years) with AI; 27 patients (29%) with bilateral (Group A) and 66 patients with unilateral adenomas (Group B). Non-diabetic patients ($n=68$) underwent a 75g-OGTT. Eighteen patients of Group A were submitted to a meal test and 15 to a posture test. The posture test was positive in 3/15 (20%) patients and the meal test in 1/18 (5.5%). The size of the largest adenoma in Group A was significantly greater compared to Group B (3.1+/−1.1 vs. 2.3+/−1.1, $P=0.01$). No significant difference regarding the mean levels of UFC, ACTH, DHEAS and midnight cortisol existed between the groups. A significantly greater proportion of Group B patients had fully suppressed cortisol levels (<1μg/dl) post-LDDST (37.9% vs. 14.8% for Group A, $P=0.023$). The prevalence of diabetes and hypertension and mean glucose levels during OGTT were similar among groups, but in Group B the HOMA-R was significantly higher (2.74+/−1.3 vs. 1.89+/−0.78 $P=0.037$) and the QUICKI and ISI-composite indices significantly lower (0.33+/−0.03 vs. 0.35+/−0.03, $P=0.046$ and 3.3+/−1.5 vs. 4.7+/−2, $P=0.016$).

In conclusion, evidence for aberrant responses to physiological stimuli, particularly to upright posture, is occasionally found in patients with bilateral AI. Although there are no major biochemical differences between subjects presenting with bilateral or unilateral lesions, bilateral lesions tend to be larger and are more often associated with lack of dexamenthasone suppression whereas unilateral adenomas are more related to increased insulin resistance.

P160

Inhibitory effect of rosiglitazone – PPARgamma receptor ligand on growth of human adrenocortical tumor cells in vitro

Katarzyna Winczyk, Hanna Lawnicka, Julita Fuss-Chmielewska, Krzysztof Kolomecki, Jacek Kuroszczyk & Marek Pawlikowski
Department of Neuroendocrinology, Chair of Endocrinology, Medical University of Lodz, Lodz, Poland.

Introduction
The peroxisome proliferator-activated receptors gamma (PPARγ) are nuclear receptors which are detected in normal and pathological tissues. Our earlier study showed the overexpression of PPARγ in human adrenal tumors and pituitary adenomas in comparison to normal glands. The *in vitro* experiments indicated that ligands of PPARγ inhibit growth of many tumors including pituitary adenomas, thyroid cancers and adrenal carcinomas. However, the data concerning the effects of PPARγ ligands on adrenal tumors is very scarce.
Objective
In the present study, we investigated the action of PPARγ ligands rosiglitazone on growth of human adrenocortical tumors in vitro.
Materials and methods
Ten surgically removed adenomas (five non-functioning adenomas, four aldosterone-secreting tumor and one cortisol-secreting adenoma) were examined. The adrenal tumors cells were exposed in the primary culture to rosiglitazone at the concentration of 10^{-3}, 10^{-4} and 10^{-5} M for 24 hours. To measure cell growth the modified colorimetric Mossman method detecting the viable cells was applied. Moreover, the immunohistochemical evaluation of PPARγ expression in paraffin sections of adrenal tumors was performed. The study protocol was approved by local Ethical Committee of Medical University of Lodz.

Results
We have shown that rosiglitazone significantly inhibited the cell growth in 9 out 10 examined adrenal tumor in a dose-dependent manner. Rosiglitazone was the most effective at concentration of 10^{-3} M. PPARγ receptors were found in all tissue, but the number of cells with positive immunoreaction was the lowest in aldosterone-secreting adenoma, which was insensitive to rosiglitazone.
Conclusions
Our results suggest that rosiglitazone may be useful in the treatment of human adrenocortical adenoma. However, the efficacy of PPARγ ligands requires a confirmation in study performed on the larger group of adrenal tumors.

P161

The modern pre- and intraoperative diagnostic algorithm of pancreatic NET with the use of 99mTc-EDDA/HYNIC-octreate scintigraphy – the impact of SRS on patients' management

Alicja Hubalewska-Dydejczyk[1], Katarzyna Frӧss-Baron[1], Renata Mikolajczak[2], Bogdan Huszno[1], Piotr Szybinski[3], Jan Kulig[3], Aleksandra Januszewska[1], Anna Sowa-Staszczak[1] & Dorota Pach[1]
[1]Chair and Department of Endocrinology, Medical College, Jagiellonian University, Cracow, Poland; [2]Radioisotope Centre POLATOM, Otwock-Swierk, Poland; [3]Gastrointestinal and General Surgery Department, Medical College, Cracow, Poland.

Pancreatic NETs often cause difficulties in imaging diagnostics and optimal diagnostic algorithm is searched for. According to the latest reports MDCT sensitivity amounts 60-90%, MR: 80-90%, SRS: 62-100%, EUS: 70-90%.
Aim
Assessment of the usefulness of $^{99\,m}$Tc-EDDA/HYNIC-octreotae scintigraphy in detection of primary and metastatic tumours of pancreatic NET in comparison to CT, EUS and IOUS and evaluation of the impact of scintigraphic results on clinical management of these patients.
Materials and methods
27 patients (aged 52.0 ± 17.3 y) with suspected or histopathologically confirmed pancreatic NET were qualified for the study. Imaging diagnostics was performed in order to detect the primary lesions, local recurrences an metastases. $^{99\,m}$Tc-EDDA/HYNIC-octreotate SRS, CT, EUS and IOUS were performed. The patients with positive SRS were qualified for RGS.
Results
On the basis of the imaging methods results and histopathologic verification: insulinoma- 8, glucagonoma-6, gastrinoma-5, somatostatinoma-2, NET with ACTH ectopy-2, non-functioning NET- in 4 pts were finally diagnosed. Primary lesions (16) and local recurrences (4) were revealed in 20pts, and metastases in 8pts. Sensitivity of SRS and CT was 85% vs 65% respectively. SRS visualized metastatic lesion in 100%, while CT in 87.5% of pts. IOUS revealed the primary tumours in all cases of insulinoma and gastrinoma (9/9). SRS and EUS detected 5/7 insulinoma and 2/2 gastrinoma (CT: 3 insulinomas, 1gastrinoma). SRS changed the diagnostic approach in 13 pts: 8 were qualified for ^{90}Y-DOTA-TATE therapy and 2pts with negative SRS were referred for chemiotherapy. 2 insulinomas and glucagonoma liver metastases were visualised only in SRS and detected with hand-held gamma-probe intra-operatively.
Conclusions
$^{99\,m}$Tc-EDDA/HYNIC-octreotate SRS is a sensitive method of pancreatic NET detection. It is particularly useful in visualisation of the small tumours of the pancreatic tail and small liver metastases. It has essential impact on patients treatment as it enables tumours'resection with RGS and selects patients for PRRT with ^{90}Y-DOTA-TATE.

P162

Segregation of P25L and S80I mutations of the *vhl* gene in an extended Hungarian family with von Hippel-Lindau syndrome

Attila Patócs[1], Katalin Balogh[1], Miklós Tóth[1], Ferenc Fazakas[2], István Likó[3] & Károly Rácz[1]
[1]2nd Department of Medicine, Semmelweis University, Budapest, Hungary; [2]Department of Clinical Biochemistry and Molecular Pathology, University of Debrecen, Debrecen, Hungary; [3]Gedeon Richter LTD, Budapest, Hungary.

Background
von Hippel-Lindau syndrome (VHL) is a rare autosomal dominant disease caused by alterations of the *vhl* tumor-suppressor gene. Patients with VHL are at risk for development of retinal, central nervous system and spine hemangioblastomas, clear-cell renal cell carcinomas, pheochromocytomas, endolymphatic sac tumors and cysts; and pancreatic islet cell tumors. Based on the presence or absence of pheochromocytoma as a phenotypic marker, VHL can be divided into different subtypes. According to Knudson's two-hit hypothesis, tumor formation in VHL requires inactivation of both copies of the tumor suppressor *vhl* gene. Some specific genotype-phenotype correlations have been recognized, but the majority of families have their own specific genetic alteration.
Objective
To identify the disease-causing *vhl* gene mutation in a large Hungarian VHL kindred and to study the genotype-phenotype correlations.
Patients and methods
32 family members spanning 5 generations were evaluated. Initial screening included medical history, physical examination, abdominal ultrasonography, abdominal and cranial CT or MRI, as well as ophthalmologic examination and laboratory tests. Mutation analysis of the vhl gene was performed in DNA samples obtained from peripheral blood. Written informed consent was obtained from all family members who participated in the study.
Results and conclusions
Two genetic alterations of the *vhl* gene (P25L and S80I), both resulting in an amino acid change were identified. The detailed medical examination confirmed that VHL-specific tumors were associated with the presence of S80I mutation. In three family members this mutation was associated with the presence of pheochromocytoma. To our knowledge, the S80I mutation has not been previously described in VHL patients who had pheochromocytoma. Therefore, this finding represents a novel genotype-phenotype association. The P25L variant was identified in clinically healthy family members, suggesting that this variant represents a sequence polymorphism rather than a real disease-causing mutation.

P163

High prevalence of novel mutations of the MEN1 gene in Hungarian patients with multiple endocrine neoplasia type 1

Katalin Balogh[1], László Hunyady[2], Attila Patócs[1], Peter Gergics[1], Zsuzsa Valkusz[3], Miklós Tóth[1], Ibolya Varga[1], Edit Gláz[1] & Károly Rácz[1]
[1]2nd Department of Medicine, Semmelweis University, Budapest, Hungary; [2]Department of Physiology, Semmelweis University, Budapest, Hungary; [3]Division of Endocrinology, University of Szeged, Szeged, Hungary.

Introduction
Multiple endocrine neoplasia type 1 (MEN 1) may present as a familial or a sporadic disorder with multiple endocrine tumours including parathyroid adenomas or hyperplasias, tumours of endocrine pancreatic and pituitary gland. Familial and sporadic MEN 1-related states which do not fulfill current diagnostic criteria but may be related to MEN 1 syndrome have been also described.
Aims
The aim of this study was to examine the prevalence and spectrum of *MEN1* gene mutations in Hungarian patients with familial and sporadic MEN 1 and in those with an MEN 1-related state.
Methods
We performed mutation analysis using temporal temperature gradient gel electrophoresis (TGGE) and direct sequencing of the entire coding and exon-intron boundaries of the *MEN1* gene. Genomic DNA was obtained from 32 patients (19 index patients with familial or sporadic MEN 1 and 13 index patients with familial or sporadic MEN 1-related state). Family screening was performed in families of patients with identified *MEN1* mutation.
Results
Ten different *MEN 1* gene mutations were identified in 10 index patients, including 5 novel mutations (A91V, G28A and E26X in exon 2, L301R in exon 6, and C354X in exon 8). All but one mutations occurred in index patients with familial or sporadic MEN 1; the prevalence of mutation was considerably higher in index patients with familial MEN 1 (6/6 patients, 100%) than in those with sporadic MEN 1 (3/13 patients, 23%). Of the 13 index patients with MEN 1-related state, only one patient with recurrent isolated primary hyperparathyroidism had *MEN1* gene mutation. Family screening indicated mutations in 6 symptomatic and in one asymptomatic first-degree relative.
Conclusions
These results confirm previous reports on the high prevalence of novel *MEN1* gene mutations among patient with MEN 1, and support the questionable efficacy of mutation screening in patients with sporadic MEN 1-related states.

P164

Analysis of germline mutations in patients with pheochromocytomas and paragangliomas

Aleksandra Krawczyk[1], Kornelia Hasse-Lazar[1], Jacek Ziaja[2], Agnieszka Pawlaczek[1], Jolanta Krajewska[1], Mariola Peczkowska[3], Aleksander Preibisz[3], Agata Kubaszek[3], Andrzej Januszewicz[3] & Barbara Jarzab[1]
[1]Department of Nuclear Medicine and Endocrine Oncology, MSC Memorial Cancer Center and Institute of Oncology, Gliwice, Poland; [2]Department of General, Vascular and Transplant Surgery, Medical Silesian University, Katowice, Poland; [3]Department of Hypertension, National Institute of Cardiology, Warsaw, Poland.

There are two types of neoplasms derived from chromaffine tissue: pheochromocytomas (tumors of adrenal core) and paragangliomas (tumors located extraadrenally). Majority of these tumors are sporadic, although according to literature, when DNA analysis is carried out, hereditary disease can be diagnosed in about 25% of patients: Multiple Endocrine Neoplasia type 2 (MEN2A and MEN2B), von Hippel-Lindau Syndrome (VHL), Pheochromocytoma/Paraganglioma Syndrome (PPS) and neurofibromatosis type 1 (NF-1), caused by DNA germline mutations in *RET* protooncogene and *VHL*, *SDHB*, *SDHD*, *NF-1* genes respectively. The aim of our study is evaluation of the frequency of hereditary chromaffine tissue neoplasms in group of apparently sporadic patients, diagnosed and treated by our cooperation. DNA was isolated from peripheral blood leukocytes. Analysis of *RET*, *SDHB* and *SDHD* was carried out in order to seek for DNA changes. DNA fragments were amplified with the use of the polymerase chain reaction (PCR). Multiplex Single Strand Conformation Polymorphism (MSSCP) analysis was used as the screening method. When a conformation change was observed, it was confirmed by sequence analysis. The whole analysis was completed in 63 patients. Germline mutations were found in 16 patients (25.5%); in the group with pheochromocytomas as the sole manifestation in 14 patients (26.4%). Most frequent germiline mutations in pheochromocytoma patients were mutations of *RET*: codon 634 (9 patients) and codon 791 (5 patients) and in paraganglioma patients – mutation in *SDHD* codon 33.

Conclusions

Our analysis confirms the significant contribution of inherited disease to the occurrence of apparently sporadic pheochromocytomas and paragangliomas.

P165

RET exon 13 germline polymorphism in patients with pheochromocytomas and paragangliomas

Kornelia Hasse-Lazar[1], Agnieszka Pawlaczek[1], Aleksandra Krawczyk[1], Malgorzata Wiench[1], Malgorzata Oczko-Wojciechowska[1], Mariola Peczkowska[2], Aleksander Preibisz[2], Agata Kubaszek[2], Andrzej Januszewicz[2], Jacek Ziaja[3] & Barbara Jarzab[1]
[1]MSC Memorial Cancer Center and Institute of Oncology, Department of Nuclear Medicine and Endocrine Oncology, Gliwice, Poland; [2]Department of Hypertension, National Institute of Cardiology, Warsaw, Poland; [3]Department of General, Vascular and Transplant Surgery, Medical Silesian University, Katowice, Poland.

Germinal mutations in protooncogene RET are associated with the inherited medullary thyroid carcinoma (MTC) which occurs as the sole manifestation of disease (FMTC) or, more frequently, as the part of multiple endocrine neoplasia (MEN2). The contribution of RET polymorphism to the occurrence of apparent sporadic MTCs is controversial. In our previous study we have found out that the frequency of RET 769 CTT>CTG polymorphism in patients with MTCs is not significantly higher when compared to control group.

In the present study we analyzed RET 769 polymorphism in 61 patients with apparent sporadic pheochromocytomas or paragangliomas, in whom known germline RET mutations and SDHB/D mutations were excluded.

DNA was isolated from peripheral blood leukocytes. Polymorphism 769 CTT>CTG was found in 39 patients (59%). Its frequency was 56% in patients with pheochromocytoma and 72.7% in the group of non functional paraganglioma. Simultaneously, its frequency was 23% in patients with true sporadic MTC and 27% in the control group of healthy patients ($P<0.05$).

Conclusions

The protooncogene RET exon 13 polymorphism is associated with the occurrence of apparent sporadic pheochromocytomas and paragangliomas

P166

Cabergoline suppression test in distinguishing the variability of response to dopamine agonists in prolactinomas

Corin BADIU[2], Daniela VOICU[4], Andra Caragheorgheopol[1], Dan Hortopan[1] & Luigi Silvestro[3]
[1]Institute of Endocrinology, Bucharest, Romania; [2]C. Davila University of Medicine and Pharmacy, Bucharest, Romania; [3]Pharmaserv, Bucharest, Romania; [4]Medicover, Bucharest, Romania.

Primary therapy in prolactinomas, the most frequent pituitary adenomas, consists in ergot derivatives dopamine agonists (bromocriptine or cabergoline) which lowers prolactin levels and shrink the tumour. Bromocriptine was the first drug used, but the therapeutic levels are attained after several days/weeks, therefore an acute suppression test is not possible. However, the biological response is variable and 10% of prolactinomas are resistant to medical therapy. In order to evaluate the degree of response to dopamine agonists, we tempted a short (48 h) cabergoline (CAB) suppression test. Twenty-nine patients with hyperprolactinemia, 21 prolactinomas (14 women and 7 men), 2 GH-PRL secreting adenomas (2 women) and 6 idiopathic hyperprolactinemia (5 women, 1 man), received a single cabergoline dose (0.5 mg) and were sampled for PRL at baseline, 12 h, 24 h and 48 h after CAB administration. Simultaneously, CAB levels were determined by mass spectrometry. Subsequently, patients were treated with Cab in doses up to 2 mg/twice a week. The final response to treatment was evaluated after completion of 6 months of therapy. According to this the 21 prolactinomas were divided into 13 sensitive and 8 resistant to dopamine agonists.

Mean PRL levels decreased from 384.37 ng/mL to 101.9 ng/ml at 12 h, 94.7 ng/mL at 24 h and 73.31 ng/ml at 48 h, in the senstitive group, and from 1508.37 ng/mL to 1060.34 ng/ml at 12 h, 755.33 ng/mL at 24 h and 600.84 ng/ml at 48 h, in the resistant group. Average cabergoline levels were similar in both groups. PRL decrease at 48 h as compared to baseline, was at 40% from basal level in resistant and at 20% in responsive cases, $P<0.005$. In acromegalic patients, co-secretion of PRL was suppressed at 65% basal level at 48 h, while in functional hyperprolactinemia, normal values were attained at 48 h. Suppression level was not influenced by the tumour size. In conclusion, cabergoline suppression test could be used as early predictor of PRL suppression and biological response in prolactinomas.

P167

Predictive value of pituitary histology on clinical outcome in acromegaly: a retrospective cohort study

Birgit Steffin[1], Wolfgang Saeger[2], Hans-Jürgen Quabbe[3], Stephan Petersenn[4], Dieter K Ludecke[5], Jürgen Honegger[6], Michael Buchfelder[7] & Martin Reincke[1]
[1]Medizinische Klinik Innenstadt, Klinikum der Universität, München, Germany; [2]Marienkrankenhaus, Hamburg, Germany; [3]Deutsches Akromegalie-Register, Berlin, Germany; [4]Universitätsklinikum, Essen, Germany; [5]Universitätsklinikum Eppendorf, Hamburg, Germany; [6]Universitätsklinikum, Tübingen, Germany; [7]Universität Erlangen-Nürnberg, Erlangen, Germany.

Immunohistochemistry is commonly performed on tumour specimen obtained during transsphenoidal surgery, but its predictive value for clinical outcome is largely unknown. The aim of this study was to compare clinical and biochemical outcome characteristics after surgery with histological tumour properties. This was achieved by matching data from the German Acromegaly Register with those of the Pituitary Tumour Registry of the German pituitary working group. From 285 out of 1543 acromegalic patients of the German Acromegaly Register (145 f,140 m), data on morphological properties analyzed by a single pathologist (W.S) in the department of pathology, Marienkrankenhaus, Hamburg were available. Using immunohisto-chemistry, the density of cytoplasmatic granules, pattern of hormone expression and mitotic activity (Ki67) were analyzed. Tumours were stratified according to growth hormone (GH) and prolactin expression and Ki67 index. Clinical and biochemical parameters predicting disease outcome such as post-surgical GH and IGF-1 were analyzed. Control of acromegaly was defined as random GH <2.5 µg/l and normal IGF-1. Results are presented as range, mean and SEM. Before surgery, GH and IGF-1 concentration did not differ between patients with sparsely ($n=93$) and densely granulated ($n=145$) adenomas. However, after transsphenoidal surgery, patients with densely granulated adenomas had significantly higher GH (0–100, 5.4 ± 1.14 vs 0–57, 2.98 ± 0.917 µg/l, $P=0.03$) and IGF-1 (94–1963.522 $\pm$ 14.81, vs.12–1002.456 $\pm$ 36.38 ng/ml, $P=0.006$) concentrations compared to sparsely granulated adenomas. These patients had a lower rate of biochemical control (31% vs 54%, $P=0.01$). Co-expression of prolactin was found in 14% of adenomas. This was associated with higher postsurgical GH and IGF-1 (GH

10.5 ± 8.31 vs 3.3 ± 1.23μg/l, IGF-1 437 ± 149.01 vs 348 ± 27.3 ng/ml) compared to tumours not expressing prolactin. Ki67 staining (Ki67 index $<1\%$ vs $>1\%$) did not have impact on clinical and biochemical variables (P = n.s.). The granulation density of GH producing adenomas is a useful parameter predicting patient's biochemical outcome in acromegaly.

P168

Somatostatin receptor immunohistochemistry in neuroendocrine tumors: a proposal of scoring system for clinical characterization and therapy selection

Antongiulio Faggiano[2], Marco Volante[1], Maria Pia Brizzi[1], Stefano La Rosa[3], Ida Rapa[1], Anna Ferrero[1], Anna De Chiara[4], Luisella Righi[1], Carlo Capella[3], Gaetano De Rosa[4], Luigi Dogliotti[1], Annamaria Colao[2] & Mauro Papotti[1]
[1]Dept. of Clinical and Biological Sciences, San Luigi Hospital, University of Turin, Orbassano, Turin, Italy; [2]Dept. of Molecular and Clinical Endocrinology and Oncology, "Federico II" University, Naples, Naples, Italy; [3]Section of Anatomic Pathology, Dept. of Human Morphology, University of Insubria and Ospedale di Circolo, Varese, Varese, Italy; [4]Dept. of General Pathology, Medicine, Human Pathology, and Clinical Pathology, "Federico II" University of Naples, Naples, Italy.

Background
Typing somatostatin receptor (SSTR) expression in neuroendocrine tumors (NETs) is of relevance to target an octreotide-based diagnostic approach and treatment. The expanding use of immunohistochemistry to detect SSTR is to date not paralleled by an accurate methodological setting and standardized interpretation of the results.
Objective
A multicentric study was designed to compare SSTR immunohistochemical expression with *in vivo* scintigraphic data and verify its usefulness in the clinical management of NETs.
Design
After methodological setting by testing different SSTR antibodies, 107 cases of NETs with available OctreoScan data and pathological material (both surgical and preoperative) were retrospectively analyzed for SSTR type 2A immunohistochemical expression, and the results combined in a four grade scoring system (0 to 3) and compared with scintigraphic images and, whenever available, with the clinical response to somatostatin analogue treatment.
Results
Restricting "positive cases" to the presence of a membrane pattern of staining (proposed scores 2 and 3), an overall SSTR type 2A immunohistochemistry/OctreoScan agreement of 77% (Chi-square test $P<0.0001$) was reached. Lower concordance ratios were detected in preoperative and metastatic tumor samples, possibly as a consequence of SSTR expression heterogeneity. Pure cytoplasmic staining showed poor correlation with OctreoScan images (54% concordance rate). In a pilot series, SSTR type 2A immunohistochemistry correlated with clinical response in 82% of 22 patients undergone to therapy with somatostatin analogs on the basis of a positive OctreoScan uptake.
Conclusions
A standardized scoring system for SSTR type 2A immunohistochemistry is proposed as a useful and reliable adjunct to OctreoScan in the clinical management of NET patients. A membranous SSTR type 2A staining well predicts clinical response to somatostatin analogue therapy and provides additional information on receptor distribution into a given tumor tissue and among primary and metastatic lesions.

P169

Prevalence of primary aldosteronism among hypertensive patients (preliminary results)

Joanna Matrozova, Sabina Zacharieva, Georgi Kirilov, Mihail Boyanov & Vladimir Hristov
University Hospital of Endocrinology 'Akad.Ivan Penchev', Sofia, Bulgaria.

Until recently primary aldosteronism /PA/ was thought to be rare, accounting for no more than 0.05–2% of the hypertensive patients. Studies published in the last decade demonstrate that primary hyperaldosteronism is a much more common cause of secondary hypertension than was previously thought, accounting for as many as 5% to 25% of hypertensives in some series. For the present, there are no data concerning the prevalence of PA in Bulgaria which determined the realization of the present study. A total of 200 patients/126 females, 74 males/were studied until now, including 160 patients, referred to the Clinical Center of Endocrinology and Gerontology, 20 patients referred to the Endocrinology Clinic, Internal Medicine Department, and 20 out-patients. The screening was effectuated using the aldosterone to renin ratio. Blood samples for aldosterone (pmol/l) and PRA (ng/ml/h) were taken under standardized sampling conditions and after correction of antihypertensive medications. We used 750 pmol/l/ng/ml/h as a cut-off for the ratio aldosterone/renin. The captopril test and the measurement of aldosterone in urine were used for confirmatory testing. The diagnosis of PA was confirmed in 13 cases, which suggests a prevalence of 6.5% among hypertensive patients. Adrenal tomography was performed in all biochemically confirmed cases of PA. The presence of different types of PA was as follows: 7 cases/54%/ of adrenal adenomas and 6 cases /46%/ of idiopathic PA. Among the confirmed cases of PA 1 normokalaemic and 12 hypokalaemic patients were found. Our study confirms the results obtained by other recent investigations for an increased prevalence of PA. In contrast to other studies in our research work the cases of Conn's adenoma are predominant, as well as the hypokalaemic forms of PA.

P170

Leptin modulates the growth of murine Colon 38 cancer and interferes with the cytotoxic effect of fluorouracil *in vitro*

Gabriela Melen-Mucha & Hanna Lawnicka
Department of Immunoendocrinology, Chair of Endocrinology, Medical University of Lodz, Lodz, Poland.

Epidemiological studies underline that obesity represents a significant risk factor for development of several cancer among them colon cancer. Moreover, multiple recent data indicate that some of adipose tissue-derived hormones may influence the growth of malignant cells. Leptin, the product of the *ob* gene, is one of them. However, research is still contradictory regarding the role of leptin in colon cancer.

The aim of our study was to examine the direct effect of leptin at various concentrations (from 10^{-5} to 10^{-12} M) applied alone or jointly with fluorouracil (the classical cytotoxic drug for colon cancer) at two concentrations (0.25 μg/ml and 2.5 μg/ml) on the growth of murine Colon 38 cancer cells *in vitro*.

Colon 38 cancer cells were preincubated in RPMI 1640 medium supplemented with fetal calf serum for 24 hours. Then the cells were cultured for 72 hours in the presence of various concentrations of the examined substances applied either alone or jointly. The growth of Colon 38 cell line was assessed by the colorimetric Mosmann method.

We have found that leptin increased the growth of murine Colon 38 cancer at the concentrations of 10^{-6}, 10^{-7} M and 10^{-10}, 10^{-11}, 10^{-12} M. Its stimulatory effect was rather slight with enhancement of cancer growth by 8% to 15% as compared to controls. Fluorouracil, at both concentrations (0.25 μg/ml and 2.5 μg/ml) inhibited the growth of Colon 38 cancer up to 28% and 40% of controls, respectively. Leptin did not modulate the cytotoxic effect of fluorouracil applied at higher concentration (2.5 μg/ml) but unexpectedly it enhanced at the concentrations of 10^{-9} and 10^{-10} M the cytotoxic effect of fluorouracil given at lower concentration (0.25 μg/ml).

These data indicate that leptin is involved in the regulation of colon cancer growth and it may even enhance the cytotoxic effect of fluorouracil.

Grant No. 502-11-295 from the Medical University of Lodz.

P171

Muscle mitochondrial function is impaired in patients with prior acromegaly

Julia Szendrödi[1], Elisabeth Zwettler[2], Albrecht Ingo Schmid[3], Marek Chmelik[3], Giovanni Pacini[4], Gertrud Kacerovsky[5], Christoph Schnack[6], Guntram Schernthaner[6], Oswald Wagner[7], Klaus Klaushofer[2] & Michael Roden[5]
[1]Karl-Landsteiner Institute for Endocrinology and Metabolism, Vienna, Austria; [2]Dept. Intern. Med. 4 and Ludwig Boltzmann Institute for Osteology, Hanusch Hospital, Vienna, Austria; [3]MR Centre of Excellence, Med. Univ. Vienna, Vienna, Austria; [4]National Research Council, Padova, Italy; [5]Dept. Intern. Med. 1 and Karl-Landsteiner Institute for Endocrinology and Metabolism, Hanusch Hospital, Vienna, Austria; [6]Dept. Intern. Med. I, Rudolfstiftung Hospital, Vienna, Austria; [7]Clinical Institute of Medical and Chemical Laboratory Diagnostics, Medical University Vienna, Vienna, Austria.

Acromegaly is amongst other symptoms associated with myopathy, alterations of energy expenditure and insulin action which are mediated by growth hormone (GH) and insulin-like growth factors (IGFs). It is unclear to which extent these abnormalities remain after treatment. Thus, we examined glucose metabolism, intracellular fat deposition and mitochondrial function in patients with prior acromegaly (AM).

Six AM (4f/2 m, age: 49 ± 10 years, body mass index, BMI: 27 ± 3 kg/m^2) with an at least 7-years history of successful treatment and age-/BMI-matched healthy volunteers (CON: 3f/3 m, 43 ± 12 years, 26 ± 4 kg/m^2) were studied. Insulin sensitivity (OGIS) and first-phase insulin secretion were assessed from the frequently sampled OGTT (insulinogenic index, ISEC). Mitochondrial function was assessed from ATP synthetic flux (fATP) during fasting using ^{31}P magnetic resonance spectroscopy (MRS) of calf muscle. Intracellular lipid contents of tibialis anterior (IMCLt) and soleus muscles (IMCLs) as well as liver (HCL) were measured with ^{1}H MRS. The protocol was approved by the local institutional ethics board.

IGF-1 did not differ between groups (AM: 177 ± 88 ng/ml; CON: 145 ± 51 ng/l). Fasting plasma glucose was ~16% higher in AM (99 ± 8, CON: 85 ± 6 mg/dl, $P <$ 0.05), OGIS was comparable (395 ± 74, CON: 415 ± 14), but ISEC was ~87% lower in AM (0.9 ± 0.9, CON: 6.7 ± 4.3, $P < 0.05$). fATP was ~22% lower in AM (10.1 ± 1.5 vs. 12.9 ± 2.4 mmol.l^{-1}.min^{-1}, $P < 0.05$) and related positively to ISEC ($r =$ 0.687, $P < 0.01$). IMCLt and IMCLs and HCL were not different different between groups. IMCLs related negatively to insulin sensitivity ($r = -0.745$, $P = 0.005$).

Successfully treated acromegaly patients exhibit reduced insulin secretion and muscle ATP synthesis despite normal insulin sensitivity. The impairment of mitochondrial function could be explained by previous long-term GH/IGF exposure and/or chronically increased plasma glucose concentrations resulting from impaired ß cell function.

P172

Diagnosis and treatment of the ACTH-secreting neuroendocrine pancreatic tumors

Gilis-Januszewska Aleksandra, Hubalewska-Dydejczyk Alicja, Trofimiuk Malgorzata, Pach Dorota, Szurkowska Magdalena, Buziak-Bereza Monika, Mazurek Elwira & Huszno Bohdan
Chair and Department of Endocrinology, Collegium Medicum, Jagiellonian University, Krakow, Poland.

Introduction
Neuroendocrine tumors secreting ACTH are a rare cause of Cushing's syndrome. Diagnostic and therapeutical difficiulties might be caused due to different clinical picture of of neuroendocrine tumors.
Patients, diagnostic and therapeutic approach
During 2004–2005 2 female patients 32-years old AL and 67-years old ZS were hospitalized in Endocrinology Department due to severe hypercorticism signs and symptoms. In both patients biochemical and functional tests revealed ACTH-dependent Cushing syndrome due to ectopic secretion of ACTH. In both patients ultrasonography and computed tomography revealed not well defined pancreas region lesions and multiple hepatic metastases. ^{99m}Tc-EDTA/HYNIC-Octreotate scinitigraphy showed the uptake of the tracer in similar locations. Neuroendocrine cells were found in bioptic examination. Due to the dissemination of the disease process and bad clinical condition in both patients no surgical treatment could be performed. Important clinical and biochemical improvement was noted after introduction of aminoglutethymide (AL, ZS) and long acting somatostatin analogue (Sandostatin LAR) (AL).

The palliative chemotherapy with 5-FU was implemented in AL. Both patients were approved for therapy with somatostatin analogue labeled with ^{90}Y (^{90}-Y DOTA-Tate). Patient ZS after three series of ^{90}Y (^{90}-Y DOTA-Tate) was approved to continous somatostatin analoque treatment; patient in relatively good condition remains under Endocrinology Outpatient Department control (actually 12 month after diagnosis). Unfortunately Patient AL before admission to the hospital, suddenly died for massive pulmonary embolism.
Conclusions
^{99m}Tc-EDTA/HYNIC-Octreotate scinitigraphy become an important localising technique in neuroendocrine tumors diagnosis.

Somatostatin analoques and ^{90}Y (^{90}-Y DOTA-Tate) therapy seem to be promising treatment methods in non-operative neuroendocrine tumor cases.

P173

Novel mutations in genes encoding succinate dehydrogenase complex subunits B (SDHB) and von Hippel-Lindau protein (VHL) in patients with nonsyndromic pheochromocytoma

Jovana Vignjevic, Jelena Nikolic, Tatjana Isailovic, Katarina Mirkovic, Milan Petakov, Jadranka Antic, Djuro Macut, Bojana Beleslin-Cokic, Bojana Popovic, Ivana Bozic, Sanja Ognjanovic & Svetozar Damjanovic
Institute of Endocrinology, Diabetes&Metabolic Diseases, Clinical Center of Serbia, Belgrade, Serbia.

Background
Several susceptibility genes have been found to be associated with development of pheochromocytoma (PHEO): RET, VHL, SDHB and SDHD. We investigate the frequency of germ-line mutations in SDHB and VHL genes in patients with apparently sporadic PHEO.
Material and methods
Fifty patients (38 women, mean age 42) with apparently sporadic adrenal and extra-adrenal PHEO were screened. DNA was extracted from whole blood and from paraffin embedded tumors using standard phenol-chloroform method. For detection of SDHB and VHL mutations PCR method followed by direct sequencing gene was used.
Results
In 5/50 (10%) patients, five novel germ-line variants were identified: four heterozygous germ-line mutations (nonsense: W218X; frameshift: c.661delG, p.Asp221ThrfsX27; splicing:c.424-12delTCTT and missense: R116M) of the SDHB gene and one heterozygous germ-line mutation (V84M) of the VHL gene. In the patient with adrenal PHEO and heterozygous germ-line W218X mutation, the same heterozygosity state in the tumor tissue was found. The patient with c.661delG mutation was found to have extra-adrenal retroperitoneal malignant PHEO. Family members were also tested and they are negative for the mutation.The patient with c.424-12delTCTT is 12 years old boy with adrenal PHEO. He inherited the mutation from his father who is clinically asymptomatic for PHEO. The patient with V84M mutation was found to have adrenal PHEO. His family history is negative and he doesn't have any other tumors associated with VHL syndrome.
Conclusion
Patients with SDHB mutations are in an increased risk for the development of extra-adrenal and malignant PHEO. Our patient with extra-adrenal disease needs careful follow-up, since he is in higher risk for the development of metastases or novel adrenal/extra-adrenal PHEO. The patient with VHL mutation (V84M) is apparently classified as 2C. Until now genotipe/phenotipe correlation is not proven. This patient may develop some other tumors than PHEO.

P174

Evaluation of plasma and urinary metanephrines as well as serum chromogranin A for the diagnosis of pheochromocytoma

Nicole Unger[1], Christian Pitt[2], Martin K Walz[2], Klaus Mann[1] & Stephan Petersenn[1]
[1]Division of Endocrinology, Medical Centre, Essen, Germany; [2]Department of Surgery and Centre of Minimally Invasive Surgery, Kliniken Essen-Mitte, Essen, Germany.

Adrenal pheochromocytomas are neoplasms characterized by catecholamine excess. We recently reported on the diagnostic value of plasma metanephrines measured by RIA for the diagnosis of pheochromocytoma. However, RIA may not be used in many laboratories.

This study evaluated plasma and urinary metanephrines determined by a newly available ELISA as well as serum chromogranin A (CgA) for the diagnosis of pheochromocytoma. Spontaneous blood samples and 24h-urine samples were collected in 154 subjects, including 24 histologically proven pheochromocytomas, 17 aldosterone-secreting and 21 cortisol-secreting adrenal adenomas, 30 nonfunctioning adrenal masses, 16 patients with essential hypertension and 42 healthy normotensive volunteers. Plasma and urinary metanephrine (MN) and normetanephrine (NMN) as well as CgA were determined and putative thresholds calculated by ROC analysis.

Plasma NMN showed highest sensitivity (89.5%) and specificity (98.3%) using a threshold of 167 pg/ml, with lower sensitivity (85.7%) and specificity (91.8%) for urinary NMN by a threshold of 318 µg/24 h. Plasma and urinary MN demonstrated a much lower sensitivity (68.4% resp. 71.4%) and specificity (90.0% resp. 77.6%) using a threshold of 26 pg/ml and 90 µg/24 h respectively. Analysis of the combination of plasma metanephrines revealed a sensitivity of 89.5% and a specificity of 90.0%. Considering both urinary parameters demonstrated a slightly higher sensitivity (92.9%) with lower specificity (77.6%). ROC analysis revealed a threshold of 215pg/l for CgA with rather low sensitivity (73.9%) and specificity (74.2%). A weak positive correlation was found between the tumor size of pheochromocytomas and plasma MN ($r = 0.53$, $P <= 0.05$) as well as CgA ($r = 0.60$, $P <= 0.01$).

In conclusion, plasma metanephrines measured by ELISA are convenient and reliable parameters for the diagnosis of pheochromocytoma. In contrast, CgA demonstrated poor sensitivity and specificity.

P175

^{11}C-5-hydroxytryptophan PET scan in diagnosis of ectopic Cushing's syndrome from typical lung carcinoid: a case report

Djuro Macut[1], Svetozar Damjanovic[1], Jelica Bjekic[2], Bojana Popovic[1], Ivana Bozic[1], Milan Petakov[1], Sanja Ognjanovic[1], Tanja Isailovic[1], Miloje Joksomovic[3], Radoslav Jakovic[4], Jelena Mirkovic[1] & Jelena Stojsic[4]
[1]Institute of Endocrinology, Clinical Center of Serbia, Belgrade, Serbia; [2]Department of Endocrinology, CHC B. Kosa, Belgrade, Serbia; [3]Institute of Neurosurgery, Clinical Center of Serbia, Belgrade, Serbia; [4]Department of Thoracic Surgery, Clinical Center of Serbia, Belgrade, Serbia.

A 34-year-old woman was initially presented with clinical signs of Cushing's syndrome (CS). On endocrinological examination, a diagnosis of ACTH dependent CS was established (serum cortisol: 08.00 h: 1245 nmol/l; 24.00 h: 275; plasma ACTH concentration 104 ng/l; inadequate cortisol suppresion on LDDST (787) and suppresion to 318 following HDDST). A magnetic resonance imaging (MRI) confirmed a microadenoma in the left part of the pituitary. Ultrasound examination confirmed hyperplastic adrenals. Hypercortisolism persisted after the transsphenoidal operation of the pituitary adenoma; immunohistochemical staining was positive only on FSH and LH. Subsequently, she developed ankle edema, hypokalemia and hormonal profile suggestive on ectopic CS (plasma ACTH 171.9 and failure to suppress serum cortisol following HDDST) confirmed by CRF and DDAVP test. Neuroendocrine origin of the ectopic ACTH production was further suspected with elevated chromogranin A (489.2 ng/ml). Normal levels of 5-HIAA and PTH were obtained. A genetical analyses excluded mutation in menin. A subsequently repeated CT/MRI scans of neck, thorax, abdomen and pelvis were negative. Scintigraphy with ^{111}In-pentreotide did not show any accumulation of the tracer in the body. Whole-body chateterization and sampling did not reveal an ectopic ACTH source. Positron emission tomography (PET) using ^{11}C-5-hydroxytryptophan (5-HTP) (Uppsala, Sweden) showed increased tracer uptake in the retrocardial region of the left lung. In meantime, octreotide in a dose of 900 μg/day s.c. was applied producing complete normalizatioon of arterial blood pressure, restoration of menstrual cyclicity, and complete normalization of cortisol and ACTH. She was successfully operated 14 months after the onset of first signs of CS with pathological confirmation of 11 mm typical lung carcinoid. We presented an unusual case of ectopic CS produced from the typical lung carcinoid that was detected only by means of 5-HTP PET, and associated with coincidentally diagnosed gonadotroph pituitary adenoma.

P176

Mutational analysis in patients with nonsyndromic MEN1

Katarina Mirkovic, Bojana Beleslin-Cokic, Jovana Vignjevic, Jelena Nikolic, Jadranka Antic, Bojana Popovic, Tatjana Isailovic, Ivana Bozic, Milan Petakov, Djuro Macut, Sanja Ognjanovic & Svetozar Damjanovic
Institute of endocrinology, Clinical Center of Serbia, Belgrade, Serbia.

Background
Mutational screening of the *MEN1* gene has been recommended for patients who fulfill clinical criteria for familial or sporadic MEN1 and those suspicious or atypical of MEN1.
Patients and methods
Eighteen apparently unrelated individuals (6 males; 12 females, age range 16–71) with clinical manifestations of MEN1 were analised. In addition, we evaluated 7 relatives. Genomic DNA from peripheral blood leucocytes was extracted using standard procedures. PCR amplification followed by bidirectional sequencing of the entire coding region and exon-intron boundaries of the *MEN1* gene was used to detect mutations.
Results
In 9/18 (50%) of the index cases we identified 9 independent germline *MEN1* mutations: 3 nonsense (R527X, Y77X, Y341X), 3 frameshift (c.1089delT, c.865del4, c.960delG), 2 missense (H317Y, G225V) and one splice-site mutation (IVS4-1G>A). Three mutations were not previously reported. In addition, we detected 3 benign polymorphisms: S145S, R171Q and D418D. The patient with c.865del4 mutation was presented with insulinoma and primary hyperparathyroidism. This mutation is in exon 4 of the MEN1 gene and is predicted to cause truncation of the protein after 28 amino-acids (p.Asp252AspfsX28). Frameshift - deletion c.960delG is located in exon 6 and creates stop codon after three amino-acids (p. A263GfsX3). Patient in whom we detected this mutation had pituitary tumor and primary hyperparathyroidism. Third novel mutation, G225V, is located in exon 4 of the MEN1 gene. This patient had hyperparathyroidism, carcinoid and adrenal gland tumor. Four out of seven relatives were found to be a mutation carriers. Patient with Y341X mutation is sixteen years old boy with mixed pituitary tumor and he is at high risk for developing other MEN1 manifestations.
Conclusion
Identification of an *MEN1* mutation allows genetic testing for family members who are at risk for developing disease. Only mutation-carriers among family members need careful follow-up for the clinical manifestations of MEN1 syndrome.

P177

Screening for mutations in exon 10, 11, 13 and 14 of the RET protooncogene associated with inherited medullary thiroid carcinoma (MTC) in Serbian population

Jelena Nikolic, Jovana Vignjevic, Katarina Mirkovic, Bojana Beleslin-Cokic, Jadranka Antic, Tatjana Isailovic, Milan Petakov & Svetozar Damjanovic
Institute of endocrinology, Clinical Center of Serbia, Belgrade, Serbia.

Background
Ret protooncogene germ-line mutations are associated with the inherited multiple endocrine neoplasia type 2 syndromes (MEN2a and MEN2b) and also with familial medullary thyroid carcinoma (FMTC). In this study, we report a large scale of mutations in exon 10, 11, 13 and 14 RET protoocogene in patients from Serbia. Our study included patients with MTC.
Methods
Our study included 180 patients. Patients were tested for RET protooncogene mutations in exons 10, 11, 13 and 14 by polymerase chain reaction (PCR) followed by restriction fragment length polymorphism (RFLP) and sequencing analyses. Sequencing analysis was performed on ALFexpress II using Thermo Sequence CY5 Terminator Cycle Sequencing Kit and Applied Biosystem Genetic Analyzer 3130 using Big Dye Sequencing Kit.
Results
In 41/180 (23%) patients 7 different heterozygous germ-line mutations were identified: (C634Y, C634R, C634F, C634W in exon 11; C618Y in exon 10; Y791F in exon 13; and V804M in exon 14). Prophylactic thyroidectomy was performed in 6 C634R germline mutation carriers. Interestingly in one family with Y791F mutation MEN 2a was found while in other three components of brachi-oto-remal syndrome were found without MTC. Two patients with V804M had MTC.
Conclusions
Base on these data in Serbian population we found similar frequencies of inherited medullary thyroid carcinoma as in other European countries.

P178

The use of ^{18}F-FDG PET/CT with or without rhTSH stimulation during follow-up of patients with differentiated thyroid carcinoma

Gelsy Arianna Lupoli[1], Annalisa Panico[1], Sara Colarusso[1], Francesco Fonderico[1], Antonio Gonnella[1], Maria Rita Poggiano[1], Luigi Pucci[1], Addolorata Martinelli[1], Emanuele Nicolai[2], Marco Salvatore[2] & Giovanni Lupoli[1]
[1]Department of Molecular and Clinical Endocrinology and Oncology – University 'Federico', Naples, Italy; [2]Department of Functional and Biomorphological Sciences – University 'Federico II', Naples, Italy.

Background
Fluorine-18 fluorodeoxyglucose positron emission tomography (FDG-PET) is a new method employed in the management of differentiated thyroid cancer (DTC). The integrated FDG-PET plus computed tomography (PET/CT) fusion imaging system seems able to provide some additional advantages over PET alone, mainly related to a better anatomical localisation of the hypermetabolic metastatic lesions. The influence of serum TSH levels on ^{18}F-FDG uptake by recurrences or metastases of DTC has not been clarified yet.
Aim
To evaluate the clinical use of PET/CT during the follow-up of patients with DTC; moreover, to ascertain whether the administration of recombinant human thyrotropin (rhTSH) can increase the sensibility and specificity of PET/CT.
Patients and methods
We selected 12 pts with positive or equivocal thyroglobulin (Tg) levels and negative or equivocal ^{131}I scintigraphy and/or conventional morphological imaging techniques (ultrasound, MRI, etc); they underwent ^{18}F-FDG PET/CT during TSH suppression (<0.05 IU/L) and after rhTSH administration (> 30 IU/L).

Results
For 4 pts both basal and rhTSH-stimulated PET/CT scans were positive: in 3 cases tumour foci were detected (confirmed also by histology in 2 cases) whereas 1 of them was false positive results (due to lymph nodes inflammation). PET/CT was completely negative in 8 pts: 6 results were true negative while 2 were false negative, since scanning following rhTSH identified metastatic lesions.
Therefore, PET/CT was able to identify the metastatic foci very efficiently and to localise previously unknown tumour relapse; moreover, in 2 out of 12 patients, rhTSH administration resulted in detection of new lesions.
Conclusions
Our data confirm that PET/CT is a valuable tool in detecting residual disease in DTC patients and suggest a potential role for rhTSH in enhancing the diagnostic accuracy of this method

P179

Abstract unavailable

Growth and development – presented on Sunday

P180

Lower catch-up growth under rGH therapy at pre-pubertal pituitary dwarves diagnosed at an older age

Dumitru Branisteanu, Corina Galesanu, Cristina Cristea, Eusebie Zbranca, Carmen Vulpoi, Cristina Preda & Voichita Mogos
University of Medicine and Pharmacy 'Gr.T.Popa', Iasi, Romania.

Growth hormone deficiency leads to profoundly decreased growth velocity and, when untreated, to pituitary dwarfism. We evaluated growth evolution for one to four years under rGH therapy (0.07 IU/kg/day, subcutaneously) at seventeen idiopathic pituitary dwarves with isolated GH deficiency, 13 boys and 4 girls, with a wide span of age at therapy onset (between 4 and 24 years old). Diagnosis was set subsequent to at least two negative GH stimulation tests. All patients were pre-pubertal, with a bone age below 13 years (Grunlich and Pyle Atlas) but had normal thyroid and adrenal function. Patients were divided into two subgroups: early-diagnosed patients (12 patients younger than 14 at therapy onset) and late-diagnosed patients (5 patients, diagnosed at a chronological age of over 16 years). Growth velocity was significantly increased in the entire group, from $0.33 +/- 0.07$ cm/month before therapy onset to $0.8 +/- 0.05$ cm/month for the whole follow-up period ($P < 0.0005$). Catch-up growth was maximal during the first year of therapy, with a velocity of $1.04 +/- 0.16$ cm/month, which decreased subsequently. Both mean growth velocities for the whole follow-up period ($0.99 +/- 0.08$ vs $0.5 +/- 0.06$ cm/month) and for the first year of therapy ($1.33 +/- 0.13$ vs $0.61 +/- 0.09$ cm/month) were significantly higher at the early-diagnosed patients ($P < 0.01$), despite present radiographic growth potential. Early therapy onset in isolated GH deficiency is therefore important not only because patients have a smaller height handicap to recuperate in order to enter the normal growth channel, but also – as our data suggest - because growth cartilage seems to loose with age its reaction potential to GH administration in pre-pubertal patients. Our data show, nevertheless, that high-dose rGH therapy is still beneficial in older pre-pubertal GH deficient patients by significantly accelerating growth speed. GH dosage should be diminished to adult substitutive levels and puberty should be triggered therapeutically once growth ceases.

P181

The growth hormone – insulin-like growth factor-I axis in adult thalassemic patients

Leila Danesi[1], Agnese Cattaneo[1], Elena Valassi[1], Massimo Scacchi[1], Emanuela D'Angelo[2], Nadia Mirra[2], Laura Zanaboni[3], Maria Domenica Cappellini[3] & Francesco Cavagnini[3]
[1]Chair of Endocrinology, University of Milan, Ospedale San Luca IRCCS, Istituto Auxologico Italiano, Milan, Italy; [2]2nd Pediatric Clinic, University of Milan, Fondazione Policlinico Mangiagalli Regina Elena, Milan, Italy; [3]Department of Internal Medicine, University of Milan, Milan, Italy.

GH deficiency (GHD) can be recognized in a not negligible proportion of thalassemic children, while data on the prevalence of this disorder in adult patients are lacking. Therefore, we elected to study the GH – IGF-I axis in a large group of adult thalassemic subjects.

Study design
Ninety-four patients (69 with thalassemia major and 25 with thalassemia intermedia on stable transfusional regimen, 39 men and 55 women, aged 31.5 ± 6.8 years, receiving sex steroid replacement when necessary) underwent a GHRH (1 μg/kg as an i.v. bolus) + arginine (0.5 g/kg as a 30 min i.v. infusion) test. Severe GHD was defined by GH peaks lower than 9 μg/l, whereas partial GHD was defined by GH peaks ranging from 9 to 16.5 μg/l. Blood samples for IGF-I, ferritin and pseudocholinesterase measurement were also performed.
Results
Severe GHD was demonstrated in 21/94 patients (22.3%), while 18 additional patients (19.1%) displayed partial GHD. No correlations were found between ferritin levels on one side and GH peaks and IGF-I SDS on the other side. GH peaks were positively correlated with IGF-I SDS ($P < 0.05$), although 1 of the 21 patients with severe GHD showed normal IGF-I SDS values, and 45 of the 55 patients with normal GH reserve displayed low IGF-I SDS. A strong positive correlation ($P < 0.0001$) between IGF-I SDS and pseudocholinesterase was shown.
Conclusions
a) This study has demonstrated a high prevalence of GHD, either partial or severe, in adult thalassemic patients. b) The lack of correlation between ferritin and both GH peaks and IGF-I SDS suggests that mechanisms other than iron overload play a major role in the pathophysiology of somatotropin-somatomedin deficiency in this clinical condition. c) The finding of a positive correlation between IGF-I SDS on one side and GH peaks and pseudocholinesterase values on the other side indicates that liver protidosynthetic activity, in addition to somatotropin secretory status, is a major determinant of IGF-I production in thalassemia.

P182

The role of BMP-3B in the establishment of zona glomerulosa in the adrenal gland

Artem Bakmanidis & Peter King
William Harvey Research Institute, London, United Kingdom.

The adrenal gland is composed of the medulla and the cortex, which 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-β (TGF-β) superfamily. In a microarray analysis of transcripts from the rat adrenal zG and zF, we have discovered that some BMPs are potentially ZG specific and BMP-3B showed exclusive expression in zG by Real-Time PCR and immunohistochemistry. Adrenal H295R cells (human adrenocorticocarcinoma cell line) were used as an in-vitro model to examine the role of BMP-3B further. The cells were differentiated into a zG (by Angiotensin II) and zF (by Forskolin) phenotype in the presence and absence of exogenous BMP-3B protein. BMP-3B was able to drive the differentiation of H295R cells into a more zG phenotype while inhibiting the differentiation into a zF phenotype as judged by the inhibition of CYP11B1 expression and the promotion of CYP1B2 expression respectively. The effect of BMP-3B on differentiation was confirmed by over-expressing BMP-3B in stable cell lines and blocking endogenous BMP-3B by siRNA. These experiments imply a role for BMP-3B in steroidogenesis and by implication in adrenal zonation.

P183

Selenium supply modulates growth spurt of selenoprotein P knockout mice

Kostja Renko, Marten Michaelis, Ulrich Schweizer, Josef Köhrle & Lutz Schomburg
Institut für Experimentelle Endokrinologie, Charité Universitätsmedizin Berlin, Berlin, Germany.

Background
Selenoproteins are playing an important role in body homeostasis and development. They control thyroid hormone metabolism and are of prime importance for intracellular redox regulation and cellular defence. The SePP knockout mouse (SePP-KO) is a model of impaired Se metabolism characterized by a disrupted distribution system for organified Se. One of the major phenotypes of the homozygote SePP-KO mice is a reduced increase in size and weight during the growth spurt that can dose-dependently be rescued by Se supplementation.

Hypothesis
Se has an effect on the growth hormone axis and affects bone metabolism by modifying either growth signal synthesis or the response of target tissues.
Materials and methods
Male and female wild-type, heterozygous and homozygous SePP-KO mice were raised on regular rodent chow. At the age of 35 days, we studied the expression of growth-relevant genes in target tissues by realtime-PCR and Nothern blot analysis. Serum markers like IGF-1 and Leptin were determined by multiplex ELISA technique.
Results
On commercial diets with Se-contents not specified, we identified disarrangements in the IGF- and IGFBP-mRNA expression levels, which appeared inconclusive. On diets with defined Se content, male SePP-KO mice had a body weight of 11.3 g ($\pm$ 0.4 g) at P35 compared to 14.8 g ($\pm$0.6 g) in heterozygous or wild-type mice ($P<$ 0.001). The diets revealed a narrow window between rescue (above 0.24 ppm Se) and lethal progression of the phenotype (below 0.15 ppm). These findings now result in a well-defined model to study the impact of Se on growth and body mass.
Conclusion
Se metabolism, Se status and Se transport have an important impact on growth and body mass. Different SePP expression levels modify growth and development in transgenic SePP-KO mice. Together with specific diets this mouse model offers an ideal way to study the interaction of Se supply and growth hormone axis.

P184

Factors affecting height velocity (HV) during GnRH analog therapy in girls with central precocious puberty (CPP)

Dimitrios Thomas[1], Elpis Vlachopapadopoulou[1], Feneli Karachaliou[1], Eirini Vourliotaki[1], Philippos Kaldrimides[2] & Stefanos Michalakos[1]
[1]Dept. of Growth and Development, Children's Hosp. "P. & A. Kyriakou", Athens, Greece; [2]Dept. of Endocrinology and Metabolism, "Metaxa" Memorial Anticancer Research Hospital, Piraeus, Greece.

Aim
To assess factors affecting HV during triptorelin suppression treatment for CPP.
Materials and methods
Forty-six girls with CPP, with mean age at diagnosis 8.4 yrs who presented with signs and symptoms of puberty before the age of 8 years and were treated with triptorelin for at least 2 years, were studied. All girls were categorized into three groups according to the difference between bone age (BA) and chronological age (ΔBA-CA): group I with ΔBA-CA $<$ 11.99 months, group II with ΔBA-CA between 12 and 23.99 months and group III with ΔBA-CA $>$ 24 months. Furthermore, girls were categorized in two groups: girls with BA before treatment initiation $\leq$10 years and girls with BA $>$10 years. Four groups were formed according to Tanner breast staging: group A,B,C,D with breasts TII, TII-III, TIII and TIII-IV respectively.
Results
A statistically significant difference in mean HV during the 2nd year of treatment was observed between group I (5.99$\pm$2.21), group II (3.87$\pm$1.46) and group III (3.09$\pm$ 1.47) (P=0.012, AN.O.VA). Mean HV during the 2nd year of treatment was statistically higher in girls with BA before treatment $\leq$10 years (5.78$\pm$1.75) compared to girls with BA before treatment $>$10 years (3.17$\pm$1.27) (P=0.0001, t-test). A statistically significant difference in mean HV during the 1st year of treatment was observed between group A (6.32$\pm$0.96), group B (5.56$\pm$0.97), group C (4.96$\pm$1.07), and group D (4.26$\pm$1.66) (P=0.05, Kruskal-Wallis AN.O.VA). HV during the second year of treatment could be statistically predicted using bone age (P=0.002) and weight (P=0.036) before treatment initiation as independent factors in multivariate linear regression model, according to the following equation: $HV_{2nd\ year}$=15,026$-$0,702X(BA)-0,0892X(W).
Conclusions
Bone age, Tanner breast stage and weight seem to be important factors affecting HV during triptorelin therapy for CPP.

P185

Auxological and IGF system parameters in African in comparison with western countries normal children

Mara Boschetti[1], Sabrina Piredda[1], Marilena Sidoti[1], Eliana Di Battista[2], Daniela Larizza[3], Diego Ferone[1], Antonina Barreca[1] & Francesco Minuto[1]
[1]Department of Endocrinology and Metabolism and Center of Excellence for Biomedical Research, University of Genova, Italy; [2]Department of Pediatrics, Ospedale Gaslini, University of Genova, Italy; [3]Department of Pediatric Sciences and IRCCS Policlinico San Matteo, University of Pavia, Italy.

Growth is an integrated process, resulting from the response of cells to nutrient availability and to hormonal status. Nutrients, in turn, are important regulators of IGF-IGFBP system which are critical regulators of growth. Genetic factors seem to be very important determinant of final stature in countries with high quality of life at variance with underprivileged countries where food intake deficiency is critical. The aim of our study was to evaluate the influence of environmental conditions on IGF-I secretion and the role of GH-IGF-I system on the generational trend of stature in a selected population of children living in conditions of low dietary intake. We analyzed the auxological parameters and the circulating levels of the different components of the GH-IGF system in 38 normal African children from Ivorian Coast (NA) and 50 normal age and sex-matched Italian children (NE). The results of this study showed that in Africans the levels of all components of the circulating 150 kDa ternary complex (IGF-I, IGFBP-3, ALS) were significantly lower as compared with Italians ($P<$0.001). However, molar ALS/IGF-I, ALS/IGFBP-3, and IGF-I/IGFBP-3 ratios in African children were comparable with those found in Italians.

Clinical and auxological data of children (Mean$\pm$Standard error)

	Age	Height sds	BMI sds	IGF-I nM/L	IGFBP-3 nM/L	ALS nM/L
NE 28M/ 22F	5.0$\pm$0.3	0.3$\pm$0.1	0.2$\pm$0.2	22.4$\pm$1.6	120.4$\pm$5.5	300.7$\pm$16.4
NA 15M/ 23F	4.0$\pm$0.2	0.2$\pm$0.2	0.0$\pm$0.2	6.7$\pm$0.8	37.7$\pm$3.9	123.3$\pm$12.7
p	0.01	n.s.	n.s.	$<$0.001	$<$0.001	$<$0.001

In conclusion the levels of IGF ternary complex parameters are maintained higher in Italian than in African children by the higher dietary intake but the molar ratios and the stature were similar in both groups. It seems therefore that an optimal concentration of total IGF-I contributes to the improvement of final stature in generational trend.

P186

X-linked neuronal T_3 transport defect: Allan Herndon Dudley syndrome

Tabinda Dugal[1], Assunta Albanese[2] & Muriel Mc Entagart[3]
[1]St Georges Hospital, London, United Kingdom; [2]St Georges Hospital, London, United Kingdom; [3]St Georges Hospital, London, United Kingdom.

Thyroid hormone is absolutely necessary for early brain development.Incidence of thyroid disorders in infancy is 1:4,000.Thyroid hormones can be deficient through hormone synthesis and action or very rarely through defective transport. Some new and exciting transporters for tri-iodothyronine (T_3) have recently come to light.MCT 8 gene encodes the protein that transports T_3 into neurons. Its mutation result in inability of T_3 to enter a developing brain neuron. This leads to peripheral elevation of T_3 and TSH and low levels of T_4. Clinically this causes a spectrum of neurological features known as Allan-Herndon-Dudley syndrome (AHDS).This X-linked mental retardation syndrome was described first in 1944.

We report a case of a male child born in 2002 with intrauterine growth retardation (IUGR).He was diagnosed with cerebral palsy with supportive MRI scan. His hypotonia, poor feeding and delayed milestones were attributed to this,although the phenotypic features of AHDS ie elongated facies, bifrontal narrowing,flat ears were also present.He had severe cognitive impairment and was not walking at 42 months. He continued to be hypotonic with athetoid movements. He was under a paediatric neurologist till his raised T_3 and TSH levels were noted.He was then transferred to endocrinologist. The diagnosis of AHDS was on genetic studies.Thyroxin treatment has normalised his T_4 and TSH.T_3 remains elevated.

Thyroid hormone replacement does not correct any neurological deficit-s.Therefore ante-natal diagnosis is important.This case is unique as the mother was a mosaic carrier with no family history.Several families have been described in literature with affected male relatives. Largest series of 6 (Schwartz *et al.* 2005).It is important to recognise the defect early to plan counselling.Sex selection can also be offered for next pregnancy. Females have 1:2 chance of being a carrier while males have a 1:2 chance of inheriting the defective gene.

P187

Cephalometric analysis and dental maturation in patients with Turner's syndrome (TS)

Rafaella Shimada Gomes, Darwin Luiz Martins Oliveira, João Carlos Ramos-Dias, Alcinda Aranha Nigri, Reinaldo José Gianini, Sérgio Yassuo Nonoyama & Maria Helena Senger
Faculdade de Medicina de Sorocaba - PUC/SP, Sorocaba, São Paulo, Brazil.

Craniofacial proportions of girls with TS, compared to normal children, show reduced size of the craniofacial complex, retrognathic profile and tendency towards advanced dental age. Growth hormone (GH) treatment in TS positively affects stature, but its effects on craniofacial growth and dental development are largely unknown. The aim of this study was to analyze and to correlate the craniofacial morphology, chronological, dental and bone ages of TS patients receiving GH or not. After the study was approved by the local Ethics Committee, we evaluated 21 cephalometric measurements (lateral cephalograms), dental age (DA) (panoramic radiograph), bone age (BA) (left hand-wrist radiograph) and stature Z-escore in 22 TS patients (9 monosomy X; 10 mosaicism; 3 structural abnormalities of the X chromosome). The GH treatment lasted from 0 to 6.8 yr. The median chronological age (CA) was 16 ± 3.4 yr ($\pm$S.D.). The variations for BA and DA were 6.8 yr to 17 yr and 6 yr to 17 yr, respectively. Stature Z-escore was -2.33 ± 1.8 (mean $\pm$S.D.). Statistics were performed using the principal component analysis, simple linear regression and Pearson correlation coefficient. P values <0.05 were considered significant. Face height and mandibular length were the most affected measures and showed correlations with BA, CA and GH treatment duration ($P<0.05$). Cytogenetic status did not influence face alterations. CA was greater than BA ($P<0.05$) and did not differ from DA, while BA was lower than DA ($P<0.05$). We observed a positive correlation between CA and BA ($r=0.7$), CA and DA ($r=0.8$) and BA and DA ($r=0.7$). In conclusion, we showed that our TS patients present a short and retropositioned face, mainly in the lower third part, conferring them a convex profile. A prospective study will provide greater knowledge of GH effects on craniofacial structures, looking for better orthodontic treatment for these patients.

P188

Craniofacial development and dental maturation in growth hormone(GH)-deficient patients

Darwin Luiz Martins Oliveira, Rafaella Shimada Gomes, Maria Helena Senger, Alcinda Aranha Nigri, Reinaldo José Gianini, Sérgio Yassuo Nonoyama & João Carlos Ramos-Dias
Faculdade de Medicina de Sorocaba - PUC/SP, Sorocaba, São Paulo, Brazil.

Growth is a complex process, influenced to a large extent by GH. Children with GH deficiency (GHD) have typical somatic features, including short stature and a reduction of the craniofacial structures. Dental age (DA) is normally delayed in relation to chronological age (CA). The effect of GH replacement on craniofacial growth is still poorly understood. We studied the craniofacial development and dental maturation in 17 patients (4F, 13M) with GHD of different etiologies. The length of rhGH treatment lasted from 0–15.2 yr. The median CA was 16.2 ± 3.9 yr ($\pm$S.D.). BA varied from 5–18 yr and DA, from 7.7–17 yr. Mean stature Z-escore was -1.8 ± 1.8 (mean $\pm$S.D.). Craniofacial morphology was analysed by standardized lateral cephalometric radiographs with 21 measurements. DA was calculated by panoramic radiographs and BA was estimated by left hand-wrist radiographs. This study was approved by the local Ethics Committee. Statistics were performed using the principal component analysis, simple linear regression and Pearson correlation coefficient. P values <0.05 were considered significant. The most affected measures were the posterior cranial base, position of the temporomandibular articulation, facial height and mandibular length, that had correlation with BA and lenght of GH treatment ($P<0.05$). BA was delayed in comparison with CA and DA. There were no significant differences between CA and DA. We observed a positive correlation between BA and DA ($r=0.8$), CA and BA ($r=0.8$), and CA and DA ($r=0.7$). In conclusion, we showed that our group of GHD patients presents with a short face (mainly in the lower third) and a retropositioned mandible, conferring a more convex face profile to them. A longitudinal study will provide a greater knowledge of the effect of rhGH treatment on the craniofacial structures, looking for earlier orthodontic follow-up and better results in these children.

P189

Developing brain as an endocrine gland secreting GnRH and dopamine to general circulation

Michael Ugrumov
Institute of Developmental Biology RAS, Moscow, Russia.

This study was aimed to test our hypothesis that the brain-derived gonadotropin-releasing hormone (GnRH) and dopamine (DA) are delivered to the general circulation in fetal and neonatal rats, i.e. before the establishment of the blood-brain barrier, that is in contrast to adult rats. The GnRH and DA concentrations were measured in plasma and in the brain on the 18th embryonic day (E18), E21, 3rd postnatal day (P3), i.e. before the establishment of the blood-brain barrier, and on P30–36 after the establishment of the barrier. Moreover, the concentrations of GnRH and DA were measured in fetal plasma after microsurgical lesion of the brain regions containing most GnRH or DA neurons or after the inhibition of DA synthesis in the brain with sterotaxically injected α-methyl-p-tyrosine. According to our data, the concentrations of GnRH and DA in plasma on E18, E21 and P3 enormously exceeded those on P30–36 being as great as those in the hypophysial portal circulation in adult rats. Reverse was true for the ontogenetic dynamics of the GnRH and DA concentrations in the brain. The lesion of the local brain regions resulted in a drop of the GnRH and DA concentrations in fetal plasma. The DA concentration in plasma also decreased significantly after the inhibition of DA synthesis in the brain. The rest of circulating GnRH and DA was shown to be insufficient to provide the regulation of the respective adenohypophysial functions.

Thus, brain-derived GnRH and DA are delivered to the general circulation in fetal and neonatal rats in amounts sufficient to influence peripheral targets and the brain itself.

Obesity and metabolism – presented on Sunday

P190

Closure by iptakalim, a cardiovascular K(ATP) channel opener, of rat islet beta-cell K(ATP) channels and its molecular basis

Sechiko Suga[1], Makoto Wakui[2], Takanori Kudo[3] & Yoshiji Ogawa[3]
[1]Center for Education and Research of Lifelong Learning, Hirosaki University, Hirosaki, Aomori, Japan; [2]Division of Clinical Research, Hirosaki National Hospital, Hirosaki, Aomori, Japan; [3]Department of Internal Medicine, Hirosaki University School of Medicine, Hirosaki, Aomori, Japan.

Diabetes mellitus is a group of diseases characterized by high levels of blood glucose resulting from defects in insulin production, insulin action, or both. Diabetes patients usually have accompanying cardiovascular disorders. Sulfonylureas have been the leading oral antihyperglycemic agents for type 2 diabetes treatment, which currently still constitute the most popular anti-diabetic drugs. Nevertheless, concern has arisen over the side effects of sulfonylureas on the cardiovascular system. Here we report that iptakalim, a novel cardiovascular ATP-sensitive potassium (K(ATP)) channel opener, closed rat islet beta-cell K(ATP) channels and increased insulin release. Using whole-cell patch-clamp recordings, iptakalim depolarized beta-cells, induced action potential firing and reduced pancreatic K_{ATP} channel currents. Using single-channel recordings, iptakalim reduced K(ATP) channel open-probability independently of intracellular ATP concentrations. We demonstrated that iptakalim elevated intracellular calcium concentrations and increased insulin release as revealed by fluorescence imaging (fura-2) and biochemical measurements, respectively. In addition, iptakalim significantly inhibited the open-probability of recombinant Kir6.2/SUR1 and Kir6.2FL4A (a trafficking mutant of the Kir6.2) channels expressed in transfected human embryonic kidney (HEK) 293 cells. Collectively, iptakalim, a cardiovascular K_{ATP} channel opener, closes rat islet beta-cell K(ATP) channels, which may result from direct inhibition of the Kir6.2 subunit. Therefore, iptakalim bi-directionally regulates K(ATP) channels in cardiovascular and islet tissues, and this unique pharmacological property suggests iptakalim could be used as a new therapeutic strategy for the treatment of type 2 diabetes with the potential benefit in alleviating cardiac and/or vascular disorders frequently associated with diabetes.

P191

Plasma adiponectin and leptin levels in obese and insulin resistant postmenopausal females with type 2 diabetes

Nona Adamia, Tea Tsotsonava & Ketevan Janberidze
Tbilisi State Medical University, Tbilisi, Georgia.

Objective

Adipocytokines appear to be important in regulating insulin sensitivity. The objective of this study was to compare the levels of adiponectin and leptin in lean, obese and obese diabetic (OD) postmenopausal female (PMF) subjects during 6 months follow-up of Metformin therapy (MT).

Methods
We examined plasma levels of adiponectin and leptin in 26 OD PMF with a mean body mass index (BMI) of 36.6±1.8, 10 obese (BMI=35.9±2.2) and 10 lean (BMI=22.3±1.9) individuals. The investigation was approved by the local ethics committees. All participants gave informed, written consent before starting the trial. Insulin resistance (IR) was assessed using the homeostasis model assessment.
Results
Baseline characteristics of all groups shown that adiponectin was significantly decreased and leptin is significantly elevated in OD PMF and obese subjects in comparison with leans ($P<0.001$ and $P=0.003$, respectively). There was a tendency for adiponectin levels to be lower in OD PMF as compared with obese individuals ($P=0.053$). OD PMF were more insulin resistant than obese and lean subjects ($P<0.001$). Results of MT shown that circulating adiponectin levels were significantly increased (16.1±3.9 vs. 19.1±6.0 ng/ml, $P=0.008$) with significant reduction of BMI and IR ($P=0.005$ and $P<0.001$, respectively). Leptin levels did not change significantly.
Conclusions
Circulating adiponectin levels is significantly reduced in OD PMF in comparison with obese and lean subjects. Hypoadiponectinemia in PMF may be explained by only IR because the amelioration of whole-body insulin action by MT causes the increase of serum adiponectin levels. Leptin levels in OD PMF are not significantly different from leptin levels of obese subjects, although they significantly differ from leptin levels of lean individuals.

P192

Pioglitazone modifies the effects of growth hormone on lipolysis and insulin sensitivity

Morten B Krag[1], Søren Nielsen[1], ZengKui Guo[2], Steen B Pedersen[3], Ole Schmitz[1], Jens S Christiansen[1] & Jens OL Jørgensen[1]
[1]Medical Department M, Aarhus University Hospital, DK-8000 Aarhus C, Denmark; [2]Endocrine Research Unit, Mayo Clinic, Rochester, MN 55905, United States; [3]Medical Department C, Aarhus University Hospital, DK-8000 Aarhus C, Denmark.

Context
Thiazolidiones (TZD) improves insulin sensitivity in type 2 diabetes via effects on fat metabolism, whereas growth hormone (GH) stimulates lipolysis and induces insulin resistance.
Objective
To evaluate the effects of TZD on fat metabolism and insulin sensitivity in GH-treated GH deficient (GHD) patients.
Design
Randomized, placebo-controlled, double-blind parallel-group study including 20 GHD patients on continued GH replacement therapy. The patients were studied before and after 12 weeks.
Intervention
Patients received either tablet pioglitazone 30 mg ($N=10$) or placebo ($N=10$) once daily for 12 weeks.
Results
12 weeks of pioglitazone treatment in GH-replaced GHD patients was associated with improved insulin sensitivity ($P=0.03$) and increased basal glucose oxidation ($P=0.004$). Change in insulin-stimulated adiponectin level after pioglitazone treatment was positive correlated to the change in insulin-stimulated total glucose disposal ($R=0.69$, $P=0.04$). Pioglitazone significantly decreased basal free fatty acid levels ($P=0.02$) and lipid oxidation ($P=0.02$). Adiponectin levels almost doubled during pioglitazone treatment ($P=0.0001$).
Conclusion
The impact of GH on lipolysis and insulin sensitivity is modified by administration of PPARγ agonists.

P193

The metabolic syndrome and associated sexual dysfunction: psycho-biological correlates

Giovanni Corona[1], Edoardo Mannucci[2], Luisa Petrone[1], Riccardo Mansani[1], Giancarlo Balercia[3], Valerio Chairini[4], Gianni Forti[1] & Mario Maggi[1]
[1]Andrology Unit, University of Florence, Florence, Italy; [2]Geriatric Unit, Diabetes Section, University of Florence, Florence, Italy; [3]Endocrinology Unit, Polytechnic University of Marche, Ancona, Italy; [4]Endocrinology Unit, Maggiore-Bellaria Hospital, Bologna, Italy.

Objectives
The aim of present study is to determine psychobiological characteristics of sexual dysfunction (SD) associated with metabolic syndrome (MS; as defined by National Cholesterol Education Program's Adult Treatment Panel III, NCEP-ATP-III criteria) in a consecutive series of 803 male out-patients.
Methods
Several hormonal, biochemical and instrumental (penile doppler ultrasound, PDU) parameters were studied, along with psychopathology scores (Middlesex Hospital Questionnaire modified MHQ). The Structured Interview on Erectile DYsfunction (SIEDY), was also applied.
Results
Among subjects studied, 236 patients (29.4%) were diagnosed as having a MS. Among them 96.5% reported ED, 39.6% hypoactive sexual desire, (HSD) 22.7% premature and 4.8% delayed ejaculation. Patients with MS were characterized by greater subjective (as assessed by SIEDY) and objective (as assessed by PDU) ED and by greater somatizated anxiety than the rest of the sample. The prevalence of overt hypogonadism (total testosterone <8 nM) was significantly higher in patients with MS. Circulating TT decreased as the number of MS components increased (B=−1.35±0.182 nmol/l; $P<0.0001$, after adjustment for age). Accordingly, the relative risk for hypogonadism was significantly higher in patients reporting 3 or more risk factors for MS. Among MS components, waist circumference and hyperglycemia were the best predictors of hypogonadism. Among patients with MS, hypogonadism was present in 11.9% and 3.8% in the rest of the sample ($P<0.0001$) and it was associated with typical hypogonadism-related symptoms, such as hypoactive sexual desire, low frequency of sexual intercourses and depressive symptoms.
Conclusion
Our data suggest that MS is associated with a more severe ED and induces somatization. Furthermore, MS is associated with a higher prevalence of hypogonadism in patients with SD. The presence of hypogonadism can further exacerbate the MS-associated sexual dysfunction, adding the typical hypogonadism-related symptoms. (including HSD, 66.7%).

P194

A comparison of NCEP-ATP-III and IDF metabolic syndrome definitions with relation to metabolic syndrome associated sexual dysfunction

Giovanni Corona[1], Edoardo Mannucci[2], Luisa Petrone[1], Francesco Lotti[1], Alessandra D Fisher[1], Giancarlo Balercia[3], Valerio Chairini[4], Gianni Forti[1] & Mario Maggi[1]
[1]Andrology Unit, University of Florence, Florence, Italy; [2]Geriatric Unit, Diabetes Section, University of Florence, Florence, Italy; [3]Endocrinology Unit, Polytechnic University of Marche, Ancona, Italy; [4]Endocrinology Unit, Maggiore-Bellaria Hospital, Bologna, Italy.

Objectives
The aim of present study was to verify possible differences in the prevalence of vasculogenic ED and hypogonadism comparing two distinct new definitions of MetS, as National Cholesterol Education Program-Third Adult Treatment Panel (NCEP-ATPIII) and International diabetes Federation (IDF) in patients with sexual dysfunction.
Methods
Several hormonal, biochemical and instrumental (penile doppler ultrasound) parameters were studied. ANDROTEST Structured Interview was also applied. This a 12-item, recently validated, inventories, which assesses the degree of androgenization in male.
Results
We studied a consecutive series of 1086 patients. The prevalence of metabolic syndrome was 32.0% and 44.7% according to NCEP-ATPIII and IDF criteria, respectively. Patients with MetS according to both criteria reported lower PGE-1 stimulated penile flow (Vpmax). At multivariate analysis, only NCEP-ATPIII was significantly associated with Vpmax (B=−7.7±3.8; $P<0.05$). Patients with MetS defined according to both criteria reported lower total (13.6±6.0 vs. 17.4±7.2 and 14.7±7.4 vs. 18.2±6.0 nmol/l,) and free testosterone levels (34.8±14.0 vs. 40.8±13.7 and 36.2±14.1 vs. 42.5±13.5 pmol/l), higher prevalence of hypogonadism (34.3 vs. 11.9 and 25.3 vs. 8.7%), and higher ANDROTEST score (9.6±3.0 vs. 7.2±3.6 and 9.2±3.2 vs. 6.0±3.2) respectively for NCEP-ATPIII and IDF; all $P<0.0001$. However, when IDF, but not NCEP-ATPIII, criteria were fulfilled, the prevalence of hypogonadism was significantly lower than that observed in patients fulfilling both criteria (15.6 vs.34.8% respectively; $P<0.0001$). Conversely, those fulfilling NCEP-ATP-III, but not IDF, criteria did not show a significant different prevalence of hypogonadism than those positive for both sets of criteria (30.8 vs.34.8%; $P=$NS).
Conclusions
In patients with ED, NCEP-ATPII criteria seem to be a better predictor of hypogonadism and impaired penile blood flow than IDF.

P195

Effect of supervised structured exercise program for 16 weeks on metabolic, pulmonary and cardiovascular parameters in obese adolescents

Juan Guízar-Mendoza, Benigno Linares, Norma Amador, Gloria Barbosa & Juan Malacara
[1]Instituto Mexicano del Seguro Social, León/Guanajuato, Mexico, [2]Universidad de Guanajuato/Instituto de Investigaciones Médicas, León/Guanajuato, Mexico, [3]Universidad de Guanajuato/Facultad de Medicina, León/Guanajuato, Mexico.

Objective
To investigate whether a supervised structured exercise program by 16 weeks improves metabolic, pulmonary and cardiovascular parameters in obese adolescents.
Material and methods
We included 38 obese adolescents between 12–15 years old. They participated in a supervised exercise program by 90 minutes, 5 days a week during 16 weeks. At baseline and at the end of the exercise program, we evaluated cardiorespiratory fitness, anthropometric measurements, lipid profile, glucose, insulin, leptin, adiponectin, and blood pressure levels. Pulmonary function was evaluated by spirometry and heart sympathetic activity by spectral analysis of the R-R interval during 60 minutes to obtain indices of heart autonomic function.
Results
The exercise program increased exercise ability ($P<0.001$), maximal oxygen uptake ($P=0.01$), forced vital capacity ($P=0.004$), and adiponectin levels ($P<0.001$); while BMI ($P=0.001$), body fat (<0.001), glucose, triglycerides ($P<0.001$ in both), leptin ($P<0.001$), blood pressure levels ($P<0.001$), and heart sympathetic activity expressed as LF/HF index ($P=0.005$) significantly decreased. The change in LF/HF index was correlated with the decrease in leptin ($r=43$; $P=0.007$), diastolic ($r=0.33$; $P=0.04$) and systolic (0.35; $P=0.03$) blood pressure levels respectively.
Conclusions
A short-term supervised structured exercise decreased adiposity and improves metabolic, pulmonary, and cardiovascular parameters in obese adolescents.

P196

Continuous administration of dihydrotestosterone or letrozole to immature female rats results in polycystic ovary syndrome characteristics at adult age

Louise Mannerås[1], Theodore Lystig[2], Agneta Holmäng[1], Malin Ottosson-Lönn[2] & Elisabet Stener-Victorin[1]
[1]Institute of Neuroscience and Physiology, Göteborg, Sweden; [2]Insitute of Medicine, Göteborg, Sweden.

Introduction
Polycystic ovary syndrome (PCOS) is a complex endocrine and metabolic disorder associated with ovulatory dysfunction, hyperandrogenism, abdominal obesity and insulin resistance. Since hyperandrogenism is a PCOS key feature, the aim was to evaluate the effects of androgen receptor activation in terms of continuously administration, beginning pre-pubertal, of either the non-aromatizable androgen dihydrotestosterone (DHT) or the aromatase inhibitor letrozole (L), on ovarian morphology, as well as on the endocrine and metabolic status were investigated.
Methods
At 21 days of age, the rats were implanted subcutaneously with a pellet releasing DHT or L continuously during 90 days. Estrus cyclicity (vaginal smear), ovarian morphology, sex steroid and leptin concentrations, body composition (DEXA, MRI, and tissue dissection), mesenteric adipocyte size (computerized image analysis), and insulin sensitivity (euglycemic hyperinulinemic clamp) were examined.
Results
DHT induced polycystic ovaries (PCO) and anovulation in 75% of the rats. DHT rats also displayed increased body weight, fat mass and weight of individual abdominal fat depots, as well as enlarged mesenteric adipocyte size with a right shifted size distribution curve. Moreover, elevated leptin levels and insulin resistance were observed in DHT treated rats. Almost all L rats developed PCO morphology with similarity to human PCO, including hyperplastic theca cell layer, and anovulation. Hyperandrogenism and increased body weight without any body composition changes were other characteristics of the L group.
Conclusions
Typical PCO morphology was induced both by DHT and L treatment. In particular DHT treatment also resulted in metabolic disorders of the syndrome, while the endocrine features of the syndrome were mainly induced by L. Both models can therefore be concluded as suitable for investigation of different aspects of the human PCOS.

P197

Neonatal sex steroid exposure of female rats results in insulin resistance and enlarged mesenteric adipocytes

Camilla Alexanderson[1], Theodore Lystig[2], Elisabeth Stener-Victorin[1], Malin Lönn[2] & Agneta Holmäng[1]
[1]Instititute of Neuroscience and Physiology, Göteborg, Sweden; [2]Institute of Medicine, Göteborg, Sweden.

Introduction
Neonatal events may contribute to the development of disorders such as type 2 diabetes and obesity at adult age. We have previously shown that neonatal testosterone (T) programming of female rats is followed by insulin resistance and changes in adipose tissue distribution with centralization of body fat. Therefore, the aim of this study was to examine the effects of neonatal injection of T, estradiol (E) or dihydrotestosterone (DHT) on insulin sensitivity and size distribution of adipocytes in intra-abdominal and subcutaneous adipose tissue in female rats.
Methods
Pups received one injection of T, E, DHT or vehicle within 3 hours after birth. At 14 wks of age the rats were exposed to a euglycemic hyperinsulinemic clamp. Intra-abdominal (mesenteric) and subcutaneous (inguinal) adipose tissues were dissected and weighed. Adipocyte size was analysed using a computerized image analysis system.
Results
All groups receiving steroids were insulin resistant in comparison with controls. The mesenteric adipocyte size distribution was shifted to the right in T- and E-rats compared with controls while adipocyte size in the inguinal depot was not affected. T-rats also displayed increased mesenteric adipose tissue weight.
Analysis of all groups together showed a negative correlation between mesenteric adipocyte size and glucose infusion rate.
Conclusions
Sex hormone exposure in early life may predispose to disturbances in insulin sensitivity and adipose tissue at adult age. Directly after birth, in particular the mesenteric adipose tissue depot seems to be vulnerable to T- and E exposure which is seen as a shift to the right of the adipocyte size distribution in adulthood.

P198

Evaluation of visceral protein malnutrition in morbid obese patients operated on laparoscopic gastric bypass

Rosa Casañ, Carlos Morillas, Eva Sola, Katherinne García, Jesus Yanini, Ana Jover, Marcelino Gomez & Antonio Hernández
Endocrinology Department, Doctor Peset University Hospital, Valencia, Spain.

Morbid obesity is associated with a decreased life expectancy and a myriad of serious medical problems. The Roux-en-Y gastric bypass (RYGBP) is the most effective procedure for the treatment of these patients, but it can be responsible of early and late complications. The aim of this prospective study was to evaluate the rate of visceral protein malnutrition (VPM) in morbid obese (MO) patients two years after laparoscopic RYGBP. Albumin (Alb), prealbumin (Prealb), transferrin (Transf), retinol binding globulin (RBG), C3-complement factor (C3) plasma levels, and lymphocyte count (Lymph) were measured before and 2 years after RYGBP. Data were evaluated using paired Student t-test. Data were available for 46 patients (9 men and 37 women). Mean age: 38.5 ± 11 years; mean follow-up time: 24 ± 9 months.
Results
No differences were observed in Prealb, RBG, Transf or Lymph count. Before surgery, 1 patient (2.2%) had C3 values under normal levels, and after surgery 4 patients (8.7%) had C3 values under normal levels.

	Baseline	24 months	*P*
Weight (kg)	124.8 ± 17.7	84.8 ± 15.9	<0.001
BMI (kg/m^2)	47.8 ± 6.7	32.5 ± 6.3	<0.001
Alb (mg/dL)	3.93 ± 0.4	3.89 ± 0.3	0.08
C3 (mg/dL)	132.5 ± 20.9	103.1 ± 16.9	<0.001

Conclusions
1- There were no changes in main visceral protein plasma levels: Alb, Prealb, Transf, and RBG in MO patients after 2 years of RYGBP. 2- A significant decrease of C3 values was observed in these patients, without changes in lymphocyte count. In spite of this decrease, C3 levels remained in most patients between the normal range. 3- RYGBP seems to be an effective procedure to treat morbid obesity which does not cause VPM, but immunity should be assessed.

P199

Overconsumption of salty and sweet foods increases blood pressure in children

Vassilios Petrou[1], Athanasia Tertipi[1], Asteroula Papathanasiou[1], Ourania Zerva[2], Vassiliki Skarpa[1], Petros Papachilleos[1], Marina Anastasacou[1], Panagiotis Nicolopoulos[1] & Charalambos Hadjiathanasiou[1]
[1]Children's Hospital "P. & A. Kyriacou" Department of Endocrinology, Athens, Greece; [2]Children's Hospital "P. & A. Kyriacou" Department of Nutrition, Athens, Greece.

Aim
To estimate the impact of overconsumption of salty and sweet foods on Body Mass Index (BMI) and Blood Pressure (BP) in children.
Patients-Methods
We studied 208 children (105 girls), aged 9.2±3.0 yr, 57 (27.4%) of normal weight, 37 (17.8%) overweight and 114 (54.8%) obese. Overconsumption of NaCl was considered >5 g/day and of free sugar >0.5 g/Kg ideal Body Weight/day. BP was measured as appropriate and BMI was estimated in all children.
Results
Children overconsuming salty and sweet foods had significantly higher BMI SDS than children consuming small amount of salty and sweet foods (2.1±1.5 vs 1.2±1.5, $P<0.001$ for salty foods and 2.1±1.5 vs 1.2±1.6, $P=0.002$ for sweet foods). Thirty-three (57.9%) of children of normal weight overconsumed salty foods versus 23 (62.2%) of overweight and 98 (86.0%) of obese ($\chi^2=18.8$, $P<0.001$). Thirty-five (61.4%) of children of normal weight overconsumed sweet foods versus 32 (86.5%) of overweight and 99 (86.8%) of obese ($\chi^2=16.5$, $P<0.001$). One hundred twenty nine children (83.8%) overconsuming salty foods had Systolic BP (SBP)>50th percentile versus 35 (64.8%) of children consuming small amounts of salty foods ($\chi^2=8.6$ $P=0.006$). One hundred thirty six children (81.9%) that overconsumed sweet foods had SBP>50th percentile versus 28 (66.7%) that consumed small amounts of sweet foods ($\chi^2=4.6$, $P=0.036$). There was no difference regarding diastolic BP (DBP) among children consuming large or small amounts of salty and sweet foods respectively. BMI SDS emerged as the most important determinant of SBP>50th percentile and DBP>50th percentile in multivariate analysis.
Conclusion
Overconsumption of salty and sweet foods is related to a relatively increased BP in children through the incremental effect on BMI SDS.

P200

Expression of PKA regulatory subunits inversely correlates with BMI and insulin resistance parameters in human adipocytes from lean and obese subjects

Sara Bondioni[1], Giovanna Mantovani[1], Luisella Alberti[2], Sabrina Corbetta[3], Luisa Gilardini[2], Cecilia Invitti[2], Marco Antonio Zappa[4], Stefano Ferrero[5], Erika Peverelli[1], Andrea G Lania[1], Paolo Beck-Peccoz[1] & Anna Spada[1]
[1]Endocrine Unit, Dpt. of Medical Sciences, Fondazione Ospedale Maggiore Policlinico Mangiagalli e Regina Elena IRCCS, University of Milan, Milan, Italy; [2]Lab. of Diabetologic Research, Istituto Auxologico Italiano IRCCS, Milan, Italy; [3]Endocrinology and Diabetology Unit, Dpt. of Medical-Surgical Sciences, University of Milan, Policlinico San Donato IRCCS, San Donato Milanese, Milan, Italy; [4]General Surgery Unit, Dpt. of Surgical Sciences, University of Milan, Fondazione Ospedale Maggiore Policlinico Mangiagalli e Regina Elena IRCCS, Milan, Italy; [5]Pathology Unit, Department of Medicine, Surgery and Dentistry, A.O. San Paolo and Fondazione Ospedale Maggiore Policlinico Mangiagalli e Regina Elena IRCCS, Milan, Italy.

In human adipocytes the cAMP-dependent pathway mediates signals originating from the activation of beta-adrenergic receptors, thus playing a key role in the regulation of important metabolic processes such as lipolysis and thermogenesis. CyclicAMP effects are mainly mediated by cAMP-dependent protein kinase (PKA), a tetrameric enzyme composed by two catalytic subunits associated with two regulatory (R) subunits. There are four different R subunit genes and proteins (R1A, R1B, R2A, R2B) expressed with a tissue-specific pattern and exerting distinct roles in cell differentiation and growth control. Recent studies indicate the R2B isoform as the most expressed in mouse adipose tissue while its presence is limited elsewhere. Moreover, R2B knock-out mice are genetically lean and protected against developing diet-induced obesity and fatty-livers. The aim of this study was to investigate the expression of the different PKA regulatory subunits in 65 human subcutaneous and visceral adipose tissue samples from 10 lean subjects (BMI<25) and 55 obese patients (BMI>30). Real-time PCR showed that, as in mice, R2B is the most abundant transcript, both in obese and normal subjects, with no differences between visceral and subcutaneous adipose tissue. Moreover, a significant negative correlation was observed between R2B expression levels and BMI, insulin levels, HOMA-IR ($r=-0.280$, $r=-0.269$, $r=-0.255$, respectively; $P<0.05$), with a positive correlation with adiponectin and adiponectin receptors 1&2 mRNA levels ($r=+0.636$, $r=+0.582$, $r=+0.631$ respectively; $P<0.001$). Moreover, among obese patients, patients with metabolic syndrome showed the lowest R2B levels. Immunohystochemistry and western-blot analysis performed in 15 of the 55 samples from obese patients and in the 10 samples from lean subjects confirmed the same expression pattern. This is the first study evaluating the relative expression of the different PKA isoforms in human adipose tissue. Our results indicating important BMI-related differences in R2B expression suggest that similar differences in PKA activity may modulate the lipolytic response to beta-adrenergic activation.

P201

Insulin resistance and fasting leptin's relationship in subjects with metabolic syndrome

T Mokhort & E Shishko
Belarusian State Medical University, Minsk, Belarus.

Background and aims
The aim of our study is to investigate the possible associations between leptin and fasting insulin and index HOMA-IR in patients with Metabolic Syndrome as Leptin is involved in regulation of body weight.
Materials and methods
The study included 100 patients (32 m, 68 f) 25–65 years. They were divided into 2 groups matched by sex, age, weight, body mass index (BMI), waist-to-hip ratio (WHR). Research group included 56 patients (24 m, 32 f) with Metabolic Syndrome: abdominal obesity, insulin resistance, hyperinsulinemia, glucose intolerance, hypertension and dyslipidemia. Control group included 44 patients (16 m, 28 f) without clinical and biochemical findings of Metabolic Syndrome. The average fasting plasma glucose, 2-hour plasma glucose concentrations following a 75-g oral glucose tolerance test, total cholesterol, triglycerides, systolic and diastolic blood pressure were also evaluated. Fasting serum leptin (FL) and fasting insulin levels (FI) were detected by sensitive and specific ELISA. Index HOMA-IR was calculated by standard formula. HOMA-IR=or> 2.7 were considered as insulin resistance.
Results
In patients of the research and control groups serum leptin levels were higher in females (median 45.1 and 27.8 ng/ml respectively) than in males (15.9 and 7.7 ng/ml respectively). But only in patients of the research group correlations were between BMI and WHR (r=0.91 $P<0.001$ vs $r=0.93$ $P<0.01$ respectively). Correlation analysis showed that FL were significantly correlated with the FI ($r=0.56$ $P<0.01$) and HOMA-IR ($r=0.52$ $P<0.01$) in research group. In subjects of the control group leptin concentration correlated with the HOMA-IR only in men ($r=0.91$ $P<0.01$) and not correlated in female. The strongest correlations were between FL and total cholesterol ($r=+0.49$ $?<0.05$ in men) and triglycerides ($r=+0.8$ $?<0.05$ in women) in research group.
Conclusion
Determine positive correlation of basal leptin and index insulin resistance confirms hyperleptinemia and leptinresistance concern in formation of metabolic syndrome.

P202

Epicardial adipose tissue, hepatic steatosis and obesity

Ayse Sertkaya Cikim[1], Ergun Topal[2], Murat Harputluoglu[3], Lezzan Keskin[1], Zeynep Zengin[1], Kerim Cikim[1], Ramazan Ozdemir[2], Murat Aladag[3] & Saim Yologlu[4]
[1]Inonu University Faculty of Medicine Department of Internal Medicine Division of Endocrinology and Metabolism, Malatya, Turkey; [2]Inonu University Faculty of Medicine Department of Cardiology, Malatya, Turkey; [3]Inonu University Faculty of Medicine Department of Internal Medicine Division of Gastroenterohepathology, Malatya, Turkey; [4]Inonu University Faculty of Medicine Department of Biostatistics, Malatya, Turkey.

Objective
The measurement of epicardial adipose tissue (EAT) sonographically is reported to be related with both obesity and insulin resistance. Hepatic steatosis is one of the best known other coincidence with obesity. We aimed to evaluate the relationships between EAT thickness, hepatic steatosis and insulin resistance in obese patients.
Methods
Obese 63 subjects were enrolled into the study. Local ethical committee approval was obtained. Patients were divided into three groups according to body mass index (BMI) as follows: 20 patients with $30 \leq BMI < 35$ kg/m^2 (Group 1, mean age 39.3 ± 12.9 yrs), 25 patients with $35 \leq BMI < 40$ kg/m^2 (Group 2, mean age 41.7 ± 9.3 yrs), and 18 patients with BMI ≥ 40 kg/m^2 (Group 3, mean age 36.8 ± 13.9 yrs). EAT thickness and grade of hepatic steatosis were assessed sonographically. Anthropometrical measurements were assessed with the foot-to-foot bioelectrical impedance analysis. Insulin resistance was assessed according to basal insulin, QUICKI and HOMA equations.
Results
hsCRP was the only metabolic parameter; which was higher in Group 3 than Group 1 significantly ($P = 0.02$). EAT thickness was similarly higher in both groups 2 and 3; but groups were found to be similar for grade of hepatic steatosis. Both EAT thickness and grades of hepatic steatosis were positively and significantly correlated with whole body fat mass and abdominal adiposity. Waist circumference was the only factor affecting EAT thickness in linear regression analysis.
Conclusion
Grade of hepatic steatosis is a lesser sensitive marker for closer obesity levels than EAT, but with its significant correlations; hepatic steatosis can also be assessed as a valuable predictor for reflecting increments of whole body fat mass and abdominal adiposity as EAT thickness.

P203

Decreased 11beta-hydroxysteroid dehydrogenase type 1 activity in obese boys

Susanna Wiegand[1], Anna Richardt[1], Thomas Remer[3], Stefan A Wudy[4], Jeremy W Tomlinson[5], Annette Grüters[1], Paul M Stewart[5], Christian J Strasburger[2] & Marcus Quinkler[2]
[1]Paediatric Endocrinology, Charite Campus Virchow, Universitaetsmedizin Berlin, Berlin, Germany; [2]Clinical Endocrinology, Charite Campus Mitte, Universitaetsmedizin Berlin, Berlin, Germany; [3]Department of Nutrition and Health, Research Institute of Child Nutrition, Dortmund, Germany; [4]Steroid Research Unit, Center for Child and Adolescent Medicine, Justus Liebig University, Giessen, Germany; [5]Division of Medical Sciences, Institute of Biomedical Research, University of Birmingham, Birmingham, United Kingdom.

Objective
The incidence of childhood obesity and type 2 diabetes has reached epidemic proportions. Glucocorticoid excess causes central obesity and diabetes mellitus as seen in Cushing's syndrome. The 11beta-hydroxysteroid dehydrogenase type 1 enzyme (11beta-HSD1), which is predominantly expressed in liver and adipose tissue, regenerates active cortisol from inactive cortisone. Increased 11beta-HSD1 may cause tissue-specific Cushing syndrome with central obesity and impaired glucose homeostasis.
Design, patients and methods
Clinical and laboratory characteristics, and anthropometric measurements were determined in 15 male (aged 12–18) and 6 female (aged 12–18) obese pubertal children. In addition, analysis of 24 h excretion rates of glucocorticoids were performed in obese and age- and sex-matched non-obese children using gas chromatographic-mass spectrometric (GC-MS) analysis.
Results
11beta-HSD1 activity (urinary THF + 5alphaTHF/THE ratio) was lower in obese compared to non-obese boys. In addition, obese children had a higher total cortisol metabolite excretion than non-obese children. 11beta-HSD1 activity was significantly related to age, but not to waist-to-hip ratio, fat mass (% of body mass), or insulin resistance index (HOMA). Standard deviation score (SDS)-BMI did not correlate with 11beta-HSD1 or −2 (urinary free F/free E ratio) activity, or with total cortisol metabolite excretion. We did not find a gender difference regarding 11beta-HSD1 or −2 activity. 11beta-HSD2 activity significantly correlated to abdominal circumference in obese children.
Conclusions
In conclusion, our findings strongly suggest that 11beta-HSD1 activity increases with age and is reduced in obese boys. In addition, obese children have a higher total cortisol metabolites excretion suggesting a stimulated HPA axis.

P204

Clinical presentation of nonclassic congenital adrenal hyperplasia (NC-CAH): from suspicion to diagnosis

Anna Casteràs Román, M[a] Concepción Páramo Fernández, Javier De la Fuente Aguado, Elena Hervás Abad, Laura Fajar Rodríguez, Beatriz Mantiñán Gil, Reyes Luna Cano, M[a] Antonia Rego Iraeta, Digna Rodríguez, Domingo González Lestón & Ricardo Víctor García-Mayor
Hospital Xeral de Vigo, Vigo, Pontevedra, Spain; Hospital POVISA, Vigo, Pontevedra, Spain.

Background
Nonclassic congenital adrenal hyperplasia (NC-CAH) caused by mutations in CYP21B gene is an inherited disorder with various clinical forms in relation to the 21-hydroxylase (21OH) activity. Classic forms are recognized early during neonatal period as salt-wasting crisis or genital ambiguity, while non-classic form presents later with wide hyperandrogenic spectrum. Genetic testing has proved to be the definitive diagnostic method.
Aim
To observe the clinical presentation in relation to the genotype among subjects with clinical suspect of NC-HAC.
Subjects and methods
Ninety-seven patients (90 female, 7 male) consulting with suggestive clinical data of NC-HAC were genotyped and classified into groups (1: no mutation $n = 54$; 2: homozygotes $n = 22$; 3: compound heterozygotes $n = 11$; 4: simple heterozygotes $n = 10$). Clinical presentation was correlated with the genetic findings.
Results
Mutations in CYP21B were present in 44,3% of patients and V281L in homozygous state was the most frequent genotype in the studied population (48,8%). In general, hirsutism and premature pubarche were the most common symptoms (32,9 and 28,8% respectively).
Conclusions
Less than 50% of hyperandrogenic patients had genetic confirmation of 21OH deficiency. We did not find clinical features associated with the genotype, but precocious pubarche, which is more common in simple and compound heterozygotes than in homozigotes or without mutation ($P < 0.05$).

P205

Daily and nightly urinary free cortisol ratio as a marker of the hypothalamic-pituitary-adrenal (HPA) axis activity in abdominal obesity

Valentina Vicennati[1], Uberto Pagotto[1], Bruno Ambrosi[2], Mauro Maccario[2], Ettore Degli Uberti[2], Marco Boscaro[2], Francesco Cavagnini[2], Franco Mantero[2], Decio Armanini[2], Roberto Vettor[2], Mario Marugo[2] & Renato Pasquali[1]
[1]Endocrinology Unit, Bologna, Italy; [2]"HPA axis and obesity" Group-SIE, Bologna, Italy.

Abdominal obese (AO) women might have a hyperactivation of the HPA axis. The limitations of previous studies have been often represented by the limited and

heterogeneous number of patients enrolled. Our aim was to asses urinary free cortisol (UFC) output during daily and nightly hours in a large cohort of AO women vs. normal weight controls (CT). 107 AO women and 37 CT were enrolled in the study. In basal condition, each subject underwent OGTT, biochemical determinations. Each subject collected daily (from 0800 AM to 0800 PM, dUFC) and nightly (from 0800 PM to 0600 AM of the day after, nUFC) urine.
Cholesterol and triglycerides levels were significantly higher ($P<0.001$) in the AO, whilst HDL were significantly ($P<0.01$) lower than in CT. AO had significantly higher HOMA index than CT. There were no differences neither in dUFC nor in the nUFC between the groups. On the contrary, AO had significantly lower dUFC/nUFC than CT.
There was a negative and significant correlation between dUFC/nUFC and waist and BMI in all subjects. When AO were analyzed separately, the correlation between dUFC/nUFC and anthropometric variables was still present. Moreover, the ratio was also positively correlated to HOMA index ($P<0.05$).
In order to assess the linkage between HPA axis activity and metabolic syndrome, a multiple regression was performed in AO. dUFC/nUFC was still negatively and significantly correlated to BMI, while the correlation with waist circumference was lost. Interestingly, dUFC/nUFC was still positively and significantly correlated to HOMA index and systolic blood pressure. On the contrary, a negative and significant correlation was found between dUFC/nUFC and both HDL and diastolic blood pressure.
In conclusion, obesity by itself is characterized by high nightly UFC excretion. The HPA axis dysregulation is strictly associated to the abnormalities of the metabolic syndrome, particularly to glucose-insulin homeostasis, dyslipidemia and hypertension.

P206

Interaction of hypothalamic receptors involved in weight regulation

Anne Rediger[1], Patrick Tarnow[1], Annette Grüters[1], Michael Schäfer[2] & Heike Biebermann[1]
[1]Charité Universitätsmedizin Berlin, Inst. Exp. Ped. Endocrinology, Berlin, Germany; [2]Charité Universitätsmedizin Berlin, Inst. Pharmacology, Berlin, Germany.

Objectives
Food intake is centrally regulated in hypothalamic nuclei where many G-protein-coupled receptors (GPCRs) are expressed which are known to be involved in weight regulation. Peripheral hormonal signals activate their corresponding receptors in the arcuate nucleus. Orexigenic signals activate POMC expression in one subset of neurons and inhibit AgRP and NPY expression in a second subset. Cleavage products of POMC, α- and β-MSH then stimulate melanocortin-4-receptors (MC4R) in the paraventricular nucleus of the hypothalamus to inhibit food intake or stimulate the melanocortin 3 receptor (MC3R) in the arcuate nucleus to activate a feedback loop. Other neuropeptides or neurotransmitters are involved in hypothalamic regulation of body weight, which also act through G-protein-coupled-receptors co-expressed with melanocortin receptors (MCR) in hypothalamic nuclei. The concept of homo and hetero-oligomerization of GPCRs today is well accepted. Recently we could show homo-oligomerization of MC4R. In a systematic approach we investigated the interaction of GPCRs that are expressed on the same neurons.
Methods
We used two different methods to investigate GPCR oligomerization: a sandwich-ELISA approach with differentially N- and C-terminal tagged receptors in COS-7 cells and the FRET-acceptor-photobleaching-technique which allows monitoring of GPCR interaction in living HEK-293 cells. Furthermore we investigated receptor co-localization on the cell surface by laser scanning microscopy.
Results
Here we report data on interaction of the MC3R and ghrelin receptor (GHSR) that are coexpressed on arcuate NPY/AgRP neurons. The usage of both methods results in a strong signal of MC3R/GHSR oligomerization.
Conclusion
We could demonstrate for the first time that GPCR from different subfamilies, that are expressed on the same neuron and are involved in weight regulation form receptor oligomeres. These findings may provide a mechanistic basis of a functional interaction between melanocortin and ghrelin receptors and thereby widen our understanding of hypothalamic signalling pathways involved in weight regulation.

P207

Association of estrogen receptor-alpha gene polymorphisms with cerebrovascular disease in patients with metabolic syndrome

Nektaria Xita[1], Sophia Markoula[2], Leandros Lazaros[3], Athanasios Kyritsis[2], Ioannis Georgiou[3] & Agathocles Tsatsoulis[1]
[1]Department of Endocrinology, University of Ioannina, Ioannina, Greece; [2]Department of Neurology, University of Ioannina, Ioannina, Greece; [3]Laboratory of Reproductive Genetics, University of Ioannina, Ioannina, Greece.

Introduction
The vascular protective effects of estrogens are known to be mediated by their binding to specific estrogen receptors (ER). However, the significance of genetic variations of the ER in vascular diseases has not been reported. We have examined the association between stroke and PvuII and XbaI polymorphisms of the estrogen receptor-alpha gene in patients with metabolic syndrome.
Methods and subjects
The study population consisted by 84 male and 46 female patients with metabolic syndrome compared with 100 healthy men and 140 healthy women respectively. The body mass index was recorded and biochemical parameters were measured. PCR-RFLP and genotyping of ER PvuII and XbaI polymorphisms were performed in peripheral blood leucocytes. Multiple logistic regression analysis was used to explore the risk factors for stroke. Local Ethical Committee approval was obtained.
Results
Both polymorphisms were in Hardy Weinberg equilibrium in the study population. Genotype distributions and allele frequencies of PvuII or XbaI polymorphisms were not significantly different between control subjects and patients. No association was found between the polymorphisms and the severity of stroke. Total cholesterol, triglyceride, or HDL-cholesterol levels were not significantly different among ER genotypes. However, men homozygous for A allele of XbaI polymorphism had a stroke at a younger age compared to other genotypes ($53,3 \pm 8,1$ years vs $56,9 \pm 9,4$ years, $P<0,05$).
Conclusion
These findings suggest that PvuII and XbaI polymorphisms of ER are not associated with the prevalence and severity of cerebrovascular disease. However, the XbaI polymorphism seems to affect the age of developing cerebrovascular disease in men with metabolic syndrome.

P208

Bone mineral density and body composition in pubescent obese children endangered by metabolic syndrome

Borbala Tobisch, Andrea Kozma, Zsuzsanna Nagy-Szakall, Ferenc Peter & Laszlo Blatniczky
Buda Children's Hospital, Budapest, Hungary.

Aims
The prevalence of type 2 diabetes due to insulin resistance is increasing in puberty. Authors have investigated whether body weight excess arises only from increased fat content of the body. Different anthropometrical data were also analysed if they are able to predict degree of insulin resistance.
Materials and methods
108 obese children (50 female, 58 male, Tanner st-s1-5, mean age 12.06 years, mean BMI: 30.97 kg/m^2) with positive familiar anamnesis of metabolic syndrome were in the study. Bone density by PQCT as well as body composition by bioelectrical impedance analyser(inBody 3.0) were measured. Waist/hip-ratio and body fat% based on skin-fold thicknesses measurement were calculated. HOMA-index and ΣC-peptide/Σ*IRI* ratio (ΣC/Σ*IRI*) during oral glucose tolerance test as markers of insulin resistance were calculated
Results
Total Z-score of bone mineral density in obese children exceeded by 0.2 SD and trabecular density by 0.65 SD those of normal population of the same age. Obese children's muscle mass exceeded by 6.8 kg in average compared with same values of "sample" population of the same age. There were slack correlations ($r=0.578$ vs. 0.682) between measured and calculated body fat% as well as measured fat% and BMI. There was no significant correlation between the anthropometrical values and HOMA-index, nor the ΣC/Σ*IRI*. Waist/hip-ratio showed a mild correlation with HOMA-index ($r=0.268$) and a moderate one with ΣC/Σ*IRI* ($r=0.462$).
Conclusions
Increased BMI-values in obese children are partially caused by both increased bone mineral content and higher muscle mass. BMI-values are less helpful to estimate inappropriate body composition. Differences between measured and calculated body fat% can indirectly indicate the degree of visceral fat. Increased waist/hip ratio predicts insulin resistance better. Anthropometrical data themselves do not

predict insulin resistance in youngsters, it has to be determined individually. The $\Sigma C/\Sigma IRI$ is a more exact indicator of insulin resistance than the HOMA-index.

P209

Influence of gaining weight on metabolic syndrome in the menopause

Aleksandra Simoncig-Netjasov, Svetlana Vujovic, Milos Stojanovic, Miomira Ivovic, Milina Tancic & Milka Drezgic
Institut of Endocrinology, Clinical Center of Serbia, Belgrade, Serbia.

Metabolic syndrome (MS) represents a prominent risk factor for cardiovascular disease. Parameters of MS were compared between obese women and controls. I: 50 women (31.92 ± 5.83 kg/m^2; 54.4 ± 3.64 y's); Controls: 37 women (23.50 ± 2.13 kg/m^2; 53.92 ± 3.95 y's). Weight, height, waist and hips circumference, sagital abdominal diameter (SAD) and blood pressure (BP) were measured. Blood was taken at 8 am for: fasting glucose, triglycerides, cholesterol, HDL, LD, Lp(a), FSH, LH, PRL, E2 and OGTT was performed. Hormone analyses: RIA. Statistics: T test, Mann – Whitney U test, ANOVA. MS: 66% in I and 22% in controls. Significant differences between groups were found for: glycose (6.22 ± 2.26 vs 5.49 ± 2.43 mmol/l, $P<0.05$), weight (86.20 ± 17.82 vs 62.81 ± 7.90 kg, $P<0.01$), waist (99.96 ± 14.65 vs 79.9 ± 8.78 cm, $P<0.01$), hips circumference (114.31 ± 11 vs 96.93 ± 11.04 cm, $P<0.01$), SAD (31.9 ± 6.83 vs 24.9 ± 9.86 cm, $P<0.01$), BMI (31.92 ± 5.83 vs 23.5 ± 2.13 kg/m^2, $P<0.01$), diastolic BP (93.08 ± 13.41 vs 85.75 ± 10.54 mmHg, $P<0.01$), Lp(a) (0.50 ± 0.36 vs 0.11 ± 0.03 g/l, $P<0.01$), FSH (54.35 ± 27.16 vs 72.32 ± 30.17 IU/L, $P<0.01$), LH (20.33 ± 11.08 vs 28.77 ± 14.16 IU/L, $P<0.01$), PRL (251.52 ± 142.60 vs 370.27 ± 237.74 nmol/l, $P<0.05$). There are positive correlations between menopausal duration and waist, BMI and BP. Negative correlation was found for BMI, menopausal duration and HDL.

Conclusion

Hypoestrogenic status in the menopausal women shows a shift to a central android fat distribution and MS that can be counteracted by HRT.

P210

Effects of physiological bell-shaped elevations of free fatty acids on glucose metabolism and insulin sensitivity in humans

Lars Gormsen, Charlotte Nielsen, Niels Jessen, Esben Vestergaard, Jens Christiansen, Jens Jørgensen & Niels Møller
Aarhus University Hospital, Aarhus, Denmark.

Background

Physiological elevations of free fatty acids (FFAs) occur in a dynamic bell-shaped fashion lasting some hours, e.g. nocturnally and during exercise. In order to define the metabolic role of physiological elevations in relation to diurnal fluctuations in insulin sensitivity, the present study was designed to identify the metabolic effects of a dynamic 4 hour elevation of FFAs during a glucose clamp.

Materials and methods

8 lean, healthy men were examined twice in a cross-over design: 1) Control (saline), and 2) 4 h graded infusion of intralipid (20%)/heparin. Insulin sensitivity and EGP were assessed by the isotope dilution (3H-glucose) technique during an 8 h hyperinsulinemic-euglycaemic clamp (0.5 mU/kg/min). Before the study, the protocol was approved by the Aarhus County Ethical Scientific Committee; the purpose and potential risks of the study were explained to all subjects; and informed, written consent was obtained from all participants.

Results

Infusion of intralipid caused a significant increase of average FFA levels (Area under the curve (AUC)) compared with saline reaching peak levels $\sim$1.9 mmol/L and markedly impairing insulin sensitivity [iAUCglucose Rate of disappearance (Rd) (mg/kg): 709 ± 25 vs. 380 ± 112, $P=0.04$). There was a lag phase of 300 minutes from initiation of intralipid infusion until glucose Rd was significantly reduced. Glucose Rd returned to control levels after a further 150 minutes. Average insulin sensitivity was negatively correlated with average FFA level ($r^2=0.52$, $P=0.002$). EGP was equally suppressed by hyperinsulinaemia regardless of treatment.

Conclusions

Our data suggest that physiological FFA elevations induce insulin resistance in the periphery after a lag of 4–5 h and that normal insulin sensitivity is restored 1–2 h after FFA values have returned to normal. It is therefore likely that FFA plays an important role in circadian variations of insulin sensitivity (e.g. the Dawn phenomenon and during exercise).

P211

The metabolic changes induced by glucocorticoids: involvement of AMP-activated protein kinase

Mirjam Christ-Crain[1], Blerina Kola[1], Francesca Lolli[1], Csaba Fekete[2], Dalma Seboek[3], Gabor Wittman[2], Sharon Ajodha[1], Judith Harvey-White[4], George Kunos[4], Beat Mueller[3], Giorgio Amaldi[5], Gilberta Giacchetti[5], Marco Boscaro[5], Ashley Grossman[1] & Marta Korbonits[1]
[1]Dept of Endocrinology, William Harvey Research Institute, Barts and The London, Queen Mary School of Medicine, University of London, EC1M 6BQ London, United Kingdom; [2]Dept of Neurobiology, Institute of Experimental Medicine, Hungarian Academy of Sciences, Budapest, Hungary; [3]Dept of Endocrinology, University Hospital Basel, Basel, Switzerland; [4]Laboratory of Physiological Studies, National Institute of Alcohol Abuse and Alcoholism, National Institutes of Health, Bethesda, United States; [5]Dept of Internal Medicine, University of Ancona, Ancona, Italy.

Background

Excess glucocorticoids result in Cushing's syndrome (CS) which is characterised by increased food intake, central obesity, dyslipidaemia and insulin resistance, leading to the metabolic syndrome. AMPK is a regulator of energy homeostasis and plays an important role in the regulation of appetite, glucose uptake, lipogenesis and gluconeogenesis. We hypothesised that the effects of corticosteroids on metabolism would be mediated by changes in AMPK activity in a tissue-specific manner.

Methods

Rats were implanted with corticosterone-containing pellets and consumed chow and 30% sucrose for 2 weeks. Control animals were implanted with cholesterol pellets consuming sucrose or saline only. AMPK activity (kinase assay), metabolic enzyme expression (qRT-PCR) and hypothalamic endocannabinoid content were measured. Human visceral fat tissue of patients with CS was analysed for AMPK activity and compared to controls. In vitro experiments using human *ex vivo* differentiated adipocytes and a human hepatoma cell line.

Results

Corticosterone-treated rats demonstrated higher insulin, leptin, cholesterol and triglyceride levels and an increase in visceral fat weight (to $129 \pm 5\%$ of controls; mean $\pm$ SEM). The AMPK activity in the visceral fat of corticosterone-treated rats and CS patients was significantly lower compared to controls. The gene expression of gluconeogenic and adipogenic enzymes was increased in adipose tissue. The data on AMPK were confirmed in human adipocytes treated with dexamethasone for 24 h. In the liver, fat content was increased concomitant with an increased AMPK activity. In the heart a decrease in AMPK was observed, consonant with the cardiomyopathy observed in humans. In the hypothalamus, AMPK and the endocannabinoid content were increased concordant with the increased appetite typical of CS.

Conclusion

We demonstrate that corticosteroids change AMPK activity in various tissues in a manner that may explain the increase in food intake, lipid deposition in visceral adipose and hepatic tissue and the peripheral cardiac effects of Cushing's syndrome.

P212

Net endogenous acid production and circulating leptin are associated with potentially bioactive free glucocorticoids in healthy lean women

Christiane Maser-Gluth[1], Triantifillia Dimitriou[2] & Thomas Remer[2]
[1]Dept of Pharmacology, University of Heidelberg, Heidelberg, Germany; [2]Research Institute of Child Nutrition, Dortmund, Germany.

Recent evidence suggests that endogenous glucocorticoids (GC) may be suppressed by adipocyte-derived leptin and elevated by dietary acidity. Therefore we examined whether these factors might be predictors of potentially bioactive free glucocorticoids independently of adrenocortical activity.

Body composition, plasma cortisol, plasma leptin, 24-h urinary excretion rates of net acid (NAE) reflecting daily diet-dependent acid load, total nitrogen, urinary free cortisol (UFF), free cortisone (UFE), the main GC metabolites tetrahydrocortisone (THE), tetrahydrocortisol (THF) and 5alpha-tetrahydrocortisol (alloTHF) were examined cross-sectionally in 30 healthy adults (15 females; 22–44 yr old; BMI: 20–25 kg/m). Adrenocortical activity (AA) was assessed by the sum of the 3 major glucocorticoid metabolites (THE+THF+alloTHF), reflecting overall daily cortisol secretion. As a measure of potentially bioactive free GCs (bioactiveGCs) the sum of free cortisol and cortisone in urine (UFF+UFE) was taken, reflecting the free fraction of circulating cortisol and cortisone. Plasma leptin (mean $\pm$ SD, 2.8 ± 1.6 vs. 7.6 ± 4.9 ng/mL) and percent body fat (%BF, 16.8 ± 4.2 vs. $26.9 \pm 4.9\%$) were lower ($P<0.01$) and body surface (BS)-corrected AA higher ($P<0.01$) in males, whereas plasma cortisol and

BS-corrected bioactiveGCs were statistically undistinguishable between the sexes. Both bioactiveGCs and AA correlated positively with %BF and leptin in males ($P<0.05$), but not in females. After adjusting for AA, NAE was a positive ($P=0.011$) and leptin a negative ($P=0.046$) predictor of bioactiveGCs in females (total explained variability $R^2=0.71$). In males only AA explained variation of bioactive-GCs ($R^2=0.49$, $P=0.004$).
Our findings indicate that – at least in females – variability of potentially bioactive glucocorticoids is not only explained by individual adreno-cortical activity, but may also be affected by circulating leptin and diet-dependent daily acid load.

P213

Serum gamma-glutamiltransferase increases in type 2 diabetes mellitus but it is not related with the body mass index

Juan Carlos Ferrer-García, Xelo García-Fabra, Raquel Albalat-Galera, Patricia Sánchez-Llópez, Agustín Herrera-Ballester & Carlos Sánchez Juan
Unit of Diabetes and Endocrinology, Department of Internal Medicine, University General Hospital, Valencia, Spain.

Objectives
We have examined the relationship between the hepatic enzymes and type 2 diabetes. We have analyzed if the levels of hepatic enzymes are associated with body weight, lipid profile and the treatment with metformin, thiazolidinediones or statins.
Methods
318 patients with type 2 diabetes were included and they were compared with 100 healthy subjects. Alanine aminotransferase (ALT), aspartate aminotransferase (AST) and gammaglutamiltranspeptidase (GGT) were measured. We studied the lipid profile, the weight and the treatment with metformin, thiazolidinediones and statins, analyzing possible differences in hepatic function.
Results
Type 2 diabetic patients showed significantly increased levels of GGT that the population control (48.3 ± 5.2 vs 25.6 ± 2.1 U/l respectively; $P<0.01$). The difference was confirmed after adjustment for age, sex and body mass index (BMI). There were no differences in the levels of ALT, AST. Levels of GGT upper the normality were presented in 33.0% of type 2 diabetic patients and 13% of healthy subjects. The diabetic subjects showed higher triglycerides and more reduced LDL-c and HDL-c levels. Lipid profile and body weight were not correlated by the hepatic function. The diabetic patients treated with metformin presented lower levels of ALT (31.3 ± 2.7 vs 22.1 ± 2.2 U/l; $P<0.05$). There were no differences in patients treated with statins or thiazolidinediones.
Conclusions
Increased levels of GGT are closely associated with type 2 diabetes, and this association is independent of the BMI. Metformin has been associated with reduced levels of ALT.

P214

Effects of pharmacological stimulation or blockade of cannabinoid receptor type 1 (CB1) on gene expression in mouse cultured adipocyte cells

Luigi Bellocchio, Cristina Cervino, Flaminia Fanelli, Valentina Vicennati, Renato Pasquali & Uberto Pagotto
Endocrine Unit and C.R.B.A. Hospital S.Orsola-Malpighi, Bologna, Italy.

The endocannabinoid system has recently emerged as an important modulator of several functions of adipose tissue by altering cell proliferation and gene expression. In this work, we investigated the effects of CB1 activation/blockade in mouse 3T3-L1 adypocite cells by using WIN55,212, a CB1/CB2 agonist and rimonabant, a specific CB1 antagonist, in different experimental settings such as acute treatment on pre-adipocytes and on mature adipocytes, and chronic treatment during differentiation process. The gene expression was first analyzed by semi-quantitative RT-PCR and then confirmed by Real-TIME PCR for selected genes. We found that CB1 and FAAH mRNAs were both up-regulated by WIN55,212 and down regulated by SR141716, this effect was stronger in pre-adipocytes than in mature adipocytes. Furthermore, in pre-adipocytes, rimonabant was able to down-regulate PPAR-γ expression, whereas WIN55,212 gave an opposite effect. Moreover, rimonabant was also able to stimulate UCP1 and UCP2 mRNA expression.
Among adipokynes, adiponectin mRNA has been shown to be down-regulated by WIN55,212 and up-regulated by rimonabant, whereas visfatin, apelin and IL-6 mRNAs resulted up-regulated by WIN55,212 and down regulated by rimonabant. In the same cells, rimonabant reduced lipogenic gene expression, in particular of FAS, ACC, LPL, SCD-1, DGAT-2 mRNAs, whereas WIN55,212 up-regulated these genes suggesting a stimulatory role of endocannabinoids on fatty acids and triglycerides biosynthesis.
All together, these results indicate that endocannabinoid system is able to stimulate differentiation of pre-adipocytes towards adipocytes and to directly influence several metabolic processes of these cells including their secretory profile.

P215

Absence of TSH-induced increase in leptin levels in patients with history of differentiated thyroid carcinoma undergoing rhTSH testing

Francesca Cecoli[1], Gabriella Andraghetti[1], Carla Ghiara[2], Lucia Briatore[1], Domenico Cavallero[1], Michele Mussap[2], Francesco Minuto[1] & Massimo Giusti[1]
[1]Dipartimento di Scienze Endocrinologiche e Metaboliche, Azienda Ospedaliera Universitaria San Martino, Genoa, Italy; [2]U.O. Laboratorio Analisi, Azienda Ospedaliera Universitaria San Martino, Genoa, Italy.

Some extra-thyroid effects of TSH have been described *in vitro* and *in vivo*. TSH has recently been suggested to induce IL-6 secretion from adipocytes. Leptin is the main secretory protein from adipose tissue. Our aim was to evaluate the acute effect of rhTSH-induced TSH surge on serum leptin levels in differentiated thyroid carcinoma (DTC) patients. Ten patients (2 m, 8 f; age range 31–66 years) with stage 1-3 DTC were evaluated during scheduled standard rhTSH testing. Leptin, thyroglobulin (Tg) and TSH were measured, before and after rhTSH administration (0.9 mg i.m. for 2 consecutive days). L-T4 therapy ranged from 575 to 1050 µg/week and f-T4 levels ranged from 8 to 23 pg/ml. According to BMI data, only 2 patients were obese. One patient presented a high HOMA-IR (>4). LDL-cholesterol levels were over 130 mg/dl in 50% of patients. Baseline leptin levels were 8.4 ± 1.3 ng/ml. Only BMI correlated significantly ($P=0.05$) with baseline leptin levels. After rhTSH administration, TSH levels increased significantly ($P<0.01$), while thyroid hormones remained unchanged. According to Tg-stimulated levels and neck sonography, all but 2 patients were considered disease-free. Two patients were considered partially ablated after post-surgical radioiodine therapy. On average, leptin levels did not significantly change during rhTSH administration. Twenty hours after the last rhTSH administration, leptin levels were 8.6 ± 1.4 ng/ml, maximal leptin levels being recorded after 1 week (8.9 ± 1.5 ng/ml). No correlation between maximal TSH and leptin levels after rhTSH was noted. In conclusion our *in vivo* experimental model suggests that acute TSH increase after rhTSH testing is uneffective on circulating leptin. These results are in contrast with some literature data reporting an *in vivo* correlation between leptin and TSH in hypothyroid, hyperthyroid and obese subjects.

P216

Serum visfatin in relation to insulin resistance and markers of hyperandrogenism in lean and obese women with polycystic ovary syndrome

Irina Kowalska, Marek Straczkowski, Agnieszka Nikolajuk, Agnieszka Adamska, Monika Karczewska-Kupczewska, Elzbieta Otziomek, Slawomir Wolczynski & Maria Gorska
Department of Endocrinology, Diabetology and Internal Medicine, Medical University of Bialystok, Bialystok, Poland; Department of Reproduction and Gynecological Endocrinology, Medical University of Bialystok, Bialystok, Poland.

Visfatin is a newly discovered protein secreted by adipose tissue, which is suggested to play a role in pathogenesis of insulin resistance. Polycystic ovary syndrome (PCOS) is heterogeneous disorder, where insulin resistance might be involved in the development of endocrine and metabolic abnormalities. The aim of the present study was to asses the relation between serum visfatin and insulin sensitivity and markers of hyperandrogenism in a lean and obese PCOS patients. The study group consisted of 70 women with PCOS (23 lean and 47 overweight or obese) and 45 healthy, normally menstruating women (25 lean and 20 overweight or obese). Euglycemic hyperinsulinemic clamp and the measurements of serum visfatin and sex hormones were performed. PCOS group had lower insulin sensitivity ($P=0.00004$) and higher serum visfatin concentrations ($P=0.026$) in comparison to controls. The decrease in insulin sensitivity was present both in

lean ($P=0.0053$) and in obese ($P=0.017$) PCOS subjects, whereas increase in serum visfatin was observed only in lean PCOS ($P=0.013$). In the whole studied group, serum visfatin was negatively related to insulin sensitivity ($r=-0.27$, $P=0.004$). This relationship was also observed in the subgroup of lean ($r=-0.30$, $P=0.038$), but not obese women. Additionally, in lean women visfatin was associated with serum testosterone ($r=0.47$, $P=0.002$) and free androgen index ($r=0.48$, $P=0.002$), independently of other potential confounding factors. Obtained results pointed out that visfatin could play a role in pathogenesis of PCOS in lean women.

P217

Relationship between serum adiponectin and oxidative and non-oxidative glucose metabolism in apparently healthy humans

Agnieszka Adamska, Marek Straczkowski, Irina Kowalska, Agnieszka Nikolajuk, Monika Karczewska-Kupczewska, Malgorzata Karolczuk-Zarachowicz, Elzbieta Otziomek & Maria Gorska
Department of Endocrinology, Diabetology and Internal Medicine, Medical University of Bialystok, Bialystok, Poland.

The pathogenesis of insulin resistance is not completely understood, however, there are data that it might be associated with altered tissue carbohydrate and lipid oxidation. Adiponectin may be a key regulator of insulin sensitivity and energy metabolism. The aim of the present study was to determine the association of adiponectin, glucose metabolism (oxidation and storage) and lipid oxidation by applying the euglycemic clamp technique and indirect calorimetry.

The study was carried out on 68 young (age 26.38 ± 6.82 yr (mean$\pm$S.D.), BMI: 29.15 ± 7.24 kg/m^2 (mean$\pm$S.D.)) people. Anthropometric and biochemical parameters were measured and oral glucose tolerance test was performed. Plasma adiponectin was measured with radioimmunoassay (RIA) kit. Insulin sensitivity was evaluated with the euglycemic hyperinsulinemic clamp technique. Whole-body fat and carbohydrate oxidation was measured by indirect calorimetry at baseline (in the fasting state) and during last 30 minutes of the clamp. Nonoxidative glucose disposal rate was calculated by subtracting glucose oxidation rate from GDR.

Plasma adiponectin was positively related to insulin sensitivity ($r=0.477$, $P=0.000038$), glucose oxidation at the steady state ($r=0.326$, $P=0.006$) and non-oxidative glucose metabolism ($r=0.424$, $P=0.0003$) and was negatively associated with FFA at the end of the clamp and fat oxidation during hyperinsulinemia ($r=-0.0309$, $P=0.0137$ and $r=-0.260$, $P=0.031$). Insulin sensitivity was positively related to fat oxidation during fasting ($r=0.241$, $P=0.04$) and carbohydrate oxidation during last 30 minutes of the clamp ($r=0.308$, $P=0.001$).

We conclude that adiponectin modulates insulin sensitivity probably through influencing both oxidative and non-oxidative glucose metabolism.

P218

Relationships between serum adiponectin, interleukin 10 and interleukin 18 concentrations and muscle lipid fractions in healthy humans

Marek Straczkowski[1], Irina Kowalska[1], Agnieszka Nikolajuk[1], Marcin Baranowski[2], Agnieszka Adamska[1], Malgorzata Karolczuk-Zarachowicz[1], Monika Karczewska-Kupczewska[1], Piotr Zabielski[2], Agnieszka Blachnio[2], Jan Gorski[2] & Maria Gorska[1]
[1]Department of Endocrinology, Diabetology and Internal Medicine, Medical University of Bialystok, Bialystok, Poland; [2]Department of Physiology, Medical University of Bialystok, Bialystok, Poland.

Intramuscular lipids, including ceramide, might be responsible for the development of insulin resistance. Insulin action is also inversely associated with circulating proinflammatory cytokines, like interleukin (IL)-18 and positively related to antiinflammatory factors, like adiponectin and IL-10. The aim of the present study was to estimate the relationships between serum adiponectin, IL-10 and IL-18 concentrations and muscle lipid fractions in healthy humans.

The study group consisted of 37 male subjects with normal glucose tolerance, without morbid obesity or other serious medical problems. Euglycemic hyperinsulinemic clamp and a biopsy of vastus lateralis muscle were performed. Muscle ceramide, sphingosine and sphinganine content and the activities of the enzymes: neutral and acid sphingomyelinase, neutral and alkaline ceramidase and serine palmitoyltransferase were measured. Muscle free fatty acid (FFA), diacylglycerol (DAG) and triacylglycerol (TG) content was also assessed.

Insulin sensitivity was related to circulating cytokines (adiponectin, $r=0.38$, $P=0.021$; IL-10, $r=0.47$, $P=0.0034$; IL-18, $r=-0.37$, $P=0.023$) and to muscle lipids (ceramide, $r=-0.45$, $P=0.024$; DAG, $r=-0.43$, $P=0.031$; TG, $r=-0.52$; $P=0.01$). It was also associated with the activities of the enzymes regulating ceramide metabolism (serine palmitoyltransferase, $r=-0.58$, $P=0.002$; alkaline ceramidase, $r=-0.37$, $P=0.025$). Adiponectin was negatively related to muscle ceramide content ($r=-0.44$, $P=0.027$) and to serine palmitoyltransferase activity ($r=-0.35$, $P=0.032$). IL-10 and IL-18 were associated, in an opposite manner, with muscle DAG (IL-10, $r=-0.46$, $P=0.022$; IL-18, $r=0.40$, $P=0.049$) and muscle TG (IL-10, $r=-0.50$, $P=0.014$; IL-18, $r=0.46$, $P=0.026$). IL-10 was also related to muscle FFA pool ($r=-0.51$, $P=0.026$).

We conclude that there are multiple associations between circulating cytokines and muscle lipid pool, which possibly might influence insulin sensitivity. Adiponectin is related to muscle ceramide content, mostly through an association with de novo synthesis pathway. IL-10 and IL-18 are associated with muscle FFA-DAG-TG pathway.

P219

Prevalence of metabolic syndrome in old men and its relation to ghrelin

Sergio Rueda Alfaro[1], Mateu Serra-Prat[2], Cristián Fernández Fernández[1], Elisabet Palomera[2], Roser Casamitjana[1] & Manel Puig Domingo[1]
[1]Hospital Clinic i Provincial de Barcelona, Barcelona, Spain; [2]Hospital de Mataró, Mataró, Spain.

Aim

To study the prevalence of metabolic syndrome (MS) and its relation with ghrelin in old men.

Material and methods

Prospective-population based study (2002–2005) in which 153 independently living men older than 70 y were included. Comorbidities, physical exam, BMI, blood pressure were recorded and blood sample taken for biochemical and hormonal determinations. Metabolic syndrome was defined using IDF criteria.

Results

MS was found in 54.9%; BMI in non-MS individuals was 25.8 ± 3.3 and in MS was 28.3 ± 3.7 ($P<0.001$). No association was found between ghrelin and MS at basal evaluation (non-MS 1185 ± 445 vs MS 1106 ± 368; p:ns), even after weight adjustment.

At 3 years follow-up ghrelin level in MS were lower than in non-MS individuals (non-MS 1165.8 ± 356.0 vs MS 988.4 ± 245.8; P:0.004). Differences between ghrelin levels at the two time-points was only statistical significant in MS group (P:0,006). Ghrelin correlated with BMI ($r=-0.22$; $P=.023$) in subjects between 70–80 years and with creatinina <1.5 mg/dl. Also a correlation was found with HDL ($r=0.21$; $P=.012$). Multiple lineal regression analysis showed than age (beta$=-12.1$; $P=.049$), BMI (beta$=-22.0$; $P=.021$) and creatinine (beta$=407.7$; $P=.002$) had an independent effect on circulating ghrelin.

Conclusions

MS in old men is associated to a decrease in circulating ghrelin over time.

P220

Antipsychotic drugs and associated metabolic disorders

Kolcsár Melinda, Nagy Réka, Nagy Imre, László Annamária & Kun Imre Zoltán
U.M.Ph – Department of Pharmacology and Endocrinology, Tg.Mures, Romania.

Obesity is a major contributor to a range of metabolic disorders responsible for much of the medical morbidity and mortality. Increasing numbers of reports concerning not only obesity, but diabetes, hyperglycaemia and lipid dysregulation in patients treated with antipsychotics have raised concerns about a possible association between these metabolic effects and treatment with these medications. The objective of our study was to investigate the prevalence of obesity and other metabolic disorders in young patients treated with different antipsychotics and in the age matched general population.

Antropometric and metabolic data of the patients treated with psychotropics, hospitalized in the Endocrinology Clinic, Tg. Mures, between years 2001–2005 were compared with the data of persons selected among patients hospitalized in the same clinic and period, without psychotropic drug use. The frequency of patients treated with antipsychotics was 10.92% (4.33% typical antipsychotics and 6.59% atypical antipsychotics) with 43.1±13.6 years of mean age. In this patients the prevalence of obesity, elevated total cholesterol and triglyceride level was significantly higher than in the control group. The blood sugar didn't present difference between the two groups, but measuring HOMA-IR in 25 patients treated with atypical psychotropics and 20 other persons without treatment with psychotropic drug we found a significant difference between them. We concluded that a complete metabolic syndrome (MS) was present in 34.2% of the patients treated with antipsychotic drugs, while the frequency of MS was only 18.7% in the age matched patients group without any psychotropic drug use. Atypicial antipsychotics causes the most severe metabolic disorders in association with a significantly elevated prolactin level, when compared with the control group. The choice of a second generation antipsychotic for a given patient depends on many factors. The likehood of developing severe metabolic disease should also be an important consideration.

P221

Associations between thyroid function parameters and adipokines in euthyroid individuals

Laszlo Bajnok[1], Ildiko Seres[2], Zsuzsa Varga[2], Sara Jeges[1], Attila Peti[1], Zsolt Karanyi[2], Attila Juhasz[2], Eva Csongradi[2], Emese Mezosi[1], Endre V Nagy[2] & Gyorgy Paragh[2]
[1]School of Medicine, University of Pécs, Pécs, Hungary; [2]University of Debrecen, Medical and Health Science Center, Debrecen, Hungary.

Adipose tissue secreted hormonal mediators, adipokines play pivotal roles in the regulation of, among others, central nervous and immune systems influencing body weight, insulin action and inflammatory responses. The aim of our present study was to investigate possible associations of thyroid function with plasma levels of three of known adipokines, i.e. leptin, adiponectin and resistin in 74 Caucasian subjects without any endocrine diseases or related therapy. In order to create broad adipokine ranges, 3 age-, and sex-matched groups were formed: Group 1 and 2 consisted of non-diabetic obese patients ($n=25$ with BMI: 28–39.9 kg/m^2, $n=25$ with BMI $\geq$40 kg/m^2, respectively), while Group 3 of 24 healthy, normal weight control subjects. Level of TSH was correlated negatively with leptin ($r=-0.26$, $P<0.05$), while positively with adiponectin ($r=0.28$, $P<0.05$). Both were independent predictors of TSH level in a multiple regression model including BMI, age, gender, FT_3 or FT_4. But when both leptin and adiponectin were included into the model, only the latter remained significant. In opposite to TSH, level of FT_3 was negatively associated with adiponectin ($r=-0.27$, $P<0.05$) and showed a positive trend with leptin ($r=0.26$, $P=0.06$) of which the latter was independent predictor in multivariate analyses, beside age, BMI and FT_4. FT_4 was not correlated with any of adipokines. In univariate analysis, neither BMI, nor resistin was significantly correlated with thyroid function parameters.
In conclusion, in individuals without thyroid illness, leptin and adiponectin plasma levels are associated with TSH and FT_3 concentrations in opposite ways, and partly independently of anthropometric parameters. Adipokines may participate in the regulation of thyroid hormone axis.

P222

Human adipose tissue derived DPP-IV regulates lipolysis through NPY in cultured abdominal subcutaneous adipocytes

Katarina Kos, Adam Baker, Alison Harte, Philip McTernan, Paul O'Hare & Sudhesh Kumar
University of Warwick, Coventry, United Kingdom.

We have previously shown that the orexigenic hormone NPY is secreted by human adipocytes. The orexigenic hormone NPY(1–36) is truncated by the dipeptidyl-inhibitor IV (DPP-IV) to NPY(3–36) as consequence its affinity changes from receptor Y1 to Y4 and 5. The aim was to investigate whether DPP-IV is expressed in adipose tissue (AT) where it could modulate adipose tissue growth through modulation of NPY activity. This is relevant in light of DPP-IV inhibitors utilised as therapeutic agents and their use for treatment in Type 2 diabetes. For this purpose *ex vivo* human abdominal AT was taken from women undergoing elective surgery (BMI: 27.5(mean±S.D.)±5 kg/m^2, Age: 43.7±10 yrs, $n=18$). Isolated AbdSc adipocytes were treated with 1–100 nM rhNPY with and without DPP-IV inhibitors; a glycerol release assay was used as an index of lipolysis and DPP-IV mRNA expression assessed in AbdScAT. Treatment with NPY reduced glycerol release which was further blunted by co-incubation with DPP-IV inhibitors (baseline 234(mean±S.E.)±23 μmol/l, NPY100: 187±30μmol/l*; NPY100 with DPP-IV: 121±14 μmol/l**, *$P<0.01$, **$P<0.01$, $n=8$). Relative DPP-IV mRNA expression was reduced in AbScAT taken from obese subjects versus lean subjects (obese: 77±6 SU versus lean: 186±29 SU*, $n=10$).
In conclusion, paracrine effects of NPY may be modulated by AT-derived DPP-IV. Thus DPP-IV inhibitors may have little effect on tissue mass regulation in the obese where endogenous DPP-IV from AT is reduced, but may enhance fat accumulation in the lean through enhanced antilipolytic effects of NPY, which requires further study.

P223

The role of nitric oxide in pathogenesis of development of arterial hypertension during obesity

Diana Giorgadze[1], Ellen Giorgadze[1], Tamar Doliashvili[1], Tamar Sanikidze[2], Ketevan Asatiani[1], Ketevan Bochorishvili[1] & Arkadi Surmava[1]
[1]N4 clinical hospital, Tbilisi, Georgia; [2]State Medical University, Tbilisi, Georgia.

Background
The cardiovascular complication is the main cause of morbidity and mortality in obese patients. Endothelial dysfunction and atherosclerosis have the goal role in development of these diseases. The aim of our study was to reveal the role of nitric oxide during obesity associated arterial hypertension.
Subject and method
200 obese patients (age 35–55) were investigated. Control group comprised 25 healthy subjects. We calculated BMI, determined lipid profile, concentration of nitric oxide, activity of antioxidant enzymes – superoxiddismutase and katalaze, evaluated arterial pressure.
Results
Systolic arterial pressure insignificantly increased in overweight group ($n=50$) compared to control group (124.3±5.6 mm/hg), but significantly ($P<0,05$) – in patients with obesity of I ($n=50$) (134.4±11.7 mm/hg), II ($n=50$) (142.6±12.6 mm/hg) and III ($n=50$) (145.7±10.3 mm/hg) degree. Diastolic arterial pressure significantly ($P<0.05$) increased in patients with obesity of II (91.8±9.4 mm/hg) and III (95.6±7.2 mm/hg) degree compared to control group (81.4±6.2 mm/hg). According to weight gain the whole lipid profile (Chol, Trig, HDL, LDL) was damaged. Concentration of nitric oxide significantly reduced in obese subjects compared to control group. Significant decrease of nitric oxide in different BMI groups was revealed (overweight-11.875±0.427, I degree-11.2154±0.3113, II degree-10.2364±0.381, III degree-9.5±0.2823 $P<0,001$). Changes in concentration of NO correlated with decrease of antioxidant enzymes activity (enzymes activity decrease compared to control group and increase according to weight gain).
Conclusion
Hyper generation of oxygen causes inactivation of antioxidant enzymes and disorders in redox-status. NO oxidative degradation, stimulated by dyslipidemia, has the main role in the pathogenesis of arterial hypertension development during obesity.

P224

Effects of PGC-1α on endothelial function and apoptosis

Joong-Yeol Park[1], Jong Chul Won[1], Ki-Up Lee[1], Woo Je Lee[2], Kyung Soo Ko[2], Kee-Ho Song[3] & In-Kyu Lee[4]
[1]University of Ulsan College of Medicine, Seoul, South Korea; [2]Inje University College of Medicine, Seoul, South Korea; [3]Konkuk University School of Medicine, Seoul, South Korea; [4]Kyungpook National University, Daegu, South Korea.

Aims
Central obesity is associated with increased cardiovascular morbidity and mortality. It has been proposed that increased lipid accumulation in vascular tissue and the consequent increase in oxidative stress may be a missing link between obesity and atherosclerosis. The peroxisome proliferators-activated receptor (PPAR)-γ coactivator 1-α (PGC-1α) is a transcriptional coactivator playing an important role in energy metabolism. PGC-1α is present in vascular

cells, but its role in vascular endothelial cells has not been established. In this study, we examined the effect of adenoviral overexpression of PGC-1α (Ad-PGC-1α) in human aortic endothelial cells (HAECs) on apoptosis induced by linoleic acid (LA).
Methods
Effect of PGC-1 on HAECs apoptosis was evaluated by ELISA, WST-1 assay, and caspase activity. Using Ad-PGC-1 and ANT-1 siRNA, effect of PGC-1 and ANT-1 on reactive oxygen species (ROS) production, fatty acid oxidation (FAO) and mitochondrial membrane potential ($\Delta\psi m$) were analyzed.
Results
PGC-1α prevented LA-induced endothelial apoptosis. PGC-1α also reduced LA-induced increases of antioxidant enzyme expression and ROS accumulation at basal state. LA decreased the activity of adenosine nucleotide translocase (ANT), and increased $\Delta\psi m$. In the Ad-PGC-1α-infected HAECs, activity and the mRNA expression of ANT-1 were increased and LA did not increase $\Delta\psi m$. siRNA against ANT-1 reversed the changes induced by PGC-1α.
Conclusion
These data suggest that PGC-1α functions as a physiologic regulator of ROS generation in endothelial cells and that part of this effect is mediated by ANT-dependent increase in FAO.

P225

Changes in serum glucose metabolism and growth hormone, cortisol, prolactin, ghrelin, leptin concentrations in normal weight patients with schizophrenia before treatment with atypical antipsychotics

Mirjana Doknic[1], Nadja Maric[2], Sandra Pekic[1], Aleksandar Damjanovic[2], Dragana Miljic[1], Marko Stojanovic[1], Marina Djurovic[1], Miroslava Jasovic-Gasic[2], Carlos Dieguez[3], Felipe Casanueva[4] & Vera Popovic[1]
[1]Institute for Endocrinology, Diabetes and metabolic Diseases, Belgrade, United States; [2]Institute for Psychiatry, Belgrade, United States; [3]Departement of Physiology, School of Medicine, and Comlplejo Hospitalario Universitario de Santiago, Santiago de Compostela, Spain; [4]Departement of Medicine, School of Medicine, and Complejo Hospitalario Universitario de Santiago, Santiago de Compostela, Spain.

Schizophrenia is a devastating mental illness associated with obesity and diabetes mellitus rates that far exceed those of the general population.
The aim was to evaluate changes in positive energy balance (serum insulin, leptin and ghrelin) and hormones involved in neuroendocrine regulations (serum cortisol, growth hormone and prolactin) before treatment with atypical antypsyhotics (SGA) in normal weight patients with schizophrenia.
Thirty patients with schizophrenia (13 males, mean age 28.9 ± 1.3 years and BMI, 23.3 ± 0.6 kg/m^2) treated with antypsyhotics first generation were investigated in this study. They had neither other diseases. The control group included 27 healthy subjects (9 males, mean age 30.7 ± 1.9 years, BMI od 22.8 ± 0.6 kg/m^2). Positive family history for diabetes mellitus was similar between groups.
A oral glucose tolerance test (OGTT) with measuring glycemia, insulin, growth hormone and ghrelin was performed in all patients. Fasting samples for leptin, cortisol and prolactin were taken. Patients had normal fasting glucose levels but significantly higher peak glucose levels during OGTT as well as glucose area under the curve (AUC) than control subjects (746 ± 25 vs 650 ± 26 mmol/L/120 min; $P < 0.01$). Fasting insulin levels, as well as insulin AUC did not differ from control subjects at baseline ($P > 0.05$) but peak insulin values were significantly higher in patients with schizophrenia (95.1 ± 14.8 vs 52.2 ± 6.5 mU/L, $P < 0.05$). Growth hormone (GH) and ghrelin levels during OGTT, and leptin concentrations did not differ between patients and control subjects ($P > 0.05$). Cortisol levels (513.3 ± 29.1 vs 441.9 ± 24.3 nmol/L; $P < 0.05$) were higher in patients. Prolactin levels were higher in patients with schizophrenia than in control subjects (821 ± 135 vs 353 ± 45 mU/L; $P < 0.01$).
Normal weight patients with schizophrenia have already some abnormalities in glucose metabolism therapy and neuroendocrine responses (cortisol, prolactin) before SGA. Thus, shizophrenia could be *per se* risk factor for diabetes mellitus.

P226

Frequency of hypogonadism in males with type 2 diabetes and its relation with erectile dysfunction and obesity

Katherinne García-Malpartida, Rosa Casañ-Fernández, Jesús Yanini-García, Ana Jover-Fernández, Marcelino Gómez-Balaguer, Eva Solá-Izquierdo, Carlos Morillas-Ariño & Antonio Hernández-Mijares
Hospital Universitario Dr. Peset, Valencia, Spain.

Introduction
The aim of our study was to determine the frequency of hypogonadism (H) in males with type 2 diabetes (T2D) and its relation with erectile dysfunction (ED) and obesity.
Methods
We studied 107 diabetic males who came successively to an Endocrine consultation. The presence of H was determined by total testosterone (T) with an immunofluerescence method and free testosterone (fT) calculated with Vermeulen's equation, defining H if $T < 2$ ng/ml or $fT < 250$ pmol/l, with LH, FSH and prolactine in the normal range. We studied ED by means of the International Index of Erectile Dysfunction (IIEF) (questions 1 to 5 and 15 that determine ED). We excluded patients taking drugs that cause ED and those diagnosed of severe autonomic neuropathy. The anthropometic parameters analyzed were weight, height, waist perimeter and the calculated body mass index (BMI).
Results
We included 107 patients, aged 55.1 ± 7.8 years (range 39–70) with an average of duration of T2D of 8.2 ± 8.1 years (range 1–32). The frequency of H was 22.4%. The average of LH was 3.7 ± 1.7 mU/ml (range 1.1–9.5), FSH 5.1 ± 2.3 mU/ml (range 1.2–13.3) and prolactine 8.5 ± 2.9 ng/ml (range 2.9–16.5). ED was present in 66.7% of hypogonadal males and 66.7% of patients not presenting H. Patients with H had more weight (93.2 ± 11.9 vs 84.8 ± 13.8 kg, $P = 0.016$), more BMI (31.8 ± 3.8 vs 29.6 ± 3.8 kg/m^2, $P = 0.025$) and more waist perimeter (111.1 ± 9.2 vs 104.7 ± 10.7 cm, $P = 0.028$), compared to patients without H. The table below show the means of T and fT according to BMI:

BMI (kg/m^2)	<25	25–30	30–35	35–40	*P*-value
T (ng/ml)	5.9	5.1	4.5	3.8	<0.05
fT (pmol/l)	440.9	336.3	309.8	296.6	<0.05

Conclusions
The frequency of H is 22.4%. ED appears in the same proportion in patients with and without H. Hypogonadal patients are more obese and there is an inverse relation between BMI and T and fT.

P227

Acute phase reactants and soluble cell adhesion molecules are associated to plasma leptin levels in obese nondiabetic children

Barbara Garanty-Bogacka, Malgorzata Syrenicz, Anhelli Syrenicz, Aneta Gebala, Grazyna Dawid & Mieczyslaw Walczak
Pomeranian Medical University, Szczecin, Poland.

There are increasing evidences that leptin, a protein secreted by adipose tissue, may be an important factor contributing to the development of atherosclerosis. In this study, the relationship between plasma leptin levels and markers of inflammation and endothelial activation was investigated in 214 obese nondiabetic children and adolescents. Fasting levels of leptin, C-reactive protein (CRP), fibrinogen (FB), interleukin-6 (IL-6), interleukin-1β (IL-1β), intercellular cell adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), von Willebrand factor (vWF), glucose and insulin were determined. Insulin resistance was assessed by the homeostasis model. At multiple regression analysis leptin predicted IL-6, FB, ICAM-1, VCAM-1 and vWF independently of obesity measures and HOMA IR. There was a trend for association between leptin and CRP concentrations. Therefore, our findings showed that leptin levels is associated with inflammation and endothelial activation markers and in such way may promote the development of attherosclerosis relatively early in life

P228

Relationship between homocysteine level and low-grade systemic inflammation in obese children with metabolic syndrome

Justyna Syrenicz & Barbara Garanty-Bogacka
Pomeranian Medical University, Szczecin, Poland.

Obesity is an independent risk factor for the development of cardiovascular disease, frequently associated with various metabolic disorders defined as metabolic syndrome. High plasma total homocysteine (Hcy) concentration is

now well established as a powerful risk indicator for a wide range of vascular diseases.
The aim of this study was to investigate total Hcy levels in obese children and their possible association with both metabolic syndrome and various inflammatory biomarkers.
The study group consisted of 61 obese children, (aged 6–18 y.) with metabolic syndrome, defined according to NCEP-ATP III criteria and 122 obese counterparts without metabolic syndrome. Both group were comparable regarding to age, sex, and pubertal development.
The obese subject with metabolic syndrome presented significantly higher values for fasting insulin ($P<.001$), HOMA IR ($P<.001$), C-reactive proteine ($P<.01$), interleukin-6 ($P<.001$), interleukin-1ß ($P<.01$), and WBC ($P<.001$). In the group with metabolic syndrome plasma Hcy concentration was positively correlated with insulin ($P<.001$), HOMA IR ($P<.01$), C-reactive protein ($P<.001$), interleukin-6 (P,.01) and WBC (P,.05), but not in the group without metabolic syndrome.
Elevated plasma Hcy level in obese children with metabolic syndrome, may be causally involved in the pathogenesis of cardiovascular disease.

Obesity and metabolism – presented on Tuesday

P229

Oxidative stress and antioxidant defense is associated with adiposity in men among the urban population of south Iran

Fariborz Haghparast & Jaffar Nourooz-Zadeh
Islamic Azad University-Larestan Branch, Larestan, Fars, Iran.

Introduction
Changes in lifestyle have resulted in an increased number of boese subjects, and obesity is currently an important causative factor of health-related problems in Iran.
Aims
To investigate the direct relationship of oxidative stress and antioxidant status with obesity in men.
Materials and methods
We measured the plasma levels of malondialdehyde (MDA) as a marker of oxidative stress and vitamin E, glutathione and superoxide dismutase as antioxidants in 44 obese and 47 no obese men and evaluated their relationship with body mass index (BMI); body fat weight; waist-to-hip ratio (WHR).
Results
Compared with controls, obese men had a significantly higher body mass index (28.97 ± 2.42 vs. 16.03 ± 1.88 kg/m^2; $P=0.0002$) and waist-to-hip ratio (WHR) (0.89 ± 0.03 vs. 0.80 ± 0.01; $P=0.0004$); vitamin E, glutathione, superoxide dismutase, vitamin C levels were significantly decreased (all $P<0.05$), whereas MDA was significantly increased (114.9 ± 21.4 vs. 64.3 ± 14.2 nmol/L; $P=0.001$). MDA significantly correlated with BMI ($r=-0.34$ ($P=0.004$)) and WHR ($r=-0.63$ ($P=0.0001$)). We calculated the amount of vitamin E per LDL-cholesterol, total cholesterol and total lipids, we found all of them, significantly lower levels in obese men as compared to controls. There was also a significant correlation between the plasma levels of MDA and vitamin E, vitamin C, glutathione and superoxide dismutase in obese men and all men (all $P<0.01$).
Conclusion
In brief, these findings showed that the circulating levels of oxidative stress are related to adiposity in men. Although correlation does not prove causation, the results of this study suggest that obesity is an important factor for enhanced oxidative stress and important role of oxidative stress deleterious impact.

P230

Ghrelin basal levels in metabolic syndrome

Tatiana Mokhort
Belarusian State Medical University, Minsk, Belarus; State Research Center for Radiation Medicine and Human Ecology, Gomel, Belarus.

Ghrelin is known to play an important role in overweight formation and glucose metabolism regulation. The aim was to assess ghrelin basal secretion features in persons with metabolic syndrome (MS).
We examined 39 patients (age 35–55years) with MS (IDF criteria) and 28 healthy persons of comparable age. Ghrelin, insulin and C-peptide serum concentrations were measured by immunoenzyme method, lipid spectrum parameters - by spectrophotometry. For IR assessment we used HOMA-IR and Reciprocal of HOMA-IR indexes.
Basal insulinemia and C-peptide levels in S significantly exceeded the ones in healthy persons: 21.3 ± 3.86 vs 9.96 ± 1.18 mU/l and 2.86 ± 0.56 vs 1.28 ± 0.76 ng/ml. HOMA-IR in MS significantly exceeded the value of control group (5.03 ± 1.03 vs 2.06 ± 0.23). Reciprocal of HOMA-IR showed the opposite results. Ghrelin level was significantly lower in MS 61.06 ± 11.9 vs 88.76 ± 16.9 ng/ml in control group. Progressive decrease of ghrelin from 71.59 ± 7.09 to 50.34 ± 6.58 ng/ml was marked at BMI increase that is confirmed at correlation analysis: ghrelin levels negatively correlated with BMI ($r=0.41$; $P<0.05$), waist-to-hip ratio ($r=0.37$; $P<0.05$) and waist circumference ($r=0.39$; $P<0.05$). Ghrelin levels also showed negative correlation with systolic ($r=0.40$; $P<0.01$) and diastolic blood pressure ($r=0.39$; $P<0.01$).
We observed significant negative correlation of ghrelin and insulin ($r=0.18$), C-peptide ($r=0.15$), HOMA-IR ($r=0.23$) and positive with Reciprocal of HOMA-IR ($r=0.22$). We revealed significant negative correlation of ghrelin and atherogenecity index ($r=0.32$), while there was no significant connection with other parameters of lipid spectrum.
Conclusion
Progressive decrease of basal ghrelin levels with increase of BMI, waist-to-hip ratio and waist circumference was revealed that can testify to ghrelin influence on formation of visceral obesity. Obtained results are proved by negative correlation of ghrelin level with basal insulinemia, HOMA-IR and positive one with Reciprocal of HOMA-IR that confirms ghrelin role in formation of insulin resistance in MS and dictates essential necessity for further studies.

P231

Pioglitazone treatment significantly decreases 5-alpha reductase activity and improves metabolic risk factors in PCOS

Dorte Glintborg[1], Anne Pernille Hermann[1], Claus Hagen[1], Johannes Veldhuis[2], Anne Schmedes[3], Jan Frystyk[4], Allan Flyvbjerg[4] & Marianne Andersen[1]
[1]Department of Endocrinology and Metabolism, Odense University Hospital, DK-5000 Odense C, Denmark; [2]Division of Endocrinology and Metabolism, Department of Internal Medicine, Mayo Clinical Research Center, Rochester, United States; [3]Department of Clinical Chemistry, Vejle Hospital, DK-7100 Vejle, Denmark; [4]Medical Research Laboratories, Clinical Institute and Medical Department M (Diabetes and Endocrinology), Aarhus University Hospita, DK-8000 Aarhus, Denmark.

Objective
To investigate the effect of pioglitazone on cortisol metabolism in PCOS.
Design
Thirty insulin resistant PCOS patients were randomized to either 16 weeks of pioglitazone (30 mg/day) or placebo treatment. Before and after intervention, patients underwent 24 h 20 min.-integrated blood sampling for measurement of cortisol and 24 h excretion of cortisol, cortisone and steroid metabolites (cortisol, corticosteron, androgen, and 17-hydroxyprogesteron) were measured in urine. Fasting insulin, adiponectin, testosterone, dihydrotestosterone (DHT), and dehydroepiandrosteronsulfate (DHAS) was measured. 5-alfa reductase activity was evaluated by alloTHF/THF and androsteron/ethiocholanolon ratios. Delta values (Δ) denoted changes during the treatment period.
Results
Pioglitazone treatment was followed by significantly decreased 5-alfa reductase activity as evaluated by alloTHF/THF levels. Δ-androsteron/ethiocholanolon showed a significant negative correlation with Δ-IGF-I and Δ-peak GH level during PD-GHRH test. Furthermore, a significant negative correlation was found between Δ- alloTHF/THF and Δ- adiponectin levels.
No significant changes were measured in 24 h mean cortisol levels or urine excretion of cortisol, cortisone or steroid metabolites.
Insulin sensitivity, GH, adiponectin, and IGF-I was significantly increased during pioglitazone treatment.
Conclusion
Pioglitazone treatment was followed by significantly decreased 5-alfa reductase activity which was inversely correlated with IGF-I, GH, and adiponectin levels. These results suggest important relations between 5-alfa reductase activity and the GH/IGF-I system as well as metabolic risk factors.

P232

Plasma adiponectin and leptin levels in menopausal metabolic syndrome

David Virsaladze[1], Nona Adamia[1], Nino Charkviani[1], Ketevan Khitarishvili[1], Tea Giorgadze[1] & David Tananashvili[2]
[1]Tbilisi State medical University, Tbilisi, Georgia; [2]E. Andronikashvili Institute of Physics, Tbilisi, Georgia.

Objective
The aim of our investigation was the study of blood adiponectin and leptin levels in patients with menopausal metabolic syndrome (MMS) and their correlation with the parameters of MMS features.
Methods
40 females with menopausa have been investigated. In 38 cases diabetes mellitus type 2 has been registered, and in 2 – impaired glucose tolerance. Mean duration of postmenopausal period was 11.1 ± 7.4. Control group consisted of 10 females of postmenopusal age. The blood content of adiponectin and leptin was measured by ELISA. For MMS diagnostics WHO classification (2002) was applied.
Results
In basic group MMS was revealed in 37 patients, in control group – in 3 cases ($\chi^2 = 19.53$, $P < 0{,}001$). It was not observed significant difference in blood adiponectin levels of basic and control groups (16.4 ± 7.6 vs. 16.3 ± 6.1, $P =$ NS), but blood leptin level was significantly higher in investigated group in comparison with control (166.7 ± 105.3 vs. 60.3 ± 51.0, $P < 0.001$). It was revealed significant correlations of blood adiponectin and leptin levels with the parameters of MMS features.
Conclusions
Obtained results show that blood adiponectin level in MMS does not differed from control value. Blood leptin level is significantly higher than control one. They significantly correlated with the parameters of MMS features.

P233

Serum interleukin 6 and soluble form of interleukin 6 receptor concentrations in obese subjects with impaired glucose tolerance

Agnieszka Nikolajuk, Marek Straczkowski, Irina Kowalska, Agnieszka Adamska, Ida Kinalska & Maria Gorska
Department of Endocrinology, Diabetology and Internal Medicine, Medical University of Bialystok, Bialystok, Poland.

Background
Obesity is associated with an increased risk of impaired glucose tolerance and type 2 diabetes. Insulin resistance is the link between obesity and disturbances of glucose metabolism. It is suggested that some substances secreted by adipose tissue might play a role in the pathogenesis of insulin resistance. One of these substances is interleukin-6 (IL-6), cytokine, which regulates synthesis of the acute-phase proteins in the liver. The aim of the present study was to estimate serum IL-6, soluble form of IL-6 receptor (sIL6-R) and C-reactive protein concentrations (hs-CRP) in obese subjects with normal and impaired glucose tolerance.
Methods
The study group consisted of 107 subjects, 28 obese with impaired glucose tolerance (IGT), 44 obese with normal glucose tolerance (obese-NGT) and 35 lean healthy controls. Insulin sensitivity was measured with euglycemic hyperinsulinemic clamp technique. The protocol was approved by Ethics Committee of Medical University, and informed consent was obtained from each subject.
Results
IGT subjects had lower insulin sensitivity index in comparison to obese-NGT and controls (both $P < 0.000001$), and obese-NGT subjects had lower insulin sensitivity in comparison to controls ($P = 0.00043$). We found higher IL-6 and hs-CRP concentrations in IGT group in comparison to obese-NGT ($P = 0.042$ and $P = 0.041$ respectively) and to controls ($P = 0.00056$ and $P < 0.000001$ respectively). Differences in sIL-6R concentration between IGT subjects and the remaining groups were approaching the level of significance (obese-NGT, $P = 0.087$, control, $P = 0.066$). We found significant correlations between insulin sensitivity index and IL-6 ($r = -0.21$, $P = 0.029$), sIL-6R ($r = -0.19$, $P = 0.049$) and hs-CRP ($r = -0.34$, $P = 0.001$). IL-6, sIL-6R and hs-CRP were also associated with fasting insulin and with post load glucose and insulin concentrations. IL-6 and hs-CRP were also related to triglycerides and HbA1c and IL-6 was related to HDL-cholesterol.
Conclusions
Our data indicate that IL-6/sIL-6R system might play a role in the development of insulin resistance in obese subjects with IGT.

P234

Association of sex hormone-binding globulin (shbg) levels with measures of adiposity and metabolic profile in apparently healthy individuals

Katerina Saltiki[2], Paraskevi Voidonikola[3], Kimon Stamatelopoulos[3], Emily Mantzou[1], Eleftheria Chryssohoou[3], Christos Papamichael[3] & Maria Alevizaki[2]
[1]Endocrine Unit, Evgenidion Hospital, Athens University School of Medicine, Athens, Greece; [2]Dept Medical Therapeutics, Alexandra Hospital, Athens University School of Medicine, Athens, Greece; [3]Vascular Laboratory, Dept of Medical Therapeutics, Alexandra Hospital, Athens University School of Medicine, Athens, Greece.

Objective
The association of SHBG levels with obesity, hyperinsulinemia and metabolic abnormalities is well recognized in both men and women. Recent data suggest that SHBG levels are an important predictor of cardiovascular disease (CVD) risk. Several methods have been used for the measurement of adiposity including ultrasonography (U/S) which is a reliable and low-cost method. We used U/S to assess regional adiposity and investigated possible associations with SHBG levels.
Methods
309 apparently healthy individuals (124 men and 185 women, mean age 43.9 ± 9) without a history of diabetes or hypertension were examined for indices of the metabolic syndrome. None of the subjects was taking hormone therapy. The thickness of abdominal subcutaneous and peritoneal fat layer was estimated by U/S. Clinical parameters of obesity such as waist and hip circumference and BMI were recorded and SHBG, insulin, glucose and lipid levels were measured.
Results
SHBG levels were inversely correlated with peritoneal fat ($P = 0.003$) whereas there was no significant association with subcutaneous fat. Lower SHBG levels were associated with increased waist circumference, decreased hip circumference, increased BMI, higher HOMA - Insulin Resistance Index and insulin levels ($P < 0.02$). Step multivariate analysis showed that peritoneal fat, hip circumference and insulin levels were independently associated with SHBG levels. Significant associations were also found with age ($P = 0.047$).
Conclusions
Peritoneal but not subcutaneous adiposity, as assessed by U/S, is inversely associated with SHBG levels. U/S seems to be a simple, low-cost method for the assessment of central adiposity in apparently healthy individuals. SHBG levels, which have been recognized as a risk factor for CVD, are highly correlated with indices of either protective type (hip) or high-risk type (peritoneal and waist) regional adiposity, indirectly supporting the importance of regional adiposity to the risk for metabolic syndrome and cardiovascular disease.

P235

Comparative analysis of adiponectin, leptin and C-peptide levels in obese non-diabetic, type 1 diabetic and lean non-diabetic children

Olga Sysoyeva, Nataly Karlovich & Tatiana Mokhort
State Rehabilitation Centre, Department of Endocrinology, Minsk, Belarus.

A range of hormones which regulate energy metabolism are secreted by adipose tissue, among which adiponectin and leptin are the main adipokines regulating insulin sensitivity.

The aim of our study was to estimate and compare levels of adiponectin, leptin, C-peptide and adiponectin-to-leptin ratio (A/L) in obese non-diabetic, Type 1 diabetic (T1D) children and lean non-diabetic controls.

BMI and SDS BMI were calculated in 88 children (46f, 42m, age 14.8 ± 3.6 yrs): 32 pts with obesity, 34 pts with T1D, 22 lean non-diabetic persons. Serum levels of C-peptide, adiponectin and leptin were measured by ELISA.

Median adiponectin levels were higher in control group (22.1 mcg/ml; $P = 0.0001$) and T1D pts (21.1 mcg/ml; $P = 0.0002$) as compared with obese pts (12.6 mcg/ml). Leptin levels were higher in pts with obesity (41.2 ng/ml) as compared with control (1.8 ng/ml; $P < 0.000001$) and T1D pts (2.8 mcg/ml; $P < 0.000001$). Leptin levels in T1D pts were higher than in control group ($P = 0.027$), while adiponectin levels were practically the same.

Highest A/L ratio was in lean controls (11.6), lowest – in obese non-diabetic children (0.5), whereas in T1D pts A/L ratio was 6.6. Differences between groups were significant ($P < 0.05$).

We did not find significant correlation of adiponectin and leptin levels, adiponectin-to-leptin ratio with age at observation, BMI, C-peptide. At the same time adiponectin level and adiponectin-to-leptin ratio negatively correlate with BMI in T1D ($r = -0.37$, $P = 0.032$; $r = -0.35$, $P = 0.049$) and obese non-diabetic children ($r = -0.55$, $P = 0.001$; $r = 0.45$, $P = 0.011$).

Surprisingly, in obese non-diabetic pts we find significant correlation of adiponectin and age at observation ($r=-0.59$, $P=0.0004$).

We concluded that the older obese pts are, the lower adiponectin level is. Adiponectin-to-leptin ratio is a more useful marker of impaired adipokines secretion than adiponectin or leptin levels alone, though further study is necessary to prove reliability of this test for assessment of insulin sensitivity.

P236

Effects of pioglitazone and metformin on body weight and the insulin resistance parameters in young patients with obesity

Olya OLeksyk, Alexander Serhiyenko & Victoria Serhiyenko
Medical University, Lviv, Ukraine.

Background and Aims
The aim of this study was to evaluate the effects of pioglitazone (PGZ) and metformin (MET) plus Hypocaloric Diet (HD) in young patients with obesity and impaired oral glucose-tolerance test (75 g glucose).
Materials and methods
49 patients (17.1 ± 1.2 yrs) were allocated in groups: A ($n=14$) received PGZ 30 mg tid plus HD, B ($n=12$) - MET 1000 mg tid plus HD, C - PGZ 30 mg plus MET 1000 mg plus HD ($n=11$), D ($n=12$) were only on HD. The duration of the study was 3 months. We investigated Body Mass Index (BMI), triglyceridaemia (TG), Systolic (SBP), Diastolic Blood Pressure (DBP), insulin resistance (IR) parameters: Homeostasis Model Assesment (HOMA) - IR index; HOMA - β-cell function (HOMA-β-CF). Statitics: ANOVA.
Results
The increase of BMI, W/H, pre- and postprandial TG, HOMA-IR index ($P<0.05$), SBP and DPB ($P<0.05$) parameters, the decrease of HOMA-β-CF ($P<0.05$) were observed. PZG lead to the decrease of postprandial TG, HOMA-IR ($P<0.05$), some increase of BMI, improvement of HOMA-βCF and did not significantly influence SBP, DBP. MET was accompanied by the decrease of BMI ($P<0.05$), postprandial TG ($P<0.05$), SDP, DBP ($P<0.05$), but in a smaller degree, than PZG. The combined administration of PZG and MET lead to more expressed positive dynamics of investigated parameters. In particular, BMI made 26.4 ± 3.6 ($P<0.05$), HOMA IR index, HOMA-βCF 0.28 ± 0.004 ($P<0.01$), postprandial TG 1.77 ± 0.03 ($P<0.01$), SBP ($P<0.05$), DBP 85.4 ± 2.5 ($P<0.05$). The use of HD only lead only to some decrease of BMI ($P<0.05$).
Conclusion
The administration of PZG and MET in young patients with obesity and impaired OGTT is accompanied with more expressed positive dynamics of IR parameters, that allows to recommend their use in such patients.

P237

Plasma visfatin levels during oral glucose tolerance test in obese women

Dragan Micic[1], Mirjana Sumarac-Dumanovic[1], Aleksandra Kendereski[1], Goran Cvijovic[1], Danica Stamenkovic-Pejkovic[1], Maja Georgiev[1], Svetlana Zoric[1], Jagoda Jorga[1], Carlos Dieguez[2] & Felipe Casanueva[2]
[1]Institute of Endocrinology, Diabetes and Diseases of Metabolism, Clinical Center of Serbia, Belgrade, Serbia; [2]Department of Endocrinology, University of Santiago De Compostela, Santiago de Compostela, Spain.

Visfatin is expressed in visceral adipose tissue and is up regulated in some animal models of obesity. Insulin-mimetic actions of visfatin may be part of the feedback regulation of glucose homeostasis, so plasma glucose or insulin may have effect on visfatin levels in humans. The aim of study was to investigate plasma glucose, insulin and visfatin during oral glucose tolerance test (OGTT, 75 gr) in obese women. 22 obese women (age: 36.73 ± 1.88 yrs; BMI 34.72 ± 0.67 kg/m^2) were studied. Plasma visfatin (EIA Phoenix, ng/ml), adiponectin and leptin (Linco RIA, ng/ml), insulin (RIA Inep, mU/l) and glucose (mmol/l) were measured in basal state, while additional visfatin and insulin were measured at the peak glucose during OGTT. Insulin sensitivity (M index:mg/kgBW/min) was measured using euglycemic 2 hr clamp. Basal glucose was 4.78 ± 0.10 and peak glucose during OGTT 8.20 ± 0.42 ($P<0.005$). There were no significant differences in visfatin between basal sample and at the peak glucose levels (72.26 ± 3.34 vs. 79.46 ± 7.15, $P>0.05$). Basal insulin was 16.57 ± 1.28 and at the peak glucose 97.88 ± 13.01 ($P<0.05$). After analysis of the individual data we found that 7 obese women (Group A) had significant decrease (44.77 ± 3.87 vs.36.19 ± 8.42, $P<0.05$) and 15 women (Group B) had significant increase in visfatin during OGTT (69.85 ± 4.49 vs. 99.66 ± 2.59, $P<0.05$). There were no significant difference between Group A and B in BMI (34.85 ± 1.12 vs. 34.65 ± 0.87), age (36.00 ± 4.64 vs. 37.07 ± 1.81), basal glucose (4.91 ± 0.26 vs. 4.73 ± 0.09), basal insulin (14.73 ± 1.82 vs. 17.43 ± 1.67), adiponectin (5.90 ± 3.19 vs. 10.97 ± 2.94), leptin (34.66 ± 6.34 vs. 33.09 ± 3.45), peak glucose (8.96 ± 1.10 vs. 7.85 ± 0.36), insulin at peak glucose (80.54 ± 20.78 vs. 105.97 ± 16.46) neither in insulin sensitivity (5.51 ± 0.87 vs. 4.81 ± 0.64). Our data demonstrate existence of two type of visfatin response during OGTT in obese women. It is still not clear which influence determines different type of visfatin response during OGTT and further studies are necessary to elucidate these mechanisms.

P238

The association of high sensitivity C-reactive protein levels with body fat mass and body fat distribution

Deniz Gokalp[1], Alpaslan Tuzcu[1], Hatice Akay[2], Senay Arikan[1] & Mithat Bahceci[1]
[1]Dicle University School of Medicine Department of Endocrinology, Diyarbakir, Turkey; [2]Dicle University School of Medicine Department of Radiology, Diyarbakir, Turkey.

Background and aim
C-reactive protein (CRP) is a sensitive marker for systemic inflammation. In this study we aimed to investigate the relationship between high-sensitivity C-reactive protein (hs-CRP) levels and BMI, body fat mass and fat distribution in healthy subjects.
Subjects and methods
A total 117 healthy subjects aged with 20–68 yr [normal weight (BMI 18.5–25.0 kg/m^2, *n*:35), overweight (BMI: 25–30 kg/m^2, *n*:27) and obese (BMI $\geq$30.0, *n*: 55)] were included to the study. Body weigt, BMI, waist and hip circumferences, skinfolds (biceps, triceps, suprailiac and subscapular region) with skinfold caliper and ultrasonography and body fat mass with bioelectric impedance of all subjects were measured. Hs-CRP concentrations were measured with immunometric assay. Analysis of variance, post hoc Benferoni test and Pearson correlation test were used for statistical analysis.
Results
Mean serum hs-CRP levels of obese group determined with BMI were higher than overweight and normal weight groups (7.3 ± 5.46, 2.5 ± 3.13, 0.66 ± 1.1, respectively, $P=0.0001$). Mean serum hs-CRP levels of overweight group was not different normal weight groups. In addition hs-CRP levels were positively correlated with BMI, waist and hip circumferences, fat mass and skinfold thickness of all 4 regions. Al data were shown in Table 1.
Conclusions
1-Hs-CRP level is high in obese patients and there was close relationship between BMI and HS-CRP serum levels. 2-Both waist and hip circumference positively correlated with hs-CRP level, these data suggest that not only android obesity but also gineoid obesity increased hs-CRP levels. 3-Skinfold thicknesses were useful methods in clinical practice and they were also positively correlated hs-CRP levels

	BMI (kg/m^2)	Waist crcum. Cm	Hip circum. (cm)	Fatmass (kg)
*Hscrp(mg/*dL	r=0.335	r=0.339	r=0.396	r=0.428
	P=0.0001	P=0.0001	P=0.0001	P=0.0001
		Skinfold	Thickness (cm)	
	Biceps	Triceps	Suprailiac	Subscapular
Hs-crp	r=0.195	r=0.358	r=0.384	r=0.376
	P=0.037	P=0.0001	P=0.0001	P=0.0001
	Biceps(USG)	Triceps(USG)	Suprailiac(USG)	Subscapular(USG)
Hs-crp	r=0.261	r=0.243	r=0.331	r=0.309
	P=0.005	P=0.008	P=0.0001	P=0.001

P239

Innervation of white and brown adipose tissue: dual viral transneuronal tracing study

Ádám Dénes[1], Miklós Darida[1], Zsolt Boldogkoi[2] & Krisztina Kovacs[1]
[1]Institute of Experimental Medicine, Budapest, Hungary; [2]Dept. Biology, University of Szeged, Szeged, Hungary.

Central control of body weight involves coordinated regulation of food intake and energy metabolism. White (WAT) and brown (BAT) adipose tissue represent functionally distinct compartments of lipid storage and fuel consumption, respectively. Both adipose tissues are innervated by the sympathetic nervous system. Tyrosine hydroxylase positive fibers were found in between fat cells. To determine the extent to which the control of different fat compartments is provided by the same pre-autonomic neurons, the central circuit innervating WAT and BAT was compared by dual viral transneuronal tracing using isogenic recombinant strains of the pseudorabies virus. BDL, expressing beta galactosidase was injected to the epididymal WAT and BDG, expressing green fluorescent protein was inoculated into the intrascapular BAT of male rats and virus reporter proteins were revealed by immunocytochemistry. In the spinal cord, BDG infected neurons were found in the intermediolateral and central autonomic nuclei of the upper thoracic segments, while BDL infection appeared in the lower thoracic and lumbar levels. Several brainstem pre-autonomic areas (C1, A5) and the gigantocellular reticular nucleus contained BDG and BDL infected neurons, but relatively few neurons were infected by both viruses. In the dorsal motor nucleus of the vagus, the periaqueductal gray matter, as well as in the dorsomedial, ventromedial, paraventricular hypothalamic nuclei and in the lateral hypothalamic area, anatomically distinct sub-regions were infected by the two recombinant viruses. Following administration of the mixture of BDG and BDL into the WAT, over 70% of the infected neurons contained both recombinant viruses. Our data suggest that neurons involved in the regulation of WAT and BAT coexist in all areas involved in the control of sympathetic outflow, although the relative proportion of these neurons vary across the regions. Double-labeled neurons may represent central command neurons that direct coordinated responses of WAT and BAT to metabolic challenges.

P240

Identification of orexin receptors in brown adipocytes: functional effects of orexin-B

Janet Digby[1], Jing Chen[1], Danijela Markovic[1], Say Viengchareun[2], Marc Lombes[2], Hendrik Lehnert[1] & Harpal Randeva[1]
[1]University of Warwick, Medical School, Coventry, United Kingdom; [2]INSERM, Paris, France.

Objective
Orexin-A and orexin-B and their G-protein coupled receptors (orexin receptor-1 & -2: OX1R, OX2R) have divergent effects on physiological behaviour, cardiovascular regulation, glucocorticoid and insulin release. Furthermore, orexins have been shown to affect both brown adipose tissue energy expenditure and thermogenesis through stimulation of sympathetic nerve activity. Despite *in vivo* studies demonstrating a role for orexins acting centrally on adipose tissue, there are no data on the expression of orexin receptors in brown adipose tissue. We therefore analyzed the expression and localization of OX1R and OX2R in mouse brown adipocytes and in the T37i brown adipocyte cell line. Furthermore, the effects of exposure to orexin-A and orexin-B were measured on the expression of key genes involved in thermoregulation and insulin sensitivity; leptin, uncoupling protein-1 (UCP-1), adipocyte-specific fatty acid binding protein-2 (AP2) and PPARγ.
Methods
Quantitative real time RT-PCR was performed using a Roche Light Cycler™ system, and genes of interest were standardised against the housekeeping gene β-actin. OX1R and OX2R were detected in differentiated T37i brown adipocytes using immunocytochemistry and confocal microscopy.
Results
mRNA expression was detected for OX1R and OX2R in mouse mature interscapular brown adipocytes, as well as in differentiated T37i brown adipocytes *in vitro*. Furthermore, mRNA expression of both receptors increased as a function of the degree of differentiation. Confocal analysis revealed intense localised staining for OX1R around intracellular lipid droplets, whereas more membrane-localised staining was observed for OX2R. T37i brown adipocytes treated with orexin-B (100 nM, 4 h), resulted in significant increases in leptin, UCP-1, AP2 and PPARγ mRNA ($P<0.05$).
Conclusions
These novel findings indicate a direct role for orexin-B in brown adipocyte tissue metabolism and thermogenesis and the potential to affect insulin-sensitivity. Furthermore, the differing cellular receptor localisation suggests divergent roles for orexins in brown adipocytes.

P241

Waist circumference and BMI as predictors of arterial hypertension in childhood and adolescence in Latvia

Inese Madrevica, Juris Krikis & Renate Ligere
Latvian University, Riga, Latvia.

Child and adolescent adiposity is a problem of major concern not only for Europe, but also for the world at large. Increase of waist circumference, BMI and arterial blood pressure are metabolic syndrome risk factors which contribute to the development of cardiovascular disease, hypertension and diabetes mellitus.

To determine whether changes in arterial blood pressure are related to the increase of waist circumference and BMI in childhood and adolescence.

We examined 1049 schoolchildren (aged 7–18), 535 of whom were included in the study. In the risk group 41 schoolchildren were observed. For the study special questionnaires including 25–28 metabolism parameters were used. The obtained data were processed with the SPSS software packages (BMDP and *Systat* 9) adapted for biological and medical studies. We also determined the insulin resistance (Caro *et al.*, 1991) and the insulin resistance index (Dunkan *et al.*, 1995).

In our study elevated arterial blood pressure for boys and girls rather correlated with BMI ($n=532$; $r=0.449$; $P=0.000$) than with the increase of waist circumference ($n=532$; $r=0.427$; $P=0.000$), whereas in the risk group arterial blood pressure for both boys and girls more closely correlated with waist circumference ($n=39$; $r=0.403$; $P<0.05$). In the child and adolescent risk group both waist circumference and BMI have a negative correlation with the blood glucose level ($n=39$; $r=-0.432$; $P=0.000$). BMI also negatively correlates with insulin resistance in the risk group ($n=39$; $r=-0.339$; $P<0.05$).

Elevation of arterial blood pressure in children and adolescents strongly correlates with increase of both waist circumference and BMI. In assessing the metabolic syndrome risk factors for children and adolescents both waist circumference and BMI should be taken into account when working out early metabolic syndrome criteria for children and adolescents.

P242

Uric acid is an important predictor of metabolic disturbances in obese women

Ayse Sertkaya Cikim, Taner Bayraktaroglu, Adil Dogan Azezli & Yusuf Orhan
Istanbul University, Istanbul Faculty of Medicine, Endocrinology and Metabolism, Capa, Fatih/Istanbul, Turkey.

Introduction
It was shown that a relationship between uric acid and cardiovascular diseases, and hyperuricemia is associated with systemic inflammation. But, determination of uric acid is widely available and inexpensive, it has been overlooked as a marker of systemic inflammation and metabolic disturbances. In this study, we aimed to evaluate uric acid value and its association with inflammation and metabolic disturbances in overweight and obese Turkish women.
Material and methods
The study population consisted of 3975 women with BMI of 25 kg/m^2 or greater, classified as overweight (BMI$>=$25 kg/m^2, $n=771$) or obese (BMI$>=$ 30 kg/m2, $n=3204$) by National Institutes of Health and WHO criteria. They divided two groups according to median uric acid levels. Demographic and anthropometric characteristics, blood glucose, insulin and lipid concentrations, and the indices of insulin resistance and inflammation were determined and compared between groups.
Findings
Median uric acid level was 4.40 mg/dl. Therefore, our patients were divided two groups according to median uric acid levels; i.e. 4.40 mg/dl, group 1(women with low uric acid levels; ‹ 4.40 mg/dl) and group 2 (women with high uric acid levels; › 4.40 mg/dl). And metabolic parameters in group 2 having higher uric acid levels were significantly different and disturbed than group 1 with lower uric acid levels.
Conclusion
In this study, we found a significant difference in various metabolic and inflammatory parameters among different uric acid levels groups. The women with high uric acid groups have had high metabolic and inflammatory markers. These findings suggest that the relationship between uric acid and inflammatory markers. However. The nature of such a relationship remains unknown. These findings support the hypothesis that uric acid may negatively impact on metabolic parameters.

P243

Fasting and postprandial plasma obestatin levels are reduced in obesity

Christina Maier[1], Dominik Haider[2], Greisa Vila[1], Michaela Riedl[1], Anton Luger[1] & Michael Wolzt[2]
[1]Department of Internal Medicine III, Division of Endocrinology and Metabolism, Medical University of Vienna, Vienna, Austria; [2]Department of Clinical Pharmacology, Medical University of Vienna, Vienna, Austria.

Obestatin has recently been identified as a peptide derived from pre-proghrelin that opposes ghrelin effects on appetite and body weight in rodents. We studied the effect of food intake on both these hormones in obese and lean subjects and recorded in parallel the subjective sensations of satiety and hunger. Eight obese (two males and six females, BMI = 31–52 kg/m^2) and eight age- and sex-matched lean subjects (BMI = 19–23 kg/m^2) were randomized to 1) take a standard breakfast and 2) time control studies after an overnight fast in a prospective cross-over study design. Obestatin and ghrelin plasma concentrations were quantified by radioimmunoassays, satiety and hunger by visual analogue scales.

Basal circulating obestatin was significantly decreased in obese as compared to lean humans and stable in both study groups during an observation period of 90 minutes. Thirty minutes after food intake, obestatin levels were markly reduced in obese subjects, but increased in lean controls. There was no correlation between ghrelin and obestatin postprandial plasma concentrations. Subjective ratings of satiety and hunger were significantly related to obestatin plasma concentrations only in lean subjects.

We conclude that obestatin concentrations are much lower in obese subjects and inversely regulated by food intake, as compared to lean subjects. Both fasting and postprandial suppression of the anorexigenic obestatin might be of relevance in the pathophysiology of the positive energy balance associated with obesity.

P244

Insulin resistance and insulin secretion in morbidly obese patients before and six months after bariatric surgery

Miriam Promintzer[1], Gerhard Prager[2], Marietta Stadler[3], Christian Anderwald[1], Martina Mandl[1], Soheila Shakeri Manesch[2], Peter Nowotny[1], Martin Georg Bischof[1], Bernhard Ludvik[1], Anton Luger[1] & Michael Krebs[1]
[1]Division of Endocrinology and Metabolism, Department of Internal Medicine III, Medical University of Vienna, Vienna, Austria; [2]Department of Surgery, Medical University of Vienna, Vienna, Austria; [3]Thrid Medical Department of Metabolic Diseases and Nephrology, Lainz Hospital, Ludwig Boltzmann Institute of Metabolic Diseases and Nutrition, Vienna, Vienna, Austria.

Recent case reports describe hyperinsulinemic hypoglycemia after gastric bypass. The aim of this study was the assessment of insulin resistance and insulin secretion in non-diabetic morbidly obese patients before and six months after bariatric surgery.

In 8 non-diabetic morbidly obese patients (OB:6f/2 m, age:42 ± 3a, BMI:47.29 ± 2.2 kg/m^2) and 6 controls (CON:4f/2 m, age:43 ± 0a, BMI:23.8 ± 0.5 kg/m^2) we performed a frequently sampled oral glucose tolerance test (75 g, 3-hOGTT). The OGTT was repeated in 4 patients (3 Roux-en-Y gastric bypass, 1 gastric band) 6 months after surgery. Before bariatric surgery fasting plasma levels of glucose were comparable between OB and CON while fasting insulin and c-peptide were higher in OB (insulin:OB:27.0 ± 5.6/CON:7.0 ± 0.6 μU/ml, $P = 0.01$; c-peptide:OB:4.3 ± 0.7/ CON:1.3 ± 0.1 ng/ml, $P = 0.003$). During the OGTT peak plasma glucose and insulin concentrations were significantly higher in OB (glucose: OB:196.1 ± 15.6/CON:130.5 ± 9.6 mg/dl, $P = 0{,}006$; insulin:OB:119.7 ± 21.6/CON:58.6 ± 9 μU/ml, $P = 0.039$). 6 months after bariatric surgery fasting and early postprandial glucose concentrations were unchanged, while insulin and c-peptide were lower at fasting and higher after glucose load. Insulin resistance, assessed by HOMA-IR and OGIS, improved after bariatric surgery. After glucose load insulin and c-peptide secretion was adapted to insulin resistance prior surgery but was excessively elevated after bariatric surgery (adaptation index: before:119 ± 16/after surgery:228 ± 53, $P < 0.05$, CON:114.5 ± 19.6 total-nmol*m-2, $P = 0.8$, for before surgery vs. CON). Conclusion: Non-diabetic morbidly obese patients exhibit preserved adaptation of insulin secretion to severe insulin resistance. Six months after bariatric surgery elevated fasting insulin and c-peptide were normalized. In the early postprandial state, however, hyperglycemia remained unchanged, while secretion of insulin and c-peptide was excessive and not adapted to improved insulin resistance. Thus, this dissociation between increase of insulin secretion on the one hand and amelioration of insulin resistance on the other hand might put patients at risk for late postprandial hypoglycemia.

P245

Growth hormone Reduces Inflammation in Postmenopausal Women with Abdominal Obesity: a 12-month randomized placebo-controlled trial

Celina Franco[1], Björn Andersson[2], Lars Lönn[3], Bengt-Åke Bengtsson[1], Johan Svensson[1] & Gudmundur Johannsson[1]
[1]Research Center for Endocrinology and Metabolism, Sahlgrenska University Hospital, SE-413 45, Göteborg, Sweden; [2]Department of Medicine, Sahlgrenska University Hospital, SE-413 45, Göteborg, Sweden; [3]Department of Diagnostic Radiology, Sahlgrenska University Hospital, SE-413 45, Göteborg, Sweden.

Context
Abdominally obese individuals have relative hyposomatotropism, elevated serum markers of inflammation, and increased risk of cardiovascular disease (CVD).
Objective
The aim was to study the effect of GH treatment on serum levels of inflammatory markers and vascular adhesion molecules in postmenopausal women with abdominal obesity.
Design
Forty postmenopausal women aged 51–63 yrs with abdominal obesity received GH (0.67 mg/d) in a randomized, double blind, placebo controlled 12-month trial. Measurements of inflammatory markers in serum: interleukin-6 (IL-6), highly sensitive C-reactive protein (CRP), and amyloid polypeptide A (SAA), and markers of endothelial function: selectin, vascular adhesion molecule-1 (VCAM-1), intercellular molecule-1 (ICAM-1) were performed at baseline and after 6 and 12 months of treatment.
Results
The GH and placebo group were comparable at baseline in terms of age, BMI, waist circumference, IGF-1, smoking habits and antihypertensive treatment. After 12 months, mean IGF-I SD score was 0.9 ± 1.5 and − 0.8 ± 0.6 in the GH and placebo groups, respectively. The 12-month GH treatment reduced serum levels of CRP and IL-6 as compared with placebo ($P = 0.03$ and $P = 0.05$, respectively), whereas the markers of endothelial function were unaffected. Within the GH treated group, serum CRP level showed a reduction from 4.3 ± 4 at baseline to 3.0 ± 3 mg/L after 12 months ($P = 0.05$) and serum IL-6 level was reduced from 4.4 ± 2 to 3.3 ± 2 ng/L ($P < 0.01$).
Conclusion
GH treatment in postmenopausal women with abdominal obesity reduced serum levels of inflammatory markers, suggesting that the risk of CVD was reduced. There was no detectable effect of the GH treatment on endothelial function evaluated using measures of vascular adhesion molecule levels in serum.

P246

Hyperactivity of the hypothalamic-pituitary-axis and adrenal hyperandrogenism in polycystic ovary syndrome: a consequence of 5 β-reductase hyperfunction

Alessandra Gambineri, Laura Patton, Giulia Forlani, Alessandra Munarini, Federica Tomassoni, Uberto Pagotto & Renato Pasquali
Endocrinology Unit, Department of Internal Medicine, S. Orsola-Malpighi Hospital, University of Bologna, Bologna, Italy.

Among the uncertainties surrounding the etiology of polycystic ovary syndrome (PCOS) the role of increased peripheral cortisol metabolism has become interesting, particularly in relationship to the pathogenesis of adrenal hyperandrogenism. The patways of cortisol metabolism include irreversible inactivation of cortisol by 5α- and 5β-reductase. To evaluate the association of 5α- and 5β-reductase activity with adrenal hyperandrogenism in PCOS, we recruited 90 PCOS women (age range: 18–45 years) classified into three groups accordingly to the responsiveness of androstenedione (A) and DHEA to $_{1-24}$ACTH: group of low responders (LR) ($n = 27$), defined by A and DHEA responsiveness to $_{1-24}$ACTH within 2 SD of mean of a group of controls; group of medium responders (MR) ($n = 43$), defined by A or DHEA responsiveness to $_{1-24}$ACTH over 2 SD; group of high responders (HR) ($n = 20$), defined by A and DHEA responsiveness to $_{1-24}$ACTH over 2 SD. Excretion of cortisol and its metabolites was measured by electron impact gas chromatography-mass spectrometry in a 24-h urine collection. Relative 5α- and 5β-reduction of cortisol was assessed by 5α-tetrahydrocortisol (5α-THF)/cortisol, and 5β-THF/cortisol and 5β-tetrahydrocortisone (THE)/cortisone, respectively. The three groups were similar for age, body weight and body fat distribution. Testosterone, A and 17OH-progesterone basal levels were also similar among the three groups, whereas DHEA-S was significantly higher in MR ($P < 0.05$) and more in HR ($P < 0.01$) respect to NR. HR presented also basal cortisol levels significantly lower and cortisol responsiveness to $_{1-24}$ACTH significantly higher than MR ($P < 0.01$) and

NR ($P<0.001$, $P<0.05$). 5β-THF/cortisol and 5β-THE/cortisone were significantly higher in HR respect to MR and NR ($P<0.05$). No differences in 5α-THF/cortisol were observed among the three groups. These data open up the intriguing possibility of 5 β-reductase hyperfunction as a new pathogenetic mechanism of adrenal hyperandrogenism in a subgroup of PCOS women.

P247

An examination of the prevalence of IDF and ATPIII defined metabolic syndrome: towards population based screening

Deirdre Waterhouse, Jayant Sharma, Frances Sheehan & Donal O'Shea
St Vincent's University Hospital, Dublin, Ireland.

Introduction
Despite the significant associated cardiovascular morbidity, as well as the significant economic implications, little consideration has been given towards population screening for the Metabolic Syndrome (MetS). Therefore, we wished to estimate the prevalence of MetS, using both the recently published IDF, as well as the previously defined Cholesterol Education Program Adult Treatment Panel III (ATPIII), criteria. Additionally, we hypothesised that simple, inexpensive anthropometric measurements offer an effective means of population based assessment for MetS.
Methods
1716 participants (1026 males, 690 females) underwent full cardiovascular assessment over a twelve month period, including detailed questionnaire, measurement of waist circumference, BMI calculation, sphygomanometry and fasting glucose and lipid profiling. Subsequently, the prevalence of the MetS was defined in accordance with both the IDF and ATPIII definitions.
Results
The prevalence of the MetS was 21.4% ($n=368$) and 13.2% ($n=227$) in accordance with IDF and ATPIII criteria respectively. Subjects identified using IDF criteria had significantly lower waist circumference ($P=0.006$) as well as significantly increased HDL cholesterol ($P=0.008$) when compared to the ATPIII cohort.The prevalence of IDF defined central obesity in our cohort was 56.8% ($n=975$); of these 37.5% ($n=368$) had MetS. The prevalence of MetS within this obese hypertensive cohort was 57.3% ($n=328$). Thus, concurrent central obesity and hypertension would identify 89.1% of the total IDF defined MetS in the population.
Conclusion
When compared to the previous ATPIII criteria, the IDF definition identifies an additional cohort of individuals with metabolic risk factor clustering despite a significantly leaner waist circumference. This leads to a higher prevalence of IDF-defined MetS. Finally, the coexistence of central adiposity and hypertension was noted in the majority of patients with MetS. This simple dysanthropometric phenomenon may potentially be used as an inexpensive means of population assessment for MetS.

P248

Neuroendocrine and genetic aspects of metabolic disturbances in women with simple obesity, polycystic ovary syndrome (PCOS) and eating disorders

Agnieszka Baranowska-Bik[1], Wojciech Bik[1], Ewa Wolinska-Witort[1], Pawel Gaj[2], Michal Mikula[2], Piotr Bragoszewski[2], Jerzy Ostrowski[2] & Boguslawa Baranowska[1]
[1]Medical Centre of Postgraduate Education, Neuroendocrinology Department, Warsaw, Poland; [2]Medical Centre of Postgraduate Education, Department of Gastroenterology, Warsaw, Poland.

Neuropeptides and adipocytokines influence metabolic homeostasis and food intake. Deregulation in their secretion leads to insulin resistance or metabolic syndrome. Adiponectin possesses anti-diabetic and insulin sensitizing properties. Expression of this gene remains under control of nuclear peroxisome proliferator-activated receptor (PPAR)-gamma. Ghrelin, an endogenous ligand for GH secratagogue receptor (GHSR), modulates metabolic homeostasis. A high amino-acid homology and transmembrane localization of G-protein coupled receptor 39 (GPR39) and GHSR suggest that ghrelin secretion can be modified by GPR39. Genetic variation found in genomic DNA sequences is a potentially important factor regulating expression level of mentioned genes. We evaluated the role of genetic factors and relationship between metabolic alterations and plasma adiponectin, ghrelin and leptin levels in women with simple obesity, PCOS (non-obese) and anorexia nervosa (AN).

The study consisted of 142 women (109 patients and 33 healthy lean controls) in similar age and was approved by the Local Ethics Committee. For SNP (single nucleotide polymorphism) analyses we genotyped all women for: (PPAR)-gamma, TNF-alpha, GPR39, GHSR, and ADIPOQ. We compared the distribution of alleles according to different clinical course vs. healthy controls. Our main findings are that in lean PCOS women insulin and HOMA-IR were higher comparing to controls but adiponectin and ghrelin did not differ significantly. Furthermore, in AN adiponectin and ghrelin were higher and leptin was lower compared with controls. The correlations between adiponectin, leptin and metabolic parameters were found. Genetic variant correlation was shown only for (PPAR)-gamma (Pro12Ala-rs1801282) locus comparing AN to healthy controls with a preference of higher level of heterozygosity among these patients. Decreased adiponectin and ghrelin levels in obesity cannot be explained by variations loci we examined. We conclude that lean PCOS women show increased insulin resistance. An evidence of genetic correlation of (PPAR)-gamma (Pro12Ala-rs1801282) locus in the group of AN patients was found.

P249

The effect of body composition and iron status on insulin resistance in hemodialysis patients

Zorica Rasic, Gordana Perunicic, Jelena Tica, Vesna Popovic, Marina Vujovic & Zoran Gluvic
University hospital Zemun, Belgrade, Serbia.

Background/Aims
High level of inflammatory cytokines was present within malnourished and chronic renal failure maintenance hemodialysis (MHD) patients, but there were conflicting data about the role of inflammation on development of insulin resistance (IR) in non-obese and overweight MHD patients.
Methods
We selected 23 well-nourished and 20 middle- to moderate-malnourished, sex and age-matched, stable MHD patients, 23 male and 20 female, with median dialysis duration of 48 months (IQR 24.5–82.0). All patients were treated at the Hemodialysis Unit. To determine the nutritional status, body composition and the presence of inflammation of MHD patients we used: subjective global assessment (SGA), anthropometrics measurements (BMI and waist circumference), bioelectrical impedance analysis (BIA) which was performed to quantify body fat and lean body mass, and biochemical parameters measurements [with serum iron, ferritin, intact parathormone (i-PTH), TNF-alpha, IL-6 and high sensitivity C-reactive protein (hs-CRP)]. All parameters were evaluated by comparisons between HOMA-IR tertiles. By backward multivariate regression analysis we identified independent variables for IR.
Results
As the tetiles of HOMA-IR increased, dialysis duration, systolic blood pressure, serum levels of glucose, insulin, and waist circumference increased, whereas HDL-cholesterol level decreased. Serum iron value was increased also. As we expected, the prevalence of the metabolic syndrome were increased significantly across the tertiles of HOMA-IR. HOMA-IR correlated with the levels of iron, ferritin, adipokine TNF-alpha, waist circumference, and total fat percentages. After adjustment for gender, age, hemodialysis duration, ferritin, BMI and total fat percentages, multivariate regression analysis was performed and the association with HOMA-IR was still strong only for serum levels of iron, TNF-alpha and waist circumference. That explains 17% of the total variation in HOMA-IR (Adjusted $R^2=0.166$, $P=0.04$).
Conclusion
Our study demonstrated that 1) serum iron had participated as independent predictor in the pathogenesis of IR on long-term MHD patients, together with 2) adipokine TNF-alpha and 3) visceral adiposity.

P250

Relationship between obesity, insulin resistance and adipokines in morbidly obese women

Rana Madani[1], Nazar Miheisi[2], Majid Hashemi[1] & Vidya Mohamed-Ali[2]
[1]University College London, Department of Surgery, London, United Kingdom; [2]University College London, Department of Medicine, London, United Kingdom.

Background
Several cytokines and chemokines are released by adipose tissue and associated with insulin resistance. We investigated the systemic and *in vitro*

release of RANTES(Regulated upon Activation Normal T-cell Expressed and Secreted), MCP-1(Monocyte Chemo-attractant Protein-1), adiponectin, leptin and IL-6 from three human adipose tissue depots and their relationship to insulin sensitivity.
Methods
Fasting blood samples were taken from obese female patients undergoing surgery($n=7$, mean age 39 years, mean BMI 46 kg. m^2). Glucose, insulin and lipid profiles and circulating adipokines named above were measured. Subcutaneous(Sc), omental(Om) and Gastric Fat Pad(GFP) adipose tissue organ culture were set up for determining *in vitro* adipokine release. Haemostasis Model Assessment for Resistance(HOMA-R) was calculated. Body fat content was measured using bioelectrical impedance. The study was approved by the hospital ethical committee.
Results
Unlike for leptin no significant correlation was observed between % body fat and other adipokines. Production rates of adipokines *in vitro* per gram adipose tissue per hr were: RANTES(Sc median = 67, Om = 29, GFP = 62 pg/ml), MCP-1(Sc median = 5, Om = 6, GFP = 4 ng/ml), leptin (Sc median = 971, Om = 212, GFP = 447 pg/ml), adiponectin(Sc median = 23, Om = 25 ng/ml) and Il-6(Sc median = 9, Om = 12, GFP = 10 ng/ml). Depot specific differences in adipokine release were not apparent except in leptin which was mainly subcutaneous. There was a direct significant correlation between % body fat and Sc leptin production and an inverse relationship with Om adiponectin. GFP release of RANTES had a negative and MCP-1 a positive relationship with % body fat. Obese subjects were significantly more insulin resistant. Serum MCP-1 was elevated in patients with worsening insulin resistance. There was a negative correlation between HOMA-R and serum adiponectin and HDL.
Conclusion
RANTES, MCP-1 and adiponectin were released *in vivo* from adipose tissue. Local production of adipokines varies between depots. Insulin sensitivity and % body fat can alter local production of the adipokines.

P251

Obesity in pre-school children –new epidemic problem?
Ewa Barg, Chmielarska Joanna, Adamska Barbara, Begar Inna, Konieczna Agnieszka & Skarzynska Malgorzata
Wroclaw medical University, Wroclaw, Poland.

In the opinion of the World Health Organization the obesity will become one of four major epidemics of the 21st century. The most important factor for its occurrence is the lifestyle.

The aim of the research was to analyse the body mass index (BMI) of pre-school children and their parents and the components of their lifestyle, such as diet, sports and free time activities.
Material and Methods
The two-stage study included 537 children (265 boys and 272 girls) aged 3 and 6 (5.63 ± 1.1). The anthropometric measurements of the children were made and the BMI was counted and referred to the percentiles. The parents were questioned about their height and weight and life style.
Results
82 (15%) children fulfilled the criteria of obesity. The fathers' overweight was stated in 54.6%, whereas obesity in 10.7%; mothers' overweight in 14.4%, obesity in 1.34%.
Children eat their first meal around 8.00 a.m., the last at 7.00 p.m.
The average number of main meals is 3.9 ± 0.9. Up to 87% of parents state that their child eats extra food (fruit, yoghurts, sandwiches) between the main meals. A major part in the diet plays the sweets. Up to 48% of children consume sweets everyday, 8.2% of them a few times daily and only 1% once a week.
Only 33% of children regularly do sports. A child spends up to a 100 minutes daily in front of a TV or a computer.
Conclusions
1. The occurrence of obesity in over 15% of pre-school children should keep parents and pediatrists alert because of the possible health consequences.
2. The incorrect nourishment and improper lifestyle may result in obesity becoming an epidemic.
3. It is vital to popularize a healthy lifestyle not only among children but also their parents.

P252

Somatostatin receptor subtype 2 inhibits glucagon secretion and regulates glucose homeostasis
Mathias Strowski, Vandana Singh, Carsten Grötzinger, Sylvia Zacharias, Bertram Wiedenmann & Ursula Plöckinger
Hepatology & Gastroenterology, Berlin, Germany.

Objectives
Somatostatin (SST) inhibits glucagon and insulin secretion. Five receptor subtypes for SST are known (SSTR1-SSTR5), all of which are expressed in the endocrine pancreas. SSTR2 inhibits glucagon secretion in vitro, however its role in vivo is not well understood. Here, we characterize the role of SSTR2 in regulating glucose homeostasis in mice with diet-induced obesity.
Methods
SSTR2-deficient ($SSTR2^{-/-}$) and control mice ($SSTR2^{+/+}$) were fed high-fat diet (HFD) for 14 weeks and the parameters of endocrine pancreas function were determined. Hepatic glycogen and lipid content was evaluated enzymatically and by histomorphology. Expression of enzymes regulating glycogen synthesis and breakdown were measured by a real-time PCR and Western blot. Insulin, somatostatin and glucose tolerance tests were performed. Glucagon secretion from isolated islets was measured by RIA, and glycogenolysis in isolated hepatocytes.
Results
Postprandial glucagon and glucose concentrations were increased in SSTR2-deficient mice. Glucose disappearance rate following administration of glucose, insulin or SST was delayed in $SSTR2^{-/-}$ mice. SSTR2-deficient mice had decreased hepatic glycogen content and decreased glucokinase mRNA. Glycogen synthase of $SSTR2^{-/-}$ mice was decreased while glycogen synthase kinase-3 was increased. Glycogen phosphorylase, phosphorylase-kinase, and CREB were increased. The hepatic lipid content of SSTR2-deficient mice was decreased. Glucose was unable to suppressglucagon secretion from pancreatic islets isolated from SSTR2-deficient mice. Hepatic glycogenolysis was inhibited by an SSTR2-selective agonist.
Conclusions
We demonstrate here that SSTR2 inhibits glucagon secretion in mice with diet-induced obesity. Deletion of SSTR2 accounts for the postprandial hyperglucagonemia. Increased glucose concentration may be due to decreased hepatic glucose utilization, lipid accumulation, and increased glycogen breakdown. SSTR2 may provide a valuable therapeutic target at improving hyperglycemia in patients with peripheral insulin resistance and obesity.

P253

The role of combined treatment of arterial hypertension in patients with obesity
Doliashvili Tamar, Giorgadze Ellen, Asatiani Ketevan & Surmava Arkadi
N4 clinical hospital, Tbilisi, Georgia.

The aim of our study was to assess the advantage of combinative therapy with lisinopril and moxonidin for the treatment of arterial hypertension in group of obese patients in comparison to the single-drug therapy with lisinopril.
Methods
26 obese patients were divided in 2 groups. They underwent a 24-hour monitoring of the arterial pressure and were diagnosed as arterial hypertension II degree (ESH-ESC). In the I group for the purpose of stabilization of arterial hypertension lisinopril was given in the daily dose of 10 mg for 2 times, in the II group we gave a combination of lisinopril in the same dose as in the I group plus moxonidinin the daily dose of 0.4 mg for 1 times. The evaluation of state of health and the ambulatory registration of the arterial pressure data were carried out every week. After 3 weeks from the beginning of the treatment repeatedly the monitoring was done and the data were compared in both of the groups.
Results
At the beginning of the treatment the mean daily indices of the arterial pressure in the groups were 165/100 mmHg and 166/98 mmHg. After 2 weeks from the treatment in both groups the data of the pressure stabilized, however in the group of combinative therapy the decrease of the daily dose of lisinopril was required on 5 mg because of more expressed lowering of the arterial pressure data, and after 3 weeks of the treatment according to repeated monitoring the mean daily indices were 142/87 mmHg and 136/85 mmHg. The state of the health was improved markedly in both of the groups.
Conclusion
Adding the agonist of imidazoline receptor in the standard antihypertension therapy significantly improves the state of the health and tolerance of the therapy, as well as enables the lowering of the other antihypertensive medications.

P254

A registry of GDM in portugal

Luísa Ruas, Tiago Rocha, Jorge Dores & Manuela Carvalheiro
Pregnancy Study Group of the Portuguese Diabetes Society, Coimbra, Portugal.

Introduction
A retrospective study of the year 2003, of 1314 women with GDM, was performed.
Patients and methods
Two groups according to pre-pregnancy BMI: Go - BMI≧30 Kg/m^2; Gno BMI< 30 Kg/m^2. Mean age 32.9±5 years, A1c<6% in both groups. Influence of BMI in different variables was analysed: family history of DM, weight gain during pregnancy; blood pressure, need of insulin, gestation age at the beginning of insulin, time and type of delivery, new-born weight and re-evaluation post-partum.
Results
Mean BMI was 26.7±5.1, 76.3%=BMI<30 and 23.8%=BMI≧30. Family history of DM - BMI 26.93 Kg/m^2, without family history −26.19 Kg/m^2; $P=0.01$. Weight gain was adequate in 41.4%, reduced in 29.9% and excessive in 28.7%. Normal arterial blood pressure −86.5%, hypertension worsened by pregnancy −6.9% and pregnancy induced hypertension −6.6%, BMI in these three groups 26.1, 30.51 and 29.33, respectively ($P<0.05$). There was statistical significant difference ($P<0.05$) between the two groups in these parameters: Insulin therapy 75.2% in Go *vs* 52.5% in Gno and its need earlier in Go −28.83 wks *vs* Gno −30.97 wks; time of delivery 38.1 wks in Go *vs* 38.4−wks in Gno; caesarean section 49.8% in Go *vs* 35% in Gno; new- born weight 3324.8 g in Go *vs* 3167.9 g in Gno; macrosomic babies 8.3% in Go *vs* 4.4% in Gno. In the re-evaluation post-partum higher BMI was related with severe degrees of carbohydrate intolerance ($P<0.05$). We didn't find any difference in the re-evaluation between the women with adequate and excessive weight gain.
Conclusions
Obesity in GDM is a risk factor for maternal and fetal outcomes, with the risk of early development in the mother of glucose intolerance.

P255

The effects of glucocorticoids on the expression of gluconeogenic and lipogenic enzymes in a rodent model of Cushing's syndrome

Francesca Lolli[1], Mirjam Christ-Crain[1], Blerina Kola[1], Csaba Fekete[2], Gábor Wittman[2], Ashley B Grossman[1] & Márta Korbonits[1]
[1]Department of Endocrinology, William Harvey Research Institute, Barts and the London, Queen Mary School of Medicine, London, United Kingdom; [2]Department of Endocrine Neurobiology, Institute of Experimental Medicine, Hungarian Academy of Sciences, Budapest, Hungary.

Background
Cushing's syndrome results from chronic exposure to excessive levels of glucocorticoids (GC). The clinical manifestations associated with hypercortisolaemia are variable and differ widely in severity, including hypertension, apparent obesity and metabolic aberrations such as diabetes, dyslipidaemia, ultimately leading to changes similar to the metabolic syndrome. We hypothesised that GC might influence the expression of the genes involved in lipogenesis and gluconeogenesis in adipose tissue and liver.
Methods
Rats were implanted with corticosterone-containing pellets, and consumed chow and 30% sucrose for two weeks according to a well-established model of glucocorticoid excess. Animals implanted with cholesterol (placebo) pellets consuming sucrose or saline only served as controls. RNA was extracted from mesenteric and subcutaneous adipose tissue and liver. Gene expression was analyzed by reverse transcription followed by real time quantitative PCR with primers specific for phosphoenolpyruvate carboxykinase (PECPK), sterol regulatory element-binding protein (SREBP1c and SREBP2), fatty acid synthase (FAS), glucose-6-phospatase (G6P) and β-actin as housekeeping gene.
Results
In the mesenteric adipose tissue GC significantly increased PEPCK mRNA expression ($P=0.01$), SREBP1c and FAS mRNA expression ($P=0.02$ and $P=0.035$, respectively). No significant changes were observed in subcutaneous fat tissue. In the liver GC significantly increased FAS mRNA expression ($P<0.0001$) and decreased PEPCK mRNA ($P=0.027$), without changes in the expression of G6P or SREBP1c.
Conclusions
GC increase the expression of lipogenic and glyceroneogenic genes in visceral adipose tissue and this could explain the increased fat storage observed in the visceral fat of Cushing's syndrome. The changes in the liver would lead to increased fat deposition with less gluconeogenesis, and this was reflected in the massive fatty liver observed experimentally. We suggest that there may be a common factor leading to these changes secondary to the excess of glucocorticoids.

P256

HRT in treatment of dislipidemia in women with hypogonadotropic hypogonadism

Vita Zektser, Irena Ilovayskaya, Aleksandr Iliyn, Nikolay Goncharov & Galina Melnichenko
Research Centre for Endocrinology, Moscow, Russia.

Hypoestrogenemia is associated with dislipidemia and is an independent risk factor for cardiovascular diseases in postmenopausal women. However, there is a cohort of young women with gonadal steroid deficit caused by the disorders of central regulation. Twenty women with hypogonadotropic hypogonadism (HH) were included in group 1. (median age - 29 years and 3 months, median duration of amenorhea - 5 years 3 months, mean BMI - 24.6±6.05 kg/cm2. Women were examined before and after the 12 months treatment with 2 mg of 17-β-estradiol and 10 mg of dydrogesteron in sequenced manner. Twenty three healthy women were included in the group 2 (control), median age 27 years, Mean BMI - 24.0± 4.37 kg/cm^2.

Dislipidemia was found in all patients with HH before the treatment. The levels of total cholesterol was 5.65±1.26 mmol/l and tryglycerides 1.63±1.0* mmol/l; HDL 1.34±1.0 mmol/l and LDL 3.9±1.1* mmol/l. In the control group total cholesterol was 4.85±0.36 mmol/l, tryglycerides 0.78±0.07 mmol/l, HDL 1.77±0.33 mmol/l and LDL 1.8±0.7 mmol/l ($^*P<0{,}05$). All of the parameters were higher in group 1, but the significant difference was in LDH and tryglyceride levels.

After 12 months treatment BMI didn't change in all of the patientes with HH, there was small but not significant reducing of cholesterol 5.2±1.23 mmol/ l and tryglycerides 1.16±0.78 mmol/l leveles and the LDL 2.96±1.1* mmol/l level reduced significantly ($^*P<0.05$).

It is important to notice that hypoestrogenemia in women of reproductive age with HH leads to dislipidemia and HRT taking can somehowe correct this unpleasant changes.

P257

Rare polimorphism in the intron of human Agouti- related protein gene is associated with obesity

Ivo Kapa[1], Ineta Kalnina[1], Valdis Pirags[1], Helgi Schioth[2] & Janis Klovins[1]
[1]Latvian Biomedical Centre, Riga, Latvia; [2]Uppsala University, Uppsala, Sweden.

Agouti related protein (AGRP) as a endogenous antagonist of melanocortin 4 receptor plays an important role in regulation of food intake and energy balance being one of the most potent orexigenic factors. We have determined complete sequence of AGRP gene and upstream promoter region in 100 patients with severe obesity (BMI>35). Three previously described polymorphisms were identified: silent mutation G538A in second exon, non-synonymous mutation G772A (rs5030980) and C662T located in second intron. Association of C662T mutation with obesity this far has not been studied. We further screened this SNP in the cohort of 1173 patients from Latvian Genome database. Carriers of C662T polymorphism had significantly higher BMI when analyzed in all subjects ($P=0.017$) and in men separately ($P=0.028$). Mean BMI levels were adjusted for other non-genetic factors including age, status of type 2 diabetes cardiovascular disease and other diseases. After adjustment BMI levels remained significantly higher in men carriers of C662T polymorphism ($P=0.035$): mean BMI value (with 95% confidence interval) was 29.768 (26.738–33.572) for CT genotype compared with 26.816 (26.432–27.208) for CC genotype. The association of C662T with higher BMI in women was not significant ($P=0.051$). The present study presents for the first time the association of AGRP polymorphism C662T with obesity in men. The possible functional effects of polymorphism are unclear and may involve splicing defects. Present study has been approved by Latvian Central Committee of Medical Ethics.

P258

The relationship between plasma androgens (testosterone and dehydroepiandrosterone sulfate), insulin resistance and visceral obesity in elderly men

Michal Rabijewski, Lucyna Papierska & Wojciech Zgliczynski
Department of Endocrinology; Medical Center for Postgraduate Education, Warsaw, Poland.

Introduction
In elderly men testosterone and DHEAS deficiency is often observed, also changes of body composition and metabolic disturbances are common disorders.
Objectives
The aim of this study was to analyze the association between testosterone and DHEAS deficiency and waist/hip ratio (WHR) and also levels of glucose, insulin, HOMA and FG/FI ratio in elderly men as well as analysis, whether these sex hormones influent on measured parameters separately.
Material and methods
Together 85 men with age from 60 to 70 years men (mean 66.3 ± 1.5 years) was analyzed. Testosterone levels <4 ng/ml or DHEA levels <2000 ng/ml and BMI <30 kg/m^2 were including criteria. Patients were divided into three groups: 52 with testosterone deficiency (L-T), 32 with DHEA deficiency (L-DHEA-S) and 67 with deficiency of both sex hormones (L-T/DHEA-S).
Results
Testosterone levels in L-T, L-DHEA and L-T/DHEA groups were respectively 3.19 ± 0.23 ng/ml, 4.89 ± 0.45 ng/ml and 3.25 ± 0.34 g/ml ($P < 0.002$). While DHEA-S levels were respectively: 2498 ± 98 ng/ml, 1435 ± 1010 ng/ml and 1501 ± 89 ng/ml). BMI values do not deferent between groups. WHR ratio values were the highest in L-T/DHEA-S group ($P < 0.05$ vs. L-T) group, significant lower in L-T group ($P < 0.005$ vs. L-DHEA-S) and the lowest in L-DHEA-S group. Insulin fasting levels were lowest in L-DHEA-S group, higher in L-T group ($P < 0.01$) and highest in L-T/DHEA-S group ($P < 0.001$ vs, L-T group). FG/FI values were highest in L-DHEA-S group, lower in L-T group (NS) and lowest in L-T/DHEA group ($P < 0.002$ vs. L-T group). HOMA ratio values similarly did not change significantly between L-T (6.6 ± 3.21) and L-DHEA-S group (5.5 ± 2.92), although tendency to higher values in L-T group was noticed, while WHR ratio values were significant higher in L-T/DHEA group (7.3 ± 2.45; $P < 0.002$ vs. L-T group).
Conclusions
DHEAS and testosterone deficiency were independently associated with higher insulin resistance and obesity and also WHR ratio is more sensitive then BMI ratio reflects androgen deficiency influence on obesity and body composition in elderly men.

P259

Prevalence of metabolic syndrome in a cohort of young Mediterranean women with polycystic ovary syndrome and association with clinical and biochemical parameters

Laura Patton, Alessandra Gambineri, Andrea Repaci, Giulia Forlani, Uberto Pagotto & Renato Pasquali
Endocrine Unit, Internal Medicine Department, S. Orsola-Malpighi Hospital, Bologna, Italy.

Aim
The purpose of the study was to evaluate the prevalence of the metabolic syndrome (MS) in a cohort of young Mediterranean women with PCOS in reproductive age and to evaluate the association of the MS with clinical and biochemical parameters.
Setting
Among 200 PCOS (17-31 years) criteria of MS in accordance with the "NCEP-ATPIII" were used to construct 3 groups: no one criteria, 1 or 2 criteria and 3 or more criteria (affected by MS). All patients underwent clinical, hormonal and metabolic assessments.
Results
36 women had no criteria, 101 women had 1 or 2 criteria, 63 women had 3 or more criteria. We found a prevalence of the MS of 31.5%. The women with MS had higher BMI, waist circumference and WHR than the other two groups. Among the 3 groups we found no differences in severity of hirsutism and menses abnormalities. However, the women with more criteria had more frequently acanthosis nigricans and less frequently acne. The group with MS respect the group without any criteria had higher levels of fasting insulin ($P = 0.014$), glucose-stimulated insulin and glucose levels ($P < 0.001$) and HOMA ($P = 0.039$) and lower levels of $HOMA_{OGTT}$ ($P < 0.001$) and QUICKI ($P < 0.001$). Moreover, we found higher levels of cortisol and androstenedione responsiveness to $_{1-24}$ ACTH ($P = 0.004$, $P = 0.040$). There were no differences for the levels of androgens at baseline except for the Free Androgen Index (FAI) which was higher in the group with MS ($P = 0.023$). Finally, the levels of SHBG were lower in patients with the MS respect to patients without any criteria ($P < 0.001$).
Conclusion
Young women of the Mediterranean area present a higher prevalence of the MS respect to the general population. Moreover, the MS is associated with a more severe insulin resistance state and hyperandrogenemia and with a hyperactivity of the hypothalamic-pituitary-adrenal axis.

P260

Obesity: diffusion weighted imaging features of brain

Alpay Alkan[1], Ibrahim Sahin[2], Lezzan Keskin[2], Hakki Muammer Karakas[1], Ayse Sertkaya Cikim[2], Zuhal Karaca[3], Nedim Turhan[3], Ahmet Kemal Firat[1], Ahmet Sigirci[1] & Ozkan Ulutas[3]
[1]Inonu University Faculty of MedicineDepartment Of Radiology, Malatya, Turkey; [2]Inonu University Faculty of Medicine, Department Of Endocrinology and metabolism, Malatya, Turkey; [3]Inonu University Faculty of Medicine, Department Of Internal Medicine, Malatya, Turkey.

Homeostasis of the body weight is maintaining by the interactions between the different sections of the brain and peripheral tissues. Diffusion weighted magnetic resonance imaging (DWI) is a method that investigates the microscopic motion of the water particules in tissues and also depends on measurement of the signal variations in tissues which is connected with the kinetic energy of the molecules called; molecular diffusion. The purpose of this work was to detect brain diffusion abnormalities in obese patients by DWI.
Method
Eighty one obese patients (68 obese (group 1),13 morbid obese (group 2)) and 29 healty control were included. ADC (Appearance Diffusion Coefficience) values were measured with DWI in the hyppocampus, corpus amygdala, insular cortex, orbifrontal cortex,middle temporal cortex and cerebellum, and compared with healty controls.
Results
The ADC values obtained from hypothalamus, hippocampal gyrus, amygdala, insula, cerebellum and midbrain were significantly increased in patients compared to controls.There were statistically significant differences for ADC values that obtained from insula between group 1 (obesity, *n*: 68) and controls.There were statistically significant differences for ADC values that obtained from insula, thalamus, hippocampal gyrus, orbitofrontal cortex, midbrain, and occipital cortex between group 2 (morbid obesity, *n*: 13) and controls. The ADC values were significantly increased in group 2. The ADC values obtained from orbitofrontal and occipital cortex were significantly increased in group 2 (*n*:13) compared to group 1 subjects.The body weight were positively correlated with hippocampal gyrus, insula, orbitofrontal and middle temporal cortex ADC values. The BMI were positively correlated with amygdala, insula, orbitofrontal and middle temporal cortex ADC values.
Conclusion
Increased ADC values in the hypothalamus, hippocampal gyrus, amygdala, insula, cerebellum and midbrain. suggest development of the extracellular water accumulation similar to vasogenic edema in these location.
Key Words: Obesity, Diffusion magnetic resonance imaging.

P261

Hepatic and brain metabolism in young adults with glycogen storage disease type 1

Martina Mandl[1], Daniel Weghuber[2], Martin Krssak[1], Michael Roden[3], Peter Nowotny[1], Atilla Brehm[3], Michael Krebs[1], Kurt Widhalm[2] & Martin Bischof[1]
[1]Division of Endocrinology and Metabolism, Department of Internal Medicine III, Medical University of Vienna, Vienna, Austria; [2]Division of Clinical Nutrition, Department of Pediatrics, Medical University of Vienna, Vienna, Austria; [3]Department of Internal Medicine I, Hanusch Hospital Vienna, Vienna, Austria.

Glycogen storage disease type 1 (GSD1) is a rare inherited defect of endogenous glucose production. While children present with severe hypoglycemia the

propensity for hypoglycemia may decrease with age in these patients. It was the aim of this study to elucidate the mechanisms for milder hypoglycemia symptoms in grown up GSD1 patients. Four patients with GSD1 (BMI: 23.2 ± 6.3 kg/m^2, age: 21 ± 3 yr) and four healthy controls matched for BMI (23.1 ± 3.0 kg/m^2) and age (24 ± 3 yr) were studied. Combined ^{1}H/^{31}P-nuclear-magnetic-resonance-spectroscopy was used to assess brain metabolism. Before and after administration of 1 mg glucagon endogen glucose production (EGP) was measured with D-[6,6-$^2H_2$]glucose while hepatic glucose metabolism was examined by ^{1}H/^{13}C/^{31}P-NMRS. At baseline GSD1 patients exhibited significantly lower rates of EGP (0.53 ± 0.04 vs. 1.74 ± 0.03 mg·kg^{-1}·min^{-1}, $P < 0.01$ vs. control) but an increased intrahepatic glycogen (502 ± 89 vs. 236 ± 11 mmol/l, $P = 0.05$ vs. control) and lipid content (16.3 ± 1.1 vs. 1.4 ± 0.4%, $P < 0.001$ vs. control). After glucagon challenge, EGP did not change in GSD1 patients (0.53 ± 0.04 vs. 0.59 ± 0.24 mg·kg^{-1}·min^{-1}; P=n.s.) but increased in healthy controls (1.74 ± 0.03 vs. 3.95 ± 1.34, $P < 0.0001$). In GSD1 patients we found an exaggerated increase of intrahepatic phosphomonoesters (PME) (0.23 ± 0.08 vs. 0.86 ± 0.19AU, $^P < 0.001$) while inorganic phosphate (P_i) even decreased (0.36 ± 0.08 vs. -0.43 ± 0.17AU, $P < 0.01$). Intracerebral ratios of glucose, glutamate, and myo-inositol:creatine were higher in GSD1 patients (at least $P < 0.05$ vs. control, respectively). Hepatic defects of glucose metabolism persist in grown up GSD1 patients. Upregulation of the glucose and lactate transport at the blood-brain barrier could be responsible for the amelioration of hypoglycemic symptoms.

P262

Body composition and GH status in morbidly obese females before and after laparoscopic silicone adjustable-gastric banding

Silvia Savastano[1], Annamaria Colao[1], Francesca Rota[1], Laura Vuolo[1], Maria Cristina Savanelli[1], Teresa Cascella[1], Francesco Orio[1], Luigi Angrisani[2], Gaetano Lombardi[1] & Carolina Di Somma[1]
[1]Department of Molecular and Clinical Endocrinology, Federico II University, Naples, Italy; [2]Department of Surgery, S.Giovanni Bosco Hospital ASL NA1, Naples, Italy.

The GH/IGF-I axis function are reported to ameliorate after weight-loss. Bariatric surgery leads to a significant weight loss in morbidly obese patients. We investigated the relationships between GH/IGF-I axis and body composition in 20 morbidly obese females (BMI: 44.8 ± 4.7; waist circumference (W) 119.5 ± 7.2 cm, age 33.7 ± 11.7 yrs) with a normal glucose tolerance, before and after laparoscopic silicone adjustable-gastric banding (LASGB). The GH axis was evaluated by GH response after GHRH + arginine test and IGF-I levels. Patients were evaluated 6 months after surgery and a well balanced mildly hypocaloric diet. Fat Mass (FM), Free Fat Mass (FFM) were evaluated by bioimpedance analysis. Before surgery, 8 (40%) subjects were GH deficient (peak GH < 4.2 μg/l), while 7 (35%) had IGF-I levels below the normal values for age and sex. Postoperatively, GH response was persistently impaired in 3 (15%) subjects, while IGF-I levels were still reduced in 9 (45%). After 6 months BMI, W, FM ($P < 0.001$) and FFM ($P = 0.03$) were significantly reduced. The percent decrement of FM was greater than that of FFM (22.4 ± 16% vs 5.6 ± 2.3%; $P < 0.001$). GH response was persistently impaired in 3 (15%) subjects, while IGF-I levels were still reduced in 9 (45%). In addition, a significant correlation was found between the decrement of FFM ($r = 0.81$; $P < 0.001$) and that of FM ($r = 0.47$; $P < 0.04$) and the decrement of IGF-I. At the multiple regression analysis, the percentage of FM and W at baseline were the major determinants of IGF-I. In conclusion, both the nutritional status and a relative malabsorption might affect IGF-I and FFM. After bariatric surgery and after the initial acute negative energy balance, a persistent deficiency in GH/IGF-I axis is present and this particular endocrine profile is also associated to unfavourable body composition changes. The low IGF-I levels might represent a possible marker of an underlying persistent catabolic state in these subjects.

P263

The importance of (TAAAA)n polymorphism of SHBG gene in the metabolic syndrome

Nektaria Xita[1], Charalambos Milionis[2], Ioannis Georgiou[3], Moses Elisaf[2] & Agathocles Tsatsoulis[1]
[1]Department of Endocrinology, University of Ioannina, Ioannina, Greece; [2]Department of Medicine University of Ioannina, Ioannina, Greece; [3]Laboratory of Reproductive Genetics, University of Ioannina, Ioannina, Greece.

Introduction
Sex hormone binding globulin (SHBG) levels have been associated with the development of the metabolic syndrome. In particular, low SHBG levels have been proposed as an indicator of increased risk for metabolic syndrome in men. The (TAAAA)n repeat polymorphism SHBG gene is believed to affect SHBG levels. In vitro experiments have shown that the allele with 6 TAAAA repeats is associated with decreased transcriptional activity of SHBG gene. The aim of this study was to examine the possible role of this polymorphism in the metabolic syndrome.
Subjects and methods
The study population consisted of 44 men with metabolic syndrome aged 51.6 ± 9.9 years and 100 healthy men. The body mass index was recorded and blood samples were obtained after overnight fasting for biochemical and hormonal tests. The fasting glucose to insulin ratio was calculated as an indicator of insulin resistance. The SHBG (TAAAA)n polymorphism was genotyped in peripheral blood leucocytes.
Results
Genotype analysis for the (TAAAA)n polymorphism of the SHBG gene in the patients and controls revealed six alleles having 6–11 TAAAA repeats. The distribution of the alleles between patients and the control group did not show statistically significant differences. However, the 6/6 genotype was more frequent in patients with metabolic syndrome compared to healthy men (22.7% vs 11%, $P = 0.05$). The small number of patients did not allow any association between polymorphism and biochemical parameters.
Conclusion
The (TAAAA)n polymorphism of SHBG gene appears to be associated with metabolic syndrome

P264

Exophthalmos and its relation to adipokines in obese men

Sinan Caglayan[1], Gokhan Ozisik[2], Melih Unal[3], Omer Ozcan[4] & Metin Ozata[2]
[1]Kasimpasa Military Hospital, Division of Endocrinology and Metabolism, Istanbul, Turkey; [2]GATA Haydarpasa Teaching Hospital, Division of Endocrinology and Metabolism, Istanbul, Turkey; [3]GATA Haydarpasa Teaching Hospital, Division of Ophthalmology, Istanbul, Turkey; [4]GATA Haydarpasa Teaching Hospital, Division of Biochemistry, Istanbul, Turkey.

Up to date, many studies were performed regarding the relationship between obesity and inflammation, and exophthalmos which is developed in thyroid-associated ophthalmopathy (Graves' ophthalmopathy). Several studies suggest that transforming orbital preadipocytes into adipocytes may cause exophthalmos because of the inflammation. Therefore, we examined the relationship exophthalmos and obesity which is also called low-grade systemic inflammation. We investigated the relationship between Hertel exophthalmometry values and plasma leptin, adiponectin, TNF-α, IL-6 and IL-1β levels in 52 obese and 34 healthy men who don't smoke and have any systemic illness.

Plasma leptin, adiponectin, TNFα, IL-6 and IL-1β levels were 25.28 ± 8.98 ng/mL, 0.41 ± 0.24 μg/mL, 305.53 ± 153.82 pg/mL, 63.99 ± 20.30 pg/mL ve 95.22 ± 69.54 pg/mL respectively, in obese group, whereas these levels were 2.66 ± 1.81 ng/mL, 1.17 ± 0.98 μg/mL, 69.31 ± 50.22 pg/mL, 18.84 ± 11.12 pg/mL ve 21.77 ± 6.84 pg/mL respectively, in control group. Hertel exophthalmometry values were found as 18.90 ± 1.63 mm in obese group and 16.88 ± 1.69 mm in control group. When obese group's variables compared to control group's variables, plasma adiponectin levels were found significantly lower whereas the other variables were found significantly higher in obese group ($P < 0.05$). In multiple regression models using backwards stepwise regression, we only found that the dependent variable, BMI, was predicted by leptin and TNF-α ($P = 0.004$ ve $P = 0.052$, respectively).

Our results suggest that the inflammation which is resulted by secreted adipokines and cytokines from adipose tissue might be associated with exophthalmos in obesity. Nevertheless, the lack of correlation between Hertel exophthalmometry values and BMI, plasma leptin, adiponectin, TNFα, IL-6 and IL-1β levels shows that there is no directly relation between exophthalmos and adipokines which causes inflammation in obesity.

P265

Influence of orlistat on adiponectin levels in obese women

Marianthi Tzoitou[1], Evridiki Papadopoulou[1], Athanasios Papageorgiou[1], A Galactidou[2], Marina Kita[1], Anargyros Kourtis[1], Pavlos Poulakos[1] & Avraam Avramidis[1]
[1]Endocrinology Clinic Ippokratio General Hospital, Thessaloniki, Greece; [2]Research Department Theagenio Hospital, Thessaloniki, Greece.

Introduction
Adiponectin is secreted by adipocytes and has been linked to glucose and lipid regulation. Obesity, diabetes and atherosclerosis have been associated with reduced adiponectin levels. Orlistat lowers lipids and improves insulin sensitivity but its effect on other metabolic parameters is not known.

The purpose of this study is to evaluate the influence of orlistat on metabolic and hormonal parameters of the adipose tissue.

Materials and methods
Thirty obese female patients with Body Mass Index >30 kg/m2 and mean aged 48.7 ± 12.9yrs and mean weight 92.47 ± 12.5 kg were included. Patients with diabetes and thyroid disorders were excluded. All patients were on a low calorie diet one month before treatment with orlistat.Blood samples for glucose, total cholesterol triglycerides, HDL,LDL,FT4, TSH, insulin and adiponectin were obtained before and three months after orlistat treatment.

Results
19/30 female (63.3%) have lost over five kilos after three months of treatment and diet. Mean body weight was 92.47 ± 12.5 kg and 85.45 ± 11.2 kg $p<0.05$ after treatment. Statistical signigicant differences between glycose triglycerides, cholesterol HDL,LDL were observed after treatment with orlistat(101 ± 31.2 vs 85 ± 14.5 mg/dl $P<0.05$, 207.5 ± 29.8 vs 196.1 ± 25.5 mg/dl $P<0.004$, 127.5 ± 50.9 vs 119.2 ± 41.4 mg/dl $P<0.001$).Insulin levels decreased significantly after three months of treatment (11.3 ± 2.4 μU/ml vs 9.19 ± 2.7 μU/ml $P<0.00$).In contrast adiponectin levels seemed to be increased significantly after treatment with orlistat.($16.284.5\pm21.640.6$ vs $41.798.5\pm64.776.1$ $P<0.00$)

Conclusion
In this study it seems that orlistat could effectively manage obesity. It decreases insulin and increases adiponectin when obese patients reduced caloric intake and lost weight after three months of treatment.

P266

Effect of omega-3 fatty acids on plasma adiponectin levels in Metabolic syndrome subjects

Dimiter Dimitrov
Medical University Varna, Varna, Bulgaria.

Background
Increased consumption of fish and fish oil as a source of *n*-3 long chain polyunsaturated fatty acids ($n-3$ LC-PUFA), mainly eicosapentaenoic acid (EPA, 20:5 *n*-3) and docosahexaenoic acid (DHA, 22:6 *n*-3) is often associated with decreased mortality (as well as morbidity) from cardiovascular disease. Treatment with n-3 LC-PUFA augments circulating adiponectin levels via a PPARγ-dependent mechanism in animal models. Given that adiponectin is known to exert antiinflammatory effects and enhance insulin sensitivity, it is conceivable that *n*-3 LC-PUFA could impede the adipose tissue switch to an inflammatory gene expression profile in response to obesity via a PPARγ- and adiponectindependent mechanism.

Aim
To evaluate the effect of n-3 LC-PUFA on plasma adiponectin levels and components of the Metabolic syndrome (Met-S).

Methods
35 overweight and obese adults ($28<BMI<36$ kg/m^2), aged 18–65 years, having developed the features of Met-S (IDF definition, 2005) were randomized to 2 gr. *n*-3 LC-PUFA daily or placebo for 3 months. All subjects were instructed to follow ad libitum diet without change in dietary lifestyle during that period. Metabolic parameters, plasma adiponectrin, insulin resistance (HOMA-IR) and CRP were measured before and after treatment.

Results
After 3 months, plasma adiponectin concentrations were increased by 44% ($P<0.001$). HDL cholesterol concentrations were increased by 10% ($P<0.001$) .Triglycerides was decreased by 39%, HOMA –IR decreased with 34% and CRP decreased with 20%. There were no significant complications resulting from treatment with n-3 LC-PUFA.

Conclusion
n-3 LC-PUFA may contribute to decreasing the burden of the metabolic syndrome, such as modulating inflammation, lipid abnormalities, endothelial function, and blood pressure via adiponectindependent mechanism.

P267

The polymorphism of PPAR and susceptibility to atherosclerosis in children with low birth weight (below 2500 g)

Ewa Barg, Anna Skoczynska, Beata Wikiera, Dorota Chacka, Ewa Glab, Arleta Lebioda & Barbara Turczyn
Wroclaw Medical University, Wroclaw, Poland.

Children, who are born with low birth weight (less 2500 g) are known to have an increased risk of developing lipid disturbances and atherosclerosis in later live.

PPAR alpha activity could play a regulatory role in the pathogenesis of hyperlipidemia and a modulatory role in the control of inflammatory response.

The aim of this study was to determine whether the presence of polymorphism in gene of peroxisome proliferators-activated receptor(PPAR) alpha is associated with lipid disturbances and susceptibility to apoptosis in children with low birth weight.

Methods
The associations between L162V polymorphism in the gene for PPAR alpha and lipid peroxidation, lipid profile, activity of caspase3 and apoptosis activation was examined in 155 children with low birth weight aged 4–11 years, and in 30 children born with normal weight as a control group.

Results
The frequency of the V allele of the L162 polymorphism gene in PPAR alpha gene in children(0.07) was similar to that in general population(0.06 in controls). In the group with polymorphism gene 4 children with LBW have the 50 Kb domain on the DNA electrophoretic profiles, but 7 children with LBW and control children haven't it.

The effect of the l162V polymorphism within PPAR alpha gene on the serum total HDL levels are observed ($P<0.001$).The levels of HDL and triglycerides and lipid peroxides were statistically higher in children with gene PPAR polymorphism ($P<0.05$) than in those children without polymorphism. Among all the children with the polymorphism, the group born with LBW presented higher level of lipid peroxides ($P<0.05$).

The linear correlations between caspasa 3 and serum cholesterol ($r=-0.999$, $P<0.05$), lipid peroxides and susceptibility of infection ($r=-0.769$, $P<0.05$),

Conclusion
In children more susceptible for atheroscerosis in adulthood due to low birth weight the L162V mutation in PPAR are connected with a protective effect on lipid pattern

P268

Visfatin, adiponectin, leptin and insulin sensitivity in severe obese women with normal and impaired glucose tolerance

Mirjana Sumarac-Dumanovic[1], Dragan Micic[1], Maja Georgiev[1], Danica Stamenkovic-Pejkovic[1], Jagoda Jorga[1], Aleksandra Kenedereski[1], Goran Cvijovic[1], Svetlana Zoric[1], Carlos Dieguez[2] & Felipe Casanueva[2]
[1]Institute of Endocrinology, Diabetes and Diseases of Metabolism, Clinical Center of Serbia, Belgrade, Serbia; [2]Department of Endocrinology, University of Santiago de Compostela, Santiago de Compostela, Spain.

Visfatin, a new adipokine, facilitates adipogenesis and has insulin-mimetic properties. There are data that hyperglycemia causes an increase in plasma visfatin levels in people with T2DM this increase gets more prominent as the glucose intolerance worsens. The aim of the study was to investigate plasma visafatin, leptin and adiponectin in obese women with normal and impaired glucose tolerance. Thirteen obese women (age: 34.50 ± 2.57 yrs; BMI 35.05 ± 0.57 kg/m2) with normal glucose tolerance (NGT) and 11 age and BMI matched obese women (age: 37.0 $2.4734.50\pm2.57$ yrs; BMI 38.20 ± 1.81 kg/m2) with normal fasting and impaired glucose tolerance during oral glucose tolerance test (OGTT) (IGT) were included in the study. Fasting plasma visfatin (EIA Phoenix, ng/ml), adiponectin (Linco RIA, ng/ml), leptin (Linco RIA, ng/ml) and insulin (RIA Inep, mU/l) were measured. OGTT (75 gr of glucose) were performed in all obese women. Insulin sensitivity (M index: mg/kgBW/min) using hyperinsulinemic euglycemic 2hr clamp was measured before and after weight reduction. There was no difference in fasting visfatin between NGT and IGT (68.65 ± 4.78 vs. 73.14 ± 5.22, $P>0.05$), fasting leptin (36.75 ± 3.79 vs. 32.06 ± 3.79, $P>0.05$) fasting adiponectin (6.82 ± 1.84 vs. 10.76 ± 4.14, $P>0.05$) and fasting insulin (17.34 ± 1.44 vs. 19.08 ± 2.65, $P>0.05$). Insulin sensitivity was reduced in obese women with IGT (5.36 ± 0.63 vs. 2.81 ± 0.39, $P<0.05$) while waist circumference were greater in the same subgroup of obese women (101.07 ± 3.12 vs. 113.18 ± 3.60, $P<0.05$). There was significant correlation between M index and waist in obese women ($r=-0.67$, $P<0.05$). In conclusion, decreased insulin sensitivity is confirmed in severe obese women with IGT. Our data suggest that impairment in insulin sensitivity precede change in adipocytokines during development of type 2 diabetes in obesity.

Signal transduction – presented on Sunday

P269

Characterization of the rat homologue of the human neuroendocrine marker secretagogin – new functional implications by *in vitro* studies

Wolfgang Gartner[1], Greisa Vila[4], Teodora Daneva[2], Felic Koc-Saral[1], Otto Majdic[3], Anastasia Nabokih[1], Anton Luger[4] & Ludwig Wagner[1]
[1]Medical University Vienna, Department of Medicine III, Vienna, Austria; [2]Bulgarian Academy of Sciences, Institute of Molecular Immunology, Sofia, Bulgaria; [3]Medical University Vienna, Department of Immunology, Vienna, Austria; [4]Medical University Vienna, Department of Endocrinology and Metabolism, Vienna, Austria.

Objective
Establishment of rodent *in vitro* cell systems for the extension of the functional data about the recently cloned neuroendocrine marker secretagogin.
Methods
1. DNA-cloning; 2. Antibody generation; 3. Immunoblotting and Immunohistochemistry; 4. Cell-transfection; 5. Luciferase Reporter Assays; 6. ELISA.
Results
1. We characterized the rat homologue of human secretagogin (rat secretagogin) and demonstrated the homologous tissue expression pattern of both proteins. 2. Highest rat secretagogin expression levels were found in rat pancreatic islets and in the rat insulinoma cell lines Rin-5F and INS-1. 3. There exists a considerable degree of sequence homology between human and rat secretagogin, indicating comparable functional properties. 4. Overexpression of rat secretagogin in Rin-5F and in INS-1 cells induced an increase in insulin secretion and expression, which is mediated mainly via the promoter elements AP-1 and CRE. 5. Insulin and rat secretagogin are secreted in an inverse ratio by Rin-5F and INS-1 cells upon incubation with dexamethasone and other agents known for influencing the insulin secretion.
Conclusion
We characterized the rat homologue of human secretagogin and present an *in vitro* system for its functional analysis, which emphasize its regulative involvement in insulin secretion and expression.

P270

Tilapia GnRH receptors: signal transduction and internalization rate

Ayelet Avitan & Berta Levavi-Sivan
Department of Animal Sciences, Faculty of Agricultural, Food and Environmental Quality Sciences, The Hebrew University of Jerusalem, Rehovot 76100, Israel.

Multiple subtypes of GnRH receptor (GnRHR) are present in individual vertebrate species. We found two distinct GnRHRs in tilapia, classified as type 1 and 3 (tGnRHR1/3). Amino-acid similarity between the receptors was calculated at 59%, with the different amino acids scattered throughout the receptors. We compared the sequence analysis and signal transduction of the two tGnRHRs, using the human GnRHR type 1 as a control. Sequence analysis revealed that all three receptors exhibit recognition motifs of Galpha q/11, while only tGnRHR3 and the hGnRHR1 revealed also, one recognition motif of Galpha s. We found that both tilapia receptors and the human receptor contain one PKA phosphorylation site. However, tGNRHR3 has five PKC phosphorylation sites whereas both tGnRHR1 and hGnRHR1 have only two sites. This diversity is further supported by the differential signal-transduction pathways: all three receptors activate the PKC pathway (as reflected by measurement of IPs accumulation), but only tGnRHR3 activates the PKA pathway (as reflected by activation of the reporter construct CRE-luciferase). All three receptors were also found to activate the phosphorylation of MAP kinase (ERK-1/2).

tGnRHR3 is highly expressed in the posterior part of the pituitary which contains LH and FSH cells. Hence, we characterized tGnRHR3 in terms of both LH release rate and receptor internalization rate in response to continuous exposure to GnRH.

Constant exposure of tilapia pituitary fragments to sGnRHa resulted in an increased secretion rate for 3 h, followed by a gradual decline to the basal secretion rate which lasted for 22 h. A chimera between tGnRHR3 and green florescence protein (GFP) was prepared and used to observe the changes in receptor distribution and translocation, activated by agonist with time. The receptor is initially localized at the plasma membrane and upon activation by sGnRHa undergoes relatively rapid endocytosis.

P271

The relationship between carotid intima-media thickness metabolic and anthropometric parameters in healthy subjects

Hatice Akay[1], Deniz Gokalp[2], Senay Arikan[2], Alpaslan Tuzcu[2] & Mithat Bahceci[2]
[1]Dicle University School of Medicine Department of Radiology, Diyarbakir, Turkey; [2]Dicle University School of Medicine Department of Endocrinology, Diyarbakir, Turkey.

Background and aim
Carotid intima-media thickness (IMT), as assessed by B-mode ultrasound, is a commonly used clinical marker that reflects systemic burden of atherosclerosis and value of IMT at or above 1 mm at any age is associated with a significantly increased risk of myocardial infarction. On the basis of such findings, we aimed to clarify the relationship between carotid intima-media thickness and anthropometric and metabolic parameters in virtually healthy subjects.
Subjects and methods
A total of 117 apparently healthy subjects were included to the study (age 20–68 year, mean age: 43 ± 12, BMI:30.1 ± 7.99 kg/m^2). Carotid Intima-media thickness (IMT) was measured with ultrasonography. Subjects were divided into two groups according to their IMT higher than 1 mm (group-1) or not (group-2). Total cholesterol, LDL-cholesterol, triglycerides, Hs-CRP, interleukin-1β, interleukin2, interleukin 6, interleukin 8, Tumour necrosis factor α, BMI, body fat mass with bioelectric impedance and body fat distribution (waist and hip circumference) of two groups were compared with independent *t* test.
Results
BMI, body fat mass, hip circumference, plasma LDL cholesterol, Hs-CRP levels of group-1 were higher than group-2 (Table 1) Interleukin-1β, interleukin2, interleukin 6, interleukin 8, Tumour necrosis factor α, triglycerides, waist circumference of the two groups were not show any statistically difference.
Conclusions
1-Carotid intima media thickness are closely related increased BMI, fatmass, hip circumference and LDL-cholesterol levels.
2-Hs-CRP is a useful marker of atherosclerosis.

	Group-1 ($n=17$)	Group-2 ($n=100$)	*P* value
BMI	37.6 ± 7.1	28.7 ± 7.3	$P=0.0001$
Body Fatmass	40.7 ± 15.2	24.9 ± 14.3	$P=0.001$
Hip circumference	122 ± 18	107 ± 14	$P=0.0001$
Hs-CRP	7.23 ± 2.95	3.67 ± 1.50	$P=0.008$
LDL-cholesterol	135.4 ± 29.8	108.8 ± 33.2	$P=0.008$

P272

CRF and the Urocortins activate NFAT and induce catecholamine production in PC12 cells

Erini Dermitzaki, Christos Tsatsanis, Ariadne Androulidaki, Maria Venihaki, Achille Gravanis & Andrew Margioris
University of Crete, School of Medicine, Heraklion, Crete, Greece.

We have previously shown that Corticotropin – releasing factor (CRF) and Urocortins (UCNs) induce the production of catecholamines from normal human and rat adrenal chromaffin and PC12 pheochomocytoma cells via induction of tyrosine hydroxylase, the rate limiting enzyme of catecholamine biosynthesis. We have also shown that CRF induces calcium ion entrance into the cytoplasm from both extracellular sources (influx) and from intracellular stores (mobilization) in PC12 cells. The transcription factor NFAT (Nuclear Factor of Activated T cells) is activated by calcium, is expressed in neuronal tissues and in PC12 cells, and is involved in neuronal cell differentiation. No information is available on its role in chromaffin cells. In the present study we have examined the effect of CRF peptides on NFAT activation, its role on catecholamine production in the PC12 pheochromocytoma cell line and the signaling pathways involved.

Our data demonstrate that: (a) CRF, UCN1 (CRF_1 and CRF_2 receptor agonists), UCN2, UCN3 (preferential CRF_2 receptor agonists) or Cortagine (synthetic CRF_1 receptor agonist) induced NFAT activity in a statistical significant manner in PC12 cells. (b) Cyclosporine A (CsA), a Calcineurin/NFAT inhibitor, abolished UCN2 or Cortagine-induced NFAT transcriptional activity in PC12 cells. (c) The effect of CRF receptor agonists on catecholamine synthesis was abolished by CsA in PC12 cells. In conclusion, our data suggest that CRF and UCNs activate the transcription factor NFAT which appears to be essential for catecholamine synthesis.

P273

Gs-dependent receptor endocytosis of melanocortin-4 receptors

Andreas Breit, Dominik Heling & Thomas Gudermann
Institut of Pharmacology and Toxicology, Philipps University Marburg, Marburg, Germany.

Melanocortin receptors (MCR), which belong to the superfamiliy 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. Recently it has been shown that the MC4R receptor undergoes, similar to most GPCR, GPCR kinase (GRK) and arrestin-mediated ligand-promoted receptor endocytosis. 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 as monitored by the bioluminescence resonance energy transfer technique in living cells, indicating that activation of the GRK/arrestin pathway does not require Gs signalling. However, despite of the key role arrestins play in regulating ligand-promoted receptor endocytosis, arrestin recruitment to the Gs signalling deficient MC4R-D90N variant was not sufficient to induce receptor endocytosis. These data indicate that although arrestin recruitmnet to the MC4R occurs independently of Gs signalling, ligand-promoted MC4R endocytosis requires the activation of Gs proteins, suggesting that so far unknown Gs signalling-dependent mechanism are involved in regulating ligand- promoted MC4R endocytosis.

P274

The endocrine disruptor DDT appears to be an uncompetitive inverse agonist for activating TSHr mutants, FSH receptor and LH receptor

Lucia Montanelli[1], Eleonora Ferrarini[1], Antonio Dimida[1], Mario Rossi[2], Patrizia Agretti[1], Franco Giorgi[2], Giovanni Umberto Corsini[2], Aldo Pinchera[1], Paolo Vitti[1], Roberto Maggio[2] & Massimo Tonacchera[1]
[1]Department of Endocrinology of University of Pisa, Pisa, Italy; [2]Department of Neurosciences of University of Pisa, Pisa, Italy.

The insecticide DDT has been shown to inhibit both the basal and the TSH stimulated accumulation of cAMP in CHO stably transfected with the TSHr (CHO-TSHr). Aim of this study was to evaluate whether the DDT has a similar effect on cells transfected with TSHr mutants displaying a high level of constitutive activity. In addition we investigate the effect of DDT on cells transfected with wtFSHr and wtLHr which share a high degree of amino-acid homology sequence with wtTSHr. In contrast with wtTSHr, wtFSHr and wtLHr do not show constitutive activity. Three TSHr mutants transiently transfected in COS cells were evaluated: S281L located in the ectodomain, I486M in the first extracellular loop and P639S in the sixth helix of the transmembrane domain. After incubation with DDT at increasing concentrations (10, 30 and 100 mcM), basal cAMP of the mutants was measured. Conversely, CHO cells stably expressing the wtFSHr and wtLHr (CHO-FSHr, CHO-LHr) were incubated with increasing concentrations of DDT (0.1, 1, 10 and 100 mcM), in presence of FSH (100 mU/ml) and hCG (1 mU/ml), respectively, and cAMP production was measured. The constitutive activity of the three activating TSHr mutants was inhibited and the maximal inhibition was obtained with the highest concentration of DDT. Similarly, DDT inhibited FSH and hCG induced cAMP activity in the two cell lines. At the highest concentration of DDT the inhibition was of 39% and 92% in CHO-FSHr and CHO-LHr, respectively. In conclusion DDT inhibited the constitutive activity of all activating TSHr mutants and the FSH and hCG stimulated accumulation of cAMP in CHO-FSHr and CHO-LHr. These effects are similar to those displayed by DDT on CHO-TSHr. Our data suggest that DDT might be an uncompetitive inverse agonist.

Steroid receptors – presented on Sunday

P275

Effect of vitamin D replacement on endothelial function and oxidative stress in vitamin D deficient subjects

Ozlem Tarcin[1], Dilek Yavuz[1], Ahmet Toprak[2], Ahu Telli[3], Meral Yuksel[4], Dilek Yazici[1], Seda Sancak[1], Oguzhan Deyneli[1], Goncagul Haklar[3] & Sema Akalin[1]
[1]Marmara University Medical School Section of Endocrinology and Metabolism, Istanbul, Turkey; [2]Marmara University Medical School Department of Internal Medicine, Istanbul, Turkey; [3]Marmara University Medical School Department of Biochemistry, Istanbul, Turkey; [4]Marmara University Vocational School of Health Related Sciences, Istanbul, Turkey.

Introduction
Vitamin D (Vit D) receptors have been shown in extra skeletal tissues. Vit D deficiency plays a role in the development of many malignant, chronic inflammatory, autoimmune and metabolic diseases. Our aim was to evaluate the effect of Vit D replacement therapy on insulin sensitivity, endothelial function and oxidative stress in Vit D deficient subjects.
Material-method
Serum 25(OH) D levels of 74 volunteer-healthy subjects (22.7 ± 2.7) were screened. Twenty subjects (22.6 ± 2.1) with 25(OH) D levels < 20 ng/ml were recruited as deficient group (D) and 20 subjects (23 ± 2.3) with 25(OH) D levels > 40 ng/ml were selected as control group (C). Monthly 300 000 IU Vit D was injected for 3 months to group D. Before and after 3 months, blood samples were collected for serum Ca, P, iPTH, thiobarbituric acid reactive substance (TBARs) and paraoxonase. Endothelial function was evaluated by measuring flow mediated dilatation (FMD) from brachial artery. Insulin sensitivity index was calculated according to 75gr OGTT.
Results
In group D, basal TBARs levels were higher compared to group C and decreased after Vit D therapy (Table 1). Basal FMD of group D were found to be lower than group C and increased after therapy. We found negative correlation between FMD and TBARs ($P=0.001$; $r=-0.51$) in group D. After therapy, 30th sec. insulin level increased during OGTT.

Table 1 Parameters before and after replacement therapy

	Before therapy	After therapy	Control
iPTH(pg/ml)	47.8 ± 22.5*	34 ± 17.6	42.8 ± 12.2
Ca(mg/dl)	9.6 ± 0.7	9.7 ± 0.4	9.8 ± 0.4
P(mg/ml)	3.8 ± 0.4	3.8 ± 0.5	3.7 ± 0.4
FMD(%)	7.2 ± 4*	10.5 ± 4	13 ± 12.6**
TBARs(nmol/mg MDA)	5 ± 1.5*	3 ± 0.7	4 ± 0.8**

*$P<0.05$ before and after therapy; **$P<0.05$ before therapy and control

Discussion
We have shown that vit D deficiency causes endothelial dysfunction. Vit D replacement led to the improvement on endothelial function and decreased lipid peroxidation which made us think that vit D deficiency could have take part in the pathogenesis of atherosclerosis.

P276

Transthyretin is up-regulated by androgens in mice liver and choroid plexus

Cecilia RA Santos[1], C Henrique Alves[1], Maria Joao Saraiva[2], Raquel Abreu[2], Graça Baltazar[1], Silvia Socorro[1], Telma P Quintela[1] & Isabel MT Gonçalves[1]
[1]Centre of Investigation in Health Sciences-CICS, Universidade da Beira Interior, Covilhã, Portugal; [2]Molecular Neurobiology, Instituto de Biologia Molecular e Celular-IBMC and ICBAS, Universidade do Porto, Porto, Portugal.

Transthyretin (TTR) is well documented as a carrier for thyroid hormones. It also binds retinol binding protein preventing its filtration trough the kidneys and therefore is involved in delivering retinol to target cells. Moreover, TTR sequesters amyloid-beta impairing its deposition in nervous tissues and possibly contributing to its removal. Despite its importance in mammalian physiology, there are few studies regarding the regulation of TTR synthesis. *In silico* analysis of the 5′ flanking region of the TTR gene allowed the identification of androgen responsive elements suggesting that androgens may regulate TTR expression in tissues where TTR and androgen receptor (AR) are co-expressed. This could assume particular relevance in the liver and choroid plexus (CP), which are the major sites of TTR synthesis. To test

this hypothesis female and male mice were either ovariectomized ($n=13$) or orchidectomized ($n=12$). Five weeks after surgery, these animals were either implanted with an alzet mini-osmotic pump delivering 419 ug/Kg/day of 5α-dyhidrotestosterone (DHT) or vehicle only, in the subscapular region. Sham operated animals (5 females and 5 males), not implanted, were also included in the experiment. After one week of hormonal stimulation, mice were euthanized and CP, livers, cerebrospinal fluid (CSF) and sera were collected and frozen at −80 °C. The levels of TTR in the CSF and sera were measured by RIA and the expression of TTR in the liver and choroid plexus was analysed by Real-Time PCR. A 3-fold increase of TTR levels in the sera and CSF of females, and a slight but significant increase of TTR levels in the sera of males were observed. As AR is expressed in liver and CP, it is likely that the observed TTR response to DHT is mediated by AR.

P277

Adrenal incidentalomas: aberrant expression of hormone receptors (preliminary results)

Elena Mena, Manel Sahun, Pedro Alià, M Ángel Navarro, Joan Soler & Carles Villabona
Department of Endocrinology and *Hormone Unit, Hospital Universitari Bellvitge, Barcelona, Spain.

Background
In some patients with adrenal tumors cortisol regulation may be under the control of abnormal or ectopic hormone receptors. The objective of this study is investigate the presence of these aberrant receptors in subjects with adrenal incidentaloma and biochemical criteria of subclinical hypercortisolism.
Patients and methods
We studied seventeen patients with adrenal incidentalomas, ten patients with a unilateral tumor (age 48–70, M/F: 4/6) and seven patients with bilateral tumors (age 53–68, M/F: 5/2), and biochemical features of subclinical cortisol hypersecretion. They were studied for plasma cortisol responses to various stimuli: upright posture, meal, terlipressin, cinitapride, combined hypothalamic-hormones (TRH and LHRH) and ACTH. Six normal controls were similarly studied. All subjects were given dexamethasone orally in order to avoid any ACTH-dependent variation of plasma cortisol. Responses to stimulation were classified as negative (increase of cortisol < 25%), partial (25–49%) and positive (≥50%).
Results
Fourteen out of seventeen patients responded to at least one stimulus other than ACTH. The most frequent cortisol response was obtained after terlipressin administration. A positive response to terlipressin was seen in 3/4 patients with bilateral tumors and in all of the patients (5/5) with unilateral incidentaloma. A partial to positive response was seen after the administration the others stimulus except to cinitapride. No response was observed in control subjects. Plasma ACTH remained supressed in all subjects throughout the study.
Conclusions
Aberrant membrane receptors detected by stimulation tests appear to be common in unilateral and bilateral incidentalomas with subclinical autonomous cortisol hypersecretion. The identification of these receptors could provide the novel opportunity to treat some of these patients with pharmacological agents.

P278

SMP30 is expressed in rat mammary gland and down-regulated by estradiol

Cláudio JB Maia, Cecilia RA Santos, Fernando Schmitt & Silvia Socorro
University of Beira Interior, Covilha, Portugal.

The SMP30 (Senescence Marker Protein 30) is involved in the maintenance of intracellular Ca^{2+} homeostasis and in the regulation of various Ca^{2+}- dependent proteins. A suppressive effect on cell proliferation, DNA synthesis and on the expression of oncogenes in rat hepatoma cells overexpressing SMP30 has been reported recently suggesting it may have a role in cancer progression. High levels of SMP30 expression have been found in liver and kidney of rats but no studies have focussed so far on the mammary gland where unbalanced calcium homeostasis and signalling is closely associated with its pathophysiology. The goal of the present study was to determine if SMP30 is expressed in rat mammary gland and to study its regulation by 17β-estradiol (E_2). For this purpose total RNA was extracted from rat mammary glands, reverse transcribed and subjected to PCR using SMP30 highly specific primers. The identity of the PCR product was confirmed by automatic sequencing. The presence of the SMP30 protein was confirmed by Western blotting of total protein extracts, which showed the presence of the protein as an intense band of ∼30 KDa, and by immunohistochemistry showing that SMP30 localizes preferentially in the citosol. To evaluate the responsiveness of SMP30 to E_2, adult females were ovariectomised ($n=10$) and 5 weeks after surgery they were either implanted with an Alzet mini-osmotic pump delivering 400 µg E_2/Kg/day ($n=5$) or vehicle only ($n=5$) for 7 days. Sham operated animals ($n=5$) were also included in the experiment but not implanted. The expression of SMP30 in the mammary gland was analysed by Real-Time PCR and the results showed its downregulation by E_2 in the rat mammary gland ($P<0.05$). These results suggest a likely involvement of SMP30 in breast physiology possibly related to estrogen dependent pathways. Further work to elucidate the SMP30 role in the mammary gland is underway.

Acknowledgements: Portuguese Foundation for Science and Technology (FCT) (SFRH/BD/13388/2003)

P279

Family mutation of PRKRA1A associated with Cushing syndrome from pigmented micronodular adrenal dysplasia

Alfonso Gentil, Tomas Martin, Milagrosa Díaz Galvez, Silvia Maraver & Miguel Lucas
Virgin Macarena University, Sevilla, Spain.

Introduction
Pigmented micronodular adrenal dysplasia (PMNAD) is an infrequent cause of Cushing syndrome ACTH-independent, and can form a part of Carney syndrome (CS). In both, regulatory subunit mutations of the protein kinase A (PRKAR1A) have been demonstrated, but without apparent genotype-phenotype correlation.
Objective
To demonstrate the mutation of PRKAR1A and its functional and clinical expression in a family affected with PMNAD.
Material and method
The index case and nine members of the family at risk were valued to demonstrate mutation of the gene PRKAR1A after diagnosed with PMNAD. DNA was extracted from the index patient and nine family members, primarily to study the segregation and linkage to locus of the PRKRA1A gene. Analysis of microsatellites was done by PCR using 32p-dCTP and autoradiograph of alleles after electrophoresis in acrylamide gel. Afterwards, the sequence was determined. Basal and post dexamethasone plasmatic and urinary cortisol and ACTH were valued.
Results
We describe a deletion of six pared sequence bases of the polypyrimidne tract [exon 7 IVS del (−7→−2)] of PRKRA1A gene in the index case and in four family members, three of them revealing PNMAD. In the remaining two family members (father and aunt of index patient), hypercorticism was not seen, although the father showed paradoxical response to dexamethasone on one occasion. Clinical manifestations of CS were not described. The other family members did not show PRKAR1A mutation.
Conclusions
A small intronic deletion of PRKAR1A gene could cause PMNAD, with a varying grade of penetration and clinical expression. This shows us the first genetic defect of PRKAR1A gene, which is associated to a specific phenotype.

P280

Non-genomic glucocorticoid effects on insulin secretion

Greisa Vila, Ludwig Wagner, Anastasia Nabokikh & Anton Luger
Department of Medicine III, Clinical Division of Endocrinology and Metabolism, Medical University of Vienna, Vienna, Austria.

Glucocorticoids act directly on pancreatic islets suppressing insulin secretion from the beta cells through a genomic mechanism of slow onset. We present here data on immediate actions of dexamethasone on two models of insulin secretion: RINm5F and INS-1 beta cell lines. Under normal glucose concentrations, dexamethasone rapidly (within minutes) decreased insulin secretion about 30%. Under hypoglycaemic conditions (glucose reduced to 50% for 1 hour) dexamethasone increased insulin release. Both these effects were present within 10 minutes and not in longer (up to 1 hour) stimulations. They were completely abolished by preincubation with pertussis toxin, slightly inhibited by the intracellular glucocorticoid receptor (iGR) antagonist mifepristone (RU486) and unaltered by the transcription inhibitor cycloheximide.

Western blotting experiments revealed that serum glucocorticoid kinase 1 (SGK1, a known early transcriptional target of glucocorticoids also known to regulate epithelial ion transport) rapidly translocated to the membrane following Dexamethasone treatment. Rapid changes were also seen in the cellular distribution of the calcium-binding protein secretagogin. Incubation with pertussis toxin 30 minutes prior to Dexamethasone stimulation, abolished not only the above effects, but also the translocation of the iGR to the nucleus and the

increase in SGK1 mRNA levels (starting 30 minutes after stimulation and measured by quantitative RT-PCR). While further mechanisms are still under investigation, we conclude that glucocorticoids act non-genomically on the beta cells affecting insulin secretion, protein distribution and possibly ion exchange. They have a dual role in homeostatic and stress conditions, similar to that seen in the fast feed-back to the HPA axis.

P281

The androgen receptor in the rat choroid plexus

CH Alves, S Socorro, IMT Gonçalves & CRA Santos
Centre of Investigation in Health Sciences-CICS, University of Beira Interior, 6200 001-Covilhã, Portugal.

The choroid plexus (CP) produces cerebrospinal fluid (CSF) and forms the blood-CSF barrier, being involved in the maintenance of the extracellular milieu of the brain and secretion of several neuroprotective factors. There are several experimental evidences showing that androgens enhance cognition and act as potential protective factors against Alzheimer's Disease. It has been shown that testosterone exerts neuroprotective actions against oxidative stress, apoptosis, and against the toxicity of β-amyloid, all via androgen receptor (AR). The AR has been identified in several regions of the central nervous system: the medial preoptic, arcurate, and ventromedial nuclei of the hypothalamus, in the medial nucleus of the amygdala, in the CA-1 hippocampus and the cortex, but not in the CP. In a first approach to study if the neuroprotective effects of CP are mediated by androgens and AR we investigated the presence of AR mRNA and protein in rat CP. Adult animals were euthanized and CPs were collected and frozen at −80 °C or fixed with 4% paraformaldehyde in PBS. The presence and levels of AR protein in the CP were studied by immunohistochemistry and Western blot, and the mRNA expression of AR in the CP was analysed by RT-PCR. The obtained results clearly demonstrate the presence of AR mRNA transcripts and protein in the rat CP with the protein levels in CP slightly higher than those found in prostate, testis, epididymis, and liver. Therefore, it is likely that some of the neuroprotective proteins secreted by the CP may also be regulated by androgens.

Acknowledgements
This work was supported by grant POCI/SAU-NEU/55380/2004 from FCT

P282

Correlation of BclI, N363S and the ER22/23EK polymorphisms of the glucocorticoid receptor gene and bone mineral density in patients with endogenous and exogenous hypercortisolism

Ágnes Szappanos, Judit Toke, Belema Boyle, Judit Majnik, Ibolya Varga, Edit Gláz, Miklós Tóth & Károly Rácz
Semmelweis University, 2nd Department of Medicine, Budapest, Hungary.

Objective
Genetic variation in the glucocorticoid receptor (GR) gene may be related to the clinical heterogenety and severity of the Cushing's syndrome. BclI, N363S and ER22/23EK polymorphisms are the three most investigated polymorphisms within the GR gene, however, the importance and magnitude of their effect in hypercortisolemic states are unclear. The BclI and the N363S variants are associated with increased, while the ER22/23EK variant is associated with reduced glucocorticoid sensitivity.
Methods
The allele frequencies of the BclI, N363S and ER22/23EK polymorphisms were investigated in 74 patients with endogenous or exogenous hypercortisolism and 172 healthy control subjects. The patient population included 31 patients with pituitary ACTH producing adenoma, 24 patients with adrenal Cushing's syndrome, 2 patients with ectopic Cushing's syndrome and 17 patients with glucocorticoid induced osteoporosis (GIO) caused by exogenously administered corticosteroids. DNA was extracted from peripheral blood leucocytes. The BclI and the N363S variants were detected by allele-specific polymerase chain reaction, and PCR-RFLP method was used to determine the ER22/23EK polymorphism. Bone mineral density was measured by DEXA at the lumbar spine and the left femoral neck (FN). This study was approved by local ethics committee, and written informed consent was obtained from all subjects.
Results
The frequency of the N363S polymorphism was significantly higher in patients with GIO than in the healthy control subjects (allele frequency 14.7% vs. 3.8%; $P<0.05$). Patients with the homozygous polymorph variant of the BclI polymorphism had significantly reduced mean FN z-score compared to patients with the wild-type variant (-1.803 ± 0.07 vs. -0.508 ± 0.944; $P<0.001$).
Conclusion
These results suggest that both of the N363S and the BclI polymorphisms of the GR gene may have an impact on the glucocorticoid sensitivity of bones.

P283

***In vitro* effects of 17 βE_2 and raloxifene on desmoid tumour derived cells**

Lucia Picariello[1], Silvia Carbonell Sala[2], Valentina Martineti[2], Alessia Gozzini[2], Maria Luisa Brandi[2] & Francesco Tonelli[1]
[1]Department of Clinical Physiopathology - University of Florence, Florence, Italy; [2]Department of Internal Medicine - University of Florence, Florence, Italy.

Desmoid tumours (DT) are a benign manifestation of familial adenomatous polyposis. The prevalent development in young fertile women, the regression during menopause or with tamoxifen treatment, underlie the potential role of estrogens and Estrogen Receptors (ERs) in the pathogenesis of these tumors. To investigate this hypothesis, the expression of ERs α and β in desmoid tumors derived cell cultures, the effects of $17\beta E_2$ and of raloxifene on DT cell proliferation has been evaluated '*in vitro*'.

Primary cultures from DT tissues obtained from seven patients were developed. RT-PCR and Western blotting analysis revealed that all the cultures expressed ERs α and β. In addition, also the RT-PCR and immunohystochemical analysis on the correspondent tissue samples confirmed the presence of ERs in these tumours. Treatment with $17\beta E_2$ (10^{-12} to 10^{-6} M) induced a dose-dependent cell growth, 10^{-9} M significantly increasing (120% to 250%) cell proliferation of the cell cultures. Raloxifene (10^{-7} to 5×10^{-6} M) reduced cell growth in a dose-dependent manner, with 5×10^{-6} M dose always significantly reducing both cell number (20% to 90%), without affecting apoptosis, as shown by DNA ladder assay. When $17\beta E_2$ and raloxifene effects were evaluated either alone or in combination, raloxifene was capable to significantly reduce (from 40% to 80%) the proliferative effects of $17\beta E_2$.

Although the study was made on a few samples these preliminary results showed that the different effects of $17\beta E_2$ and raloxifene on cell proliferation were probably linked to a different expression of ERα or Erβ in these cells.

These findings support on estrogenic role in the control of DT proliferation providing evidence for raloxifene effect on '*in vitro*' DT cell growth and viability. All together the '*in vitro*' and the previous '*in vivo*' studies encourage future investigation into a possible role of this SERM in the prevention and/or treatment of DT.

P284

ESR2 genotypes are associated with a reduced relative risk for sporadic colorectal cancer

Valentina Martineti[1], Lucia Picariello[2], Francesca Del Monte[1], Rosa Valanzano[2], Debora Strigoli[1], Silvia Carbonell Sala[1], Giuseppe Canonico[2], Francesco Tonelli[2] & Maria Luisa Brandi[1]
[1]Department of Internal Medicine, School of Medicine, University of Florence, Florence, Italy; [2]Department of Clinical Physiopathology, School of Medicine, University of Florence, Florence, Italy.

According to incidence data from Italian cancer registers, colorectal cancer is the third most common cancer in both men and women even considering skin non-melanoma cancers, lung and breast cancers. Moreover it represents the third absolute leading cause of cancer death in women and the fourth in men. Although data on Italian population regarding the role of estrogens in colorectal cancer have not yet been collected, several strands of evidence from international epidemiologic datasets indicate their protective role against the development of colon cancer. The effect of estrogens are mediated by oestrogen receptor (ERs), ERα and ERβ but ERβ has been identified as the predominant ER subtype in human colon, been expressed at higher levels in normal mucosa and significantly decreasing along tumour progression. According to the existence of a genetic predisposition to sporadic colorectal cancer, which is based on the carriage of common, low-penetrance polymorphic alleles, including those of PPARγ, NAT and VDR genes, polymorphism analysis of colorectal cancer has been recently attempted but none of the studies took into consideration the analysis of ERβ polymorphisms, dealing only with the most common estrogen receptor. On the basis of experimental and epidemiological data reported in the literature we thought that polymorphisms of ERβ had to be considered, as well as those of ERα, and indeed we performed an association study on 166 subjects affected by sporadic colorectal cancer and 197 healthy controls matched for age and sex. All enrolled subjects signed the informed consent. No association was ascertained between nor ERα *Pvu*II or *Xba*I polymorphisms, while a significant association emerged between ERβ *Alu*I genotype and colorectal cancer. In particular homozygous AA genotype was associated with a reduced risk (RR 0.57, $P<0.005$) and the homozygous opposite genotype aa with a higher risk (RR 1.59, $P<0.05$) for sporadic colorectal cancer. No further association was detected between ERα or ERβ genotypes and tumour features like Duke's staging, and histopathology.

P285

Frequency of three major glucocorticoid receptor gene polymorphisms in patients with adrenal incidentalomas

Jadranka Antic, Bojana Beleslin Cokic, Sanja Ognjanovic, Jovana Vignjevic, Katarina Mirkovic, Jelena Nikolic, Ivan Paunovic & Svetozar Damjanovic
Institute of Endocrinology, Diabetes and Metabolic Diseases, Belgrade, Serbia.

Background
Several polymorphisms of glucocorticoid receptor (GR) gene, including *Bcl*I, N363S and ER22/23EK, which may have an influence on glucocorticoid sensitivity, have been reported. *Bcl*I and N363S polymorphisms have been associated with clinical characteristics of increased and ER22/23EK of decreased glucocorticoid (GC) effects. On the other hand, metabolic syndrome has been described in patients with adrenal incidentalomas.
Objective and participants
We investigated the relation between *Bcl*I, N363S and ER22/23EK polymorphisms in GR gene in 31 patients with adrenal incidentalomas who underwent unilateral adrenalectomy (26 women; 36–76 yr old) and 117 healthy subjects (38 women; 20–76 yr old). The study was approved by the Institutional Ethical Committee.
Material and method
Several metabolic and anthropometric parameters were determined in order to correlate them to the genotype. Constitutive DNA was isolated from blood leucocytes. Genotyping was performed using PCR-RFLP, allele-specific PCR method and direct DNA sequencing.
Results
The larger allele frequency of the *Bcl*I variant was significantly lower in control subjects than in patients (4.3 vs 41.9%). Similarly, N363S (2.6 vs 16.1%) and ER22/23EK (0.9 vs 3.3%) variants of GR gene were less frequent in controls. Of several variables that were significant in univariate logistic regression analyses including age, gender, BMI, hyperlipidemia, hypertension, and diabetes mellitus, independent predictors of adrenal incidentaloma were *Bcl*I genotype [$P<0.001$, odds ratio (OR) 22.7; 95% confidence interval (CI) 6.7–77.0] and homeostatic model index (R_{HOMA}) ($P=0.028$, OR 1.5; CI 1.1–2.1).
Conclusion
*Bcl*I variant of GR gene is associated not only with metabolic syndrome but also with higher frequency of adrenal incidentalomas in population.

Thyroid – presented on Sunday

P286

An analysis on delays in diagnosis of papillary thyroid cancer

Gianlorenzo Dionigi[1], Renzo Dionigi[1], Luigi Bartalena[2], Maria Laura Tanda[2], Eliana Piantanida[2], Luigi Boni[1] & Francesca Rovera[1]
[1]University of Insubria Department of Surgery, Varese, Italy; [2]University of Insubria Department of Clinical Medicine, Varese, Italy.

Background
Thyroid carcinoma represents the most frequent form of cancer of the endocrine glands. In Italy, temporal trend shows a significant increase of incidence rates. Papillary thyroid cancer is the most common thyroid malignancy. Papillary thyroid carcinoma happens to be a multicentric tumor and trends to spread to the lymph nodes in the early stage of the disease. Thus early diagnosis is vital to improve the outcome for patients with thyroid cancer. The aim of this study was to determine the impact of delays in the diagnosis and treatment of this cancer.
Methods
43 patients [median age 42 (range 19–67), male to female ratio 1:8] with papillary thyroid cancer initially referred by a general practitioner and treated within this Unit from 2002 to 2005 were evaluated. Other histologic type were excluded from the study. Incidental microcarcinomas found in a multinodular goiter were also excluded. Details of referral, investigation and treatment were obtained by patient interview and cross-referenced with the case notes. Subjects completed an utilization questionnaire. The primary outcome variable was the time duration from cancer diagnosis to the time of cancer treatment.
Results
The overall median delay from the onset of symptoms to definite treatment was 13 weeks comprising patient delay in consulting a doctor (59%), delay in referral (19%), delay in diagnosis (10%), and delay in commencing treatment (12%). No significant reduction in total delay vs or change in the stage of disease at diagnosis was identified.
Conclusion
Long-term survival rate for papillary carcinoma is more than 90%, but this varies considerably among subsets of patients. A long delay in initiating this therapy has an adverse and independent effect on prognosis. In our experience the major delay occur prior to referral (patient delay), this has traslated into a significant raise in the overall delay. To achieve this, patient awareness must also be targeted. Patients with symptoms of these diseases should be initially referred for further care or followed up.

P287

Post-treatment effects of maternal hypothyroidism and thyroxin therapy on the subiculum neuronal density of the newborn rats

Morteza Behnam-Rassouli[1], Zahra Delshad[1] & Alireza Fazel[2]
[1]Dept. of Biology, Ferdowsi University, Mashhad, Iran; [2]Dept. of Medicine, Mashhad Medical School, Mashhad, Iran.

Studies in mice and rats suggest that legions of hippocampus interfere with memory for space and context and can have a significant effect on memory storage. The goal of the present study was to investigate the effect of maternal hypothyroidism, and thyroxin therapy on the neuronal density of the subiculum.

Twenty five adult female Wistar rats were divided into experimental groups (Exp) 1 and 2 and control. The Exp groups made hypothyroid (500 mg/l PTU in drinking water). The Exp 2 received levothyroxin as well (1 mg/l in drinking water). The treatment regimes were the same throughout the experimental period. Two 20 days old offspring were randomly selected from each litter, deeply anesthetized (0.2 ml of 2% xylazine), perfused by 10% formaldehyde, their brains processed for histological preparation and the parasagittal sections (9 μm) stained in tuloidin blue. By using the dissector method, the numerical density (N_V) of subicular region of the left hemisphere were estimated and statistically analyzed by JMP software in all groups.

The results show significant differences in subicular N_V in Exp 1 when compared with control and/or Exp 2 ($P<0.0001$). It seems that thyroxin therapy may improve the effects of hypothyroidism on the neuronal growth and extension of dendritic arborization of subicular neurons.

P288

Abstract unavailable

P289

The effects of subclinical hypothyroidism and replacement therapy on paraoxonase-1 (PON-1) and common carotis intima media thickness

Levent Kebapcilar[1], Abdurrahman Comlekci[1], Pinar Tuncel[2], Ahmet Solak[2], Mustafa Secil[3], Omur Gencel[3] & Sena Yesil[1]
[1]Dokuz Eylul University Endocrinology and Metabolism, IZMIR,Inciralti, Turkey; [2]Dokuz Eylul University Biochemistry, IZMIR,Inciralti, Turkey; [3]Dokuz Eylul University Radiology, ZMIR,Inciralti, Turkey.

The mechanism of atherosclerosis in patients with subclinical hypothyroidism (sHT), which has been partly attributed to lipid abnormalities, is still controversial. There is substantial evidence that ox-LDL plays an important role during the atherosclerosis process and paraoxonase-1 (PON-1) significantly inhibits generation of lipid peroxidation and thus plays a role in against atherosclerosis. The aim of the study was evaluate qualitative changes in lipoprotein metabolism, hs-CRP concentrations and PON1 activities with respect to common carotid artery intima-media thickness (CIMT) in 25 sHT (aged

48.96 ± 8.42 yr) patients before and after 4 months of levothyroxine substitution therapy and 24 normolipidemic healthy individuals (aged 42.79 ± 8.12 yr) comprised with the control group. There were no significant differences between controls and patients with sHT for age ($P = 0.05$). At baseline, compared to controls, patients with sHT showed similar levels of total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides levels. PON1 activities, hs-CRP concentrations and mean CIMT were similar between sHT and control group. Levothyroxine treatment had no effect on serum PON-1 activities and hs-CRP concentrations but resulted a significant reduced mean CIMT in the subgroup of patients with TSH levels > 10 mIU/L ($P = 0.017$).

In multiple linear regression analysis, we found the decrement in mean-CIMT was directly related to the decrement of waist circumference ($r = 0.532$, $P = 0.006$). In conclusion, monitoring of PON-1 activities and hs-CRP concentrations did not offer additional arguments for treating patients with sHT. However, the fact that levotiroxine replacement therapy was able to reduced CIMT suggests that beneficial effects of levotiroxine treatment for decreasing the risk of atherosclerosis in the subgroup of patients with TSH levels > 10 mIU/L.

P290

Elevated plasma FABP4 (aP2) levels in hypothyroidism: potential implication for accelerated atherosclerosis

Gokhan Ozisik[1], Sinan Caglayan[1], Mustafa Gultepe[2] & Metin Ozata[1]
[1]GATA H.pasa Teaching Hospital, Div.of Endocrinology and Metabolism, Istanbul, Turkey; [2]GATA H.pasa Teaching Hospital, Div.of Biochemistry, Istanbul, Turkey.

Context
FABP4 (adipocyte-specific fatty acid-binding protein 4, also known as aP2) is a cytoplasmic lipid chaperon involved in lipid metabolism, glucose homeostasis, and the regulation of inflammatory response. Its expression is limited to adipocytes, macrophages, skeletal muscle, and bronchial epithelia. Recently, a polymorphic allele of the *aP2* promoter (−87T→C) has been shown to be associated with decreased FABP4 expression in fat tissue, lowered triglyceride levels, and reduced risk for cardiovascular disease as well as type 2 diabetes (Proc Natl Acad Sci USA, 103:6970, 2006). However, circulating FABP4 levels in various disease states remains to be investigated.
Objective
The aim of this study was to determine circulating FAP4 levels in hypothyroidism.
Design
After having obtained local Ethical Committee approval, circulating FABP4 levels were measured in 38 adult patients with hypothyroidism before and two months after restoration of euthyroid state, and were compared to those levels in 34 age- and sex-matched control subjects.
Main outcome measures
Plasma FABP4 is measured using an ELISA kit (Human FABP4 ELISA, BioVendor-GmbH, Heidelberg). We also measured thyroid hormones, plasma lipids, insulin, and glucose levels. As FABP4 levels were not normally distributed data are given as "median (interquartile range)".
Results
We found that plasma FABP4 levels are elevated in hypothyroidism (0.67 ng/ml vs. 1.23 ng/ml; $P < 0.001$), and restoration of euthyroid state is associated with normalization of FABP4 levels. Hypothyroid state was also associated with elevated LDL-cholesterol, triglycerides, and HOMA-IR all of which decreased significantly following thyroid hormone replacement ($P < 0.001$, $P < 0.01$, and $P = 0.004$; respectively). We did not detect any correlation between plasma FABP4 levels and lipid parameters or HOMA-IR.
Conclusions
This is the first study to report plasma FABP4 levels in hypothyroidism. Our findings suggest that elevated FABP4 levels may be involved in the atherosclerotic process associated with hypothyroidism.

P291

Markers of REDOX system at autoimmune diseases of thyroid gland

Tsotsonava Tea, Adamia Nona & Chitava Irina
Tbilisi State Medical University, Tbilisi, Georgia.

Objective
Oxidative stress is developing by disequilibrium between antioxidative and oxidative mechanisms. In these conditions dysfunction of thyroid gland (TG) has been reported. It is related with deranged biosynthesis of thyroid hormones, in particular, with the absorption of iodine in thyrocytes. The objective of our investigation was to study the impact of oxidative stress on autoimmune diseases (AD) of TG.
Methods
38 patients [group 1 - with diffuse-toxic goiter (DTG, $n = 19$), and group 2 – with chronic autoimmune thyroiditis (ChAT, $n = 19$)] have been investigated. 10 healthy subjects serve as controls. The investigation was approved by the local ethics committees. The parameters of blood redox-system were investigated by electron-paramagnetic resonance. The AD was diagnosed by ultrasonography, function of TG and thyroid autoantibodies.
Results
Ceruloplasmin in group 1 was significantly higher than in controls (18.6 ± 1.3 vs. 16.0 ± 1.1 mm/mg, $P < 0.001$) and lower than in group 2 (18.6 ± 1.3 vs. 20.0 ± 2.0 mm/mg, $P = 0.015$). Fe^{3+}-transferrin in group 1 and 2 was significantly lower than in controls (19.2 ± 1.2 and 18.5 ± 1.3 vs. 22.0 ± 0.9 mm/mg; $P < 0.001$ in both cases). The difference between nitric oxide EPR-signals in groups was not significant. EPR-signals of Mn^{2+}, methemoglobin and lipid peroxyradical ions were appeared in investigated groups. Ceruloplasmin EPR-signals significantly inversely correlated with plasma thyroxine levels in main group and thyroid volume.
Conclusions
The results of our investigation suggest that oxidative stress occurs at AD of TG and expressed: a) by increase of blood ceruloplasmin levels; b) by decrease of blood Fe^{3+}-transferrin levels; c) by appearance of Mn^{2+}, methemoglobin and lipid peroxyradical ions in blood. These changes demonstrate possible association between AD of TG and REDOX-system.

P292

Selenium and its relation to thyroid antibodies, volume and ultrasound texture

Daniel Smutek[1], Ivana Cermakova[2], Jan Jiskra[1], Eliska Potlukova[1] & Ludvik Tesar[3]
[1]1st Medical Faculty, Charles University, Prague, Czech Republic; [2]Institute of Endocrinology, Prague, Czech Republic; [3]Inst. of Information Theory and Automation, Academy of Sciences, Prague, Czech Republic.

Objective
To find a relation between thyroid parameters (thyroxin serum level, thyroid antibodies, thyroid gland volume and ultrasound texture) and serum level of selenium.
Background
Selenium deficiency can lead to a decrease of triiode-thyronine in peripheral tissues. Changes in thyroid hormone production can be reflected in followed thyroid parameters.
Methods
In 33 patients ultrasound examination of thyroid gland was performed, volume was determined and texture features (spatial and second-order co-occurrence texture properties) were computed. Also free thyroxin, anti-thyroglobulin, anti-thyroperoxidase, anti-thyrotropin receptor (TRAK) and selenium serum levels (Se) were measured.
Results
A correlation between TRAK and Se with a very high correlation coefficient 0.95 ($P = 0.01$) was found. Furthermore significant correlation between Se and thyroid volume was found with correlation coefficient −0.54 ($P = 0.001$). Additionally we found several correlations between Se and following texture features: Euclidean distance from standard deviation to the median of original pixel gray levels and their four gray-level transformations ($r = -0.38$, $P < 0.05$), Euclidean distances from average deviation of original pixel grey levels and their four gray-level transformations to their mean and median ($r = -0.38$, $P < 0.05$).
Conclusion
We have found that there is a relation between selenium serum level and volume of thyroid gland. This is in concordance with known fact that selenium deficiency impairs normal thyroid metabolism. Our finding suggests that selenium supplementation, in addition to well-established iodine prophylaxis, may protect against goiter growth and optimize the function of thyroid axis. This is in concordance with other authors' findings. Another interesting finding is that selenium levels were also related to texture features representing thyroid morphological structure and TRAK. This suggests that selenium deficiency might have a role in development of autoimmune thyroid disorders.
The study was supported by of Czech Academy of Sciences (IET101050403)

P293

Which prognosis criteria for thyroid anaplastic carcinoma?

Guillaume Larroumets[1], Frederic Somda[1], Beatrice Roche[1], Catherine Dejax[2], Francoise Desbiez[1], Fabrice Kwiatkowski[2], Philippe Thieblot[1] & Igor Tauveron[1]
[1]CHU Endocrinologie-Groupe Thyroide Auvergne, Clermont-Ferrand, France; [2]Centre Jean Perrin, Clermont-Ferrand, France.

The prognosis of thyroid anaplastic carcinoma is poor. Yet can prognosis criteria change the therapeutic options?
Method
From a series of 26 cases from a single group recorded between 1990 and 2006, we analyzed the outcome after treatment based on surgery, radio and chemotherapy and looked for prognosis criteria.
Results
All but one patients died with a mean survival of 273 days (median survival of 130 days). Over 50% of patients had died within 6 months, and 80% within 12 months. Most deaths are related to loco regional tumour progression ($n=15$), but general dissemination (6) and drug toxicity (2) are also responsible.
Increased age, poor general condition at admission, rapid tumour growth (evaluated by pre-diagnosis duration of symptoms), compressive tracheal or oesophageal symptoms, and metastasis are associated with poorer prognosis while the concomitant presence of another histological thyroid carcinoma seems of better outcome.
Treatment can also influence the prognosis: complete surgery (563 vs 123 days) and multimodal treatment improve survival.
Conclusion
Despite poor overall prognosis, accurate evaluation of TAC at diagnosis is needed to evaluate outcome. Intensive treatment should always be applied.

P294

Clinical-epidemiological characteristics of thyroid cancer (TC) in the Crimea

Olexandr Butyrsky, Oraz Hajiyev & Oleg Bezrukov
Crimean State Medical University, Simferopol, Crimea, Ukraine.

We retrospectively analyzed case reports of patients been operated within 50 yrs. Our investigation demonstrates increasing tendency of TC. Total frequency of TC has increased from 0.76% in 1953–1964 to 8.48% in 2001–2005. Analysis revealed prevalence of TC in women (87.6%), sharp increase of morbidity after 30 years (14.6%), peak of morbidity – after 60 years (27.4%). Frequency of TC among adolescents is not increased (1.6–2.3%) that's associated with relative prosperity on pollution with iodine isotopes. TC is more frequent in town-dwellers (72.2%) due to higher pollution of environment that's a factors of thyroid hyperplasia.

Analysis of CT morphology demonstrates prevalence of differentiated forms: papillary (24.9%), follicular (15.5%), papillary-follicular (20.4%), microcarcinoma is revealed in 32%, medullary - in 4.5%, anapl?stic - in 1.9%, non-epithelial tumors - 0.8%.

We occupy active position for treatment of thyroid nodes, especially in doubtful cytological results, elderly women, children/adolescents, after radiation in the past.

Thyroid surgery isn't indifferent to patients. Baseless thyroidectomy worsens life quality (constant replacement therapy, intensifies accompanying diseases, provides background for other tumors), increases risk of complications. Therefore in differentiated TC we prefer sparing surgery – hemithyroidectomy, resection of isthmus & medial part of another lobe. Thyroidectomy and fat dissection is indicated in non-differentiated TC if tumor is extended out one lobe, multifocal growth in both lobes, distant metastases before iodine-therapy. Crile's operation is performed if TC proliferates into sternocleidomastoid muscle/internal jugular vein.

Conclusions

1. Thyroid surgery must be provided in specialized clinics.
2. Differentiated TC is indication for sparing surgery. Thyroidectomy must be adequately based.

All thyroid nodes should be operated with following histological identification and adequate post-surgery management.

P295

Fine needle aspiration biopsy of the thyroid. Cytohistologic correlation: experience in a central military hospital

Ema Lacerda Nobre, Helena Vilar, Zulmira Jorge, Mafalda Marcelino, Saudade André, Paulo Guerra, Carlos Lopes & João Jácome de Castro
Hospital Militar Principal, Lisboa, Portugal.

Background and aims
Thyroid nodular disease (TND) is a common condition in the general population. Malignant nodules occur in 5% of patients with thyroid nodules. Fine-needle aspiration biopsy (FNAB) is considered to be the most reliable method of differentiating benign and malignant thyroid nodules. The purpose of this study was to assess the accuracy of FNABs performed in our Hospital.
Methods
We retrospectively reviewed the medical records of patients submitted to thyroid surgery in our Hospital between June 1999 and June 2005.
Results
FNABs were performed in our Hospital since 1999. We included in our study 98 patients who had undergone thyroid surgery for TND. To the 98 patients a total of 142 FNABs had been performed. 80% were considered benign, 7% malignant and 13% suspicious. The discrepant cases were: 4 false-negative and 1 false-positive. The 4 false-negative cases had a cytologic diagnosis of nodular hyperplasia and found to be papillary thyroid carcinomas on histologic findings. The false positive case had a cytologic diagnosis of papillary carcinoma that revealed to be an Hürthle cell adenoma on histology. Our results showed a sensitivity of 60% and a specificity of 98.6%.
Discussion
All patients with false-negative results had multiple nodular goitre in which carcinoma was found in non dominant nodules on histology. None of these patients performed FNABs guided by ultrasound, consequently, aspirations were only done on the larger, palpable nodules. We suggest to perform ultrasound-guided FNAB in all supracentimetric nodules, in patients with multinodular goitre.

P296

Thyroid nodules in the elderly: role of ultrasound (US) and ultrasound-guided fine-needle aspiration biopsy (US-FNAB)

Vincenzo Triggiani, Edoardo Guastamacchia, Francesco Resta, Carlo Sabba & Emilio Tafaro
University Of Bari, Bari, Italy.

The prevalence of thyroid nodules is increased in the elderly. The aim of this study was to evaluate the accuracy of US and US-FNAB in the diagnosis of thyroid cancer in a population of elderly subjects, all of whose thyroid nodules were subjected to US-FNAB, with no prior selection based on dimensions or echo-pattern. Over a three-year period, 276 consecutive patients (64 males and 212 [76.8%] females), aged 65 to 87 (mean 70 ± 4.4), underwent US evaluation and US-FNAB of all their thyroid nodules. A total of 507 nodules were analyzed. Diameter: 5 to 70 mm (19.7 ± 12 mm). Solitary in 97 cases (19.1%), more than one in 410 cases (80.9%). Echographic pattern: hypoechoic in 255 cases (50.3%), isoechoic in 147 (29%), hyperechoic in 46 (9.1%), anechoic in 19 (3.7%), mixed in 40 (7.9%). Halo was present in 194 cases (38.3%). Microcalcification in 64 cases (12.6%). Cytology: negative in 448 nodules (88.4%), suspicious or indeterminate in 11 (2.2%), positive in 27 (5.3%), non-diagnostic in 21 (4.1%). Twenty-two patients underwent surgery (8%): 13 carcinomas (10 papillary, 2 anaplastic, 1 medullary), and 9 struma/adenomas. A total of 44 excised nodules were finally examined: 17 hyperplastic nodules (38.6%), 3 adenomas (6.8%), 24 carcinomas (54.5%): 19 papillary, 4 anaplastic and 1 medullary carcinoma. Malignant nodules were solitary in 8.3%; more than one in 91.7%; benign nodules were solitary in 10%, more than one in 90% (NS). Malignant nodules were hypoechoic in 21 (87.5%), isoechoic in 3 cases (12.5%). Benign nodules were hypoechoic in 6 cases (30%), isoechoic in 12 cases (60%), mixed in 2 (10%) ($P<0.0005$). A hypoechoic pattern had 87.5% sensitivity, 70% specificity and 79.5% accuracy in the diagnosis of thyroid cancer. A halo sign was present in 12.5% of malignant nodules vs 40% of benign nodules ($P<0.04$). The absence of a halo sign had 87.5% sensitivity, 40% specificity and 66% accuracy in the diagnosis of cancer. Microcalcifications were present in 12.5% of malignant and 10% of benign nodules (NS) (sensitivity 14%, specificity 90%, accuracy 48%). A hypoechoic pattern, microcalcifications and absence of a halo were simultaneously present in 1/20 benign nodules (5%) and in 3/24 malignant nodules (12.5%) (NS). Diameters were not statistically different (M:21.5 ± 14.3 vs B:19.4 ± 8.8 mm). For positive and suspicious or indeterminate cytological results considered in the same category, US-FNAB had 100% sensitivity, 100% specificity and 100% accuracy in this group of subjects.

P297

Clinical and pathological characteristics of thyroid anaplastic carcinoma: a regional survey in Auvergne

Guillaume Larroumets[1], Igor Tauveron[1], Frederic Somda[1], Beatrice Roche[1], Francoise Desbiez[1], Catherine Dejax[2], Fabrice Kwiatkowski[2], Philippe Thieblot[1] & Groupe Thyroide Auvergne[1]
[1]Endocrinology CHU, Clermont-Ferrand, France; [2]Centre Jean Perrin, Clermont-Ferrand, France.

Thyroid anaplastic carcinoma (TAC) is rare among thyroid cancers. Few surveys on these diseases are available.
Aim
To analyze the clinical and pathological characteristics of TAC in Auvergne, France, from a series of 26 patients among a cohort of over 1500 thyroid carcinomas (i.e. 1.7% of total thyroid cancers).
Results
Mean age was 72.1 years (range 42–91 years), with a sex ratio of 19 women to 7 men. A previous history of thyroid disorder is reported in 77%. 17 patients had goitre (among which 3 previously underwent surgery for nodular disease). 21 patients were euthyroid, 4 hyperthyroid and one presented with hypothyroidism. Recent onset (<6 month) of clinical symptoms is the rule. 92% of patients present with rapidly growing cervical mass. Other common symptoms include dyspnoea (50%), dysphagia (46%), dysphonia (42%). Occasionally pain ((8%), superior vena cava syndrome (19%) or poor general condition is reported. Tumour size is large, 8 cm (range 1–19 cm) with capsular overlap in 69%. Muscular extension occurs in 38%. Lymphadenopathies are reported in 38% and metastasis in 15% at admission. Pathological analysis of TAC reveals spindle cell carcinoma (54%), giant cells (46%) or occasionally squamous cells.In conjunction, 9 patients presented other thyroid carcinomas (7 papillary, 1 follicular and 1 sclerous occult).
Conclusion
TAC remains rare, occurs in the elderly with rapid growth and major compressive disorders. Spindle cell and giant cells are the most common pathologic findings, and association with other thyroid carcinomas appear in over 1/3 patients.

P298

Abstract unavailable

P299

Serum n-terminal pro-b-type natriuretic peptide (NT-proBNP) levels in patients with hyper- and hypothyroidism. Hyperthyroidism may affect NT-proBNP levels as independent of cardiac dysfunction

Senay Arikan, Alpaslan Tuzcu, Deniz Gokalp, Mithat Bahceci & Ramazan Danis
University of Dicle, School of Medicine, Department of Endocrinology, Diyarbakir, Turkey.

Background and aim
It was known that NT-proBNP levels increased in cardiac failure. But, NT-proBNP levels in different thyroid status still unclear. We aimed to evaluate serum NT-proBNP levels in both of hyperthyroid and hypothyroid patients without cardiac insufficiency.
Subjects and methods
Thirty nine patients with hyperthyroidism (43.0±16.5 yr), 25 patients with hypothyroidism (35.4±13.9 yr) and 34 ages matched euthyroid subjects (41.4±13.8 yr) were included to study. After all anthropometric evaluations, body fat analyses were determined with bioelectrical impedance. Electrocardiography and echocardiography were used in cardiac evaluations. Serum NT-proBNP was measured with immunoassay.
Results
Mean serum NT-proBNP levels in hyperthyroid patients was higher than both of control subjects ($P=0.02$) and hypothyroid patients ($P=0.03$). But, mean serum NT-proBNP levels in hypothyroid patients was not different from control subjects. There was a positive correlation between serum NT-proBNP and thyroid hormones (NT-proBNP and fT3: $r=0.316$, $P=0.002$; NT-proBNP and fT4: $r=0.284$, $P=0.006$, respectively). Serum NT-proBNP levels were positively correlated with left ventricle end-diastolic diameters ($r=0.317$, $P=0.006$), interventricular septum thickness ($r=0.395$, $P=0.001$), left ventricle posterior wall thickness (systolic) ($r=0.301$, $P=0.01$), left atrial dimension ($r=0.609$, $P=0.0001$) and negatively correlated with left ventricular ejection fraction ($r=-0.338$, $P=0.003$).
Conclusions
Hyperthyroidism may affect serum NT-pro-BNP levels independent of cardiac insufficiency. NT-proBNP values were increased in hyperthyroidism. Hyperthyroidism may lead to cardiac dysfunction undetermined with conventional echocardiography and these undetermined changes in cardiac functions may lead to elevation of NT-proBNP levels.

P300

Soluble intercellular adhesion molecule-1(sICAM-1) levels and different schemes of Graves' ophthalmopathy (GO) treatment

Elena Bogomazova & Tatjana Mokhort
The Republican Clinical Hospital of Medical Rehabilitation, Minsk Region, Belarus, The State Medical University, Minsk, Belarus.

Aim
To evaluate the base-line serum sICAM-1 levels among patients with GO and levels sICAM-1 at the end of 6 month follow up after different schemes of GO treatment.
Material and methods
72 patients with GO have been surveyed. Patients have been put into 4 groups depending on spent treatment: **1st group** – 26 patients received methylprednisolone per os 1 mg/kg in a combination with plasmapheresis; **2nd group** – 14 patients received pulse-therapy by methylprednisolone; **3rd group** – 18 patients received methylprednisolone per os 1 mg/kg in a combination with plasmapheresis and autogemomagnitotherapy; **4th group** – 14 patients received methylprednisolone per os 1 mg/kg.
We used «Human sICAM ELISA, BMS 201» kits for measured serum sICAM-1 levels.
Results
Serum levels sICAM-1 were 48.13±12.61 in the control group.

	1st group	2nd group	3rd group	4th group
Baseline serum sICAM-1 (M±σ, ng/ml)	81.82±33.3[b]	93.03±33.32[b]	79.04±21.94[b]	90.34±29.53[b]
sICAM-1 (M±σ, ng/ml) after 6 month follow up after GO treatment course	56.16±10.99[a, c]	50.82±13.77[c]	55.02±12.89[a, c]	64.27±29.87[a, c]
Δ sICAM-1 (Me)	17.55[d]	41.16	20.32[d]	23.07[d]

[a]$P<0.05$, [b]– $P<0.00001$ –*vs* control group; [c]$P<0.05$ –*vs* same groups before GO treatment; [d]$P<0.05$ – *vs* 2nd group).

Conclusions
The base-line serum sICAM-1 levels was significantly higher in all groups *vs* control and sICAM-1 levels significantly decreased in all groups at the end of 6 months follow up after the complete course of GO treatment. The treatment of GO with used pulse-therapy by methylprednisolone was better among different schemes of Graves' ophthalmopathy treatment, taking into account that ΔsICAM-1 was significantly higher in this group.

P301

The influence of smoking upon the incidence of Graves' disease and severity of Graves' ophthalmopathy

Eusebie Zbranca, Dumitru Branisteanu, Bogdanel Scurtu & Voichita Mogos
University of Medicine and Pharmacy "Gr.T.Popa", Iasi, Romania.

In order to investigate the relationship between Graves' disease, its associated ophthalmopathy and smoking, we followed retrospectively a group of 270 patients with Graves' disease (233 females and 37 males). Smoking incidence within this group was compared to that found in a control, thyroid disease-free group of 120 patients. The incidence of smokers was significantly higher in the group with Graves' disease (145 out of 270, 54%) when compared to the control group (42 out of 120, 35%, $P<0.01$). The 143 patients with Graves' disease having clinically obvious ophthalmopathy included a higher percentage of smokers than those without significant ophthalmopathy (63% compared to 43%, $P<0.01$). This difference was due mainly to female patients (76 smokers out of 123 female patients with ophthalmopathy – 62%, compared to only 44 smoking ophthalmopathy-free Graves' patients out of 109 −40%, $P<0.001$). Forty-four out of 90 (49%) tobacco users having ophthalmopathy were heavy smokers (i.e. over one pack per day for over 20 years), an incidence significantly higher than that of heavy smokers found in the smoking Graves' patients without ophthalmopathy (19 out of 55, 35%), or in the smoking patients from the control group (13 out of 42, 31%) ($P<0.05$). The data obtained support the hypothesis of tobacco influence upon Graves' disease evolution. Smoking seems to trigger both thyroid disease and ophthalmopathy appearance, especially in females. The risk of ophthalmopathy development and its severity might be dependent of the amount of cigarettes smoked.

P302

Fewer and fewer thyroidectomies in the treatment of Graves' disease

Mihai Radu Diaconescu, Mihai Glod & Ioan Costea
Umf "Gr.T. Popa", Iasi, Romania.

Background
Surgery which was until sixty years ago the only treatment available for Graves' disease (Gd) is now the last recommended therapeutical option, the number of thyroidectomies (Tx) being in continous decrease. American physicians prefere radioiodine use while their European and Japanese colleagues like better long-term administration of antithyroid drugs.
Patients and methods
A retrospective study carried out on 52 consecutive patients with Gd [female/male rate of 46/6 and age range at 28–65 (mean 44 years), representing 38.7% from all cases of thyrotoxicosis surgically treated in our clinic in the last two decennies, the annual number of such interventions is gradually diminished each year from 8 to only one. In all the cases a large subtotal Tx was performed (Dunhill's technique in three patients) conserving less of 5 g of functional tissue. The weight of resected gland varied between 40 to 260 (range 80) g. We had not neither postoperative crisis nor mortality, but permanent recurrent palsy and hypoparathyroidism was noted each in only one case. None of operated patients developed hypothyroidism or recurrent thyrotoxicosis and exophtalmy – present in half of our cases – diminished in 5 patients and was stablished in the rest of them.
Discussion
The better understanding of biologic behavior and natural history of Gd and the availability of effectiveness of another modalities of treatment refined our own philosophy about indications for surgery. So we operated on only patients with failure, major adverse reaction or poor compliance at medical therapy and consumpting clinical syndrome, large size (third or fourth degree) of diffuse goiter eventually with presence of a dominant cold nodule with suspicious FNAB or refusal of radioiodine administration.
Conclusion
In the absence of the golden standard therapy of Gd and in spite of increased worldwide preference for medical and radioiodine treatment with correspondent reduction of number of thyroidectomies, surgery attentive indicated in selected cases proved yet to be a safe and highly efficient solution which quickly restores euthyroidism with minimal risk of anatomic and functional complications.

P303

The prevalence of thyroid cancer in Albania

Agron Ylli[1], Florian Toti[1], Thanas Furreraj[1], Gerond Husi[1], Evis Sinjari[1], Medi Shehu[2] & Edmond Celiku[3]
[1]UHC "Mother Theresa" Service of Endocrinology & Metabolic Diseases, Tirana, Albania; [2]UHC"Mother Theresa" Service of Morphology, Tirana, Albania; [3]UHC"Mother Theresa" Service of Surgery, Tirana, Algeria.

Introduction
The prevalence of thyroid cancer is increasing worldwide as well as in Albania. For the first time we have created the National Thyroid Cancer Register, including the period 2000–2005.
Aim
Define the prevalence of thyroid cancer in Albanian population, the prevalence of different histopathologic forms of cancer and the probable risk factors.
Results
During this period 83 patients were diagnosed of Thyroid cancer. 62 (74.6%) were females. According to histopathologic form we found: papillary form 32 (38.6%), follicular 29 (34.9%), papillo-follicular 6 (7.3%), anaplastic 4 (4.8%), medullary cancer 4 (4.8%), other forms (metastases and lymphoma) 8 (9.6%). The clinical diagnosis at admission was: multinodular goiter 39 cases (46.9%), cold nodule 25 (30.1%), suspected thyroid cancer 11 (13.4%), toxic adenoma 4 (4.8%), benign adenoma 2 (2.4%), Graves' disease 1 (1.2%). According to the age-group: 20–30 yrs old 12 (14.4%), 30–40 yrs 21 (25.3%), 40–50 yrs 18 (21.6%), 50–60 yrs 17 (20.4%), > 60 yrs 15 (18.3%). The papillary form was more frequent in the age group 30–40 yrs old. It was present in M/F 42.8/37%, whilst follicular form was present in M/F 14.2/41.9%.
Conclusions
The thyroid cancer in Albania is more frequent in females than in males, with a 3:1 ratio. The follicular form is more frequent in females, while in general the papillary form is the more frequent one. Almost half of our patients (46.9%) belong to the age group of 30–50 years old. More efforts have to be done for a better and faster diagnosis where the FNA could play an important role.

P304

The prevalence of anti-C1q antibodies is increased in autoimmune thyroid disorders

Eliska Potlukova[1], Jan Jiskra[1], Petra Sandova[2], Zdenka Limanova[1], Daniel Smutek[1], Helena Mareckova[2], Marie Antosova[1] & Marten Trendelenburg[3]
[1]3rd Clinic of Medicine, General Teaching Hospital, 1st Faculty of Medicine, Charles University, Prague, Czech Republic; [2]Laboratory of Clinical Immunology and Allergology, General Teaching Hospital, Prague, Czech Republic; [3]Internal Medicine B and Clinical Immunology laboratory, University Hospital Basel, Basel, Switzerland.

Background
Autoantibodies directed against complement C1q (anti-C1q) have been described in a number of systemic autoimmune disorders. In systemic lupus erythematosus, they are strongly associated with proliferative lupus nephritis. However, no study has focused on the presence of anti-C1q in organ specific autoimmune disorders. The aim of this study was to determine the prevalence of anti-C1q in autoimmune thyroid disorders (AITD).
Methods
Serum levels of anti-C1q were measured using a commercially available ELISA kit (Bühlmann Lab. AG) in 23 patients with Graves' disease (GD) and 51 patients with Hashimoto's thyroiditis (HT). As controls, 16 patients with polynodular goitre and 72 normal blood donors were included. The patients underwent standard endocrinological evaluation.
Results
Positive serum concentrations of anti-C1q (>15 U/ml) were found in significantly more patients with AITD than in controls: 7/23 patients with GD (30.4%; $P<0.005$) and 10/51 patients with HT (19.6%; $P<0.05$), compared to 0/16 with polynodular goitre and 6/72 blood donors (8.3%). In patients with HT, anti-C1q correlated significantly with autoantibodies against thyroglobulin (Spearman $r=0.3312$, $P<0.01$) and against thyroid peroxidase ($r=0.2339$, $P<0.05$). Interestingly, in HT anti-C1q correlated also with thyroid stimulating hormone (TSH) ($r=0.2684$; $P<0.05$). In contrast, in patients with GD we found a negative correlation of anti-C1q with TSH ($r=-0.4169$, $P<0.05$) and a positive correlation with free thyroxine ($r=0.4365$, $P<0.05$).
Conclusions
Anti-C1q antibodies have an increased prevalence in patients with AITD. Their concentration correlates with autoantibodies against thyroid autoantigens and with some of the parameters of thyroid function.
Supported by the grant of the Czech Health Ministry IGA NR 8352-3.

P305

The analgesic efficacy of lidocaine/prilocaine (EMLA) cream during the fine-needle aspiration biopsy of thyroid nodules

Alptekin Gursoy, Derun Ertugrul, Mustafa Sahin, Neslihan Bascil Tutuncu, Asli Nar Demirer & Nilgun Guvener Demirag
Baskent University Faculty of Medicine, Department of Endocrinology and Metabolism, Ankara, Turkey.

Pain is one of the few drawbacks of fine-needle aspiration biopsy (FNAB) in patients with nodular thyroid disease (NTD). Lidocaine/prilocaine cream, an eutectic mixture of local anesthetics (EMLA), is a frequently used topical anesthetic. Despite its well-documented efficacy for the relief of pain associated with other cutaneous procedures that involve needle insertion, the analgesic role of EMLA has not been previously reported in patients with NTD who are undergoing FNAB. The aim of this study was to determine the analgesic efficacy of EMLA for FNAB-associated pain in patients with NTD. This double-blind, placebo-controlled clinical trial was conducted at a thyroid outpatient clinic. We studied 99 patients with NTD. Patients with NTD were allocated to receive either 2.5 g of EMLA ($n=50$) or placebo ($n=49$) 60 minutes before ultrasonographically guided FNAB. A series of 4 biopsies of each nodule was performed. Patients rated pain associated with the procedure according to a 100-mm visual analog scale (VAS), an 11-point numeric rating scale (NRS), and 4-category verbal rating scale (VRS). When the EMLA group was compared with the placebo group, there were no significant differences with respect to age, sex, thyroid volume, nodule size, or nodule site. Significant differences were noted in the pain ratings of those 2 groups according to all 3 pain scales. When the effectiveness of EMLA was compared with that of placebo, the mean VAS score was 25.0 ± 22.3 mm versus 40.0 ± 30.5 mm ($P=.006$) and the mean NRS score was 2.9 ± 2.3 points versus 4.0 ± 2.6 points ($P=.02$). The absolute numbers according to VRS score in each group was also significantly different ($P=.01$). No adverse events from the use of EMLA were reported. To our knowledge, this is the first study demonstrating that a topical anesthetic, EMLA, provides effective and noninvasive analgesia during the FNAB of NTD.

P306

Radioactive iodine in the treatment of type 2 amiodarone-induced thyrotoxicosis

Alptekin Gursoy[1], Neslihan Bascil Tutuncu[1], Arzu Gencoglu[2], Hamiyet Yilmaz[1], Asli Nar Demirer[1] & Nilgun Guvener Demirag[1]
[1]Baskent University Faculty of Medicine, Department of Endocrinology and Metabolism, Ankara, Turkey; [2]Baskent University Faculty of Medicine, Department of Nuclear Medicine, Ankara, Turkey.

Objective
Amiodarone-induced thyrotoxicosis (AIT) is usually classified into 2 types (type 1, in which a high iodine content triggers the autonomous production of thyroid hormone; and type 2, in which destructive thyroiditis causes the release of preformed thyroid hormone). A mixed form of AIT has also been also described. AIT is a difficult management problem that sometimes requires ablative thyroid therapy. The use of radioactive iodine (RAI) therapy in patients with type 1 AIT who had a 24-hour radioactive iodine uptake (RAIU) value of more than 10% has been previously reported. Despite its documented efficacy at usual doses (10–30 mCi) in patients with type 1 AIT, the efficacy of RAI in those with type 2 AIT has never been questioned, because type 2 patients usually have low RAIU. We thought that high adjusted-dose RAI (an adjustment made in accordance with the patient's 24-hour RAIU value and thyroid weight) might be an attractive alternative to thyroid gland ablation in patients with type 2 AIT.
Patients and methods
Four patients with type 2 AIT who required thyroid ablation were included in the study. These individuals were either poor candidates for surgery or had refused surgery. The size of the thyroid gland in all subjects was within normal limits, and each thyroid was characterized by a homogenous echotexture on ultrasonography, the absence of vascularity on Doppler sonography, a low ($<4\%$) 24-hour RAIU value, and the absence of thyroid autoantibodies, all of which are characteristic of type 2 AIT.
Results
The patients were initially treated with thionamides and glucocorticoids. All patients except 1 were euthyroid before RAI therapy. All 4 patients received 1 dose of RAI (range, 29–80 mCi), and followed-up for 12 months. No exacerbation of thyrotoxicosis was noted after RAI therapy. Hypothyroidism (in 3 patients) or euthyroidism (in 1 patient) was achieved in first 6 months.
Conclusions
In patients with type 2 AIT, RAI treatment may be the therapy of choice for thyroid gland ablation.

P307

The prevalence of hyperprolactinaemia in overt and subclinical hypothyroidism

Zeliha Hekimsoy, Sabriye Kafesçiler, Feyzullah Güçlü & Bilgin Özmen
Celal Bayar University Medical Faculty Department of Internal Medicine, Division of Endocrinology and Metabolism, Manisa, Turkey.

Hyperprolactinaemia can occur in patients with primary hypothyroidism. Although its prevalence in overt hypothyroidism varies from 0 to 40%, its prevalence and clinical significance in subclinical hypothyroidism has not been studied. In this prospective, observational study, serum levels of prolactin (PRL) were measured in 167 consecutive patients presenting to our endocrinology clinic for evaluation of hypothyroidism, and correlation of PRL levels with the severity of hypothyroidism (overt or subclinical) was performed. Forty three patients (37 women, 6 men, mean age 46.18 ± 12.98 years) had overt hypothyroidism. One hundred twenty four patients (112 women, 12 men, mean age 44.14 ± 12.19 years) had subclinical hypothyroidism. The other potential causes of the PRL elevation were evaluated. Serum levels of thyrotropin (TSH), free thyroxine, free triiodothyronine and PRL were measured in all patients before L-thyroxine treatment and after TSH normalization. PRL elevation was found in 10 patients (23.25%) with overt hypothyroidism, and in 29 patients (23.39%) with subclinical hypothyroidism. PRL levels decreased to normal levels after thyroid function normalised with L-thyroxin treatment. In both overt and subclinical hypothyroid patients, no relationship was found between TSH and PRL levels. In conclusion, our data confirm that in overt and subclinical hypothyroidism PRL regulation is altered, and that PRL levels return to normal with appropriate L-thyroxine treatment.

P308

The role of parathyroid hormone monitoring after total thyroidectomy in predicting post-thyroidectomy related hypocalcaemia

Evangelos Athanassiou, Gregory Christodoulidis, Constantinos Hatzitheofilou, Constantinos Tepetes, Demetrios Zacharoulis, George Tzovaras & Manthos Euthymiou
University of Thessaly, Larissa, Greece.

Objective
Clinically apparent hypocalcaemia following total thyroidectomy occurs in 20–25% of patients subjected to total thyroidectomy, in 2-4% of these patients the hypocalcaemia is permanent. Treatment by per-os or iv administration of calcium supplements and vitamin D is suggested when the serum calcium concentration falls bellow a critical level, either before or whenever the patients develop clinical symptoms of tetany. The purpose of this study is to evaluate the parathyroid hormone (PTH) serum levels, measured 6, 12, and 24 hours postoperatively, as predictors of hypocalcaemic symptoms in patients undergoing total thyroidectomy.
Materials and methods
In a period of 2 years (2004–2006) 108 patients were subjected to total thyroidectomy for benign or malignant thyroid pathology. Serum PTH, ionized serum calcium, and serum phosphorus levels were measured prior to surgery, 6, 12, and 24 hours postoperatively.
Results
In thirty-one out of 108 patients postoperative hypocalcaemia was observed (28,7%). In four of the 31 patients permanent symptomatic hypocalcaemia occurred, requiring vitamin D and calcium supplements indefinitely (3.7%). Tetany in 22/31 patients with PTH levels lower than 8pg/ml (normal range 8 pg/ml–75 pg/ml). These patients required vitamin D and calcium supplements for a few weeks or months (transient hypocalcaemia). Although 5/31 patients with clinical symptoms of hypocalcaemia (tetany) had the PTH levels recorded postoperatively within the normal range, an abrupt decline of serum PTH of more than 50% of the initial preoperative value was observed. These patients were also treated with vitamin D and calcium supplements for a few weeks postoperatively until the normal function of the parathyroid glands recovered (transient hypocalcaemia). In 77 out of 108 patients with normal calcium, phosphorus and PTH levels no symptoms of hypocalcaemia were noticed.
Conclusion
Following total thyroidectomy, an abrupt decrease in PTH serum levels either within or below the lower value of the normal range, a few hours postoperatively, serves as a reliable predictor of the development of clinically significant hypocalcaemia. Further studies are required however for validation of post-op PTH levels assay, in identification of a group of operated patients requiring prompt early therapy before tetany occurs.

P309

Abstract unavailable

P310

Intraorbital tissues effects of rituximab (RTX) treatment in patients with thyroid-associated ophthalmopathy (TAO)

Guia Vannucchi[1], Irene Campi[1], Stefania Rossi[2], Paola Bonara[3], Claudio Guastella[4], Nicola Currò[5], Simona Simonetta[5], Clara Sina[6], Roberto Ratiglia[5], Paolo Beck-Peccoz[1] & Mario Salvi[1]
[1]Endocrine Unit, Department of Medical Sciences, Fondazione Policlinico IRCCS, University of Milan, Milan, Italy; [2]Pathology Unit, Department of Medicine, Surgery and Dentistry, University of Milan, Ospedale San Paolo, Milan, Italy; [3]Internal Medicine, Fondazione Policlinico IRCCS, University of Milan, Milan, Italy; [4]Otolaryngology, Fondazione Policlinico IRCCS, University of Milan, Milan, Italy; [5]Ophthalmology, Fondazione Policlinico IRCCS, University of Milan, Milan, Italy; [6]Neuroradiology, Fondazione Policlinico IRCCS, University of Milan, Milan, Italy.

We previously described a significant response to RTX treatment in patients with active TAO, with no effect on TRAb and hyperthyroidism. In order to study the effect of RTX in the orbit, we analyzed the orbital tissues of 9 patients with TAO at decompression after RTX (n.2) or other treatments. Decompression was carried out in 2 patients for sight threatening optic neuropathy and in 7 for correction of proptosis. Of the RTX treated patients, one was decompressed after 12 months because of optic neuropathy, while the other after 23 months with burnt out disease. Of the other 7 patients, one was decompressed for the second time because of relapse of optic neuropathy that did not respond to steroids and 6 had burnt out disease of 15-175 months of duration. Immunohistochemistry of orbital fat and muscle showed presence of infiltrating immune cells in all patients. Infiltrates were present independently of the duration and the type of treatment of TAO and of thyroid disease. Interestingly, in the orbital fat of the patient who underwent decompression twice, we observed a typical lymphoid aggregate with CD3+ and CD20+ cells. In patients treated with RTX immunohistochemistry and cytofluorimetry were performed. While no cells were observed in the orbital fat of the patient with burnt out disease, we found persistence of CD3+ cells in the muscle of the patient with optic neuropathy at immunohistochemistry. In this patient, RTX induced peripheral CD20+ depletion, but persistence of 3 and 6% CD19+ after the first and a second cycle of treatment, respectively. Cytofluorimetry showed that almost all of these cells were CD19+5+ both in periphery and the orbital fat, suggestive of autoreactive clones. An increase of the absolute and relative numbers of CD19+5+ was observed in relation to the worsening of optic neuropathy, despite the absence of CD20+. These findings suggest that: 1) immune infiltrates are present in the orbital tissues of TAO patients even in long standing disease; 2) RTX may act by depleting CD20+ in the orbit; 3) persistence of autoreactive CD19+5+ clones in the orbit may correlate with an only temporary and partial response to RTX in TAO patients.

P311

Thyroid and gastric autoimmune diseases

Stéphanie Morel, Agnès Georges, Laurence Bordenave & Jean-Benoît Corcuff
Hôpital Haut-Lévêque, Pessac, France.

Background & aim
Autoimmune thyroid disease (AITD) is frequently accompanied by other organ-specific diseases. The aim of this study was to estimate the frequency of the association AITD-Biermer's disease (pernicious anemia) by investigating the presence of intrinsic factor antibodies (IF-Ab) in the serum of patients with AITD.
Methods
Sera from patients with biological signs of AITD (increased serum TSH levels associated to detectable thyroid peroxydase autoantibodies ($N=55$) or very low serum TSH levels associated to detectable TSHR autoantibodies ($N=58$)) were screened for the presence of type I IF-Ab with an automated chimiluminometric immunoassay based on a competitive method (Access IF Ab). Matched sera from patients with hypothyroidism ($N=66$) or hyperthyroidism ($N=47$) but no detectable peroxydase or TSHR autoantibodies, respectively, were similarly tested.
Results
Sera from 4 patients were tested positive for IF-Ab. All of them suffered from an autoimmune thyroid disease (2 Graves' disease, 2 Hashimoto's thyroiditis). Biermer's disease was previously known for 2 of them. Biermer's disease is strongly suspected in the 2 other patients: for the first, presence of parietal cell autoantibodies, normal serum vitamin B12 concentration and for the second, presence of type I diabetes and vitiligo and low serum B12 concentration. Sera from patients with non autoimmune thyroid dysfunction were all IF-Ab negative.
Conclusion
The incidence of detectable IF-Ab is significantly higher (3.5%) in patients with AITD than in patients with non autoimmune thyroid disease. Testing sera for the other IF-Ab (type 2) should uncover even more patients at risk for vitamin deficiency as the presence of type 2 IF-Ab could occur alone (no type I IF-Ab) in half Biermer's disease (thus potentially doubling the incidence). A prospective study looking for evidence of gastric autoimmunity and vitamin B12 deficiency in patients with AITD should establish whether the need to routinely test the patients is clinically useful or purely academic.

P312

VEGF, FGF and HGF in differentiated thyroid cancer

Elwira Przybylik-Mazurek & Bohdan Huszno
Collegium Medicum of Jagiellonian University in Krakow, Krakow, Poland.

Pathogenesis of thyroid cancer involves a number of biological, and environmental factors. The growth factors have mitogenic, proliferative and dedifferentiating effects. Some of the cytokines: Vascular Endothelial Growth Factor (VEGF), Fibroblast Growth Factor (FGF), Hepatocyte Growth Factor (HGF) are detected in a neoplasmatic tissue. Moreover, there are affected thyroid cancer cell growth and function *in vitro*.
Aim of the study
The aim of the study was to detect if the mitogenic cytokines level is higher in patients with differentiated thyroid cancer (DTC) than in healthy subjects.
Material and methods
There was two groups analysed in this study: 59 patients with DTC (follicular and papillary histotype) age 28 to 68 year and 21 healthy person in the similar age. The personal and familial history of thyroid disease and other chronic diseases was excluded by anamnesis. Blood level of VEGF, FGF and HGF were measured by ELISA kits R&D Systems USA in both groups.
Results
In DTC patients VEGF was significantly higher than in control group: 362.24 pg/ml, vs 198.24 pg/ml. There were no statistic differences between patients wit papillary and follicular histotype. VEGF was highest (413.35 pg/ml) in metastatic patients. FGF was higher in patients (8.37 pg/ml) than in controls (4.10 pg/ml) and in patients with follicular histotype (9.19 pg/ml) than in papillary histoptype (7.85 pg/ml). There were no differences in patients with or without metastases: 7.51 pg/ml vs. 7.37 pg/ml. HGF level in DTC patients was 1434.70 pg/ ml, and in controls 1294.18 pg/ml respectively.
Conclusions
The growth factors: VEGF, and FGF could be sensitive but perhaps not specific peripheral markers of thyroid gland cancer especially in metastatic patients.
Keywords
differentiated thyroid cancer, growth factors, VEGF, HGF, FGF.

P313

Fas and FasL expression on peripheral lymphocytes in patients with autoimmune thyroid disease

Stelios Fountoulakis[1], George Vartholomatis[2], George Philippou[1] & Agathocles Tsatsoulis[1]
[1]Department of Endocrinology, University of Ioannina, Ioannina, Greece; [2]Laboratory of Hematology, Unit of Molecular Biology, University HIospital of Ioannina, Ioannina, Greece.

Objective
The Fas/Fas ligand (FasL) apoptotic pathway is activated in patients with autoimmune thyroid disease (AITD). It is believed that Fas and FasL expression in intrathyroidal T lymphocytes and thyrocytes is regulated in a manner resulting in thyroid cell apoptosis in Hashimoto's thyroiditis (HT) or lymphocyte apoptosis in Graves' disease (GD). The hypothesis that Fas and FasL may be differentially expressed on peripheral lymphocytes in patients with HT and GD was investigated in the present study.
Methods
A total of 45 patients with untreated HT, 30 with subclinical hypothyroidism (mean age 34.9 ± 14.9 years) and 15 with clinical hypothyroidism (mean age 37.0 ± 18.4 years) as well as 13 hyperthyroid patients with untreated GD (mean age 35.8 ± 14 years) were studied and compared with 20 healthy controls (mean age 37.4 ± 15.3

years. Fas and FasL expression on $CD4^+$ and $CD8^+$ peripheral T lymphocytes were evaluated using two- and three-color flow cytometry on FACScan and the appropriate software (CELL Quest, Becton Dickinson).
Results
The proportion of $CD4^+$ T cells expressing Fas was increased in both GD ($64.1\% \pm 14.2$, $P<0.05$) and HT patients ($61.1\% \pm 15.1$ in those with clinical and $61.4\% \pm 13.0$ in those with subclinical hypothyroidism, compared to controls ($49.9\% \pm 7.7$, $P<0.05$). The proportion of CD8+T cells expressing Fas was also increased in patients with HT ($77.4\% \pm 16.6$ in those with clinical and $74.4\% \pm 14.4$ in those with sublclinical hypothyroidism, $P<0.05$) while no significant increase was observed in patients with GD ($67.2\% \pm 10.7$) compared to controls ($59.8\% \pm 14.0$). FasL expression on peripheral $CD4^+$, $CD8^+$ lymphocytes was below 3%.
Conclusion
Fas expression is upregulated in peripheral $CD4^+$ and $CD8^+$ T lymphocytes in patients with untreated AITD with no significant differences between patients with HT and those with GD. This may reflect the activation of the Fas mediated apoptotic pathway in AITD.

P314

A novel pro-migratory action of TGFb in papillary carcinoma

María Elena Rodríguez García-Renueles[1], Susan Belen Bravo López[1], Rafael Seoane[2], Carmen Gil Puig[1], José Cameselle Teijeiro[3], Francisco Barreiro[4] & Clara Álvarez Villamarín[1]
[1]Department of Physiology, School of Medicine, University of Santiago de Compostela, Santiago de Compostela, Spain; [2]Department of Microbiology, School of Medicine, University of Santiago de Compostela, Santiago de Compostela, Spain; [3]Department of Pathology, Hospital Clínico Universitario, Santiago de Compostela, Spain; [4]Department of Surgery, Hospital Clínico Universitario, Santiago de Compostela, Spain.

Neoplastic thyroid diseases (multinodular goiter (MNG), follicular adenoma, differentiated (DTC) and undifferentiated thyroid carcinoma) have a higher incidence in women than in men. In fact, in the last ten years, DTC is the only cancer increasing the frequency in women, with an incidence similar to ovarian carcinoma or lymphomas.

TGFb is a secreted factor important in the normal function of the thyrocyte. It has two independent actions: a fast antiproliferative action, inhibiting cell division through Smads and p15Ink, and an apoptotic action, decreasing p27Kip1 levels and activating Cdk2. In PC2 we have demonstrated that p27Kip1 overexpression blocked TGFb-induced apoptosis and induced a new slow-proliferating action, leading to a slow, but steady cell cycle that increases cell numbers in presence of TGFb.

In this study we have performed microarrays expression study in PC2 cells transiently tranfected with p27Kip1-expressing vectors (or the corresponding empty vector as control), with or without TGFb treatment.

In summary our results show that TGFbeta, apoptotic or anti proliferating genes are increased at the same time that anti-apoptotic genes are decreased in response to TGFb treatment. Interesting, p27Kip1expression reversed this signature causing induction of anti-apoptotic genes and reduction in apoptotic or antiproliferative genes after TGFb treatment. For example, BAX beta is increased in TGFbeta-treated cells but decreased in presence of TGFbeta in p27kip1-overespressin cells. Moreover, we discovered that the experimental condition p27Kip1+TGFbeta induced 12 migration genes and repressed 7 genes whereas mock-transfected cells exposed to TGFbeta increased 2 anti-migration genes and repressed only one.

A new pro-migratory action of TGFbeta in thyroid Papilar Carcinoma suggested by this fingerprint will be discussed.

P315

Significance of accurate serum thyroglobulin cut-off values in the postoperative follow-up of differentiated thyroid carcinoma

András Boér, Borbála Vincze, István Sinkovics, Sándor Keresztes, Bence Kapuvári, Mariann Boldizsár, Éva Remenár & Szabolcs Otto
National Institute of Oncology, Budapest, Hungary.

The present study was undertaken to evaluate the diagnostic performance of a high-sensitive thyroglobulin (Tg) immunoradiometric assay (BRAHMS Tg-S) in the follow-up of papillary and follicular thyroid cancer patients treated with total/nearly total thyreoidectomy and radioiodine ablation therapy. During TSH suppression serum Tg concentration was measured 6 weeks prior to the radioiodine ablation (onT4-Tg before ablation) as well as 3 months following treatment (onT4–Tg after ablation) in 54 tumour-free and 43 metastatic TgAb-negative patients, and accurate cut-off values were calculated. The selectivity and specificity of the measurement were determined by ROC curve analysis (MedCalc statistical software). The cut-off values calculated from the serum Tg levels of 'onT4–Tg before ablation' and 'onT4–Tg after ablation' were 1.9 ng/mL and as low as 0.6 ng/mL respectively. Medical history of 894 patients (differentiated papillary $n=715$ and follicular thyroid carcinoma $n=179$) were compared with the serum levels of Tg, TgAb and TSH at regular intervals. Serum Tg concentrations of clinically tumour-free, TSH-suppressed (TSH <0.3 mIU/L) patients ($N=774$) treated with total/nearly total thyroidectomy was below the threshold level of the kit (<1.9 ng/mL). The sensitivity of Tg determination in TSH-suppressed thyroid cancer patients with local recurrences or lung metastases was 86% and in bone metastases was 100%. The number of false negative data (11/29) was high in patients with papillary cancer and lymph node metastases. The sensitivity of Tg determination could be increased considerably even in case of patients with lymph node metastases by excluding TgAb positive patients. Measuring of Tg and TgAb, with IRMA and RIA methods applied proved to be effective for monitoring differentiated thyroid tumours. The determination of TgAb is highly recommended for the adequate interpretation of serum Tg levels. During the follow-up of patients the most accurate cut-off value should be selected according to the applied therapy.

P316

Thyroid ultrasonography and ultrasonography-guided fine-needle aspiration biopsy of thyroid nodules in correlation with pathological findings

Anna Poplawska-Kita[1], Janusz Dzieciol[2], Urszula Lebkowska[3], Anna Zonenberg[1], Arkadiusz Kita[4], Katarzyna Siewko[1] & Maria Górska[1]
[1]Department of Endocrinology, Diabetology and Internal Medicine, Medical University of Bialsytok, Bialystok, Sklodowskiej 24A, Poland; [2]Department of Human Anatomy, Medical University of Bialystok, Bialystok, Sklodowskiej 24A, Poland; [3]Department of Radiology, Medical University of Bialystok, Bialystok, Sklodowskiej 24A, Poland; [4]Orthopedic Department Regional Hospital, Bialystok, Sklodowskiej 26, Poland.

Introduction
Ultrasonography-guided fine-needle aspiration biopsy (ug-FNAB) is recommended as the first and most important step in the management of nodular thyroid disease.
Material and methods
We compared the results of ultrasonography examination (US) and the ug-FNAB of the thyroid gland with postoperative histpathological findings in 387 patients with thyroidectomy operated on (61 cytological and 326 clinical indication).
Results
Cytological diagnoses included 298 benign nodules (BN) (77%), 40 suspicious of follicular (FN) or 16 of Hurthle cell neoplasm (HCN), 21 papillary carcinoma and 8cysts.The incidence of thyroid carcinomas in the population studied was 8.5%. The size of the nodule was not related to the probability of getting an adequate specimen for cytological diagnosis. All patients were divided into four groups. Group I subjects with BN-97.8% were confirmed on histological results, whereas 6 of them were malignant (4 papillary, 1 follicular, 1 Hurthle cell). Group II histological confirmation of malignancy was 8 (20%) out of 40 patients with a diagnosis of FN (5 follicular, 3 papillary carcinoma). In this group we found also 17 follicular adenoma and 15benign nodules. Group III in the ug-FNAB diagnosed group of HCN after histological verification were 18.7% of carcinoma. GroupIV-in the 21 patients with diagnosis of papillary carcinoma, 16 cases were confirmed, 1 was FN and 4 benign. Correlation of cytology and histology showed that 76.2% ug-FNAB results correlated with the histological diagnoses, whereas 23.8% was discrepant. The smallest papillary carcinoma diagnosed by ug-FNAB had a diameter of 0.4 cm and 30% of all papillary cancer <1 cm displayed stage pT4.
Conclusion
Nodules with non-suspicious ug-FNAB results can be safety followed-up by US and ug-FNAB. However, FN and HCN remain the limitation of ug-FNAB, as the cytology cannot distinguish between benign and malignant nodules. Clinical characteristics, such as gender, age and nodule size, are not useful predictors for the presence of malignancy.

P317

Different prevalence of type 1 and type 2 amiodarone-induced thyrotoxicosis over a 30-year period

Luca Tomisti[1], Enrica Dell'Unto[1], Chiara Cosci[1], Chiara Sardella[1], Luigi Bartalena[2], Fausto Bogazzi[1] & Enio Martino[1]
[1]University of Pisa, Pisa, Italy; [2]University of Insubria, Varese, Italy.

Amiodarone induced thyrotoxicosis (AIT) may develop in patients with either underlying thyroid disorders (type 1) or normal gland (type 2). The latter is

considered a drug-induced destructive thyroditis, usually responding to glucocorticoids. Further treatments after restoring euthyroidism are often not necessary. The former is a true form of iodine-induced hyperthyroidism the management of which includes thionamides, potassium perchlorate and thyrodectomy. The prevalence of the two forms of AIT is unknown.
Objective
To study the prevalence of type 1 and type 2 AIT.
Patients
Two hundred and fifteen consecutive patients with AIT referred to our Department over a 30-year period.
Results
Type 1 AIT was more prevalent at the beginning of the study(67%). During the middle 80's the prevalence of the two AIT forms crossed each other. Thereafter prevalence of type 2 AIT progressively increased (up to 88% in 2006; $P<0.0001$) while that of type 1 AIT decreased. Type 2 AIT patients had a male preponderance, higher serum FT4/FT3 ratio ($P<0.002$), lower thyroidal 3 °h and 24 °h RAIU values($P<0.0001$) and received a higher cumulative dose of amiodarone than type 1 AIT patients ($P<0.0001$).
Conclusions
Over a 30-year period, the prevalence of type 2 AIT progressively increased and that of type 1 decreased. Thus, endocrinologist will face mostly with type 2 AIT patients, who will have a potentially self-limiting destructive thyroiditis, often successfully treated with glucocorticoids. On the other hand, a more aggressive (total thyrodectomy) therapeutic option might be necessary in patients unresponsive to glucocorticoids. Finally, after restoring euthyroidism, patients should be followed for late-developing hypothyroidism.

P318

Carbamazepine and risk of hypothyroidism: a prospective study

Jiri Horacek, Julius Simko & Gerhard Waberzinek
[1]Charles University, Faculty of Medicine and University Hospital, Dept. Internal Medicine, Hradec Kralove, Czech Republic; [2]Charles University, Faculty of Medicine and University Hospital, Dept. Neurology, Hradec Kralove, Czech Republic.

While carbamazepine (CBZ) treatment may affect serum thyroid hormone concentrations it rarely leads to clinically important hypothyroidism. This study was aimed to evaluate an early effect of CBZ on thyroid status in hypothyroid patients with thyroid hormone replacement, as compared with patients without a thyroid disorder.

Twenty-nine patients indicated for CBZ treatment were followed prospectively. Their thyrotropin (TSH), total thyroxine (TT4) and free thyroxine (FT4) serum levels were assayed before the start of CBZ medication (150 mg/d in the 1st week, then 450 mg/d), and then at week intervals for 7 weeks. Nineteen patients had no thyroid disorder before CBZ treatment (control group A), whereas 10 patients were treated with L-thyroxine (median 100 ug/d) for hypothyroidism and were stable before CBZ treatment (group B). The fluctuations of thyroid status after the start of CBZ treatment were compared between the groups.

In the control group, TT4 was significantly decreased by ca. 15 to 25%, starting from the 1st week of treatment (Friedman, $P<0.001$), while FT4 was decreased by only ca. 10 to 15%, and the significance ($P<0.001$) was delayed till the 2nd week. There was a concomitant increase in FT4/TT4 ratio ($P<0.001$) and a mild, non-significant increase in TSH ($P=0.073$) never exceeding normal range. Conversely, in group B with hormonal replacement, a similar TT4 and FT4 decline was followed by significantly increasing TSH levels ($P=0.011$), while the FT4/TT4 ratio was not significantly changed ($P=0.218$). In 3 of 10 patients TSH rose over 5 mU/L in the 3rd and 4th week, and the treatment had to be modified.

In patients with no thyroid disorder, CBZ caused subtle hormonal changes of no clinical relevance, due to adaptive response. In hypothyroid patients with replacement therapy this adaptation is lacking, and CBZ may precipitate subclinical or overt hypothyroidism. In this group, thyroid function monitoring early in the course of CBZ treatment seems advisable.

The study has been supported by research project MZO 00179906

P319

Epidemiology of thyroid cancer in the north eastern region of Poland

Anna Zonenberg[1], Beata Telejko[1], Agnieszka Nikolajuk[1], Mikolaj Rybaczuk[2], Janusz Dzieciol[3], Lech Zimnoch[4], Maciej Kokoszko[5], Jacek Dadan[5], Mieczyslaw Wasielica[6], Jacek Borawski[1], Zbigniew Puchalski[5], Marzena Juczewska[7], Ida Kinalska[1] & Maria Gorska[1]
[1]Department of Endocrinology, Diabetology and Internal Medicine, Medical University, Bialystok, Poland; [2]Department of Business, Informatics and Logistics, Technical University, Bialystok, Poland; [3]Department of Human Anatomy, Medical University, Bialystok, Poland; [4]Department of Clinical Pathomorphology, Medical University, Bialystok, Poland; [5]Department of General Surgery, Medical University, Bialystok, Poland; [6]Voivodship Hospital, Bialystok, Poland; [7]Oncological Hospital, Bialystok, Poland.

Before the introduction of mandatory salt iodination in 1997 the North-Eastern Region of Poland was known to be a moderate iodine deficiency area. Moreover, it was one of the mostly contaminated regions after the Chernobyl accident in 1986. The aim of our study was to evaluate the descriptive epidemiological features of incident thyroid cancers diagnosed among the residents of this area between 1996 and 2005. The Regional Cancer Surveillance Program was used to collect data on 691 newly diagnosed thyroid cancers registered during a 10–year period. The average annual incidence of all types of thyroid cancer per 100 000 residents rose from 3.9 in 1996 to 5.5 in 2005 (mean – 5.8 cases per 100 000 inhabitants). Thyroid cancer was more frequently diagnosed in women (82%) than in men. The majority of all cases was diagnosed in the age group of 46–55 years. There were 12 newly diagnosed cancers in children under 15 years of age (3 cases among children born after the Chernobyl disaster). The commonest histological type was papillary carcinoma (74.5%). Follicular type accounted for 10.9%, oxyphilic – 5.4%, medullar - 4.5%, anaplastic - 3.0% and other types – for 1.7% of cases. Conclusion: The increased incidence of thyroid cancers between 1996 and 2005 is most likely explained by the improvement in diagnostic techniques, but the effect of ionizing radiation after the Chernobyl accident has also to be taken into account. The influence of iodine deficiency seems to be a less probable factor in view of the predominance of the papillary type of carcinoma.

P320

The Na^+/I^- symporter (NIS) transports two of its substrates, I^- and ClO_4^-, with different stoichiometries

Orsolya Dohan[2], Carla Portulano[1], Cecile Basquin[1], L Mario Amzel[3] & Nancy Carrasco[1]
[1]Albert Einstein College of Medicine, Bronx, New York, United States; [2]Institute of Experimental Medicine Hungarian Academy of Sciences and Szent Rokus Hospital and Institutions, Budapest, Hungary; [3]Johns Hopkins School of Medicine, Baltimore, MD, United States.

The sodium/iodide transporter (NIS) mediates active I^- uptake in thyroid, lactating breast, salivary gland, and stomach epithelial cells. NIS-mediated I^- transport is electrogenic with a 2:1 Na^+:I^- stoichiometry, i.e. when NIS activity is measured electrophysiologically in NIS-expressing oocytes, inward positive currents are recorded upon addition of I^- or other anions that are NIS substrates. However, no currents are detected when perchlorate (ClO_4^-), a competitive inhibitor of NIS is used. This suggests that ClO_4^- either blocks NIS function without being transported, is translocated with a 1:1 stochiometry, or is transported at an extremely slow rate. ClO_4^-, which is used in military industry as a component of jet fuel, is a well known environmental contaminant of water supplies. The possible impact of environmental ClO_4^- exposure on the thyroid function of adults and nursing newborns is widely debated.

We report a thorough analysis of NIS-mediated ClO_4^- transport *in vivo* and *in vitro*. When lactating rats received ClO_4^-, both mothers and suckling pups exhibited a ~50% decrease in thyroidal I^- uptake relative to controls. For *in vitro* studies, we used a polarized MDCK epithelial monolayer setup in which NIS is expressed on only one side. Simultaneous addition of I^- and perchlorate markedly slowed NIS translocation of I^- to the opposite side, as compared to the control with I^- alone, because perchlorate was translocated first.

Hill plot analysis of NIS-mediated Na^+-dependent perrhenate transport revealed that perrhenate, an analogue of ClO_4^-, is transported with a 1:1 stoichiometry, explaining the absence of electrical currents observed with perrhenate also. Taken together, these observations provide novel mechanistic information on NIS, i.e. that NIS catalyzes substrate transport with different coupling ratios. In addition, that perchlorate is unequivocally transported by NIS and therefore actively concentrated in the milk, suggests that ClO_4^- water contamination may be more serious than previously thought, particularly for the most susceptible population, pregnant and lactating women and nursing newborns.

P321

Estimation of influence radioiodine treatment on course of Graves' ophthalmopathy

Olga Nechaeva, Alexander Dreval, Tatiyana Shestakova, Yulia Bardeeva, Irina Komerdous, Oksana Perepelova & Irina Chzikh
Moskow Regional Research Clinical Institute, Moskow, Russia.

Aim
To estimate influence of radioiodine treatment of Graves' disease (GD) on course of Graves' ophthalmopathy (GO).
Material and methods
9 patients with GD and mild or moderate GO were included to the study (3 (33%) men and 6 (67%) women); the anamnesis of smoking had 4 persons (44%). Diagnosis of GO was evaluated by determination of severity and activity of disease with CAS, presents of diplopia, orbital ultrasound. CAS before radioiodine treatment (RIT) and glucocorticoid pulse – therapy was 2.7 ± 0.7 points. The thickness of rectal extraocular muscles were (right/left eyes): upper – $5.44 \pm 0.37/5.4 \pm 0.4$ mm, low – $5.6 \pm 0.3/5.5 \pm 0.08$ mm, lateral – $5.1 \pm 0.3/5.1 \pm 0.3$ mm, medial – $5.2 \pm 0.5/5.2 \pm 0.5$ mm.
5 (55.6%) patients were underwent of prevention intravenous pulse therapy with glucocorticoids in a mean doze of 4.4 ± 2.3 gr. This therapy was spent 0.5–1.5 months prior to RIT. CAS in all patients after pulse therapy was 1.5 ± 0.7 points. The median of activity of 131I was 10.4 mCu.
Results
Right after treatment periorbital edema was determined in 2 cases (22%), burning of cornea – 2 (22%). All symptoms were stopped within 10 days. We did not find significant changes of eye muscles thickness.
In 1.5 months after RIT 7 (77.8%) patients were without worsening of GO. There was increasing of CAS to 2.5 points in other cases, but all these patients were hypothyroid. Symptoms of activity were decreased without additional treatment after administration of L-T4. Diplopia was kept in 1 patient without worsening.
Conclusions
After RIT worsening of GO was observed only in hypothyroid patients. In all cases it was not required to special therapy. In some cases symptomatic therapy was appointed.

P322

Influence of a subclinical thyrotoxicosis on heart in various age-grades

Alina Babenko, Elena Grineva & Natalia Vashrameeva
Pavlov Medical State University, St.Petersburg, Russia.

Subclinical thyrotoxicosis (ST) characterized by low serum TSH and normal FT_4 and FT_3 concentrations. ST may cause changes of geometry of heart and developments of diastolic dysfunction. Influence of ST on this evolutions depending on age of patients, duration of S?, effect of TSH level is not clear. In present research the effects of ST on changes of EchoCG at a different age were studied. The present study includes 66 normotensive patients with ST without any CVD (the age of 20–60 years, 6 men and 60 women) The patients were examined echocardiography by standard method. The patients were distributed on 3 age-grades: 1st group (gr1) ($n=20$) – 20–35 years; 2-nd group (gr2) ($n=24$) – 35–45 years and 3-rd group (gr3) ($n=22$) – 45–60 years. The parameters EchoCG were normal in patients of gr1 and gr2: relative wall thickness (RWT) ($0.34+0.009$ and $0.35+0.01$ cm), left atrial diameter (LAD)($3.8+0.09$ and $3.8+0.07$ cm), isovolumic relaxation time (IVRT) ($93.8+1.93$ and $92.7+3.1$ msec) left ventricular mass index (LVMI) ($83.6+3.24$ and $90.5+5.1$ g/m^2). However, the mean RWT ($0.41+0.01$ cm, $P<0.05$), LAD($4.1+0.18$ cm, $P<0.05$), IVRT($100.6+4.1$ msec, $P<0.05$) and LVMI ($103.2+7.3$ g/m^2 $P<0.05$) in patients gr3 was higher than that in gr1 and gr2. The frequency of left ventricular hypertrophy (LVH) was in gr1 – 10%, in gr2 – 8.3%, in gr3 – 36.4%, left atrial enlargement (LAE) was in gr1 – 25%, in gr2 – 20.8%, in gr3 – 35.5%, diastolic dysfunction (DD) was in gr1 – 30%, in gr2 – 31.8%, in gr3 – 47.4%, increase pulmonal pressure >30 (IPP) was in gr1 – 19%, in gr2 – 59%, in gr3 – 19%. The level T3, T4 was highly positive correlated with LAD($r=0.32$, $P<0.05$) and pLA ($r=0.55$, $P<0.01$) and level TSH was highly negative correlated with pLA($r=-0.31$, $P<0.05$). The LVMI and IVRT were positive correlated both with age ($r=0.49$, $P<0.01$ and $r=0.34$, $P<0.05$) and level T3($r=0.32$, $P<0.05$ and $r=0.25$, $P<0.1$). Specific attributes of influence of ST on a heart were appearanced of IPP, LAE and DD, which were meet at any age with high often. The LVH was less characterised at ST and frequency of its development at young age is similar as in a comparable population on age. Frequency of LVH was significantly higher in patients >45 years old.

P323

Thyroid abnormalities during treatment with PEGIFNα-2a and PEGIFNα-2b in patients affected by HCV-related chronic disease: a prospective randomized study

Francesco Scavuzzo[1], Guido Di Monda[1], Domenico Serino[1], Maria Teresa Tartaglione[2], Filippo Lampasi[2], Antonio Ascione[2] & Raffaele Volpe[1]
[1]Endocrinology Unit – Cardarelli Hospital, Naples, Italy; [2]Liver Unit – Cardarelli Hospital, Naples, Italy.

Background
Correlation between therapy with interferons, thyroid autoimmunity and dysfunction is widely reported. Currently we used two different pegilated-interferons (α-2a, α-2b).
Aim
Evaluate a probable different behaviour of two PEG-IFN responsible of thyroid abnormalities.
Patients and methods
We observed 236 consecutive naïve-patients with HCV-related chronic-disease undergoing a treatment with antiviral therapy from June 2003 to June 2005; we enrolled 54 females and 68 males alternatively to α-2a (median age 49.03, chronic hepatitis 98, cirrhosis 24) and α-2b (median age 48.3, chronic hepatitis 106, cirrhosis 16). Thyroid autoimmunity (TgAb, TPOAb) and function (FT_4, FT_3, TSH) were evaluated before, during treatment (3, 6, 9, 12 months) and in follow up (12 months).
Results
At the end of treatment 21 patients (8,6%), median age 48.03, 10 females, all chronic hepatitis without cirrhosis, 16 without preexisting thyroid dysfunction, 5 with low positivity for thyroid autoantibodies (Abs+), developed thyroid disorders:

	pts	hypothyroidism hyperthyroidism in autoimmune thyroiditis		Subacute thyroiditis	Abs+
A-2a	10	4	2	1	3
A-2b	11	9	2	0	0

Therapy was discontinued for thyroid abnormalities in 3 patients: 2 for hyperthyroidism to VI month, (one with α-2a, one with α-2b, Abs+ before therapy), confirmed in the follow up; 1 for subacute thyroiditis to VI month with α-2a, with euthyroidism in the follow up. At the end of follow up 6 patients were Abs−, 3 was Abs+; for 8 patients hypothyroidism, for 4 patients hyperthyroidism remained.
Conclusions
Thyroid disease appear to III,VI and IX month of therapy in: **α-2a** 4,4 and 2 patients (8 females; median age 47); **α-2b** 1,7 and 3 patients (5 females; median age 44.3). Two PEG-IFN don't show significative differences for induced thyroid dysfunction; furthermore none cirrhotic patients developed thyroid abnormalities.

P324

The effect of L-thyroxin theraphy on left ventricular diastolic dysfunction in patients with subclinical hypothyroidism

Tayyibe Saler[1], Zuhal Aydan Saglam[1], Kazim Korkmaz[1], Hayriye Esra Ataoglu[1], Mustafa Yenigun[1] & Berrin Oguzhan[2]
[1]Haseki Training and Research Hospital 4th Clinic of Internal Medicine, Istanbul, Turkey; [2]Brussels Free University, Department of Hormonology, Brussels, Belgium.

Aim
Subclinical Hypothyroidism (SH) is associated with cardiovascular disorders which may include increased risk for atherosclerosis, endothelial dysfunction and myocardial dysfunction.To investigate the prevalence of left ventricular systolic and diastolic dysfunction in patients with subclinical hypothyroidism and the effect of L-thyroxin therapy on myocardial performance using conventional echocardiographic parameters.
Method
The study includes 95 patients (F/M:83/12, age: 40.91 ± 10.07 years) with SH as judged by elevated serum thyroid-stimulating hormone (TSH) levels (>4.2 mIU/l) and FT3 and FT4 within the normal range and 44 healthy controls (F/M: 39/5, mean age 38.77 ± 9.59 years). None of the participants had hypertension or BMI >25 kg/m^2. All patients and the control group underwent standart echocardiography and Doppler imaging. E/A ratio [early (E) and late (A) mitral peak velocities] and the interventricular septum thickness (IVST) were determined. 25 SH patients with E/A ratio <1 were diagnosed as myocardial diastolic dysfunction and received LT4 replacement therapy during 6 months in order to establish euthyroidism. The biochemical and echocardiographic measurements were repeted six months later.
Results
The E/A ratio was significantly different among SH and control group.At the baseline the SH patients showed significantly lower E (0.83 ± 0.25 vs 0.99 ± 0.17, $P<0.0001$), E/A ratio 1.18 ± 0.33 vs 1.33 ± 0.23, $P<0.003$) and IVST (0.98 ± 0.12 vs 0.91 ± 0.08, $P=0.001$). Left ventricular end systolic and diastolic diameters were comparable between the two groups ($P=0.025$ and $P=0.494$ respectively). After 6 months of follow-up with LT4 replacement therapy, 25 patients with SH had significantly higher

E/A ratio (1.09±0.22 vs 0.75±0.23, $P<0.0001$) and reduced (1.05±0.14 vs 0.95±0.10, $P<0.0001$) IVTS measurements. With the comparison of all groups with Pearson test, TSH levels show a parallelism with IVST ($r=0.194$; $P=0.031$).
Conclusions
LT4 replacement therapy may reverse the impairment of left ventricular dysfunction and IVST observed in SH patients and should be advised to prevent the alteration of myocardial function.

P325

Risk factors for thyrotoxic cardiomyopathy

Veronika Shulgina[1], Galina Melnichenko[1], Valentine Fadeev[2] & Sergey Shornikov[2]
[1]National Research Centre of Endocrinology, Moscow, Russia; [2]Sechenov Moscow Medical Academy, Moscow, Russia.

Objective of the study
To examine risk factors for thyrotoxic cardiomyopathy (ThC).
Methods
In retrospective study (1975 to 2003) 272 patients aged 54 [43; 62] years with different forms of toxic goiter in combination with cardiac rhythm disturbances with or without heart failure (HF) were included. Atrial fibrillation (AF) and/or atrial flutter and/or ventricular premature beats accompanied with HF were diagnosed in 80.5% (219/272) patients (group 1), whereas 19.5% (53/272) patients had sinus tachycardia and/or atrial premature beats without HF (group 2).
Results
The prevalence of demographic and clinical characteristics of two groups was compared by use of χ^2-test. The factors associated with ThC $P<0.05$ (age at onset of thyrotoxicosis, age at hospitalization, period from onset of thyrotoxicosis until first treatment, period from onset of thyrotoxicosis until hospitalization, ophthalmopathy, relapse of Graves' disease, familial history of hypertension and coronary heart disease, such cardiovascular characteristics as previous history of rhythm disturbances, angina and HF) were retained as potential confounders. Then, binominal logistic regression was performed to identify those factors most associated with ThC using a probability value of $P<0.05$ and to calculate odds ratios (ORs) and 95% confidence intervals (CIs).
After adjustment for above-mentioned factors period over 1 year from onset of thyrotoxicosis until first treatment (OR=1.8, CI 95% 1.06–3.13; $P=0.02$) and age at hospitalization (OR per 1-year increment=1.1, CI 95% 1.02–1.15; $P=0.01$) remained independently associated with ThC. Weak positive interaction was observed between these two factors ($r=0.16$; $P=0.007$).
Conclusion
The data on natural history of patients with thyrotoxicosis and cardiovascular symptoms allowed us to identify risk factors for ThC. The frequency of ThC is increased in older patients with period from onset of thyrotoxicosis until first treatment over 1 year.

P326

Partial withdrawal of levothyroxine to stimulate serum thyroglobulin (TG) in the follow-up of differentiated thyroid carcinoma (DTC)

Francesca Ianni, Carlo Antonio Rota, Salvatore Maria Corsello, Annapina De Rosa, Francesca Gallo, Rosa Maria Paragliola, Francesca Ingraudo, Maria Pia Ricciato, Maria Ludovica Maussier, Massimo Salvatori & Alfredo Pontecorvi
Catholic University of Sacred Heart, Rome, Italy.

Aim
We compared effectiveness of partial withdrawal of levothyroxine (L-T4) to the use of recombinant human TSH (rhTSH) in preparation for Tg testing. We also evaluated clinical aspects and quality-of-life (QOL) during both regimens.
Materials and methods
Ten consecutive patients, previously treated with total thyroidectomy and radioiodine ablation for DTC, underwent rhTSH protocol and, after 15 days, reduced their L-T4 dose by 50% for 5 weeks. At the fourth week TSH was tested (predictive cut-off >10 μUI/ml), and at the fifth week TSH and Tg were measured (cut-off TSH >25 μUI/ml). Patients who did not reach the last cut-off were asked to continue half-dose protocol and to repeat TSH and Tg dosage at the sixth week.
At baseline and at the end of both rhTSH and "half-dose" protocols, all patients filled out questionnaires for QOL (SF-36) and symptoms and signs of hypothyroidism (Zulewski score). The study was approved by local ethical committee.
Results
Adequate stimulation of Tg was obtained in all patients after rhTSH. At half-dose protocol, 5/10 patients had TSH >25 μUI/ml at the end of the fifth week and 2/10 attained cut-off at the end of the sixth week. One patient left the study, another patient had limited compliance because of depression, and the last one completely withdrew L-T4 to receive radioiodine treatment because of high stimulated-Tg levels although not attaining TSH cut-off.
Tg levels were slightly more sensitive in the partial withdrawal scheme than in the use of rhTSH, but without any statistically significant difference. During the partial withdrawal period 5/7 patients reported no disease-specific morbidity, while 2/7 had just minimal discomfort. On the SF-36 health survey no statistically significant differences were found.
Conclusion
Partial L-T4 withdrawal seems to be an effective, simple, economical and well-tolerated procedure for Tg stimulation during follow-up for DTC.

P327

Selected markers of endothelial dysfunction in patients with subclinical and overt hyperthyroidism

Anna Modzelewska, Malgorzata Szelachowska, Anna Zonenberg & Maria Gorska
Department of Endocrinology, Diabetolygy and Internal Medicine Medical University of Bialystok, Bialystok, Poland.

Objective
The aim of this study was to evaluate the influence of subclinical and overt hyperthyroidism on the chosen markers of endothelial function.
Material and methods
The groups studied consisted of 97 hyperthyroid subjects (51 with subclinical and 46 with overt hyperthyroidism) and 39 healthy controls matched for age, gender and body mass index. The following parameters were measured: TSH, FT3, FT4 (by MEIA), VCAM-1 (vascular cell adhesion molecule 1), ICAM-1 (intercellular adhesion molecule 1), von Willebrand factor (vWF) and PAI-1 (plasminogen activator inhibitor 1) (by ELISA). Statistical analysis was performed using the computer program STATISTICA 6.0. The local ethical committee aproved the study.
Results
Among hyperthyroid patients 71 had toxic goiter (42 with subclinical and 29 with overt hyperthyroidism) and 26 had Graves' disease (9-subclinical, 17-overt hyperthyroidism). Significantly higher VCAM-1 levels were found in patients with overt and subclinical hyperthyroidism in comparison with the control group (1336.5+608.5 and 1168.9+508.4 vs 835.5+302.6 ng/ml, $P<0.001$ and $P<0.001$, respectively); vWF concentration was also significantly higher in patients with overt and subclinical hyperthyroidism than in the controls ($P<0.001$ and $P<0.01$, respectively), and in patients with overt hyperthyroidism in comparison with the subclinical group ($P<0.01$). The highest PAI-1 values were observed in patients with overt hyperthyridism (68.07 ng/ml, $P<0.001$ in comparison with subclinical hyperthyroidism and $P<0.001$ in comparison with the control group). There were not significant differences in ICAM-1 levels between the groups studied.
Conclusion
Our results suggest that endothelial dysfunction occurs in patients with overt as well as subclical hyperthyroidism.

P328

The changes in the incidence of nodular goitre, thyroid cancer and urine excretion of iodine in the inhabitants of north eastern Poland in 1997 and 2005

Anna Zonenberg[1], Wieslaw Zarzycki[1], Beata Telejko[1], Anna Modzelewska[1], Agnieszka Nikolajuk[1], Mikolaj Rybaczuk[2], Janusz Dzieciol[3], Katarzyna Siewko[1], Ida Kinalska[1] & Maria Gorska[1]
[1]Department of Endocrinology, Diabetology and Internal Medicine, Medical University, Bialystok, Poland; [2]Department of Business, Informatics and Logistics, Technical University, Bialystok, Poland; [3]Department of Human Anatomy, Medical University, Bialystok, Poland.

A higher incidence of nodular goitre and less differentiated types of thyroid carcinoma have been observed in iodine deficient regions. North-Eastern Poland was an area with a moderate deficiency of iodine until the introduction of the mandatory salt iodination in 1997 (30±10 mg KJ/kg NaCl). The aim of our study was to compare the incidence of goitre, thyroid carcinoma and urine excretion of iodine in the inhabitants of the North-Eastern Region of Poland in 1997 and 2005. In 1997 816 persons were investigated, 431 (52.8%) of whom reported for follow-up investigation in 2005. The study consisted of a questionnaire. thyroid ultrasonography and the measurement of iodine concentration in random urine sample. Parenchymatous goitre was found in 267 persons (32.7%) in 1997 and in 37 persons (8.6%) in 2005 ($P<0.001$. chi^2-58.165). The incidence of nodular

goitre was 12.75% (104 persons) and 24.59% (106 persons), respectively ($P<0.001$. chi^2-19.557). In 1997 three cases of papillary carcinoma were diagnosed. and in 2005 – 1 case. Decreased iodine excretion was observed in 71.28% subjects in 1997 and in 19.1% in 2005 ($P<0.001$. chi^2-105.748). Conclusion. During the last 8 years, the incidence of parenchymatous goitre in the North–Eastern Poland significantly decreased, whereas the percentage of nodular goitre increased in the period analysed. Prospective analysis did not reveal an increase in thyroid carcinoma incidence. The observed changes may be due to the introduction of the mandatory iodination of table salt in Poland in 1997.

P329

The role of deiodinases in thyronamine biosynthesis

Susanne Piehl[1], Balizs Gabor[2], Heberer Thomas[2] & Josef Köhrle[1]
[1]Institut für Experimentelle Endokrinologie, Charité – Universitätsmedizin, Berlin, Germany; [2]Bundesinstitut für Risikobewertung, Fachgruppe 55, Berlin, Germany.

Deiodinases (5′-D1, 5′-D2, 5-D3) control the systemic and local bioavailability of thyroid hormones by removing iodine from their substrates. Thyronamine (T0AM) and 3-iodothyronamine (3-T1AM) are possible novel metabolites of classical thyroid hormones which have been demonstrated to occur endogenously and to display unique effects such as reducing body temperature in mice and activating the plasma membrane bound G-Protein coupled receptor TAAR1 (Scanlan *et al.*, 2004). As the pathways of thyronamine biosynthesis are still unknown, we reasoned whether deiodinases might be involved.

In preliminary experiments using classical ^{125}I release assays the HepG1 cell line was found to express a specific 5′-D1 activity of $1.2+0.29$ pmol iodide released$\times$ mg$^{-1}\times$min^{-1} but not to exhibit 5′-D2 or 5-D3 activity at all. Thus, HepG2 cells were used to study the ability of 5′-D1 to accept thyronamines as substrates. Cells were homogenized in HEPES buffer containing sucrose, EDTA and DTT. Homogenates were incubated for 2 h at 37 °C in the absence or presence of 1 mM PTU in 100 mM sodium phosphate buffer at pH = 6.8 containing 1 mM EDTA, 20 mM DTT and various concentrations of the following substrates: thyronamine (T0AM), 3-iodothyronamine (3-T1AM), 3,5-diiodothyronamine (3,5-T2AM), 3,5,3′-triiodothyronamine (3,5,3′-T3AM), 3,5,3′,5′-tetraiodothyronamine (T4AM) as well as rT3 and 3′,5′-diiodothyronine (3′,5′-T2) as positive controls. Deiodination products were analysed using a newly established selected reaction monitoring (SRM) based liquid chromatography tandem mass spectrometry (LC-MS/MS) method.

5′-D1 from HepG2 cells did not deiodinate any of the thyronamines at the substrate concentrations tested (50 nM to 20 μM). Thus, a role of 5′-D1 in thyronamine deiodination is rather unlikely. The ability of 5′-D2 and 5-D3 to accept thyronamines as substrates still remains to be tested.

Supported by Graduate College 1208, DFG

P330

Thyroid disease prevalance in Cushing's disease

Tayfur Toptas, Ozay Tiryakioglu, Demet Ozgil Yetkin & Pinar Kadioglu
Istanbul University Cerrahpasa Medical Faculty Division of Endocrinology and Metabolisim, Istanbul, Turkey.

Purpose
To determine the prevalence of nodular thyroid disease, autoimmune thyroid disease, goiter and primary thyroid dysfunction in patients with active Cushing's disease.
Patients and methods
Nineteen patients with active Cushing's disease (17 female, 2 male, mean age 43.16 ± 3.55 years) and forty, age and gender matched healthy volunteers who served as the control group (34 female, 6 male, mean age 47.28 ± 2.31 years) were included in the study. The diagnosis of active Cushing's disease was determined by, 24 hour urine free cortisol levels, 1 mg dexamethasone suppression test and loss of diurnal rhythm. fT3, fT4, TSH, anti TPO, anti TG measurements and thyroid ultrasound were performed in both groups.
Results
Thyroid gland volume was smaller in patients with Cushing's disease (11.84 ± 1.5 ml vs 17.85 ± 1.84 ml). The prevalence of goiter was 2/19 (11%) and 12/40 (30%), the prevalence of nodular thyroid disease was 10/19 (52%), and 20/40 (50%), the prevalence of autoimmune thyroid disease was 7/19 (58%) and 20/40 (50%), the prevalence of primary thyroid disease was 6/19 (27%) and 10/40 (25%) in Cushing's disease and in control group respectively.
Conclusion
The prevalence of nodular thyroid disease, goiter, autoimmune thyroid disease and primary thyroid dysfunction in Cushing's disease was found similar to control group.

P331

From sampling to analytics: experience and diagnostic consequences with some thyroid markers

Erzsebet Toldy[1], Zoltan Locsei[2], Istvan Szabolcs[3], Erzsebet Toldy[4] & Gabor L. Kovacs[5]
[1]Central Laboratory Markusovszky Teaching Hospital of County Vas, Szombathaly, Hungary; [2]1st Department of Medicine, Markusovszky Teaching Hospital of County Vas, Szombathely, Hungary; [3]National Medical Centre, Budapest, Hungary; [4]Department of Practical Diagnostics, Institute of Diagnostics and Management University of Pecs, Szombathely, Hungary; [5]Institute of Laboratory Medicine, University of Pecs, Pecs, Hungary.

In the clinical laboratory practice, endocrine biomolecules are mainly measured by immunoassay. Storage of the samples can not be avoided in many cases. Measurement in the low concentration ranges require exact knowledge on how storage would influence the functional sensitivity of the measurement.
Aim
To evaluate the effect of storage of serum samples on their stability and the functional sensitivity of the applied method.
Methods
The biomolecules parathormone intact (PTHi), thyroglobulin (Tg) and thyroglobulin antibody (TgAb) were studied.The measurements were performed by an electrochemiluminescence immunoassay (Elecsys 2010, Roche). The stability of Tg and TgAb were studied in serum ($N=71$) and that of PTHi in plasma ($N=31$) as well. The parameters were measured in the fresh samples as well as after 4 and 8 hour of storage at room temperature and after 48 hour of storage at 4–10 °C. A longer-term storability test was also performed by keeping the samples for 1–4 weeks in deep freezer. The functional sensitivity of the methods was calculated from the results of deep frozen samples.
Results
In the first 8 hours the immunoreactivity of Tg, TgAb and PTHi changed only marginally (2–8%). During 48 hours storage, the Tg immunoreactivity increased by 23%, the PTHi molecule by 5–12% and the TgAb immunoreactivity decreased by 8–13%. During the long-term deep freezing, the immunoreactivity of all biomarkers decreased by 12–39%. A stronger degradation of molecules was observed in the lower range. PTHi appeared to be more stable in plasma than in serum samples. The functional sensitivity of the PTHi (2.6 pg/ml) and Tg (0.66 ng/ml) methods were excellent, but the TgAb (85 IU/ml) sensitivity makes questionable the application as a tumor marker.
Conclusions
The immunoreactivity of Tg, TgAb and PTHi is not influenced by a short storage at room temperature, but freezing even for longer-term significantly alters the analytic results.

P332

$G_{q/11}$-dependent signaling of the tyhrotropin receptor regulates metallothionein 1 expression in human thyroid carcinoma cells

Thomas Buech[1], Christer Baeck[1], Nicola Nowak[2] & Thomas Gudermann[1]
[1]Department of Pharmacology, Philipps University, Marburg, Germany; [2]CRBA Gynecology & Andrology, Schering AG, Berlin, Germany.

Metallothioneins (MT) are cystein-rich intracellular proteins which exert anti-apoptotic effects by protecting cells against oxidative stress and DNA damage. Previously, expression of MT in normal and neoplastic thyroid tissue has been demonstrated. However, the thyroidal regulation of MT expression is widely unsettled. Thus, we investigated the expression of MT isoform 1 in human thyroid carcinoma cells (FTC-133-TSHR) upon stimulation with thyrotropin (TSH). Using quantitative RT-PCR we found that TSH led to a dose-dependent increase in MT-1 mRNA levels in these cells. To further characterize the signaling pathway involved in MT-1 induction we investigated thyroid carcinoma cells expressing a mutated TSH receptor incapable to couple to $G_{q/11}$ proteins (FTC-133 Y601H cells). In these cells, TSH still led to a marked increase in intracellular cAMP levels wheras an increase in inostiol phosphates was completely absent. Interestingly, TSH did not induce MT-1 in these cells, giving evidence that regulation of MT-1 was cAMP-independent but dependent on $G_{q/11}$-coupling. This finding was further corrobated by the fact that TSH-promoted incuction of MT-1 in FTC-133-TSHR cells was blocked by inhibitors of phospholipase C, whereas treatment with phorbol esters mimicked the effect of TSH. Finally, we investigated changes in MT-1 protein levels. Immunoblots and immunocytochemistry with MT-1 specific antibodies revealed a TSH-induced up-regulation of MT-1 in FTC-133-TSHR cells whereas no effect of TSH occurred in FTC-133 Y601H cells. The finding of $G_{q/11}$-dependent regulation of MT-1 by TSH adds further complexity to possible cAMP-independent functions of the TSH receptor.

P333

Association of cytokine gene polymorphism with Graves' disease in Turkish population

Faruk Kutluturk, Sema Yarman, Fatma Oguz Savran & Cigdem Kekik
Istanbul University, Istanbul, Turkey.

Cytokines play a crucial role in the pathogenesis of autoimmune thyroid disease, and recent studies have demonstrated an association between cytokine gene polymorphisms and Graves' disease (GD) in different ethnic groups.The aim of the present study was to investigate the relationship of IL-6, IL-10, TNF-α, TGF-β, and INF-γ gene polymorphisms with the development of GD in Turkish population. A total of 224 subjects were included in the study comprising of 100 patients with GD (70F/30M; mean age, 43.9 ± 13.8 years) and 124 healty subjects (81F/43M; mean age, 37.8 ± 10.2 years) without antithyroid autoantibodies or family history of autoimmune disorders.Genotyping was done by using PCR and sequence-specific primers. Statistical analysis showed a significant association between high TNF-α −308GA and IL-6 −174CC gene polymorphisms in patients with GD compared to control subjects ($P=0.044$, $P=0.016$, respectively). On the otherhand, the frequency of TNF-α −308GG genotype was significantly increased in control subjects compared to patient ($P=0.049$). However, no differences were observed between GD and control subjects for IL-10, TGF-β, and INF-γ gene polymorphisms. In conclusion, these results suggested that TNF-α −308GA and IL-6 −174CC gene polymorphisms are involved in susceptibility for GD, whereas TNF-α −308GG gene polymorphism has a protective effects against the development of GD in Turkish population.

P334

Hashimoto's encephalitis: role of diagnostic SPECT

Tomas Martin, Alfonso Gentil, Alberto Torres, Silvia Maraver & Milagrosa Diaz-Galvez
University Hospital "Virgen Macarena", Seville, Spain.

In autoimmune thyroid disease some diverse neurological alterations like dementia, psychosis or peripheral neuropathy, are described. Hashimoto's encephalopathy (EH) is a serious form of these neurological alterations. We describe three cases with different presentation and morphologic normal tests where cerebral SPECT was diagnostic.

Case n°1. A 32-year-old male diagnosed of autoimmune hypothyroidism which presents paresthetics and muscular stiffening, what do not improve with oral levotiroxine. The analyses shows a TSH> 200 and T4L of 0.2 ng/dl, with Ac. antiTPO> 4500 U/ml. After substitution, TSH 9, T4 l 1.80 ng/dl. RMN cranial and EEG were no diagnostic, SPECT shows cortical diffuse hypoperfusión, starting therapy with deflazacort 60 mg/24 h with evident improvement, worsening when the was reduced. Treatment was restored by 2 mg/kg. with resolution of the clinic.

Case n°2. 39-year-old female presents migraine, confusion and agitation with hallucinations and fever treated with aciclovir and antibiotics. A normal thyroid function with Ac. antiTPO >3000 U/ml was found and SPECT show patched cortical affectation in temporal lobe. Therapy with prednisona to 1.5 mg/kg was established, with successful results.

Case n°3. 33-year-old male with hiperthyroidisim autoimmune, in treatment with carbimazole, present a convulsive stroke. Increase TSI (TSI> 40 U/ml) and Ac antiTPO: 5850 U/ml, with normal thyroid function was found (TSH: 0,025 mU/ml, T4L 1,90 ng/dl). A treatment with carbamazepine (800 mg/24 h), discharging him. One month later he shows recidivants convulsive attacks again. Normal RMN, slow wave diffuse EEG without epileptic foci. SPECT showed a decrease of cortical perfusión. Therapy with steroids achieved disapparing the convulsions.

Conclusions

EH's diagnosis must be considered in subacute presentation, high levels of antithyroid antibodies (even with thyroid normal function) and absence of another pathology. The practice of cerebral SPECT a and a fast response to steroids are important confirmation signs in this pathology.

P335

Usefulness of Ki-67, PCNA, c-erbB-2 and CK 19 in the diagnosis of some thyroid follicular tumors

Ioana Zosin[1], Marioara Cornianu[2], Ioana Golu[1], Melania Balas[1] & Aurora Milos[1]
[1]University of Medicine and Pharmacy Victor Babes, Clinic of Endocrinology, Timisoara, Romania; [2]University of Medicine and Pharmacy Victor Babes, Department of Morphopathology, Timisoara, Romania.

This study represents a complex evaluation of a group of 26 cases with thyroid nodular disease (TND), using different diagnostic methods. Clinically and by means of ultrasonography, 12 patients were suspected of malignancy. Fine-needle aspiration biopsy (FNAB) detected mainly suspicious (12 cases) and malignant smears (5 cases). The microscopic examination of surgical specimens established the following diagnosis: follicular adenoma – FA (4 cases), papillary hyperplasia – PH (2 cases), papillary carcinoma – PC (10 cases), follicular carcinoma – FC (2 cases) and Hürthle cell tumors – HCT (8 cases). PCs were represented by occult, classic forms and variants, HCT included adenomas, carcinomas and some adenomas showed an uncertain malignant behaviour. Metastases were diagnosed in 6 cases.The expression of Ki-67 antigen, proliferating cell nuclear antigen (PCNA), cytokeratin (CK) 19 and c-erbB-2/neu oncogene was evaluated by IHC (DAKO LSAB method) in all surgical specimens. For IHC we used paraffin-embaded sections and monoclonal antibodies (mAb): MIB-1, PC10, mAb against c-erbB-2 and mAb CK 19. The most interesting conclusions regard the expression of CK 19 and c-erbB-2. CK 19 was diffusely and intense expressed in all cases of PCs, 1 case of Hürthle cell carcinoma (HCC), but never in PH. There was no apparent difference in immunostaining reactivity between tumors with or without metastases. Follicular and oxyphilic cell neoplasia showed at best a focal staining. Regarding the expression of c-erbB-2, 50% of PCs presented a cytoplasmic staining pattern and the rest a mixed one (cytoplasmic and membranous). Some FC and HCC showed also a mixed staining. The epithelial malignant tumors with metastases presented more expressed reactivity versus the cases without metastases.

The used corroborated investigations helped us to obtain an accurate diagnosis in some peculiar epithelial thyroid tumors.

P336

Soluble CTLA-4 is increased in Graves' disease and not related to thyroid status or ophthalmopathy severity

Jacek Daroszewski[1], Edyta Pawlak[2], Marek Bolanowski[1], Miroslaw Slowik[3] & Irena Frydecka[4]
[1]Dept. of Endocrinology, Diabetology and Isotope Treatment, Medical University, Wroclaw, Poland; [2]Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wroclaw, Poland; [3]Dept. of Ophthalmology, Medical University, Wroclaw, Poland; [4]Dept. of Hematology, Blood Neoplastic Diseases and Bone Marrow Transplantation, Medical University, Wroclaw, Poland.

Cytotoxic T-lymphocyte antigen-4 (CTLA-4) is a B7-binding protein that plays an important role in the down-regulation of T-cell activation. CTLA-4 function is closely associated with predisposition to autoimmune diseases. A native soluble form of CTLA-4 (sCTLA-4) is reported to be present in the sera of patients suffering from autoimmune thyroid disease. In this study we report data on sCTLA-4 concentrations in patients with clinical expression of Graves' disease.

The study group consisted of 102 patients with Graves' disease (83 females and 19 males, mean age: 50 ± 11 years). Of these, 47 were euthyroid, 38 were hyper-, and 9 were hypothyroid. Nine patients were without clinical signs and symptoms of ophthalmopathy, while 42 presented mild and 51 severe ophthalmopathy. The control group was 38 apparently healthy volunteers. Study was approved by a local Ethical Committee.

Soluble CTLA-4 was measured in serum by means of ELISA.

sCTLA-4 was not measurable in 13 samples from the control group, while it could be estimated in all the patient serum samples and was higher than in control group (range: 0.02-1983.94 ng/ml, median: 7.48 ng/ml, dispersion: 11.2 ng/ml vs. range: 0.16-35.49 ng/ml, median: 3.2 ng/ml, dispersion: 3.98 ng/ml, respectively, $P=0.03$).

Soluble CTLA-4 concentration was not related to FT4 or to FT3 level ($r=0.026$ and $r=-0.034$, respectively). Regression analysis of factors describing the severity of the course of disease (thyroidectomy, ^{131}I treatment, or methylprednisolone treatment in the past) did not reveal any link with sCTLA-4 concentration ($P=0.15$). Soluble CTLA-4 serum level was also not related to the severity of ophthalmopathy.

In our group of 102 patients with Graves' disease, sCTLA-4 was higher than in the control subjects. Soluble CTLA-4 was a sensitive marker of the disease and appeared to be related neither to metabolic status nor to clinical course of the disease or the severity of eye changes.

P337

The relationship of epicardial fat thickness with carotid intima media thickness and endothelial function in subclinical and overt hypothyroidism

Dilek Yazici[1], Hasan Aydin[1], Beste Özben[2], Ahmet Toprak[2], Dilek Yavuz[1], Ozlem Tarcin[1], Seda Sancak[1], Oguzhan Deyneli[1] & Sema Akalin[1]
[1]Marmara University Medical School, Section of Endocrinology and Metabolism, Istanbul, Turkey; [2]Marmara University Medical School, Deparment of Cardiology, Istanbul, Turkey.

Hypothyroidism is associated with increased cardiovascular mortality. Epicardial fat thickness (EFT) has been found to be correlated with visceral fat accumulation and is thought to be a novel cardiovascular risk factor. The aim of this study was to determine EFT and its relationship with carotid intima media thickness (CIMT) and flow-mediated vasodilation (FMD) in subclinical and overt hypothyroid patients.

Ten patients with overt (Group H) (42.2±15.1 y; F/M:9/1) and 18 patients with subclinical hypothyroidism (Group SH) (34.7±10.3 y; F/M:16/1) and without any other systemic disease were included. 28 healthy volunteers were recruited as controls. EFT was determined by M-mode echocardiography and FMD and CIMT were evaluated by Doppler echocardiography. The study was approved by local Ethical Committee.

EFT, FMD and CIMT results and the comparisons between the groups are shown in the table. EFT was weakly correlated with CIMT ($r=0.33$; $P=0.11$) and FMD ($r=-0.26$; $P=0.22$). TSH was also weakly correlated with CIMT ($r=0.33$, $P=0.11$) and FMD ($r=-0.38$; $P=0.06$).

	GROUP H ($n=10$)	GROUP SH ($n=18$)	CONTROLS ($n=27$)	P
Epicardial fat thickness (mm)	4.42±2.41[a]	2.41±1.49	3.28±0.31	P<0.05
FMD (%)	6.63±4.05[b]	11.33±6.07	9.99±5.44	NS
CIMT (mm)	0.60±0.18[c]	0.51±0.05	0.52±0.07	NS

[a]$P<0.05$; [b]$P=0.06$; [c]$P=0.05$ compared to group SH

Epicardial fat accumulation is greater in subclinical and overt hypothyroid patients than healthy controls. This finding is more prominent in overt hypothyroid patients. Although larger studies are needed to confirm this preliminary finding, EFT seems to be a promising marker for early atherosclerotic changes in this group of patients.

P338

Prevalence of thyroid antibodies in gestational diabetes mellitus

Luísa Ruas[1], Sandra Paiva[1], Maria Campos[1], Elvira Marta[2], António Lobo[2], Annette Cravo[2] & Manuela Carvalheiro[1]
[1]Endocrinology Dept – University Hospital of Coimbra, Coimbra, Portugal; [2]Obstetrical Dept – University Hospital of Coimbra, Coimbra, Portugal.

Background
Pregnancy alters the natural history of autoimmune thyroid disorders. The incidence rate of positive thyroid antibodies (T-abs +) in asymptomatic women during pregnancy has been reported to be between 6 and 19.6%.
Aim
To determine the prevalence of thyroid antibodies (T-abs) in Gestational Diabetes Mellitus (GDM).
Subjects & Method
In 408 women, at the time of diagnose of GDM, TSH, free thyroxine, free tri-iodothyronine and anti-thyroid antibodies (T-abs) (thyroperoxidase and thyroglobulin) were measured. In these women we evaluated: previous thyroid disease, maternal age, BMI, spontaneous abortion, first degree relatives with D.M., Sullivan and OGTT values, insulin needed for diabetes control, new-born weight, gestational age at the time of GD diagnose and at delivery, evaluation of glucose tolerance after delivery. Statistical analysis involved SPSS (Statistical Analysis for Social Sciences); $P<0.05$ was considered to indicate statistical significance.
Results
From the women (408) who were enrolled in the study 21(5.1%) had positive T-abs. Only 20 women had thyroid disease (2%), with no direct relation with the presence of T-abs. The presence of T-abs + had a positive correlation with type 1 DM abs ($r=0.202$, $P<0.001$). There was no correlation between T-abs + and TSH, free thyroxine and free tri-iodothyronine values, as well as with the other maternal and fetal variables.
Conclusion
The results revealed a prevalence of autoimmune thyroid disease of 5.1% in women with GDM, identical to normal pregnant women, thus this measurement should not be systematic in women with GDM during prenancy. However, in the sub-group of GDM with type 1 DM positive abs, the positive correlation founded, suggests a systematic screening for T-abs. These data reinforce the importance of screening of latent pluri glandular auto immune disorders during pregnancy in women prone for those.

P339

Thyroid investigation profile in patients with Hashimoto's thyroiditis associated with other autoimmune disorders

Catalina Poiana[1], Dan Peretianu[2] & Mara Carsote[3]
[1]"Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania; [2]SCM "Povernei", Bucharest, Romania; [3]"C.I.Parhon" Institute of Endocrinology, Bucharest, Romania.

Introduction
The prevalence of autoimmune disorders (AID) is more frequent in patients with Hashimoto's thyroiditis (HT).
Aim
To see if the clinical and biochemical aspects are different among the patients with HT and if they change related to the systemic or organ specific AID.
Material and method
A. HT was diagnosed on antithyroperoxidase antibodies (ATPO) over 34 UI/ml. **B**. 491 patients with HT were investigated; 67 (15.8%) of them associated other known AID. **C.** AID were also searched in 404 patients with ATPO less than 34UI/ml, as control group; 21 (5.19%) of them had at least one AID. **D.** TSH, antithyreoglobulin antibodies (ATG) and the thyroid echographic pattern – spilt into 7 subtypes, according to our original classification, were also investigated. **E.** Statistical analysis was performed using student' t test and χ^2 test, as appropriately.
Results
1. Prevalence of AID in HT patients is higher than in control group ($P<0.001$, $\chi^2=17.82$, 56 degrees of freedom). **2**. The most frequent AID were vitiligo, immune hepatitis, rheumatoid arthritis, drugs allergies and premature ovarian failure. **3.** The mean age at diagnosis was not statistically different between patients with HT and AID and patients with HT, but without AID, respectively 50.97 years vs. 50.06 years, $P=0.6$. **4**. The sex ratio in HT-AID patients and HT-nonAID patients was the same (96% women). **5**. Average of ATPO levels in HT-AID patients was statistically significant higher than in HT-nonAID patients (respectively 964.47 UI/ml vs. 587.44 UI/ml, $P=0.054$). **6**. The mean values of TSH were not different between the two subgroups (8.81 μUI/ml vs. 9.76 μUI/ml, $P=0.75$). **7**. The difference between mean ATG levels was small and non significant ($P=0.34$). **8**. There was a certain difference between echographic patterns ($P=0.025$, $\chi^2=16.06$, 7 degrees of freedom), but without the predominance of a specific subtype.
Conclusions
1. In HT, AID are more frequent than in control group. **2**. Vitiligo is by far the most frequently AID associated with HT. **3**. Higher ATPO levels are found in patients with HT associated with other AID.

Thyroid – presented on Monday

P340

Ultrasound patterns in patients with autoimmune thyroiditis

Dan Peretianu[1], Mara Carsote[2] & Catalina Poiana[3]
[1]SCM "Povernei", Bucharest, Romania; [2]"C.I.Parhon" Institute of Endocrinology, Bucharest, Romania; [3]Carol Davila University of Medicine and Pharmacy, Bucharest, Romania.

Objectives
To analyze the conceptual frame for a correlation between thyroid echographic description and antithyroid peroxydase antibodies (ATPO) levels in Hashimoto's thyroiditis (HT).
Material-methods
A. 783 patients: 396 with HT (ATPO > 34 UI/ml), 386-control (ATPO < 34 UI/ml). **B**. Ultrasound aspects were described in 8 patterns: 0-thyroid lack; 1 – hypoechogenic and pseudonodular; 2 – hypoechogenic and homogenous; 3 – hypoechogenic micronodular; 4 – macronodular (>10 mm), 5 – inhomogeneous hypoechogenic and pseudonodular; 6 – anechogenic micronodular; 7 – diffuse hyperechogenic (normal). **C**. ATPO was split into 9

intervals as: 0 – 34, 35 – 100, 101 – 350, 351 – 550, 551 – 800, 801 – 999, 1000 – 3000, 3001 – 5000, > 5000 UI/ml.
Results
A. In HT cases, pattern - number: 0 – 2; 1 – 186; 2 – 53; 3 – 25; 4 – 75; 5 – 39; 6 – 10; 7 –6. In controls: 0 - 1, 1 – 21; 2 –46; 3 –55; 4 –150; 5 –20; 6 – 21 7 – 73. **B**. Sensibility, specificity and positive predictive value (PPV): pattern 0: 51%, 99.74%, 66.67%; pattern 1: 46.97%, 94.56%, 89.86%; pattern 2: 12.28%, 88.08%, 53.54%; pattern 3: 6.31%, 85.55%, 31.25%; pattern 4: 18.04%, 61.14%, 34.44%; pattern 5: 9.85%, 24.82%, 66.1%; pattern 6: 2.53%, 94.56%, 33.26%; pattern 7: 1.52%, 81.09%, 7.54%. **C.** Correlation between serum ATPO and ultrasonographic patterns: χ^2 test (54 degrees of freedom) = 100.94; $P = 0{,}0002$.
Conclusions
1. There are differences in sensitivity, specificity and positive predictive values for the 7 patterns. **2**. When PPV is near 90%, as in pattern 1, the test may be very suggestive for HT. Therefore, "hypoechogenic-pseudonodular" type means HT in 90% cases. **3.** PPV around 30% as in 3, 4, 6 patterns reveals low probably of HT. **4**. In type 7, PPV of 7.54% reflects a very low possibility of HT.

P341

Iodine intake in Portugal: preliminary results in pregnant women
Edward Limbert[1], Susana Prazeres[1], Ana Miranda[1], João Castro[2], Francisco Carrilho[3], Henrique Reguengo[4] & Fatima Borges[4]
[1]Portuguese Cancer Institute, Lisbon, Portugal, [2]Military Hospital, Lisbon, Portugal, [3]University Hospital, Coimbra, Portugal, [4]Sto Antonio Hospital, Porto, Portugal.

Introduction
Iodine is the key element for the synthesis of thyroid hormones and its intake modulates the physiology and physiopathology of thyroid gland. In Portugal, endemic goiter has disappeared, but some data make us consider that iodine intake, as in other European areas, is far from being sufficient. Taking into account the potential harmful effects of moderate iodine deficiency during pregnancy, when needs are increased, and the absence of recent data on iodine intake in Portugal, a countrywide study on urine iodine was undertaken. Preliminary results of this on going study from pregnant women are presented
Material and Methods
Target Population-Pregnant women from maternity hospitals and school children from strategic geographical areas (coast line and inland);1911 urines from 8 maternity hospitals were analysed.
Urinary iodide-A fast colorimetric method (Gnat *et al*, Clin Chem 2003) is being used
Statistical methods-Central methods and proportional comparison tests
Global Results
Median urinary iodide concentration was 88.9 μg/L, being 21.3% below 50 μg/L.19% had values above. 150 μg/L
Results by Hospital
Median urinary iodine varied from 78 to 124 μg/L; 13.9% to 29.6% of women had values below 50 μg/L and 12.5 to 34% had values above 150 μg/L In South Portugal the proportion of women with values below 50 μg/L was significantly lower in Greater Lisbon than in other cities.
Conclusions
Although this results are preliminary they point out to an inadequate iodine intake in pregnant women, from most Portuguese regions. Considering these preliminary results the on going study needs to be completed (data from pregnant women and also from school children) and more detailed analysis is warranted in order to explain the observed differences between regions. Taking into account the potential deleterious effects of inadequate iodine supply during pregnancy, iodine supplementation is recommended in this period of life.

P342

"False negative" results of Tg in patients with DTC (differentiated thyroid carcinoma)
Vassilios Voliotopoulos[1], Vassilios Gianakopoulos[2], Ioannis Valais[1] & Isaia Charalabidou[1]
[1]Iatroplis Magnitiki Tomografia, Athens, Greece, [2]Sotiria Hospital, Athens, Greece.

Purpose
The long-term monitoring of patients with differentiated thyroid carcinoma is essential throughout the patient's life after total or near total thyroidectomy followed by I-131 ablation after thyroid suppression using recombinant TSH (Thyrogen).
As known the administration of rhTSH increase the sensitivity of Tg concentrations measurements. Antithyroglobulin antibodies are common clinical problem in patients with differentiated thyroid carcinoma. Because the presence of these antibodies usually interferes with serum globulin.
Methods and materials
We used the recombinant human TSH in 20 patients one year after the ablation therapy. All patients underwent WBS I-131 scan and thyroglobulin (Tg) and antithyroglobulin antibodies (ATG) were measured using eclia assay technique.
Results
8 patients had positive anti-Tg antibodies and in these patients the result was confirmed using the Tg confirmatory test (Roche Cobas 6000 eclia method).
In 3 patients the percentage recovery wasn't in our laboratory's expected values (a finding of 70–130% indicates correct recovery). In these patients we suggest another I-131 therapy.
Conclusion
Our data suggest that ATG determination and the following recovery test may determine some additional information to the follow-up of patients with DTC. We have to improve our ability to predict and monitor wich patients are likely to be harmed by their disease or oppose to those who will live unaffected by theirs.

P343

The incidence of Hashimoto's thyroiditis in the differentiated thyroid carcinoma
Vassilios Voliotopoulos[1], Vassilios Gianakopoulos[2] & Ioannis Valais[1]
Iatropolis Magnitiki Tomografia, Athens, Greece, [2]Sotiria General Hospital, Athens, Greece.

Purpose
Hashimoto's thyroiditis is medical disease witch affects more than 5% of the population and represents the most common cause of hypothyroidism. The possibility of an immunological and autoimmune mechanism in the pathogenia of the disease has been suggested.
Methods and Materials
In 200 patients, who received iodine 131 therapy after total or near total thyroidectomy for one or more cold nodules,in our department last year (71% with papillary and the rest with follicular carcinoma) 50 (25%) had Hashimoto's thyroiditis, based in the cytological analysis of the surgical resects thyroid gland. In 25 patients the diagnosis of Hashimoto's thyroiditis was not reached before the surgery.
Conclusion
An adequate follow-up of the patients with Hashimoto's thyroiditis may permit an early diagnosis of the differentiated thyroid cancer and its appropriate management, because the increased incidence of DTC and HT may indicate that HT is a precursor of thyroid cancer.

P344

Thyroid cancer radioidine therapy using recombinant human TSH
Vassilios Voliotopoulos[1], Vassilios Gianakopoulos[2] & Ioannis Valais[1]
[1]Iatropolis Magnitiki Tomografia, Athens, Greece, [2]Sotiria General Hospital, Athens, Greece.

Purpose
The use of recombinant TSH (Thyrogen) has already entered in the clinical routine in order to avoid the discomfort and the morbidity associated with the withdraw of the thyroid hormone.
Methods and Materials
We used the recombinant human TSH in 20 patients (age > 50 years) totally or near totally thyroidectomized who came in our clinic to receive radioiodine therapy for locally invasive differentiated thyroid cancer. All patients were treated, while euthyroid on L-4, after rhTSH administration with to consecutive daily injections (0.9 mg) of rhTSH. Half of them underwent diagnostic –before therapy diagnostic whole body scan using again rhTSH administration and after that iodine therapy using an identical second course of rhTSH.
Results
Administration of Thyrogen promoted I-131 therapy uptake in all patients as demonstrated with the post-therapeutic whole body scan. As known the administration of rhTSH increase the sensitivity of the Tg (thyrogobulin) concentrations measurements. About 12 months after therapy we performed whole body I-131 scan and we show a complete remission of the residual sites and in two patients reduction in one metastatic site.
Conclusion
Administration of rhTSH is safe and a very useful tool for inducing I-131 uptake in local or metastatic differentiated thyroid cancer avoiding L-T4 withdrawal.

P345

Characterization of facilitative glucose transporters (GLUT) in human thyroid carcinoma cell lines

Raffaele Ciampi[1], Agnese Vivaldi[1], Pamela Piampiani[1], Laura Agate[1], Piero Salvadori[2], Alberto Del Guerra[3], Aldo Pinchera[1] & Rossella Elisei[1]
[1]Department of Endocrinology, University of Pisa, Pisa, Italy, [2]Institute of Clinical Physiology (C.N.R.), Pisa, Italy, [3]Department of Physics "E. Fermi", University of Pisa, Pisa, Italy.

18FDG-PET is based on the capability of tumor cells to take up glucose. An increment in expression of the glucose transporter 1 (GLUT1) has been observed in thyroid tumors with poor prognosis but very few data are available about the expression of other glucose transporters in thyroid. Here, we study the expression and function of GLUT isoforms 1, 2, 3, 4, 6, 8, 10, 12 in human thyroid carcinoma cell lines ARO and FRO (anaplastic carcinoma), NPA (poorly-differentiated papillary carcinoma), WRO (follicular carcinoma) and TT (medullary carcinoma). We studied expression of GLUTs by conventional and quantitative RT-PCR, we evaluated cell 2-Deoxy-D-[^{3}H]-glucose uptake and we studied GLUT1 protein on cell membrane fractions. We confirmed that GLUT1 is the predominant isoform in thyroid carcinoma with higher expression in ARO and FRO. By contrast, GLUT3 expression is lower in these two cell lines but comparable to GLUT1 in WRO, NPA and TT. GLUT4 and GLUT10 are barely expressed in all cell lines. We also observed GLUT6 and GLUT8 expression in all cell lines and GLUT12 in ARO, TT and FRO. Western blot shows GLUT1 protein in ARO and FRO membrane fractions. All lines studied but TT display different levels of glucose uptake; surprisingly, NPA and WRO uptake is higher than in ARO and FRO although these latter show higher levels of GLUT1 expression. In conclusion, we confirm that GLUT1 is the predominant form in thyroid tumors but other isoforms can be present and its protein is abundant in anaplastic carcinoma cell membranes. Medullary carcinoma cell line TT, despite the expression of some GLUT isoforms, is not able to take up glucose. Finally, the high rate of glucose uptake observed in NPA and WRO could be justified by presence of other forms of GLUT not considered in this study.

P346

Genotype/phenotype relation for toxic thyroid nodules with or without TSH receptor mutations

Hulya Gozu[1], Sandra Mueller[2], Rifat Bircan[3], Knut Krohn[4], Mehmet Sargin[1], Haluk Sargin[1], Cem Gezen[5], Turgay Erginer[5], Nimet Karadayi[6] & Ralf Paschke[1]
[1]Dr. Lutfi Kirdar Kartal Education and Research Hospital, Section of Endocrinology and Metabolisim, Istanbul, Turkey; [2]Leipzig University, Department of Internal Medicine III, Leipzig, Germany; [3]Marmara University, School of Medicine, Department of Medical Biology, Istanbul, Turkey; [4]Leipzig University, Interdisciplinary Center of Clinical Research, Leipzig, Germany; [5]Dr. Lutfi Kirdar Kartal Education and Research Hospital, Section of 1.General Surgery, Istanbul, Turkey; [6]Dr. Lutfi Kirdar Kartal Education and Research Hospital, Department of Pathology, Istanbul, Turkey.

Constitutive activation of the cAMP pathway by activating TSHR mutation stimulates both thyrocyte proliferation and function. Thus they lead to formation of toxic thyroid nodules (TTNs) and ultimately hyperthyroidism. The *in vitro* activity of the various TSH-receptor mutation varies from 2–7 fold cAMP increase over the wild type TSH receptor. One previous study invrestigated a possible genotype to phenotype relation in TTNs with somatic TSHR mutation with a negative result.

TSHR mutations have been identified in 52(70.2%) of 74 TTNs in a recent study. In order to investigate the genotype-phenotype relation in TTNs we compared the clinical and laboratory findings of these patient (nodules) with or without TSHR mutation.

Most strikingly, nodule volume was found significantly higher in the mutation + groups (Z:-2,803;*P*:0,005). No significant difference between iodine sufficient and deficient regions of Turkey was established for all of the clinical and laboratory findings. Genotype-phenotype relation was also evaluated for the different *in vitro* basal cAMP fold increases of the somatic TSH receptor mutations over the wild type TSH-receptor. No statistical difference was noticed for the clinical (age, time for euthyroidism, cumulative dose of propylthiouracil (PTU), nodule and thyorid volume) and laboratory (TSH, FT4, FT3)) findings between groups of different basal cAMP fold (basal fold ≤ 2, $n=5$, fold 2–5, $n=15$ fold ≥ 5, $n=8$).

TSH at the start of PTU treatment was found significantly lower in the mutation (+) group (Z:-2.058; *P*: 0,040). FT3 level was also found higher in the mutation positive groups, but it was not significant (Z:-1.755; *P*: 0,079). No significant difference was found between mutation +/− groups for serum level of FT4, age, gender, thyroid volume, time to euthyroidism until the end of PTU treatment and cumulative dose PTU for establishment of euthyroidism.

No significant difference was found between TSHR D727E variant +/− nodules for thyroid and nodule volume, time to euthyroidism after begin of PTU treatment, cumulative dose of PTU for establishment of euthyroidism and serum levels of FT3, FT4. Serum level of TSH was found significantly lower in the variant positive groups (Z:-2,385; *P*:0,017).

Our results suggest that hot nodules with a somatic TSHR mutation are larger than those without a TSHR mutation.

P347

High prevalence of ER22/23EK polymorphism of the glucocorticoid receptor gene in patients with Graves' orbitopathy

Belema Boyle, Katalin Korányi, Rita Bertalan, Károly Rácz & Csaba Balázs Semmelweis University, Budapest, Hungary.

Objective

To investigate whether three polymorphisms of the glucocorticoid receptor gene known to influence the sensitivity to glucocorticoids could be implicated in the pathomechanism of Graves' orbitopathy.

Methods

Allelic frequencies of the ER22/23EK, Bcl I and N363S polymorphisms of the glucocorticoid receptor gene were investigated in 99 patients with Graves' orbitopathy (mean age, 47.8 ± 13.4 years) and in 175 healthy individuals (mean age 54.4 ± 14.2 years). DNA was isolated from whole blood. Genotypes for the N363S and the Bcl I variants were determined by allele-specific polymerase chain reaction (PCR) and the ER22/23EK polymorphism was genotyped by PCR-RFLP analysis. The study was approved by local ethics committee, and written informed consent was obtained from all subjects.

Results

A significantly higher frequency of the ER22/23EK polymorphic allele was detected in patients with Graves' orbitopathy compared to that found in healthy control subjects (allelic frequency 5.05% *vs.* 2.0%, $P<0,05$), whereas the allelic frequencies of the Bcl I and N363S polymorphisms were similar in the two groups.

Conclusion

In this study we found that the ER22/23EK polymorphic allele of the glucocorticoid receptor is significantly overrepresented in patients with Graves' orbitopathy compared to healthy individuals. This polymorphism is known to be associated with a decreased sensitivity to glucocorticoids and, therefore, its high prevalence could increase the risk for the development of tissue-specific autoimmune inflammation underlying Graves' orbitopathy.

P348

Removal of tick box for TFT in pathology request forms reduces TFT performed during acute medical admission

Milan Piya, Biparna Belbase, Meenakshi Thapa, Anu Koppada, Martin Myers, Susan Baxter & Satyan Rajbhandari
Lancashire Teaching Hospitals NHS Trust, Lancashire, United Kingdom

Joint UK guideline (2006) recommends that routine testing of thyroid function (TFT) in patients admitted acutely to hospital is not warranted unless specific clinical indications exist. Despite this, TFT is frequently requested during acute medical admission. In our previous audit in 2002, during a 1 month period from 18th September, 458 subjects were admitted to medical assessment unit (MAU) and 183 (40%) were offered TFT. 39 (29%) results were beyond the laboratory reference range but this changed management only in 2 (1.1%) subjects. We recommended that TFT during acute medical admission should not be checked routinely as it known to alter transiently during acute illness. To implement this, the tick box for TFT was removed from the standard pathology request form but could be requested in the free text box without restriction. We re-audited the effect of the change in 2005 in the same MAU over the same month starting 18th September, and found that there had been a 55% reduction ($P<0.0001$) in request for TFT during acute medical admission. Out of 698 subjects admitted to MAU during this period, only 127 (18.2%) had TFT checked and 30 (23.6%) had results beyond the laboratory reference range. When these notes were reviewed, 7 (5.5%) had their management changed ($P=0.03$). In comparison to the previous audit, the removal of TFT tick box from the standard pathology form reduced routine testing by 3 fold (odds ratio 3.0 & 95% Confidence Interval 2.3 to 3.9), and improved efficiency by 5 fold (OR 5.3 & 95% CI 1.1 to 25.9). Our audit suggests that it is possible to reduce unnecessary TFT request during acute medical admission simply by removing tick boxes from the standard pathology request form. This helped reduce unnecessary TFT requests, in keeping with the 2006 UK guidelines for thyroid function tests.

P349

Thyroid hormones in serum and cerebrospinal fluid in patients with brain tumor and acute stroke

Darko Katalinic, Martina Matovinovic, Miljenko Solter, Andreja Maric, Jure Murgic, Milan Vrkljan & Karolina Dobrovic
Sestre milosrdnice University Hospital, Zagreb, Croatia.

We studied levels of T_3, T_4, FT_3, FT_4, rT_3 and TSH concentrations in serum and FT_3, FT_4, rT_3 and TSH concentrations in cerebrospinal fluid (CSF) in 10 patients with brain tumor and 20 patients with acute stroke and compared them to 7 patients in control group (further clinical evaluation in control group did not show brain lesions). All patients were euthyroid. The study was approved by local Ethical Committee. Serum T_3 and T_4 levels were similar in all three group. The values of FT_3, FT_4 and TSH did not significantly differ to control group neither in serum nor in CSF. On the contrary, significantly elevated rT_3 was found in serum and CSF, at both group of patients. The rT_3/FT_3 ratio were the highest in patients suffering from brain tumor and were significantly elevated compared to control group (serum, CSF), as well as compared to the patients with acute stroke. The values were particularly high in CSF (4 times higher) which would suggest that changes connected with "low T_3 syndrome" in patients with brain lesion are more obvious in CSF than in serum and identify brain tumor as a prototype of serious "local" nonthyroid illness. Serum and CSF test showed positive correlation for FT_4 and FT_3 in patients with acute stroke and for rT_3 in patients with brain tumor. This suggests that hormones are passing through still functional blood-brain barrier. The study did not show correlation between elevated rT_3 or rT_3/FT_3 ratio and poor prognosis. Thyroid hormones are present in CSF at concentration lower than in serum. There are probably two mechanisms responsible: hormones are partly crossing the blood-brain barrier from serum, but also T_3 and rT_3 may derive from local conversion of T_4 within the central nervous system. The impairment of this conversion which occurs in different brain lesions could be responsible for the changes in hormones level known as "low T_3 syndrome", which are particularly evident in CSF.

P350

The influence of universal salt-iodization on the iodine status of County Mures, detected through TSH determinations in newborns between 2001–2006

Zsuzsanna Szanto[1], Ildiko Kun[2], I. Kun[1], Ligia Coros[3] & Manuela Cucerea[4]
[1]Endocrinology Clinic, UMPh, Tg.Mures, Romania; [2]Clinic of Internal Medicine No. II, UMPh, Tg.Mures, Romania; [3]Central Laboratory of Emergency Clinical Hospital County Mures, Tg.Mures, Romania; [4]Clinic of Obstetrics & Gynecology No. I, UMPh, Tg.Mures, Romania.

Iodine deficiency in a geographical area can be quantified not only by urinary iodine excretion, but by the frequency of elevated TSH-levels in newborns, too. We compared the TSH-levels obtained between 2001–2003 with those collected after extension of universal salt-iodization with increased iodine-content (KIO_3 34 ± 8.5 mg-/kg) in the whole country (2004–2006). The governmental decision was adopted in 2002, implemented in practice in December 2003, and extended only in 2004 (the iodized salt was used in 96% of households). We observed TSH-levels (10 μIU/mL (WHO-criteria) at 8.23% of 2454 newborns tested between 2001–2003, in comparison with the 9.91% from 555 subjects borned between 2004–2006. Accordingly to the upper normal TSH-level (12 μIU/mL) used at the Central Laboratory of Emergency Clinical Hospital County Mures, 6.07% and 6.31% of the newborns seen between 2001–2003, and 2004–2006, respectively, had elevated TSH-levels. The difference between the two periods was not significant. Based upon these results, County Mures can be characterized at present as a moderate/mild iodine-deficient area.

However, we observed an important change: the mean TSH-level obtained in the period of 2001–2003 ($19.81 + 12.63$ μIU/mL) was reduced significantly in the second period ($15.63 + 7.35$ μUI-/mL), i.e. a decrease with 4.18 μIU/mL ($P = 0.02$). In conclusion, after increasing the iodine-content of the alimentary salt and applying the measures for the universal iodization, the incidence of elevated TSH-level did not decrease, but its mean value was reduced statistically significant, showing an impro-ve-ment of iodine supple-mentation.

While the moderate increased TSH-levels (10–12 μIU/mL) are considered as indicators of the iodine deficiency, the higher concentrations ((20 μIU/mL) usually indicate the coexis-ten-ce of hypothyroidism due to reduced iodine supply. We observed an important reduction of the hypothyroidism induced by iodine-deficiency: if in the first period its incidence was 2.49%, in the second it decreased to 1.46%.

P351

The evolution of hypothyroidism in pregnant women in County Mures between 2001–2006

Ildiko Kun[1], Zsuzsanna Szanto[2], I Kun[2], B Szabo[3] & Ligia Coros[4]
[1]Clinic of Internal Medicine No. II, UMPh, Tg.Mures, Romania; [2]Endocrinology Clinic, UMPh, Tg.Mures, Romania; [3]Clinic of Obstetrics & Gynecology No. I, UMPh, Tg.Mures, Romania; [4]Central Laboratory of Emergency Hospital County Mures, Tg.Mures, Romania.

County Mures is a moderate/mild iodine-deficient area, the iodine-prophylaxis having an important role in prevention of IDD. Between 2001–2003 we made a partial screening at 320 pregnant women to detect thy-roid dysfunctions, and in 13% (43 cases) we observed hypo-thy-roidism, the majority being subclinical forms. The most frequent compli-ca-tions were threatened abortion or premature birth, and dys-gra-vidia. We found that even the subclinical hypothyroidism can cause se-ve-re complica-tions in preg-nan-cy or may contribute to their development.

The governmental decision from 2002 regarding the universal iodization of alimentary salt was put in practice from December 2003, while in 2004 was decided the obligatory iodization of the salt used in the baking industry. Consequently, in 2004 the iodized salt was used in 96% of households, according to some authors. Our aim was to evaluate the influence of these new measures on the thyroid function of pregnant women, so we restarted the TSH- and FT_4-determinations between 2004–2006, and compared the results with those obtained bet-ween 2001–2003. In the period of 2004–2006 from the 205 pregnant women 7.3% (15) presented hypothyroidism (increased TSH-levels and/or decreased FT_4-values), a much more reduced percentage as in the first period (13%). Thus, between 2004–2006 the frequency of hypo-thyro-idism decreased significantly comparing with 2001–2003 ($P < 0.05$). However, the values of urinary iodine excretion of the two periods did not differ significantly, in concordance with the similar data obtained in whole country in 2004. So, other factors could contribute to the better results, i.e. a more rigurous follow-up of the thyroid function and a more adequate treatment of hypothyroidism in pregnancy, taking into account that this dysfunction can be determined besides the IDD by other thyroid disorders (especially by chronic thyroidites), too, or can be a consequence of an inadequately treated thyroid ablation.

P352

The use of perchlorates in the treatment of some special forms of hyperthyroidism (report of two cases)

I Kun, Zsuzsanna Szanto & Emilia Let
Endocrinology Clinic, UMPh, Tg.Mures, Romania.

The perchlorates block the enzyme NIS, inhibiting iodine accumulation in the thyroid and favour the elimination of intrathyroidal iodine unused for hormone synthesis. Their therapeutical utilisation actually is limited due to the toxicity. In the literature there are different opinions regarding the adverse effects (nephrotic syndrome, irreversible aplastic anemia etc.), but several authors sustain that these appear only after high doses, and after the development of therapeutical actions.

Perchlorates are used rarely in the treatment of hyperthyroidism, mainly in iodine-, especially amiodarone-induced forms. They are indicated also to prevent these forms, using perchlorates pre- and postinterventionally with iodine-containing substances (e.g.contrast agents). In hyper-thyroidism induced by amiodarone, perchlorates are usually associated with thioamides. Similarly, these drugs can be attempted in cases of intolerance to other antithyroid drugs, e.g. thioamides, when can not be applied ablative measures.

We report two cases of hyperthyroidism treated with perchlorates, obtaining good therapeutical results. In both cases perchlorates were introduced after (hema-to-lo-gic and CNS) adverse effects produced by methimazole, alone and associated with lithium carbonate. Taking into account the recommended short duration of the therapy with perchlorates (not excee-ding 1 month) and lacking the possibilities for other efficient and durable conservative treatment (both pa-tients presented Hashitoxicosis aggravated through iodine intake, and had thioamide-intolerance), we indicated thyroidectomy after obtaining euthyroidism with perchlorates. At 7–10 days after surgery their thyroid status evolu-a-ted to hypothyroidism, so now they are receiving thyroxine substitution under longitudinal follow-up.

P353

Iodine deficiency detected through urinary iodine excretion in school-children living in goiter prevalent regions of County Mures (2005–2006)

I Kun[1], J Balazs[1], Anisie Nasalean[1], Camelia Gliga[1], Gabriela Detesan[2], Mihaela Simescu[3], Ligia Coros[4], Anca Ionescu[1], Gabriela Madaras[1], Zsuzsanna Szanto[1] & C Macarie[1]
[1]Endocrinology Clinic, UMPh, Tg.Mures, Romania; [2]Municipal Hospital, Reghin, Romania; [3]Institute of Endocrinology C. I. Parhon, Bucuresti, Romania; [4]Central Laboratory of Emergency Clinical Hospital County Mures, Tg.Mures, Romania.

Our previous studies made between 1999–2003 demonstrated that County Mures is a moderate/mild iodine-deficient geogra-phical area. In 2002 a governmental decision was given for universal iodization of alimentary salt with increased iodine-content, realized during 2004. The aim of our study was to investigate the effect of in-creased iodine-supplementation at school children living in different iodine-deficient areas in County Mures, through urinary iodine excretion (UIE). In December 2005 were tested 50 school-children from a rural mountain area, while in October 2006 other 133 children from surrounding villages: 55 from Casva, 28 from Glajarie and 50 from Ibanesti.

The group tested in 2005 had mean UIE of 56.00±38.07 μg/L, only 6% of children having normal values. The group studied in October 2006 had mean UIE of 85.37±60.05 μg/L, only 30.8% having normal values, 38.3% between 50–99 μg/L, 22.6% between 20–49 μg/L (mild and moderate dec-re-a-se), and 8.3% under 20 μg/L (very low levels). Thus, 69.2% of child-ren had subnormal levels, and the percentage of UIE <50 μg/L reached 30.8%, which is above 20%, the upper admitted limit for an adequate iodine-intake. Our results from 2005 are similar with those obtained by Balazs *et al.* in 1999 in the superior and middle hydrographic basin of the river Mures (mean value 59.95±30.22 μg/L, normal UIE in 6.9%) at a group of 58 school-children from zone of locality Deda. At the same time, our recent results (October 2006) are much better: the mean value rose to 85.37±60.05 μg/L and 30.8% of children had normal UIE. Ana-lysing separately the groups of villages, the results are somehow different: 72.90±48.63 μg/L in Casva, 75.42±60.30 μg/L in Glajarie and 109.83±73.22 μg/L in Ibanesti.

In conclusion, the rural mountain zones of County Mures known before as moderate/mild iodine-deficient areas, became mild deficient, due to the new measures of iodine prophylaxis. In these areas is necessary to apply permanently special prophylactic measures, too.

P354

Endothelial function and hemostasis factors in hypothyroidism and subclinical hyperthyroidism

Annamaria Gazdag[1], Endre Nagy V[1], Emma Varga[1], Laszlo Samson[1], Annamaria Erdei[1] & Zoltan Jenei[2]
[1]Division of Endocrinology, Department of Medicine, Health and Science Centre, Universitiy of Debrecen, Debrecen, Hungary; [2]Division of Nephrology, Department of Medicine, Health and Science Centre, Universitiy of Debrecen, Debrecen, Hungary.

Long-term follow-up of differentiated thyroid cancer (DTC) patients requires yearly measurements of serum thyroglobulin (Tg) levels is endogenous (4 weeks off-thyroxin) or exogenous (human recombinant TSH injection) high TSH state. Subclinical hyperthyroidism is maintained in the patients throughout the rest of the year. We examined the endothelial function, hemostasis factors and metabolic parameters in short-term iatrogenic hypothyroidism (HO) and in subclinical hyperthyroidism (SH) in patients with DTC. In seventeen patients who had undergone total thyroidectomy and radioiodine ablation, blood pressure (RR), Tg, thyroid function, lipid parameters, homocystein, CRP, fibrinogen, von Willebrandt factor activity (vWF), flow-mediated vasodilatation (FMD) and nitroglycerin-mediated vasodilatation of the brachial artery in HO (TSH=89.82±29.36 mU/L) and in SH (TSH=0.24±0.11 mU/L) were measured. The study protocol has been approved by the institutional ethics committee. In HO the FMD was markedly lower than in SH (6.79±4.44 vs.14.37±8.33%, $P<0.001$), whereas the vasodilatation in response to nitroglycerine was not different between HO and SH (28.20±8.33 vs. 29.27±14.19%, ns). RR did not significantly differ in HO and SH (128.62±7.17/82.29±3.98 vs. 125.8±7.05/85.2±5.8 Hgmm, ns). Total cholesterol (7.34±1.23 vs. 4.75±/1.24 mmol/L, $P<0.002$), LDL-cholesterol (4.55±1.10 vs. 2.70±0.89 mmol/L, $P<0.001$) and homocystein (12.95±4.49 vs. 9.62±2.3 μmol/L, $P<0.01$) were significantly higher in HO than in SH. Triglyceride (1.79±1.12 vs. 1.03±0.73 mmol/L) and HDL-cholesterol (1.95±0.47 vs. 1.58±0.42 mmol/L) were similar in HO and SH. Fibrinogen (3.23±0.50 vs. 4.38±0.84 g/L, $P<0.01$), vWF activity (90.09±25.92 vs.130.62±29.97%, $P<0.001$) and CRP (4.12±4.67 vs. 5.32±5.15 mg/L, $P<0.05$) were lower in HO. In conclusion, FMD, fibrinogen and vWF activity was found to be lower in HO than in SH. Thyroxin normalizes the low FMD in HO patients.

P355

The relevance of thyroid echography in diagnostic of subclinical autoimmune thyroiditis during pregnancy and postpartum

Gorduza Carmne[1], Mogos Voichita[2], Gorduza Eusebiu Vlad[2] & Zbranca Eusebie[2]
[1]St. Spiridon Hospital Iasi, Iasi, Romania; [2]University of Medicine and Pharmacy Iasi, Iasi, Romania.

Thyroid diseases are more frequent in women. We made a longitudinal study at 112 healthy pregnant women from Iasi county, Romania. The aim of study was to determine incidence and evolution of autoimmune thyroiditis in pregnancy and postpartum. The proceedings of study imposed to verify clinical aspect of thyroid, the volume of thyroid measured echographic, echostructure of thyroid, function of thyroid (TSH, FT4) and autoimmune modification of thyroid (anti-thyroid peroxidase autoantibodies - AAT-anti-TPO). Knowing that in postpartum it exists a risk to develop a subclinical autoimmune thyroiditis, even in normal women, we tried to find a specific element that can be use like "signal" to identify such disease risk during pregnancy. In these conditions we followed in progress the echostructure of thyroid during pregnancy and in the first 3 months after delivery. We observed an hypoechogenity of thyroid correlated with the levels of AAT-anti-TPO, TSH and FT4. The prevalence of hypoechogenity was 12.5% in I trimester, 16% in 2nd trimester, 23% in the end of pregnancy and 25% after delivery. Majority of cases with thyroid hypoechogenity (accentuated in 3rd trimester) presented some degree of autoimmunity, despite of reduction of AAT anti-TPO level in the end of pregnancy. The conclusion of our study is that the echography of thyroid can represent a screening method for detection of subclinical autoimmune thyroiditis during the pregnancy.

P356

Outcomes of a fixed dose of 370 MBq of radioiodine in hyperthyroidism

Isabel Howat, Hilary Sawers & Stewart Sawers
Cumberland Infirmary, Carlisle, United Kingdom.

In 1995 the Royal College of Physicians issued guidelines for the use of radioiodine in hyperthyroidism. They recommended administration of enough radioiodine to achieve euthyroidism, with acceptance of a moderate rate of hypothyroidism e.g. 15–20% at 2 years and 1–3% per annum thereafter. Guide activity was 400–550 MBq for standard hyperthyroidism (mainly Graves' disease), at least 550 MBq for toxic multinodular goitre, and 300–500 MBq in toxic adenoma.

We wished to see if we were achieving the recommended outcomes. We conducted a retrospective audit over 5 years from January 2000 to December 2004. During that time we used a fixed dose of 370 MBq. 351 patients received 390 doses of radioiodine. Mean follow-up was 35 months (1–66). We reviewed the outcomes of patients who had a diagnosis documented in their case records.

114 patients had documented Graves' disease. During follow-up 75 (65.78%) became hypothyroid, 73 (64.03%) within 2 years, 2 (1.75%) within 3 years. 17 (14.91%) remained euthyroid at follow-up. 18 (15.78%) remained hyperthyroid or required up to 2 further doses of radioiodine. 4 patients were lost to follow-up.

57 patients had multinodular goitre. During follow-up 8 (14.03%) became hypothyroid, all within 2 years, 39 (68.42%) remained euthyroid, 10 (17.54%) remained hyperthyroid or required 1–3 further doses of radioiodine.

16 patients had toxic adenoma, 6 (37.5%) became hypothyroid, all within 2 years, 7 (43.75%) remained euthyroid, 3 (18.75%) required 1 further dose of radioiodine.

Despite using a dose less than that stipulated in the guidelines, our rate of hypothyroidism was higher than recommended for patients with standard hyperthyroidism and within the recommendation for toxic multinodular goitre. Our rate of hypothyroidism was also high for toxic adenoma although there were only 16 patients.

P357

The lymphocyte interactions in thyroid tissue in graves' disease

Iwona Ben-Skowronek[1], Jadwiga Sierocinska-Sawa[2], Leszek Szewczyk[1] & Elzbieta Korobowicz[2]
[1]Dpt. Pediatrics Rndocrinology and Neurology Medical Academy of Lublin, Lublin, Poland; [2]Dpt. Pathomorphology Medical Academy of Lublin, Lublin, Poland.

Introduction

The T,B and antigen presenting cells play a key role in the pathogenesis of autoimmune diseases.

The aim of the studies was to analyse different regulatory cells subsets interaction in patients with Graves' disease.

Material and methods
We have studied paraffin thyroid specimens obtained from 10 children with Graves' disease after thiamazole treatment. The thyroid tissue was stained with hematoksylin-eosin (HE). The mononuclear T-cells were detected by CD3+, CD4+, CD8+ antibodies and the presenting antigen's dendritic cells with CD1a and CD35 and antibodies (DakoCytomation Denmark).
Results
Thyroid tissue infiltrates were observed in HE staining. Intensivity of infiltrates was correlated with time of thiamazole treatment.
The B cell CD3+ and T suppressor/cytotoxic cell CD8+ between thyroid follicles. In the 4 patients with thiamazole short treatment (<6 mc) the lymphocytes have formed the lymphatic follicles in thyroid tissue. We have observed dendritic cells presenting antigen (APC) CD1a+ in reproduction centre. On the edges of lymphatic follicles were present lymphocytes T-helper CD4+, T-suppressor CD8+ and B-cells CD79+. In 6 patients after long thiamazole therapy the B and T cells were rarely observed in interstitium. It was interesting, that thyrocytes revealed positive reaction with CD1a monoclonaly antibody, which detected transmembrane α-chain connected with β-l microglobulin.
Conclusions
In the active states of Graves' disease, lymphocytes T, B and antigen presenting cells are present in big amount in interstitium and in lymphatic follicles. Thiamazole treatment leads to reduction of their amount.
Thyrocytes can have in their structure components similar to α-chains connected with β-microglobulins, which are characteristic for APS.
Grant 2P05E04327 Min. Science and Inform. Poland

P358

Water purification technology reduces iodine content of drinking water and contributes to iodine deficiency

Laszlo Samson, Ildiko Czegeny, Endre V Nagy, Emese Mezosi & Annamaria Erdei
Division of Endocrinology, Department of Medicine, University of Debrecen Medical and Health Science Center, Debrecen, Hungary.

Drinking water is the major natural source of iodine in many European countries. In the present study, we examined possible sites of iodine loss during the usual water purification process. Water samples from 6 sites during the technological process were taken and analyzed for iodine content. Under laboratory circumstances, prepared iodine in water solution has been used as a model to test the effect of the presence of chlorine. Samples from the purification sites revealed that in the presence of chlorine there is a progressive loss of iodine from the water. In the chlorine concentrations employed in the purification process, twenty four hour chlorine exposure eliminated more than 50% of iodine when the initial iodine concentration was 250 μg/L or less. Iodine was completely eliminated if the starting concentration was 16 μg/L. We conclude that chlorine used during water purification may be a major contributor to iodine deficiency in European communities.

P359

The ultrastructural changes of thyroid tissue in recipient of bone marrow graft with Graves' disease

Iwona Ben-Skowronek[1], Jadwiga Sierocinska-Sawa[2], Leszek Szewczyk[1], Elzbieta Korobowicz[2] & Zofia Karpinska[2]
[1]Dpt. Pediatrics Endicrinology and Neurology Medical Academy of Lublin, Lublin, Poland; [2]Dpt. Pathomorphology Medical Academy of Lublin, Lublin, Poland.

Introduction
In connection with usage of allogeneic bone marrow transplantations (BMT) there rises the problem of transfer of lymphocytes capable of induction autoimmunological reactions in recipient.
The aim of our study
Is a presentation of ultrastructural changes in thyroid tissue as the pathogenesis of autoimmunological thyroid disease in a recipient following BMT from donor with Graves' disease after total surgical treatment.
Material and methods
The thyroid gland tissue removed during surgery was routinely fixed and stained with hematoxylin and eosin. The immunohistochemical investigation of lymphocyte subsets was performed using DAKO monoclonal antibodies. Fluorescence *in situ* hybridization studies (FISH) was performed using a commercially available CEP X/Y DNA Probe (Vysis). Histological specimens were routine estimated and investigated in electron microscope.
Case report
The 14 -year boy who underwent bone marrow transplantation (BMT) for severe aplastic anemia from his HLA matched sister, who had been diagnosed with Graves' disease 5 years before transplantation. After 2 years of BMT, the same disease was diagnosed in the recipient. Thyreoidectomy was performed after achieving a euthyroid state. The thyroid gland contained interstitial lymphocytic infiltrates: T, B and antigen presenting cells. FISH showed that at least some of the lymphocytes were of donor origin and these could be seen among the recipient's thyroid cells. In the ultrastructural investigations were noticed numerous lymphocytes such as plasmocytes between thyroid cells in contact with thyrocytes. It was observed the lymphocytes in contact with plasmocytes and the lymphoblasts and lymphocytes in lymphatic follicules. The thyrocytes were very active and in numerous places were proliferated.
Conclusions
In thyroid were ultrastructural changes typical for AITD observed. The transfer of donor immunocompetent cells to the recipient of hematopoetic stem cells has been proposed as a mechanism of inducing autoimmune thyroiditis post BMT.
Grant 2P05E04327 Min. Science and Inform. Poland

P360

Do patients and clinicians agree about which aspects of quality of life are relevant when evaluating the impact of thyroid diseases?

Torquil Watt[1], Laszlo Hegedüs[2], Åse Krogh Rasmussen[1], Mogens Groenvold[3], Steen Joop Bonnema[2], Jakob Bjorner[4] & Ulla Feldt-Rasmussen[1]
[1]Copenhagen University Hospital Rigshospitalet, Copenhagen, Denmark; [2]Odense University Hospital, Odense, Denmark; [3]Institute of Public Health, Copenhagen, Denmark; [4]Quality Metric Inc., Lincoln, RI, United States.

Objective
During the development of a thyroid-specific quality of life (QoL) questionnaire, patients and clinicians rated the relative relevance of a list of possibly relevant QoL issues. In this study we compare the patient and clinician ratings.
Methods
Fifteen thyroid experts and 80 thyroid outpatients (14 with non-toxic goitre, 12 nodular toxic goitre, 21 Graves' disease, 17 thyroid associated ophthalmopathy (TAO) and 16 primary hypothyroidism) were interviewed, using semi-structured interviews.
The relevance of 138 thyroid disease related issues was rated. Patients' rating of importance was combined with prevalence of the issue in question to calculate a mean relevance rank for each patient category. Experts rated the relevance directly. Patient and expert relevance ratings were compared using nonparametric correlation. To explore the (dis-)agreement in greater detail, the 15 issues considered most relevant by the patients were compared to the 15 issues considered most relevant by the clinicians.
Results
The Spearman correlations between patient and expert ratings were: Graves' disease 0.69, TAO 0.48, toxic nodular goitre 0.60, non-toxic goitre 0.35 and primary hypothyroidism 0.46 ($P < 0.0001$ for all coefficients). This corresponds to substantial agreement regarding Graves' disease, moderate agreement about TAO, toxic nodular goitre and primary hypothyroidism and only fair agreement in non-toxic goiter.
For most disease categories, less than half of the 15 issues considered most relevant by the patients were also among the 15 most relevant to clinicians. Generally, issues among the 15 most relevant according to clinicians only were physical symptoms characteristic of the diagnosis in question. Issues among the 15 most relevant according to patients only were generally non-physical aspects of HRQL such as emotional susceptibility and nervousness as well as general physical symptoms.
Conclusions
When evaluating possibly relevant QoL-issues, clinicians focused more on specific symptoms, whereas patients focused more on emotional, mental and social aspects of QoL.

P361

ret/PTC oncogene expression in papillary thyroid carcinoma

Dilek Arpaci[1], Yersu Kapran[2], Ayse Kubat Uzum[3], Ferihan Aral[3], Nese Ozbey[3] & Faruk Alagol[1]
[1]Istanbul University, Istanbul Faculty of Medicine, Department of Internal Medicine, Istanbul, Turkey; [2]Istanbul University, Istanbul Faculty of Medicine, Department of Pathology, Istanbul, Turkey; [3]Istanbul University, Istanbul Faculty of Medicine, Department of Internal Medicine, Division of Endocrinology and Metabolism, Istanbul, Turkey.

Objective
We aimed to investigate the expression of ret/PTC oncogene in PTC and to determine the relationship of this expression with clinical parameters and the prognosis.

Materials and methods
Surgical specimens of 45 PTC and adjacent normal thyroid tissues were obtained from the files of the Department of Pathology at Istanbul Faculty of Medicine, Istanbul University. All of the patients had definite diagnosis of PTC between 1995–2003 and had adequate clinical information and a continous follow-up. ret/PTC expression was studied with the immunohistochemistry method. Correlation between ret/PTC expression positivity and the pathologic parameters at initial diagnose and during the follow up were examined.
Results
Study group consisted of 39 (86.7%) female, six (13.3%) male patients. Mean age was 44.4 ± 11.28 years, follow-up time was 59 ± 25 (24–120). Mean tumor size was 18.13 ± 15.75 mm (3–80 mm). According to TNM staging % 22 (n = 10), %13.3 (n = 6), %8.9 (n = 4) and %55.6 (n = 25) of the tumors were T1, T2, T3 and T4 respectively. Lymph node metastasis, capsule invasion, vascular invasion, soft tissue invasion, multicentricity, and relaps rates were 24.4% (n = 11), 71.1% (n = 32), 40% (n = 18), 51.1% (n = 23), 42% (n = 19) and %6.8 (n = 3) respectively. In 17 (37.8%) of the 45 specimens, ret/PTC was found positive immunohistochemically. There was no significant difference in ret/PTC expression rate according to gender, stage of tumor, invasion of lymph node, capsule, soft tissue and vascular invasion, multicentricity and relaps ($P > 0.05$). Ret/PTC expression positivity was not different between patient < 40 and ≥ 40 years old. No correlation was found between ret/PTC positivity and tumor size (< 10 mm and ≥ 10 mm) ($P = 0.160$) as well as between the histologycal subtypes ($P = 0.60$).
Discussion
In our study, ret/PTC expression had no influence on initial clinicopathological findings and the prognosis.
(The present work was supported by the Research Fund of Istanbul University. Project No:950,T-601/170320)

P362

Prognostic value of polymorphisms and somatic RET proto-oncogene mutations in sporadic medullary thyroid carcinoma (MTC)
Barbara Cosci[1], Cristina Romei[1], Pamela Piampiani[1], Mariangela Sculli[1], Paolo Miccoli[2], Clara Ugolini[3], Michele Minuto[2], Aldo Pinchera[1] & Rossella Elisei[1]
[1]Department of Endocrinology, University of Pisa, Pisa, Italy; [2]Department of Surgery, University of Pisa, Pisa, Italy; [3]Department of Oncology, University of Pisa, Pisa, Italy.

Germline point mutations of the RET proto-oncogene are causative of hereditary MTC. Somatic mutations are described in 40% of sporadic MTCs. Although a relationship between somatic mutations and bad prognosis has been described, data are controversial.No date on the prognostic value of RET polymorphisms are available.
Aim of the work was to verify if the presence of a somatic RET mutation and or a polymorphisms can influence the prognosis of MTC. This study was performed in a large series of MTC with a mean follow-up of 10 years.
Seventy MTC cases, known to be sporadic on the basis of genetic analysis, were studied. RET point mutations and polymorphisms were analysed by direct sequencing.
We identified a total of 28 somatic RET mutations (40%). In particular 1 (3%) 48 bp deletion in exon 10, 1 (3%) 883 mutation in exon 15, 3 (10.7%) 634 mutations in exon 11 and 23 (82%) 918 mutations in exon 16 were described. RET mutations were correlated with TNM and outcome. Among 28 mutated patients, 6 were free of disease and 22 were affected by MTC or dead. On the contrary among the 42 not mutated patients, 23 were free of disease and 19 were affected by MTC or dead ($P = 0.006$). In the group of mutated tumors we found 16 patients (57%) with lymph-node metastasis. On the contrary only 12 (28.5%) cases of lymph-node metastasis were identified among not mutated patients ($P = 0.004$). No statistically significant correlation between RET mutation, the size of the tumour and the presence of distant metastasis was found. RET polymorphisms did not show any correlation with clinico-pathological features of the tumor.
In conclusion our study show that RET somatic mutation is a negative prognostic factor for MTC and is significantly correlated with lymph-node metastasis. Although Met918Thr mutation is the most frequent, somatic RET mutations can be found in different exons.

P363

Partial redifferentiation of thyroid carcinoma cell lines treated with decitabine and retinoic acid
Agnese Vivaldi[1], Miasaki Fabiola[1], Raffaele Ciampi[1], Laura Agate[1], Valeria Bottici[1], Alessandra Capodanno[2], Paola Collecchi[2], Aldo Pinchera[1] & Rossella Elisei[1]
[1]Department of Endocrinology and Metabolism, University of Pisa, Pisa, Italy; [2]Division of Pathology, University of Pisa, Pisa, Italy.

In a previous study we demonstrated that retinoic acid (RA) decreased the growth only of thyroid carcinoma cell lines expressing RA receptor β (RAR β) and that decitabine (5-Aza-CdR) re-induced RARβ expression. The aim of this study was to analyze the effects induced by the combined treatment with RA and 5-Aza-CdR in the same thyroid cancer cell lines.
We studied the effect of 5-Aza-CdR 800 nM and RA 1 μM on the expression of thyroid specific genes and RARα, β and γ by quantitative RT-PCR and the effect of the two drugs on cell growth by cell counting, cytotoxicity, bromodeoxyuridine, apoptosis assays and FACS analysis.
After the combined treatment, we observed the induction of the RARβ mRNA expression in all cell lines, of NIS mRNA in ARO, FRO, WRO and TT, of TTF-1 in ARO, Tg in FRO and Pax-8 in WRO and TT. However, no cell line was able to actively take up ^{125}I despite of NIS mRNA re-expression. Accordingly, immunofluorescence showed NIS protein expression only in the cytoplasm.
The combined treatment determined an inhibition of the growth curve in all cell lines: after 24 h in FRO and NPA, after 48 h in WRO, after 72 h in ARO and after a week in TT. We observed inhibition of DNA synthesis in NPA and WRO and apoptosis in ARO and, NPA and TT. Finally, FACS analysis showed a G0/G1 increase in FRO and WRO.
In conclusion, the combined treatment with 5-Aza-CdR and RA reduces the tumoral growth speed *in vitro* by means of apoptosis in ARO, NPA and TT and of inhibition of DNA synthesis in NPA and WRO. The combined treatment can also partially re-differentiate the analyzed thyroid cancer cell lines, inducing NIS mRNA expression. The cytoplasmic localization of NIS protein explains the inability of cells to take up radioiodine.

P364

Comparison between serum calcitonin (CT) levels following Pentagastrin (Pg) and Calcium (CA) stimulus
Valeria Bottici, Mariangela Sculli, Giulia Renzini, Paolo Passannanti, Fabiana Lucchetti, Lucia Grasso, Aldo Pinchera & Rossella Elisei
Department of Endocrinology, University of Pisa, Pisa, Italy.

Serum CT is the most specific marker of Medullary Thyroid carcinoma (MTC). Although Pg test is the most frequently used to induce CT secretion, the poor availability of Pg makes it necessary to look for different agents. Aim of this work was to compare the induction of CT secretion following 2 different stimuli in the same patient. We studied 25 patients (14 females, 11 males; mean age 50 ± 15 yrs; range 12–77 yrs). All patients were subjected to both tests by injection of 0.5 μg/kg of Pg and 2 mg/kg of CA in 5 fminutes. Thirteen/25 patients showed undetectable basal CT (< 10 pg/ml); these cases were already treated with total thyroidectomy. Six/13 patients showed undetectable CT levels both after Pg and CA stimulation and were disease-free. In 2 patients CT was elevated both after Pg (mean 37 pg/ml, range 11–63) and CA (mean 22 pg/ml, range 21–23). Imaging was negative (biochemical persistence of disease, BP). In 5/13 patients CT was undectable after CA but not after Pg (mean 33 pg/ml, range 11–114); all of them were BP. In12 patients basal CT was detectable (mean 980 ± 1782 pg/ml, range 62-4590 pg/ml). In all patients CT peak after Pg and CA was higher than basal CT (mean 3196 pg/ml, range 65-17990; mean 1522 pg/ml, range 60-9650, respectively). Six/12 patients had a metastatic disease, 3/12 showed a BP, 3/12 were under presurgical investigation for MTC. In summary, we demonstrated that Pg and CA test give similar results in 20/25 cases, although CT levels after CA injection are lower than after Pg. In 5 cases the CA test was negative while Pg test was positive with moderate levels of CT. These patients were already been subjected to thyroidectomy for MTC and they would be considered erroneously as disease free on the basis of CA test. In conclusion, Pg test is more sensitive than CA test in patients with basal undectable CT levels. It has a similar sensitivity in patient with elevated basal CT. Although CA stimulation induces a lower secretion of CT than Pg, we propose that CA test is useful in the diagnosis and follow-up of these patients. Patients, already treated by surgery, showing a negative CA test should repeat this test before declaring them as disease-free.

P365

Usefulness of calcitonin (CT) measurement in wash-out fluid from fine needle aspiration biopsy in thyroid nodules of patients with dectectable serum CT

Giulia Renzini, Teresa Rago, Paolo Passannanti, David Viola, Barbara Cosci, Eleonora Molinaro, Lucia Grasso, Fabiana Lucchetti, Paolo Vitti, Aldo Pinchera & Rossella Elisei
Department of Endocrinology, University of Pisa, Pisa, Italy.

Up to now there isn't any study to validate the CT measurement in wash-out-fluid from FNAB in the diagnosis of medullary thyroid cancer (MTC). To demonstrate the usefulness of CT measurement in wash-out fluid from FNAB in thyroid nodules, we have retrospectively analyzed 25 cases with detectable serum CT in which CT measurement in wash-out-fluid from FNAB, citology and histological examination were available. In 7 cases CT level was <10 pg/ml: cytology was negative in 4 cases and not diagnostic in 3 cases. In 6 cases C cell iperplasia (ICC) or MTC was identified at histology but in a different nodule and in one case a focus of MTC was found in the punctured nodule. In 6 cases the CT level was $10<CT<1000$ pg/ml: an MTC was found in 5 cases at histology; in one case a papillary thyroid carcinoma (PTC) was found both at histology and citology. Citology described a MTC in 2 cases and was not diagnostic in 3 cases. In 6 cases CT level was $1000<CT<10000$ pg/ml. In all cases the histology described a MTC with the exception of one case in which there was a PTC. Citology found 4 cases of MTC, but it was not diagnostic in 2 cases. In 6 cases CT levels was >10000 pg/ml: in all cases a MTC was described both at histology and citology.
In conclusion CT level <10 pg/ml in wash-out-fluid from FNAB was indicative of absence of cancer in 86% of cases. The citology identifies only 57% of benign nodules. CT level >10 pg/ml in FNAB was indicative of presence of malignant or premalignant in 100% of cases (15 MTC; 1 ICC; 2 PTC), while cytology only in 72% of cases. We conclude that CT measurement in wash-out-fluid from FNAB increases diagnostic sensibility of citology from 65% to 95% and it represents an useful diagnostic tool to associate with citology when an MTC is suspected.

P366

Prognostic significance of BRAF mutation in patients affected by papillary thyroid carcinoma with a follow up of 20 years

David Viola[1], Cristina Romei[1], Agnese Biagini[1], Laura Agate[1], Eleonora Molinaro[1], Raffaele Ciampi[1], Giulia Renzini[1], Riccardo Giannini[2], Clara Ugolini[1], Piero Berti[3], Aldo Pinchera[1] & Rossella Elisei[1]
[1]Department of Endocrinology, University of Pisa, Pisa, Italy; [2]Department of Oncology, University of Pisa, Pisa, Italy; [3]Department of Surgery, University of Pisa, P, Italy.

$BRAF^{V600E}$ is the most common mutation in papillary thyroid carcinoma (PTC). Anatomo-patology and clinical features of PTC with $BRAF^{V600E}$ are well described in literature.
Aim of this study was to examine the prognostic significance of $BRAF^{V600E}$ in patients with PTC and a follow-up of 15-20 years.
Genomic DNA was purified from 67 paraffin-embedded tumoral tissue. A PCR-SSCP analysis of exon 15 of BRAF was performed. Direct sequencing of SSCP positive cases was made. $BRAF^{V600E}$ was found in 23/67 cases (34%): 18 females (78%) and 5 males (22%), with a mean age of 48.9 ± 16.2 yrs (median: 50 yrs). Ten were in class 1 (43.6%), 6 in class 2 (26%), 4 in class 3 (17.4%) and 3 in class 4 (13%). Among the 44 patients without $BRAF^{V600E}$ 37 were females (84%) and 7 were males (16%), with a mean age of 42.2 ± 15.36 (median: 39.5 yrs). Twenty-seven were in class 1 (61.4%), 12 in class 2 (27.3%) and 5 in class 3 (11.3%). At the end of the study 54 patients (80.5%) were free of disease, 9 (13.5%) had persistent disease and 4 (6%) died of thyroid carcinoma. Among the 44 patients without $BRAF^{V600E}$ mutation, 41 (93.2%) were free of disease, 2 (4.5%) had persistent disease and only 1 (2.3%) died for PTC at the end of follow-up. Between the 23 patients $BRAF^{V600E}$, 13 (56.5%) were free of disease, 7 (30.5%) had persistent disease and 3 (13%) died for thyroid carcinoma. The statistical analysis showed a positive correlation between $BRAF^{V600E}$ mutation and the outcome of the patients ($p=0.0003$). Older age, male sex, advanced tumoral class, loco-regional and/or distant metastasis were more frequent in the patients with $BRAF^{V600E}$ without statistically significant correlation.
In conclusion our data suggest that $BRAF^{V600E}$ is an unfavorable prognostic factor in patients affected by PTC.

P367

Expression of folate receptor is down-regulated in somatotropinomas

Jadwiga Zebracka[1], Adam Rudnik[2], Kornelia Hasse Lazar[1], Dawid Larysz[2], Michal Jarzab[3], Beata Lubieniecka[1], Grzegorz Gala[1] & Barbara Jarzab[1]
[1]Department of Nuclear Medicine and Endocrine Oncology; MSC Cancer Center and Institute of Oncology, Gliwice Branch, Gliwice, Poland; [2]Department of Neurosurgery, Silesian University School of Medicine, Katowice, Poland; [3]Department of Tumor Biology; MSC Cancer Center and Institute of Oncology, Gliwice Branch,, Gliwice, Poland.

Introduction
Pathogenesis of pituitary adenomas is largely unknown thus, identification of genes specific for various types of pituitary tumors should enable better understanding of their biology.
The aim of our study was to analyze differences in gene expression between functional (FA) and non-functional (NFA) pituitary adenomas. For this goal, we considered folate receptor (*FOLR1*) shown by previous study (Evans *et al.* 2003) to be overexpressed in NFA, as well as some other genes reported for its changed expression.
Material and methods
Analysis of gene expression was performed by real-time quantitative PCR (QPCR) with the use of fluorescent probes, based on 5′-nuclease assay (TaqMan). Within the 54 pituitary adenomas collected there were 16 nonfunctioning and 38 functioning ones, among them 7 GH and 13 PRL- secreting adenomas. Expression of the examined genes was normalized to the reference index, obtained by calculation of geometric mean of reference genes expression: *GUS-B, B2M, ACTB, EIF3S10, UBE2D2 and ATP6V1E.*
Results
Folate receptor gene (*FOLR1*) was not significantly overexpressed in NFA compared with FA but was significantly overexpressed when NFA were compared to GH (but not PRL) adenomas. Also, we observed a 3-fold decrease of *CCND1* expression in GH adenomas compared with NFA. Again, the change in expression was not significant at the comparison PRL/NFA. *hPTTG1* and *MEN1* expression was similar in all tumors analyzed.
Conclusions
Folate receptor expression and cyclin D1 expression are down-regulated in somatotropinomas when compared to non-functioning pituitary tumors while prolactinomas do not show such a distinct change in their expression.

P368

Initiating mutations of BRAF gene in papillary thyroid carcinoma and their relation to gene expression profile

Dagmara Rusinek[1], Malgorzata Wiench[1], Malgorzata Oczko-Wojciechowska[1], Jadwiga Zebracka[1], Malgorzata Kowalska[1], Daria Handkiewicz-Junak[1], Sylwia Szpak-Ulczok[1], Ewa Chmielik[2], Agnieszka Czarniecka[3], Michal Jarzab[4], Grzegorz Gala[1], Elzbieta Gubala[1] & Barbara Jarzab[1]
[1]Department of Nuclear Medicine and Endocrine Oncology; Maria Sklodowska-Curie Memorial Cancer Center and Institute of Oncology, Gliwice, Poland; [2]Department of Tumor Pathology; Maria Sklodowska-Curie Memorial Cancer Center and Institute of Oncology, Gliwice, Poland; [3]Department of Oncological Surgery; Maria Sklodowska-Curie Memorial Cancer Center and Institute of Oncology, Gliwice, Poland; [4]Department of Tumor Biology; Maria Sklodowska-Curie Memorial Cancer Center and Institute of Oncology, Gliwice, Poland.

Introduction
Discovery of V600E ($BRAF^{T1799A}$) mutation in papillary thyroid carcinoma (PTC) widened our knowledge about mechanisms of its molecular initiation. It has been revealed that activating mutations of the BRAF kinase are much more frequent in PTC than *RET* rearrangements.
Aim of the study
Estimation of V600E *BRAF* mutation frequency in PTC and analysis of differences in gene expression profile between papillary thyroid carcinomas activated by various molecular events with particular consideration of age of the patients.
Material and methods
The analysis of frequency of *BRAF* mutation was carried out in 77 PTC tumors. In the collection of 45 of these tissues *RET/PTC* rearrangements were analyzed and gene expression profiles were previously obtained (Genechip, Affymetrix). Total RNA was extracted from postoperative tumor tissues, cDNA was synthesized by gene-specific primers. Exon 15 of the *BRAF* gene was amplified by PCR and analyzed by automated sequencing.

Results
The V600E mutation was detected in 54.5% cases of PTC whereas *RET/PTC* rearrangements were identified in 11/42 cases (we identified $BRAF^{T1799A}$ mutation in two patients with previously detected *RET/PTC* rearrangement). The frequency of the V600E mutation was the highest in patients older than 40 years (67% of cases). Patients below 21 years harbouring $BRAF^{T1799A}$ mutation constituted only 7%, in contrast to *RET* rearrangements which were more often found in young patients. Meta-analysis of our own microarray data and these published by Giordano et al., 2005, showed significant differences in gene expression profiles dependent on the type of initiating mutation in PTC. Genes specified by this analysis were subsequently validated by QPCR.
Conclusions
The frequency of *BRAF* mutation in PTC is almost two times higher than of *RET* rearrangements. The occurrence of these genetic alterations is age-dependent. The meta-analysis of PTC gene expression profiles indicates a distinct difference between BRAF-induced and RET-induced papillary thyroid cancers.

P369

CRABP2 expression is up-regulated in parathyroid adenomas in correlation with HRPT2 down-regulation

Jadwiga Zebracka[1], Janusz Waler[2], Malgorzata Kowalska[1], Grzegorz Gala[1], Jacek Gawrychowski[2] & Barbara Jarzab[1]
[1]Department of Nuclear Medicine and Endocrine Oncology; MSC Cancer Center and Institute of Oncology, Gliwice Branch, Gliwice, Poland; [2]Department of General Surgery, Silesian University School of Medicine, Bytom, Poland.

Introduction
The molecular events involved in the formation of parathyroid adenomas are not well understood. Two genes, cyclin D1 (*CCND1*) and *MEN-1*, have been established as having major roles in parathyroid tumorigenesis. Tumor suppressor gene *HRPT2* is frequently mutated in parathyroid carcinoma. The aim of our study was to analyze *HRPT2* expression in parathyroid adenomas and in residual normal/atrophic parathyroid tissue and to relate it to other molecular markers – *CCND1* (cyclin D1) and *MEN-1* expression. We also put the question whether *CRABP2* (cellular retinoic acid binding protein 2), a gene selected on the basis of the microarray study by Forsberg *et al.*, 2005, does show the change in expression in parathyroid adenomas when analyzed by Q-PCR.
Material and methods
The analysis of *HRPT2, CRABP2, c-JUN, CCND1* and *MEN-1* was carried out in 19 parathyroid adenomas taken intraoperatively, and 56 normal/atrophic parathyroid samples. Analysis of gene expression was performed by real-time quantitative PCR (QPCR) with the use of fluorescent probes, based on 5′-nuclease assay (TaqMan). Expression of the examined genes was normalized to the reference index, obtained by calculation of geometric mean of reference genes expression: *EIF3S10, UBE2D2 and ATP6V1E.*
Results
We observed a 1.5-fold, non significant decrease of *HRPT2* expression in adenomas in comparison to normal/atrophic parathyroids. The expression of the gene was significantly correlated with *c-JUN* expression but not with *CCND1* and *MEN-1*. *CRABP2* expression was significantly increased ($P<0.05$) in adenomas and the change in expression (mean: 1.3-fold) was correlated with *HRPT2* expression.
Conclusion
CRABP2 expression is up-regulated in parathyroid adenomas in correlation with *HRPT2* down-regulation.

P370

Hypothyroid Graves' ophthalmopathy: a case report

Jasmina Ciric, Milos Zarkovic, Biljana Beleslin, Mirjana Stojkovic, Slavica Savic & Bozo Trbojevic
Institute of Endocrinology, Belgrade, Serbia.

Background
Hypothyroid Graves' ophthalmopathy is a rare condition, present in about 3% of all cases. Since thyroid-stimulating antibodies could be detected in a majority of euthyroid and hypothyroid Graves' patients, the most probable explanation for unincreased thyroid function is a reduction of tissue capability to response to stimulation.
Case report
A 57-yr-old man visited the hospital with signs and symptoms typical of hypothyroidism. Since TSH was 77 IU/ml, FT4 6.8 pmol/l and TPO Ab 4828 IU/ml, the treatment with 100 mcg/day T4 was started. Three months later, when euthyroid, he developed Graves' ophthalmopathy with slight proptosis, moderate palpebral edema, conjuctival injection and chemosis, reduction of visual acuity to 0.7, diplopia and secondary glaucoma. He had no palpable goiter and ultrasound revealed small (V 5 cm^3), diffuse hypoechoic thyroid. Orbital computed tomography (CT) showed a pronounced enlargement of all extraocular muscles (9–15 mm). TSH receptor antibodies were 65 U/l. Patient was treated with two doses of 0.5 g intravenous methylprednisolone during three days, followed by oral prednisone 40 mg/day tapered to 10 mg/day in four weeks. Six courses of therapy were performed. There were no significant side effects during the treatment. A prompt improvement of visual acuity, intraocular pressure and inflammatory signs was noticed, but diplopia became permanent. Orbital CT revealed a significant reduction of all rectus muscles (2–10 mm). TSH receptor antibodies were 10 U/l, TPO Ab 8603 IU/ml. He developed cataract on his left eye and refused extraocular muscle surgery since he lost diplopia.
Conclusion
Hypothyroid Graves' disease reflects a subtle relation between destructive changes in the thyroid gland and autoimmune mechanisms involved in thyroid pathology.

P371

Increased risk of cardiovascular events in subclinical hyperthyroidism

Marianne Schultz[1], Caroline Kistorp[2], Ilan Raymond[1] & Jens Faber[2]
[1]Dep. of Cardiology & Endocrinology E, Frederiksberg University Hospital, Frederiksberg, Denmark; [2]Dep. of Endocrinology J, Herlev University Hospital, Herlev, Denmark.

Objective
Untreated overt hyperthyroidism is known to predispose the patient to cardiovascular diseases, while predisposition in subclinical hyperthyroidism has been debated. The1 cut-off point of TSH for initiating treatment in subclinical hyperthyroidism is still undefined.
Method
A community-based prospective study including non-hospitalised participants, aged 51 to 91 years, living in Copenhagen, Denmark were examined between September 1998 and January 2000 and provided blood and urinary samples on inclusion. All participants had normal left ventricular ejection fraction (LVEF > 50%), estimated by echocardiography, and were without heart or renal failure. The follow-up period was up to 5 years (to December, 2003). The local ethical committee approved the study.
Results
609 participants were included in the study, 549 (90.1%) were euthyroid (TSH 0.4–4.0 mU/L), 34 (5.6%) had TSH >4.0 mU/L and 26 (4.3%) had TSH <0.4 mU/L. Three were overt hypothyroid and one overt hyperthyroid. Of the participants having TSH ≤4.0 mU/L, 86 died and 59 had first major cardiovascular event during follow-up. In the subclinical hyperthyroid group, the mean value of TSH was 0.2 mU/L (range 0.0–0.4 mU/L). The incidence of major cardiovascular events incl. cardiovascular dead ($r=0.8$, $P=0.04$), as well as the incidence of stroke ($r=1.4$, $P=0.01$) was increased among the subclinical hyperthyroid participants. The TSH < 0.4 mU/L were independently associated with the risk of stroke ($r=1.2$, $P=0.03$), hazard ratio 3.28, even after adjusting for sex, age and atrial fibrillation.
Conclusion
Subclinical hyperthyroidism was a risk factor for developing major cardiovascular events including cardiovascular dead, in particular stroke, in a group of 575 non-hospitalised individuals with TSH ≤4.0 mU/L, aged 51 to 91 years. On this perspective, we recommend the condition subclinical hyperthyroidism to be treated as a disease instead of a condition to be observed.

P372

Predictive factors for thyroid function abnormalities in patients with chronic hepatitis C treated with pegylated interferon and ribavirin

Cristina Cijevschi[1], Catalina Mihai[1], Eusebie Zbranca[2] & Paul Gogalniceanu[1]
[1]Institute of Gastroenterology and Hepatology, Iasi, Romania; [2]Endocrinological Clinic, University Hospital 'St.Spiridon', Iasi, Romania.

Introduction
Chronic hepatitis C has a high incidence in our country being a major public health issue.
Aims and methods
To establish prognostic factors for developing thyroid dysfunction in patients with chronic hepatitis C receiving pegylated interferon and ribavirin therapy. A prospective study of patients with chronic hepatitis C on antiviral therapy was undertaken. 68 patients started on antiviral therapy in the period 1st January 2003 – 1st January 2005 were enrolled in the study. Patient with pre-existing thyroid pathology were excluded from the study. Patient follow-up occurred at 3, 6, 8 and 12 months after commencement of treatment. Follow-up consisted of thyroid echography, TSH, fT3 and fT4 measurement, as well as anti-thyroid anti-body (anti-thyroglobulin and anti-peroxidase) and anti-TSH receptor antibodies assessment. The patients were divided into two groups: group A – patients who developed thyroid dysfunction; group B – patients who did not develop thyroid dysfunction. The following parameters were recorded: age, gender, family history of thyroid disease, initial viral load, cytolysis, histology, early viral response and type of interferon used. Viral genotyping was not performed, as Hepatitis C genotype 1b is present in over 90% of cases diagnosed in our country.
Results
11 patients (16.7%) developed thyroid dysfunction (7 hypothyroid, 4 hyperthyroid), forming group A. The remaining patients (57) formed group B. Statistically significant factors associated with thyroid dysfunction were: female gender (8 patients group A, 29 group B), family history of thyroid disease (6 patients group A, 13 group B), severe hepatic fibrosis (6 patients group A, 19 group B).
Conclusions
Thyroid dysfunction in more common in elderly patients, being associated with female gender, family history of thyroid disease and degree of hepatic fibrosis. Thyroid dysfunction is not associated with initial viraemia, cytolysis, early viral response, type of pegylated interferon used.

P373

Aetiology and therapeutic guideines in thyrid dysfunction at the patients with chronic hepatitis C treated with pegylated interferon and ribavirin

Cristina Cijevschi[1], Catalina Mihai[1], Eusebie Zbranca[2], Andreea Brumaru[1] & Paul Gogalniceanu[1]
[1]Institute of Gastroenterology and Hepatology, Iasi, Romania;
[2]Endocrinological Clinic, University Hospital'St.Spiridon', Iasi, Romania.

Introduction
Chronic Hepatitis C is highly prevalent in our country.
Management involves combination treatment with pegylated interferon and ribavirin. Thyroid disease in affected patients can be caused by the hepatitis C virus or by the interferon therapy.
Aims and method
The study aims to investigate thyroid dysfunction and optimal management strategies for patients with chronic hepatitis C treated with pegylated interferon and ribavirin. A prospective study of 68 patients with chronic hepatitis C was undertaken. Patients commenced treatment between1st January 2003 – 1st January 2005. Patients with previous thyroid pathology were excluded from the study. All patients were followed up at 3, 6, 8 and 12 months from starting therapy. Patients were investigated using thyroid echography, TSH, fT3 and fT4 measurement, as well as anti-thyroid anti-body (anti-thyroglobulin and anti-peroxidase) and anti-TSH receptor antibodies assessment.
Results
11 patients (16.17%) developed thyroid pathology: 7 patients developed hypothyroidism and 4 developed hyperthyroidism. Of the latter, 3 developed destructive thyrotoxicosis and one developed Graves' disease.6 patients (54.54%) were asymptomatic (especially those with hypothyroidsim), whilst 75% of those with hyperthyroidism were symptomatic. 3 out of 7 patients with hypothyroidism developed antithyroid antibodies, probably due to an undiagnosed destructive thyroiditis. Only 2 patients (18.18% of those with thyroid pathology and 2.94% of all patients) stoped peginterferon treatment due to the thyroid related side effects.
Conclusion
The prevalence of thyroid dysfunction in chronic hepatitis C treated with pegylated interferon and ribavirin is 16.17%, mostly manifesting as hypothyroidism. The majority of patients are asymptomatic. Few patients required cessation of antiviral treatment. Monitoring of thyroid function during antiviral therapy is compulsory.

P374

Thyroid function in pregnancy

Emma Varga[1], Zita Molnar[2], Zoltan Szabo[1], Annamaria Gazdag[1], Katalin Jenei[3], Attila Jakab[3], Emese Mezosi[4], Judit Boda[1], Zsolt Karanyi[1] & Endre Nagy V[1]
[1]University of Debrecen Medical and Health Science Center 1st Department fo Medicine, Debrecen, Hungary; [2]BAZ County Hospital, Miskolc, Hungary; [3]University of Debrecen Medical and Health Science Center Department of Obstetrics and Gynecology, Debrecen, Hungary; [4]Pécs University Medical School 2nd Department of Medicine, Pécs, Hungary.

Pregnancy induces hormonal and metabolic changes that result in profound alterations of thyroid hormone economy and regulation. Adaptation of the pituitary-thyroid axis may be influenced by the iodine supply, especially iodine deficiency.

The aim of the study was to define characteristics of changes in certain biochemical parameters and regulation of thyroid function during pregnancy in a mildly iodine-deficient region of Hungary. Thirty-eight healthy pregnant women were enrolled in the study. The local ethical committee approved the study. Serial TSH, free thyroid hormone, total thyroid hormone, chorionic gonadotropin (hCG) and thyroid autoantibody levels were determined 5 times during gestation and 6 months after delivery. Data of 19 individuals were analyzed. To study the influence of pregnancy on the results of free thyroxin measurement, kits of five manufacturers were compared on 40 samples of women with varying gestational ages.

An increase of total T3 and T4 levels was observed parallel with changes of TBG concentration during the first 4 months of gestation. Serum TSH time-curve showed a transient fall in the first trimester, thereafter it returned to the non-pregnant values. Curves of serum TSH and hCG created clear mirror images. Free T4 concentrations elevated in line with the hCG peak at the beginning of gestation, thereafter it clearly followed the course of serum TSH. Free T3 levels gradually decreased throughout pregnancy.

The negative correlation between hCG and TSH levels, and the clear identity of the hCG+TSH and free T4 curves, suggest that thyroid function in pregnancy is the result of the two glycoprotein hormones, TSH and hCG. In pregnancy, total T3 may not be substituted for free T3 in thyroid function estimation, as total and free T3 levels do not correlate. Manufacturers' non-pregnant reference ranges do not apply to pregnancy.

P375

Painful Hashimoto's thyroiditis – 2 cases report

Carmen Vulpoi, Delia Ciobanu, Cristina Preda, Dumitru Branisteanu, Maria-Christina Ungureanu, Elena Livadaru, Roxana Dinu & Eusebie Zbranca
University of Medicine and Pharmacy 'Gr.T.Popa', Iasi, Romania.

Hashimoto's thyroiditis (HT) is usually characterized by goiter and/or hypothyroidism. Thyroid pain and tenderness are rare and suggest an alternative diagnostic of subacute thyroiditis (SAT).

We present two cases of painful HT, who had temporary relief with corticosteroids and required surgical intervention for persistent pain. Both patients were middle-aged women with painful goiter, fever, and inflammatory syndrome. Thyroid function was normal, and ultrasonography showed a hypoechoic inhomogeneous pattern. Corticosteroid treatment was started with rapid amelioration of both pain and inflammatory syndrome, but with relapse in about two months. First patient (MR, 52 y) had moderate hypothyroidism and restarted the corticosteroid treatment in association with l-thyroxine, with a new amelioration. Six months later, she presented relapse of intense pain with inflammatory syndrome, with no response to corticoids, and she was operated. Pathology confirmed lymphocytic thyroiditis, with diffuse fibrosis. She had a favourable evolution for the next 10 years. On her second episode, second patient (MD, 50 y) had high antibodies titre with normal thyroid function. Corticosteroids induced a new amelioration but with relapse at smaller doses. Ultrasonography showed a left thyroid nodule with suspicious cytology after FNAB. She was

operated, with favourable evolution until nowadays. Pathology found a rare association of lymphocytic thyroiditis with giant cells, suggesting the association of subacute thyroiditis.

The overlapping of the symptoms may lead to confusion between painful HT and SAT. Thyroid function is variable and antibodies titre are not always elevated. There are few small series of painful HT published in the literature, in which surgery was imposed by the evolution of the disease. In front of a clinical picture of SAT with no or little response to anti inflammatory treatment, painful HT must be considered. Thyroidectomy seams to be the best option, with relief of the symptoms.

P376

Increase of L-thyroxine requirement during pregnancy

Laura Russo, Massimo Tonacchera, Elena Gianetti, Paolo Vitti & Aldo Pinchera
Department of Endocrinology of Pisa, Pisa, Italy.

In pregnant women with a thyroid disease 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 138 women treated with LT4 divided in two groups: 47 euthyroid (E) (affected by nodular goiter (NG) under LT4 suppressive therapy) and 91 hypothyroid (H). This last group was divided in two subgroups: women with a residual functioning thyroid tissue (R-H) and women without residual thyroid tissue (NR-H). 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. 21 E and 48 R-H and 19 NR-H pregnant women respected these criteria during the entire pregnancy.

Only 11 out of 21 (52%) E had to increase LT4 in order to maintain serum TSH in the appropriate range. The mean increase was 125% at 3rd trimester with respect to pre-gravidic dose. In 32 out of 48 (66%) R-H and in 14/19 (74%) NR-H an increase of L-T4 was necessary to maintain serum TSH in the appropriate range. The mean increase was 134% in R-H and 140% in NR-H 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 and 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 is higher in hypothyroid with respect to NG pregnant women.

P377

Safety of pharmacological treatment of thyroid diseases during pregnancy

Elena Gianetti, Massimo Tonacchera, Laura Russo, Paolo Vitti & Aldo Pinchera
Department of Endocrinology of Pisa, Pisa, Italy.

Pregnant women may require treatment of hypo- or hyperthyroidism, L-thyroxine (LT4), propylthiouralcil (PTU) and methimazole (MMI) being the most frequently used drugs. Aim of this study was to verify the consequences of pharmacological treatment during pregnancy. We retrospectively evaluated 379 pregnancies: 124 patients under MMI treatment, 35 of whom still hyperthyroid in spite of treatment (H-MMI) and 89 euthyroid (E-MMI); 52 GD patients under PTU, 20 of whom still hyperthyroid (H-PTU) and 32 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 nodular goiter or autoimmune thyroid disease. 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: 9/89 (10.1%) E-MMI, 3/35 (8.5%) H-MMI, 4/32 (12.5%) 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. In 2 H-PTU newborns a fetal goiter and a hypertrophic pyloric stenosis 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 that currently there are not contraindications for the use of LT4, MMI and PTU treatment during pregnancy.

P378

Fine-needle aspiration biopsy – possibilities and limitations

Delia Ciobanu[1], Carmen Vulpoi[1], Niculina Florea[2], Lidia Andriescu[1], Alexandru Grigorovici[1], Voichita Mogos[1], Bogdan Galusca[1], Cristina Cristea[1], Letitia Leustean[1] & Eusebie Zbranca[1]
[1]University of Medicine and Pharmacy 'Gr.T.Popa', Iasi, Romania; [2]University Hospital "Sf.Spiridon", Iasi, Romania.

The critical problem of thyroid nodules is to identify the malignant ones Fine needle aspiration biopsy (FNAB) plays a crucial role in this diagnosis and enables the number of surgical operations to be reduced. We have evaluated the performances of FNAB in comparison with the histological examination in 1971 consecutive patients who suffered both fine-needle puncture and surgery in a 5 years interval at a University Hospital. FNAB was malignant or suspicious in 8.4% patients, and the histology confirmed thyroid cancer in 8.7% (confirming all those diagnosed by FNAB). Statistical analysis revealed a sensitivity of 77% and a specificity of 95%, better than the admitted inferior limit of the literature data (71% respectively 72%). Papillary thyroid carcinoma was the easiest to diangose by the cytology, the efficacy of the method being 97%. For anaplastic and medullary carcinoma, FNAB is a good method to diagnose the malignancy (concordance of 97%) but has not the capacity to confirm the type of the neoplasia. In the follicular carcinoma, the positive predictive value is lower than for the other forms (27% vs 99%) although the efficacy is not significantly modified (94%). These data justify the introduction of morphometric methods and of the cytochemistry, able to enhance the accuracy of FNAB. These methods are time-consuming and we were using them only in controversial cases. With a very good sensitivity and specificity, FNAB is a reliable method of diagnosis in thyroid nodules, easy to perform and permitting to avoid unnecessary surgery.

P379

Association of p53 codon 72 polymorphism with thyroid cancer

Sinan Caglayan[1], Cenk Aral[2], Sirin Massaumilary[2], Gokhan Ozisik[3], Huseyin Baloglu[4], Mustafa Akkiprik[2], Ayse Ozer[2] & Metin Ozata[3]
[1]Kasimpasa Military Hospital, Division of Endocrinology and Metabolism, Istanbul, Turkey; [2]Marmara University, School of Medicine, Department of Medical Biology, Istanbul, Turkey; [3]GATA Haydarpasa Teaching Hospital, Division of Endocrinology and Metabolism, Istanbul, Turkey; [4]GATA Haydarpasa Teaching Hospital, Department of Pathology, Istanbul, Turkey.

Tumors of thyroid gland are one of the most prevalent forms of human cancers. Despite the various molecular mechanisms, mutations or polymorphisms of p53 have a potential role in the development and/or progression of human malignancies including thyroid. A common variation in p53 that results in adenine to proline change in codon 72 has been identified as a predisposing factor for various cancers since controversial results have been reported. In this study, we investigated codon 72 polymorphism in 58 thyroid cancer patients and 115 healthy individuals. Genomic DNAs were extracted from paraffin embedded tumor tissues of patients and blood samples of healthy individuals. PCR-RFLP method was applied for determination of codon 72 polymorphism. Genotype frequencies of arg/arg, arg/pro and pro/pro were 0.293, 0.483, 0.224 for patients and 0.461, 0.452, 0.087 for healthy controls, respectively. Proline allele frequencies of patients and healthy controls were 0.466 and 0.313, respectively. A significant difference was found between genotypes of patients and controls ($P=0.006$). Also, proline allele frequency was significantly higher in patients group than healthy control ($P=0.005$) (Odds ratio=0.527, 95% CI=0.341–0.817). No difference was found between 16 follicular adenoma and 18 papillary carcinoma patients ($P>0.05$). Additionally, no significant difference was found for TNM classification of papillary carcinoma patients for codon 72 status ($P>0.05$). In conclusion, p53 codon 72 polymorphism is a significant contributor of thyroid malign and benign lesions and proline allele is significantly increasing the risk of thyroid cancer.

P380

Adiponectin in patients with Graves' ophthalmopathy (GO)

Helena Jastrzebska[1], Malgorzata Gietka-Czernel[1], Jadwiga Janik[1], Izabella Czajka-Oraniec[1], Wojciech Jeske[1], Regina Karczmarzyk[2] & Wojciech Zgliczynski[1]
[1]Medical Centre of Postgraduate Education, Department of Endocrinology, Warsaw, Poland; [2]Institute of Oncology; Department of Teleradiotherapy, Warsaw, Poland.

Adiponectin is a soluble protein produced solely by matured adipocytes. Adipogenesis contributes to the pathogenesis of GO in many ways including a direct effect on proptosis due to increased volume of mature adipocytes.

The aim of the study was to estimate an influence of immunosupresive and anti-inflammatory treatment using systemic corticotherapy combined with orbital radiotherapy on serum adiponectin level in GO patients. The study was accepted by Ethical Commitee.

Material consisted of eight previously untreated euthyroid women aged 53.62±4.89 yrs. Corticotherapy was aplicated once a week, intravenously following a protocol: methylprednisolone in a dose of 0.5 g for the first 6 weeks, therafter the dose was reduced to 0.25 g for another 6 weeks, and from the third week was combined with weekly orbital irradiation (2 Gy) over 10 weeks.

Clinical examination with estimation of clinical activity score (CAS), proptosis, ophthalmopathy index (OI), BMI as well as blood sampling for adiponectin estimation were performed before therapy, after second methylprednisolone injection and after last orbital irradiation. Adiponectin was measured using RIA kits (Linco Research). Treatment resulted with significant clinical improvement and decrease in CAS of 3 points ($P<0.01$), reduction of proptosis >2 mm ($P<0.01$) and OI from 6.5 points±1.19 to 4.0±0.53 ($P<0.01$). BMI did not change during the study (mean 26.64±3.90 kg/m^2 vs. 26.43±3.37 kg/m^2). Serum levels of adiponectin were in normal range in all patients: before therapy mean 16.10±6.10 mcg/ml, during therapy mean 16.42±6.03 mcg/ml and after therapy mean 17.08±7.48 mcg/ml.

No significances were observed in adiponectin concentration during the treatment in all subjects.

Our results may suggest that changes in proptosis in GO patients during anti-inflammatory and immunosupressive therapy are not associated with any significant changes in serum adiponectin level.

P381

Resistin levels in hypothyroid patients before and after treatment with thyroxin

Marianthi Tzoitou[1], Evridiki Papadopoulou[1], A Galactidou[2], Athanasios Papageorgiou[1], Anargyros Kourtis[1], Marina Kita[1], louiza ventzi[1] & Avraam Avramidis[1]
[1]Endocrinology Department, Hippokratio General Hospital, Thessaloniki, Greece; [2]Research Department of Theagenio Hospital, Thessaloniki, Greece.

Introduction
Resistin is a peptide hormone that is secreted from fat cells and its secretion is regulated from hormonal and dietary factors. In hypothyroid patients its levels are decreased.

The purpose of this study is to evaluate the levels of circulating resistin in hypothyroid patients before and after thyroid function is normalized with thyroxin therapy.

Materials and methods
Twenty (20) hypothyroid patients (2M, 18F) mean aged 49.9±12.4 and mean weight 75.1±19.4 Kgr) were studied.

FT4, TSH, AMA, ATA, Resistin were measured before and three months after thyroxin therapy.

Results
Resistin levels do not change significantly (5.8±4.1 vs 5.1±3.4 μg/lt).All patients became euthyroid after three months of treatment and TSH, FT4, AMA, ATA levels were changed significantly(16.7±3.4 miu/lt vs1.2±0.2 miu/lt, 0.8±0.07 ng/dl vs1.3±0.07 ng/dl and 1579.6±653 vs 412±219. 441.8±205 vs 264.8±111). The body weight of the patients was not change significantly during therapy(75.1±19.4 vs 74.1±17.2 Kgr).

Conclusions
Normalization of thyroid function did not affect resistin levels significantly. Possibly this is because there was no change IN the patients weight during treatment.

P382

Increased need for oral thyroxine in total thyroidectomized patients: a prospective analysis

Lucilla Gargano, Camilla Virili, Nicola Viceconti, Maria Giulia Santaguida, Serena Nardo & Marco Centanni
Endocrinology Unit, University of Rome 'La Sapienza', Latina, Italy.

Increasing evidence suggests a relevant role for thyroid gland in maintaining hypophysis-thyroid homeostasis even in patients treated with oral thyroxine. Aim of the study was to compare the daily dose of thyroxine required to attain subnormal serum TSH levels in patients with nontoxic goitre before and following total thyroidectomy. To address this question we have studied: a) 15 patients (8 women and 7 men; median age=53 years) with nontoxic goitre (NTG) and no evidence of autonomous functioning nodule, prospectively analyzed before and after total thyroidectomy for differentiated thyroid carcinoma and b) a cohort of 45 randomly selected patients (35 women and 10 men; median age=51 years) with similar characteristics submitted to total thyroidectomy. Thirty-nine randomly selected T4-treated patients with NTG (33F, 6M; median age=46 years) represented the reference group. In all these patients we compared the dose of thyroxine (normalized by Kg body weight/day) required to stably attain plasma TSH levels to within 0.1–0.2 mU/l. No patients were taking drugs or had evidence of other diseases, known to interfere with the absorption of thyroxine. In the patients prospectively studied the median dose of thyroxine required to obtain low TSH (median=0.11 mU/l) was 1.41 μg/Kg/day. Following thyroid removal, being the thyroxine dose maintained to pre-surgical levels, median TSH significantly rose to 2.94 mU/l ($P=0.031$). Low serum TSH (median=0.16 mU/l) was restored in all patients by increasing the median dose by 37% (1.94 μg/Kg/day; $P=0.0013$). Similarly, in the randomly selected patients the median dose of thyroxine required was higher in thyroidectomized patients (1.83 μg/Kg/day) than in those with nontoxic goitre (1.50 μg/Kg/day; $P<0.0001$). These data indicate that, both in the same patient and in different groups of patients, the daily dose of thyroxine required to lower plasma TSH is 1/3 higher when the thyroid is absent.

P383

Efficacy and safety of radiofrequency thermal ablation in the treatment of thyroid nodules with pressure symptoms in elderly patients

Antongiulio Faggiano[1], Carolina Di Somma[1], Francesco Milone[1], Valeria Ramundo[1], Gaetano Lombardi[1], Annamaria Coloa[1] & Stefano Spiezia[2]
[1]Dept. Of Molecular And Clinical Endocrinology And Oncology, 'Federico Ii' University Of Naples, Naples, Italy; [2]Unit Of Ultrasound-Guided And Neck Pathologies Surgery, 'Sm Del Popolo Degli Incurabili' Hospital, Naples, Italy.

Background
Nodular goiter is one of the commonest endocrinopathy. Its incidence increases with age accounting for more than 50% of subjects older than 60 years. Elderly subjects more frequently suffer from pressure symptoms. Loco-regional treatments, like laser photocoagulation and percutaneous ethanol injection, are a potentially useful tool to treat TNs but their efficacy is still debated. Radiofrequency thermal ablation (RTA) has been applied to several benign and malignant tumors proving to be a safe procedure, potentially helpful to stabilize or decrease tumor growth. Recently, RTA proved to be safe and to induce short-time effects in the treatment of patients with thyroid nodules.
Objective
The aim of this study is to evaluate safety and efficacy of RTA in elderly patients with compressive thyroid nodules followed-up for 1 year.
Patients and methods
Thirty-nine elderly patients with cytologically benign compressive TNs were enrolled in the study. Twenty-seven of them were affected with nontoxic goiter, five with pre-toxic goiter, four with toxic goiter, three with toxic adenoma. Thyroid surgery was contraindicated in 22 and refused in 17 cases. RTA was performed by using a RITA © Starbust needle inserted under ultrasonographic real time guide. Efficacy and safety of RTA were followed-up at 1, 3, 6, 12 month.
Results
After treatment, all TNs showed a significant decrease during the follow-up. Mean TN volume decreased from 24.3±2.6 to 6.4±1.6 ml ($P<0.001$) with a mean percent decrease of 78.6±2.5% 12 months after RTA. Compressive symptoms improved in all cases and disappeared in 82%. The treatment was well tolerated by all patients. No major complications were observed.
Conclusions
RTA seems to be a valid and safe approach in the treatment of benign thyroid nodules with pressure symptoms. RTA may be of great benefit in elderly patients in whom surgery or radio-iodine therapy are contraindicated or refused.

P384

The effect of nodule size on diagnostic efficacy in fine needle aspiration biopsy of thyroid nodules

Dimitrios Thomas[1], Ifigenia Kostoglou-Athanassiou[1], Emmanouil Vassiliou[1], Vassilios Liakos[1], Fotini Chatzimarkou[1], Anastasios Pappas[1], Panagiotis Athanassiou[2] & Philippos Kaldrymidis[1]
[1]Department of Endocrinology, Metaxa Hospital, Piraeus, Greece; [2]Department of Rheumatology, Asclepeion Hospital, Athens, Greece.

Fine needle aspiration is used extensively in the diagnostic evaluation of thyroid nodules. However, its diagnostic efficacy may be reduced by non-diagnostic findings. The aim was to study the effect of nodule size on the diagnostic efficacy of ultrasound-guided fine needle aspiration biopsy in thyroid nodules.

Ultrasound-guided fine needle aspiration biopsy was performed in 210 patients with thyroid nodules. Cytology results were compared to nodule size. Patients were stratified in 5 groups according to nodule size, group A ($n=41$) nodule size 0.1–0.426 cm^3, group B ($n=43$) nodule size 0.427–0.816 cm^3, group C ($n=42$) nodule size 0.817–1.593 cm^3, group D ($n=43$) nodule size 1.594–3.382 cm^3 and group E ($n=41$) nodule size >3.39 cm^3. Ultrasound guidance of the fine needle aspiration biopsy was performed in all patients using the same linear modifier (6–12 MHz) attached in a Toshiba ultrasound apparatus (SSA–550A). Statistical evaluation of the results was performed using x^2 test and ANOVA.

In group A thyroid nodule fine needle aspiration biopsy was successful in 43.9%, in group B 79.1%, in group C 76.2%, in group D 69.8% and in group E 58.5% ($P=0.004$, x^2 test). The number of cystic nodules and the pattern of vascularization (central, peripheral or both) differed significantly between the groups studied.

Diagnostic efficacy of fine needle aspiration biopsy seems to increase in parallel to nodule size. However, this relationship was not apparent in very big nodules, nodule size >3.38 cm^3, possibly due to confounding factors, such as the presence of cystic areas and increased vascularization within the very large thyroid nodules.

P385

The effect of thyroxine suppression therapy on the diagnostic efficacy of fine needle aspiration biopsy in thyroid nodules

Dimitrios Thomas[1], Ifigenia Kostoglou-Athanassiou[1], Emmanouil Vassiliou[1], Vassilios Liakos[1], Fotini Chatzimarkou[1], Anastasios Pappas[1], Panagiotis Athanassiou[2] & Philippos Kaldrymidis[1]
[1]Department of Endocrinology, Metaxa Hospital, Piraeus, Greece; [2]Department of Rheumatology, Asclepeion Hospital, Athens, Greece.

Ultrasound-guided fine needle aspiration biopsy is used extensively in the diagnostic evaluation of thyroid nodules. However, its diagnostic efficacy is hampered by the presence of non-diagnostic cytology results.

The aim was to study the effect of previous thyroxine suppression therapy on the diagnostic efficacy of fine needle aspiration biopsy in thyroid nodules.

Ultrasound-guided aspiration biopsy was performed in 45 patients, 31 patients on thyroxine suppression therapy and 14 patients without current or previous thyroxine therapy. Ultrasound guidance of the fine needle aspiration biopsy was performed in all patients using the same linear modifier (6–12 MHz) attached in a Toshiba ultrasound apparatus (SSA-550A). Statistical evaluation of the results was performed using Student's t test and x^2 test.

Patient characteristics did not differ significantly between the 2 groups studied. In 13 of 14 (92.9%) patients without current or previous thyroxine therapy the cytology result of fine needle aspiration biopsy was diagnostic, whereas the cytology result of the biopsy was diagnostic in 20 of 31 (64.5%) patients on thyroxine suppression therapy ($P=0.046$). The diagnostic efficacy was not found to differ according to the duration of thyroxine therapy, possibly due to the small number of patients studied.

It appears that thyroxine suppression therapy in patients with thyroid nodules is related to lower diagnostic efficacy of ultrasound-guided fine needle aspiration biopsy. Thyroxine suppression therapy may induce changes in thyroid cell structure and size, thus modulating the diagnostic efficacy of fine needle aspiration biopsy in thyroid nodules.

P386

Congenital hypothyroidism – results of a protocol implemented 1993–2006

Miguel Melo[1] & Charles Buchanan[2]
[1]Endocrinology Department, University Hospital of Coimbra, Coimbra, Portugal; [2]King's College Hospital, London, United Kingdom.

Introduction
A new Regional Paediatric Endocrine Service was established in 1993 which implemented a protocol for Congenital Hypothyroidism (CH) management. Its aim is to confirm the diagnosis, establish aetiology and start treatment within 48 hours after the result of the screening test.
Objective
To audit the results of this protocol.
Methods
Case note and laboratory data review for all Neonates referred since 1993 after a positive TSH screening test. The following issues were considered: age at screening, time to lab. Receipt and processed the blood sample, child's age when results known, time from referral to first appointment at, clinical presentation, presence of associated disorders, family history of thyroid diseases, presence of thyroid auto-antibodies in mother's blood, child's age when treatment was started, starting dose of L-T4, time to normality of TSH, diagnostic group – agenesis, dysgenesis, dyshormonogenesis, transient or other; presence of learning difficulties, assessed with Griffiths scale.
Results
A total of 28 patients were included; 7 (36.8%) were premature. Median age at screening was 9 days. Medians of time to laboratory for sample and processing were 6 and 3 days, respectively. By the time the screening test results' were known, children had a median age of 16 days. Median time from referral to first visit was 1 day (mean age 22.0 ± 18.2 days). Median age start treatment was 18 days; mean starting dose 8.8 ± 3.6 mcg/kg/day. At presentation, 15 (54%) babies had jaundice. A ^{99m}Tc scan was done in the first visit in 19 (68%) patients. 22% had thyroid agenesis, 39% dysgenesis, 30% dyshormonogenesis (all normal hearing tests) and 9% were transient 3 patients had Down's syndrome and 1 a CNS malformation. 3 mothers had thyroid antibodies. Median time to normal TSH was 91 days and there was no a statistically significant difference between the aetiological groups. 2 patients had learning difficulties.
Conclusions
The objectives of this protocol were largely achieved, since most of the patients had a full aetiological workup and started treatment in the first 24 hours after the screening test is known.

P387

Relationship of treated maternal hyperthyroidism and perinatal outcome

Amjed Khamis[1], Sharon Murray[1], Sean Daly[2], Richard Firth[1] & Brendan Kinsley[1]
[1]Diabetes and Endocrine department, Mater Misercordea University Hospital, Dublin, Ireland; [2]Coomb's women Hospital, Dublin, Ireland.

Hyperthyroidism in pregnancy is associated with increased foetal and maternal morbidity. Hyperthyroidism occurs in 0.2–0.6% of pregnancies. This suggests that based on 23,000 deliveries in the 3 major Dublin maternity hospitals that 40–60 cases per year would be expected to be at risk of a poor outcome from hyperthyroidism.

To clarify those factors associated with poor outcome in hyperthyroidism in pregnancy we undertook an audit of 53 cases of hyperthyroidism in pregnancy attending from 2004–2005. Women with hyperthyroxinaemia secondary to hyperemesis were excluded. Demographic data, maternal thyroid function tests, doses of anti-thyroid medications were noted. Pregnancy outcomes, birth weight and neonatal TFTs were noted. Cases were divided according to those Delivering pre 37 weeks (Group A, $n=11$) and at term, post 37 weeks (Group B, $n=42$).
Results
Mean age was 31 ± 5 years. Mean booking to OPD at 13 ± 5 weeks gestation. Mean delivery gestation was 39 ± 1 weeks in-group A, 35 ± 3 weeks in group B ($P<00.1$). Mean birth weight 3.3 ± 0.7 kg. One neonatal death occurred in-group A.
In Group A, baseline TSH was 0.09 ± 0.1, $P<0.05$ vs Group B (1.1 ± 1.3). By end of the second trimester, TSH in Group A was 0.17 ± 0.2, $P<0.05$ vs Group B (0.88 ± 1.0). By end of third trimester TSH was 0.34 ± 0.5 (GROUPA), $P<0.05$ vs. Group (0.98 ± 0.9). Average BW in Group A was 2.5 ± 0.9 kg, $P<0.01$ vs Group B (3.44 ± 0.6 kg)

Conclusion
TSH levels were significantly lower in those women with pre-term delivery. This suggests that sub-optimal control of hyperthyroidism during pregnancy is associated with increased infant morbidity or mortality

P388

Characteristics of locally advanced differentiated thyroid cancer in a cohort of patients surgically treated at one oncological institution

R Baldelli, G Pasimeni, A Barnabei & M Appetecchia
Regina Elena Cancer Institute, Rome, Italy.

Differentiated thyroid carcinomas (DTC) (papillary, follicular and follicular type of papillary) are characterized by a favourable prognosis, but a part of patients can develop recurrences and eventually die of the disease. We retrospectively reviewed 108 DTC patients affected by locally advanced thyroid carcinoma (77 females, 31 males) (49 ± 15 years), in order to evaluate validate known prognostic factors that enable them to be recognised as having a low or a high risk of death related to the tumor, by reference to the staging classifications systems. The TNM classification was as followed: T2b (0.9%), T3 (62%), T3b (30%), T4a (5.5%), T4B (1.8%). The mean diameter of tumor was 24 ± 1 mm. In particular the histology was papillary (62%), follicular (8%), follicular type of papillary (28%), Hurtle (1%), Hurtle+ papillary (1%). Lymph nodes status was N0 (9.2%), N1a (13.8%), N1b (26.8%), Nx (50%) while metastases were present in 3.7% of patients. With the regards of stage patients were stage I (50%), stage III (33.3%), stage 4a (12%), stage IV b (3.7%). Seven of them (6.4%) had local or distant recurrences. Thyroiditis was found in 30% at the histology. No deaths were reported regarding our group of patients. Papillary and follicular thyroid carcinoma, referred to as differentiated thyroid carcinoma (DTC) cover the majority of thyroid carcinoma cases. The prognosis for DTC is usually excellent, but even so a proportion of the patients develop recurrences and eventually die of the disease. In particular the majority of our patients (50%) were in the stage I explaining the good prognosis of this group of patients. These previous data show that age at the time of diagnosis, histological type, tumour size and extrathyroidal invasion are associated with a good clinical outcome.

Bone/calcium – presented on Monday

P389

Abnormal calcium metabolism as shown by the Ellsworth-Howard test and its relation to pseudohypoparathyroidism II

Levent Kebapcilar, Sena Yesil & Tevfik Demir
Dokuz Eylul University, IZMIR, Inciralti, Turkey.

Peudohypoparathyroidism is a heterogenous group of disorders characterized by hypocalcemia, hyperphosphatemia, increased serum concentration of parathormone, and insensivity to biological activity of parathormone.

A 45-year-old female was admitted to the regional hospital for check up and routine laboratory studies revealed slightly decreased level of calcium. Her neurologic examination was negative for Chvostek's and Trousseau' signs. Laboratory testing revealed low calcium (8.1 mg/dL; reference: 9.5–10.5 mg/dL) with elevated PTH (388 pg/mL; reference: 12–72 pg/mL) and phosphate levels. 25 hydroxycholecalciferol (56 ng/mL; reference: 7.6–75 ng/mL), and 1.25 dihydroxycholecalciferol levels were normal (40 pg/mL; reference: 30–60 pg/mL). This laboratory tests indicative of PTH resistance and suggested PHP. We therefore applied Ellswoth- Howard (EH) test, which shows receptor function and the presence of intracellular signal transduction disorder in renal tubular cells and to determine the type of PHP.

Both the phosphaturic (Δ) and urinary c AMP (Uc AMP) responses were estimated. The Δ P responses in the patient was significantly lower than normal response (18 mg/2 h) but its UcAMP response did not differ (Δ c AMP ≥7.9 μmol/h and after/ before c AMP ratio: 13.2) from normal subjects. This was suggested us that the patient had PHP type II. We started treatment with calcium (2000 mg daily) and 1.25- vitamin D3 (0.5 μg daily).

Many individuals affected by pseudohypoparathyroidism type II (PHP-II) have no apparent clinical symptoms and may show only a mild PTH elevation as evidence of PTH resistance. Patients with pseudohypoparathyroidism type II lack the features of Albright's hereditary osteodystrophy and may manifest hormonal resistance limited to target tissues.

Peudohypoparathyroidism is a heterogenous group of disorders characterized by hypocalcemia, hyperphosphatemia, increased serum concentration of parathormone, and insensivity to biological activity of parathormone.

A 45-year-old female was admitted to the regional hospital for check up and routine laboratory studies revealed slightly decreased level of calcium. Her neurologic examination was negative for Chvostek's and Trousseau' signs. Laboratory testing revealed low calcium (8.1 mg/dL; reference: 9.5–10.5 mg/dL) with elevated PTH (388 pg/mL; reference: 12–72 pg/mL) and phosphate levels. 25 hydroxycholecalciferol (56 ng/mL; reference: 7.6–75 ng/mL), and 1.25 dihydroxycholecalciferol levels were normal (40 pg/mL; reference: 30–60 pg/mL). This laboratory tests indicative of PTH resistance and suggested PHP. We therefore applied Ellswoth- Howard (EH) test, which shows receptor function and the presence of intracellular signal transduction disorder in renal tubular cells and to determine the type of PHP.

Both the phosphaturic (Δ) and urinary c AMP (Uc AMP) responses were estimated. The Δ P responses in the patient was significantly lower than normal response (18 mg/2 h) but its UcAMP response did not differ (Δ c AMP ≥7.9 μmol/h and after/ before c AMP ratio: 13.2) from normal subjects. This was suggested us that the patient had PHP type II. We started treatment with calcium (2000 mg daily) and 1.25- vitamin D3 (0.5 μg daily).

Many individuals affected by pseudohypoparathyroidism type II (PHP-II) have no apparent clinical symptoms and may show only a mild PTH elevation as evidence of PTH resistance. Patients with pseudohypoparathyroidism type II lack the features of Albright's hereditary osteodystrophy and may manifest hormonal resistance limited to target tissues.

P390

The role of non-calcemic analogs of vitamin D in differentiation of cultured rat bone marrow into osteoblast-like cells: age and sex differences

Dalia Somjen[1], Alvin M Kaye[3], Michal Ofer[2] & Ilan Bleiberg[2]
[1]Tel-Aviv Sourasky Medical Center, Tel-Aviv, Israel; [2]The Sackler Faculty of Medicine, Tel-Aviv Univ, Tel-Aviv, Israel; [3]The Weizmann Institute of Science, Rehovot, Israel.

We have previously demonstrated that rat bone cells *in vivo* and *in vitro*, responded sex-specifically to gonadal steroids in stimulation of the specific activity of creatine kinase (CK). Pretreatment with vitamin D analogs upregulated the sex-specific responsiveness and sensitivity to gonadal steroids. We also found that mice cultured femoral bone marrow (BM) in the presence of dexamethasone (DEX), 1.25(OH)2D3 (1.25D) or both, differentiated into osteoblast-like cells (Obs), acquiring sex-specific responsiveness to gonadal steroids. We now examined the effect of age, sex and vitamin D non-hypercalcemic analogs on differentiation of rat femoral BM into Obs. In female or male, BM from intact but not gonadectomized rats DEX and DEX + 1.25D increased the constitutive levels of CK. BM from old females showed lower stimulation of CK than BM from young females by estradiol-17β (E2) or raloxifene (Ral) in the presence of both DEX and 1.25D. The non-hypercalcemic analogs of vitamin D: CB 1093 (CB), EB 1089 (EB) and MC 1288 (MC) were more effective than 1.25D in both age groups in stimulating CK in the absence of DEX. In the presence of DEX, CK was further increased with the same differential effectiveness. BM from gonadectomized male or female rats, lost the sex-specific response namely responding to both E2 and dihydrotestosterone (DHT). BM derived from intact and gonadectomized males and females, growing with DEX or DEX + 1.25D showed increased activity of basal alkaline phodphatase (AP) with no stimulation by gonadal steroids. These findings suggest that manipulation of the hormonal milieu in early stages of differentiation into Obs determines the subsequent selective responsiveness of the developing bone tissue to sex steroids. Non-calcemic vitamin D analogs were more effective than 1.25D and showed activity even in the absence of DEX and may be applied for bone tissue engineering.

P391

Mutual modulation of the vitamin D system and estrogen receptors in human bone cells in culture

Dalia Somjen[1], Sara Katzburg[1], Orly Sharon[1], Rona Limor[1], David Hendel[3] & Gary H Posner[2]
[1]Tel- Aviv Sourasky Medical Center, Tel-Aviv, Israel; [2]The Johns Hopkins University, Baltimore, United States; [3]Sharei-Zedec Med. Ctr., Jerusalem, Israel.

Vitamin D receptors are widely expressed in the skeletal system, and vitamin D and its metabolites and analogs, exert a variety of biological activities such as regulation of cellular proliferation and differentiation, cell calcium transients and energy metabolism in vitro. The latter is exerted through the control of the brain type isozyme of creatine kinase specific activity (CK), which serves to provide a readily available reservoir for ATP generation under increased workload.

We have previously reported that pretreatment with the less-calcemic analog of vitamin D JKF 1624F_2-2 (JKF) upregulated the responsiveness to estrogenic compounds via modulation of the expression of mRNA for ERs. In the present study we analyzed the mutual modulation of the vitamin D system and estrogens in human cultured female bone cells (hObs). We compared the effects of the different hormones on the expression of mRNA for both ERα and ERβ and 1α 25 vitamin D hydroxylase in hObs. In pre-menopausal hObs all hormones tested increased 1α 25 vitamin D hydroxylase mRNA expression whereas in post-menopausal hObs biochainin A had no effect and genistein is decreasing this mRNA expression. All these compounds increased the expression of mRNA for ERα in pre-menopausal hObs whereas in post-menopausal hObs biochainin A had no effect and estradiol and raloxifene decreased this mRNA expression. ERβ in both hObs was increased only by carboxy-biochainin A and raloxifene and all other hormones decreased ERβ. In conclusion vitamin D analogs and estrogens modulate each other's activity in hObs. The different hormones modulate the response to estradiol by direct modulation of ERs mRNA expression and by indirect modulation via increasing vitamin D in bone cells leading to modulation of responsiveness by this system as well. Whether or not this property can be utilized to achieve better bone protection remains subject to further studies.

P392

Less-calcemic vitamin D analogs enhance biological responses and modulate responsiveness to gonadal steroids in skeletal tissues

Dalia Somjen[1], Gary H Posner[2], Yosef Weisman[1] & Alvin M Kaye[3]
[1]Tel- Aviv Sourasky Medical Center, Tel-Aviv, Israel; [2]The Johns Hopkins University, Baltimore, MD., United States; [3]The Weizmann Institute of Science, Rehovot, Israel.

Vitamin D metabolites modulate creatine kinase specific activity (CK) in cultured skeletal cells. In this study we assess the effect of vitamin D metabolites on CK in rat epiphyseal cartilage (Ep) and diaphyseal bone (Di). Female or male Wistar-derived rats were used either as intact or after gonadectomy (Ovx or cast respectively), and treatments started 2 weeks post surgery. Rats were injected daily for 1, 2 or 8 weeks with the less-calcemic vitamin D analogs CB 1093 (CB), JKF 1624F_2-2 (JKF) or QW 1624F_2-2 (QW) and 24hrs after the last analog injection, rats were injected with E_2, raloxifene (Ral) or tamoxifen (TAM) or both in females or dihydrotestosterone (DHT) in males, and organs were collected for CK measurements and western blot analysis for estradiol receptor ((ERα) 24hrs after last injection. CK was lower in Ep and Di from vitamin D- depleted than in vitamin D- replete rats. Moreover E_2 or DHT, which increases CK in Ep and Di of intact female or male rats, stimulated CK to a much lower extent in vitamin D-depleted rats. Treatment of intact female rats for 2 or 8 weeks with JKF or QW, upregulated the E_2 – or DHT- response of CK in Ep and Di, without affecting constituent levels. All vitamin D analogs enhanced the CK response to Ral and TAM in these organs, but the inhibitory effect of Ral or TAM on E_2-induced CK was lost. CB induced also ER(protein in Ep and Di from intact and Ovx female rats. In conclusion, vitamin D induces CK and upregulates the response and sensitivity of CK to E_2 and SERMs, possibly via increased ER(protein. These results corroborate our *in vitro* studies in human bone cells and provide evidence that vitamin D is crucial to maintain normal skeletal energy metabolism.

P393

The predictive role of body mass and composition upon bone mineral content: differences between premenopausal and postmenopausal women

Dumitru Branisteanu, Anca Piciorea, Eusebie Zbranca & Voichita Mogos
University of Medicine and Pharmacy 'Gr.T.Popa', Iasi, Romania.

Gravitational stress influences bone mass. Adipose tissue represents a supplementary source of estrogens at postmenopausal women, *via* aromatization of androgens. We evaluated the importance of weight and fat tissue on bone mass at premenopausal or postmenopausal women in a cross-sectional study upon a group of 138 women between 25 and 77 ys old and with a BMI between 17.1 and 44.3 kg/m^2. Fifty six women were menstruating, 15 were perimenopausal and 68 were postmenopausal. We assessed the correlation between lumbar bone mineral content (Z and T scores, measured by dual X ray absorptiometry) and: body mass, adipose and lean tissue mass (measured by electric impedance). Postmenopausal women had a significantly lower bone mass than premenopausal women (mean T score of $-1.87+/-0.14$ vs $-0.91+/-0.16$, $P<0.05$). Lean (BMI <24 kg/m^2) postmenopausal women had an even lower mineral content (T score $=-2.17+/-1.23$, $P<0.01$ when compared to premenopausal women), whereas overweight postmenopausal women (BMI >26 kg/m^2) had an intermediate T score between premenopausal and postmenopausal lean women ($-1.63+/-0.19$, $P<0.05$). Total body mass, lean and fat mass were all correlated to bone mineral content, having comparable predictive powers in premenopausal women. When applied to postmenopausal women, correlation significance of fat mass with the Z score augmented ($R^2=0.329$ vs $R^2=0.253$ for premenopausal women), whereas correlation significance between total or lean body mass and Z score decreased (in the case of total body mass - $R^2=0.148$ vs $R^2=0.28$ for premenopausal women). Adipose tissue mass seems therefore to be an important BMD predictive factor. Its predictive value increases in postmenopausal women, whereas total and lean body mass are correlated to BMD especially in premenopausal women, which are not yet submitted to estrogenic depletion.

P394

Increased cortisol level in type 1 diabetic patient may lead decreasing of bone mineral density

Alpaslan Tuzcu[1], Semir Bayer[1], Senay Arikan[1], Deniz Gokalp[1], Mithat Bahceci[1] & Naime Canoruc[2]
[1]Dicle University School of Medicine Department of Endocrinology, Diyarbakir, Turkey; [2]Dicle University School of Medicine Department of Biochemistry, Diyarbakir, Turkey.

Objective
In this study we aim to investigate the association of osteoporosis and type 1 diabetes in 43 type 1 diabetic subjects and 41 control subjects.
Subjects and methods
Bone mineral density of both groups was measured by DEXA. Age, BMI, waist/hip ratio, daily calcium consumption of both groups were determined. Twenty-four hours urinary calcium, phosphorus, deoxipyridinoline and pyridinoline were measured. Osteocalcin ALP, IGF-1, IBF-BP3. HbA1c, cortisol, albumin, LDL and triglyceride were measured in both groups. Independent t-test and chi- square test were used to the groups.
Results
Age, body weight, BMI, waist/hip ratio, daily calcium consumption of diabetics were not different from the control group ($P>0.05$). Total lumbal BMD (0.88 ± 0.1; 0.93 ± 0.1 g/cm^2 respectively; $P<0.05$) total femur BMD (0.93 ± 0.14, 0.99 ± 0.1 g/cm^2 respectively; $P<0.05$) and total femur Z- score (-0.16 ± 1, 0.53 ± 0.7 respectively; $P<0.005$) of the diabetic group were statically lower than control group. Urine DPD/ creatinine level (7.6 ± 6.1, 4.9 ± 3.8 pmol/μmol respectively; $P<0.05$), serum ALP level (113 ± 62, 74 ± 18 U/L respectively; $P<0.001$), IGF-BP3 level (5.4 ± 0.9, 4.7 ± 1 μg/ml respectively; $P<0.001$) of diabetic groups were statically higher than control group. Serum cortisol levels in diabetic group were statically higher than control group (14.7 ± 3, 12.8 ± 2.7 μgr/dl respectively; $P<0,005$).
Conclusions
Bone mineral density of type 1 diabetic patient were decreased due to increased bone turnover.

P395

Body fat concentration is a poor predictor of bone mineral content in hyperthyroid women

Carmen Vulpoi, Dumitru Branisteanu & Eusebie Zbranca
University of Medicine and Pharmacy 'Gr.T.Popa', Iasi, Romania.

Hyperthyroidism has significant impact upon both bone turnover and body composition. The present study was designed to investigate whether there is a connection between changes in body composition and bone mineral content at female patients with perturbed thyroid function. Sixty-seven long standing (over 6 months) overt hyperthyroid women had significantly lower bone mineral content as expressed by the Z score measured by quantitative ultrasonography ($-0.86+/-0.69$ compared to $-0.08+/-0.37$ in the age- and BMI- matched euthyroid control group of 82 women, $P=0.01$) and a modified body composition (evaluated by the bioelectrical impedance technique), with lower body fat percentage ($39+/-2\%$ compared to $44+/-1.9\%$ in controls, $P=0.01$). Bone mineral content of hyperthyroid women was significantly correlated to serum alkaline phosphatase ($R^2=0.545$, $P<0.001$), but not to the percentage of body fat ($R^2=0.0069$, NS). Body fat percentage was however a good predictor for the bone mineral content of control euthyroid women ($R^2=0.176$, $P=0.027$). We conclude that loss of bone mass in hyperthyroid women is caused rather by an increase in bone turnover, under the direct action of thyroid hormones, than by a thyroid hormone-induced decrease of body fat mass.

P396

Vitamin D receptor gene polymorphisms: influence on bone metabolism in type 1 diabetic patients

Dilek Gogas Yavuz[1], Dilek Yazici[1], Meral Yüksel[2], Sinem Kiyici[3], Murat Sert[4], Ibrahim Sahin[5], Lerzan Keskin[5], Oguzhan Deyneli[1], Hasan Aydin[1], Ercan Tuncel[4] & Sema Akalin[1]
[1]Marmara University Medical School, Istanbul, Turkey; [2]Marmara University School of vocational, Istanbul, Turkey; [3]Uludag University Medical School, Bursa, Turkey; [4]Cukurova University Medical School, Adana, Turkey; [5]Inonu University Medical School, Malatya, Turkey.

Patients with type 1diabetes mellitus are at higher risk of developing osteoporosis. Among the genetic factors related to the development of osteoporosis, a possible association between vitamin D receptor (VDR) gene polymorphism and bone mineral density (BMD) has been described in some populations.

The aim of this study is to investigate the distribution of vitamin D receptor (VDR) polymorphisms and relation to bone turnover parameters and bone densitometry in Turkish type 1 diabetic patients/

One hundred nine type 1 diabetic patients (M/F 59/50, 30±7 yrs) and 109 healthy controls (M/F 62/47, 29±8 yrs) were included in the study. Duration of diabetes was 8.1±6.3 yrs in patients. Bone mineral density (BMD) of the lumbar spine (L2–L4) and femoral neck were evaluated by DEXA scans. VDR genotype was assessed by polymerase chain reaction amplification followed by BsmI, Apa, Taq and Fok digestion on DNA isolated from peripheral blood leukocytes. Serum levels of calcium, osteocalcin, parathyroid hormone, ctx, 25-OH-vitamin D levels, and A1c, urinary deoxypyridinoline levels were measured Data were analyzed using the chi.2-test and students *T* test where appropriate.

Genotypes FF, Ff and ff were 55.9%, 36.6%,7.3% vs 37.6%, 32.6%, 8.4%; BB Bb and bb were 20.1%, 39.4%, 40.3% vs 15.5%, 53%, 31.5%; TT, Tt, tt were 33.9%, 58.6%,18.4% vs 28.4%, 55.9%, 15.5% for diabetic and control groups respectively. And distributions did not differ between the groups. Genotype AA, Aa, aa were 32.1%, 47.7%, 20.1% for diabetics and 24.7%, 62.5%,12.8% for controls and significantly different ($P=0.04$). Type 1 diabetic group had a lower BMD at femoral and lomber areas compared with the control group. BMD at the head of femur and serum osteocalcin levels tend to be lower at ff genotype in diabetic patients compared to controls.

These findings suggest a small influence of VDR gene polymorphism on BMD in our group of type 1 diabetic patients. Fok1 polimorfisms may have interaction on bone metabolism and requires further studies of larger cohorts.

P397

Change in bone mass due to hyperthyroidism

Florian Toti[1], Dorina Ruci[2], Vilson Ruci[2], Artur Kollcaku[2], Argjend Tafaj[2] & Ergita Nelaj[1]
[1]UHC'Mother Theresa' Service of Endocrinology & Metabolic Diseases, Tirana, Albania, [2]UHC'Mother Theresa' Service of Reumatolgy, Tirana, Albania.

Objectives
To evaluate the presence and the degree of osteoporosis and osteopenia in a group of patients with established hyperthyroidism from at least six months.
Material and methods
This study was based on a quantitative measurement of bone mineral density with and heel ultrasound densitometer (Type Pegassus). Each selected patient was recorded for the weight, height, BMI, age, gender. No patient was previously treated for osteoporosis or osteopenia. A control group with similar data was selected form the general population, with no personal or familiar history of hyperthyroidism. None of them had a known history for osteoporosis or had received any medication for this condition. The criteria for osteoporosis were those recommended from the WHO. Osteoporosis =T-score <-2.5.
Results
We studied 64 patients with confirmed hyperthyroidism from at least six months. There were 22 males and 42 females (33/67%). Mean age 59.9±12.1 years, weight 74±2 kg, BMI 27.8 kg/m^2. For the control group: 38 patients (18/20 M/F), age 60.5±11.1 years, weight 69.9±12.3 kg, BMI 26.2 kg/m^2. The mean values of T-score for the hyperthyroid patients were -3.7 ± 1.4 and -2.0 for the control group. 55% of patients with hyperthyroidism had severe osteoporosis, compared with only 9.5% of control group ($P<0.001$). The gender itself was no significant.
Conclusions
The silent osteoporosis and osteopenia is relatively frequent in hyperthyroidism, significantly more than in normal population. The stimulation of osteoclasts more than osteoblasts and alteration of remodeling cycling from thyroid hormones is believed to be the causative factor.

P398

Correlation between bone minerale density and bone turnover in delayed puberty

Iulia Bistriceanu, Marian Bistriceanu, Elena Neacsu, Magda Preda & Alida Pruna
Craiova University of Medicine and Pharmacy, CRAIOVA / DOLJ, Romania.

It is established that the delayed puberty is the lack of development of sexual maturation in boys and girls at a chronological age that is 2.5 standard deviation above the mean age. Some possible causes of delayed puberty are: hypothalamic defects, pituitary defects or the gonads.
Objectives
Early diagnosis of the gonadal insufficiency; identification of the bone mass and the bone turnover at the pacients with delayed puberty; prophylaxis measures of the bone modification still in pre, puberal and postpuberal stage which lead to a maximal bone mass in correlation between sex and age.
Materials and methods
The study group includes 23 patients with age under 17–22 years with next forms of delayed puberty: Turner syndrome (8), gonadotropin deficiency (8), growth hormon deficiency with gonadal defects (5), nonsecreting pituitary tumors - the chromophobe adenoma (2). The diagnosis of osteoporosis was besed on BMD measurement using dual energy X-ray absorptiometry (DEXA). The cases were evaluated and diagnosed using the determination of levels seric of bone resorption represented by C-terminal telopeptide of tip I procollagen (CrossLaps) and as marker of bone formation represented by osteocalcine.
Results
Osteoporosis was found in 9 (T-score between -2.73 and -3.50), 7 presents osteopenia (T-score between -1.70 and -2.30) and 7 have normal BMD. The Crosslaps (1.054–2.1 ng/ml) and the calcitonina (47–149 ng/ml) were increased in osteoporosis and the results are comparative with postmenopausal women value, the pacients with osteopenia had identical results with premenopausal women value (osteocalcine 22.91–24.94 ng/ml, Crosslaps 0.179–0.250 ng/ml).
Conclusion
Early diagnosis of gonadic failure in order to stabilize/increase the bone mass and to reduce the fractures' incidents, osteoporosis/osteopenia therapy associates estroprogestative/androgenic substitution with specific means of the bone remineralization (biphosphonates, calcium formulas and vitamin D derivates)
Keywords: delayed puberty, BMD, osteocalcine, Crosslaps.

P399

Prevalence of primary hyperparatiroidism in treated and untreated breast cancer

Claudio Giani, Emilio Fiore, Elisa Giustarini, Ilaria Muller, Edda Vignali, Paola Fierabracci & Aldo Pinchera
Department of Endocrinology and Metabolism, University of Pisa, Pisa, Italy.

Hypercalcemia is a frequent metabolic disorder in metastatic breast cancer (BC). Aim of this study was to evaluate the incidence of hypercalcemia due to PHPT in BC patients. The study group included 271 consecutive BC, mean age±S.D. 57.7± 11.96 yrs. 100/271(36.9%) evaluated at different times after mastectomy (A) and 171(63.1%) before surgery (B), with no distant metastases. Age matched control group included 108 healthy women (Co) and 70 women with thyroid cancer(TC) before thyroidectomy. PTH and total serum calcium were measured in BC, Co and TC. The increment of serum calcium and PTH at the initial observation, indicated PHPT. Subjects with PHPT were selected for parathyroid surgery according to NIH consensus conference. PHPT was diagnosed in 12/271 BC(4.42%) and in none Co or TC. PHPT incidence in A was 7/100(7%). 2/7(28.6%) were submitted to adjuvant radiotherapy, 2/7(28.6%) to adjuvant chemotherapy two years before, and 4/7(57.1%) were on Tamoxifene therapy. A parathyroid adenoma was histologically confirmed in all 7 BC at surgery. The prevalence of PHPT in BC was significantly higher than in Co and TC ($P=0.005$, $P=0.004$ respectively). In the remaining 93 patients with no evidence of PHPT mean values of serum calcium (9.6±0.5 mg/dl) and PTH (38±16.4 pg/ml) were significantly greater than in both Co (PTH 27.9± 10.6 pg/ml, $P=0.0001$; calcium 9.3±0.5 mg/dl, $P=0.001$) and TC (PTH 26.2± 11.0 pg/ml, $P=0.003$; calcium 9.2±0.6 mg/dl, $P=0.001$). PHPT incidence in B was 5/171(2.92%), and in 2/5(40%) a parathyroid adenoma was histologically confirmed. In B mean serum PTH and calcium were similar to Co and TC. This study indicates an increased prevalence of PHPT in BC. The highest frequency of PHPT in A may be explained by the interferences of Tamoxifene or previous X-Ray adjuvant treatment on parathyroid cells activity. The significant increase of mean serum PTH and calcium levels in treated BC patients with no evidence of PHPT seems to confirm this hypothesis.

P400

Relationship between antropometric and metabolic parameters and parathyroid hormone levels in women

Ayse Sertkaya Cikim, Taner Bayraktaroglu, Adil Dogan Azezli & Yusuf Orhan
Istanbul University, Istanbul Faculty of Medicine, Endocrinology and Metabolism, Istanbul, Turkey.

Aim
Increased levels of fasting parathyroid hormone(PTH) have been hypothesized to influence increased levels of body fat mass. Preliminary studies show that serum PTH levels are higher in obese than in non-obese young adults and decline with weight loss. In the present study, it was aimed to evaluate relationship between antropometric and metabolic risk parameters and PTH levels in Turkish women.
Materials and methods
Analyses were performed on 710 Turkish women without hyperparathyroidism. They were enrolled to tertils of PTH levels (group I, <42 pg/ml; group II, 42–62 pg/ml; group III, >63 pg/ml and above) to the study. Body compositions, plasma lipids and lipoprotein levels, glucose homeostasis were determined and compared between groups.
Results
There were 227 patients in group I, 246 in group II and 237 in group III. Mean body mass index (BMI), body fat mass, waist circumferences were highest in group III, and increased with PTH. Mean PTH levels were significantly highest in patients having high BMI (48.6 ± 22.1 pg/ml in patients with <25 kg/m^2, 56.3 ± 35.1 pg/ml within 25–30 kg/m^2, 61.8 ± 30.3 pg/ml within 30–35 kg/m^2, 63.8 ± 29.9 pg/ml within 35–40 kg/m^2)($P < 0.05$). Mean values of total cholesterol, triglycerides, fasting glucose, insulin, HDL-cholesterol, LDL-cholesterol and HOMA were not different between groups ($P > 0.05$). Mean systolic and diastolic blood pressure in group II and III were significantly higher than group I ($P < 0.05$).
Conclusion
Preliminary studies suggest that PTH excess may promote weight gain by impeding catecholamine-induced lipolysis. Our data support a relationship between fasting serum PTH and fat mass in women. Fasting PTH is associated with increased fat mass and BMI.

P401

Serum calcium levels and metabolic disturbances in obese women

Taner Bayraktarogl, Ayse Sertkaya Cikim, Adil Dogan Azezli & Yusuf Orhan
Istanbul University, Istanbul Faculty of Medicine, Endocrinology and Metabolism, Istanbul, Turkey.

Aim
Serum calcium levels have a relation glucose homeostasis and weight management, and controversies in findings. This study carried out relationship between serum calcium levels and various metabolic parameters in obese Turkish Women.
Materials and methods
Subjects for this study were 3544 overweight or obese Turkish women with mean level of serum calcium 9.4 md/dl. According to mean calcium level, they divided group I having >9.4 mg/dl and group II having ≤ 9.4 mg/dl. Thereafter, we determined and compared body compositions (body mass index, abdominal fat mass), resting blood pressures, plasma lipids and lipoprotein levels, glucose homeostasis.
Results
A total of 715 (20.2%) patients were identified as overweight (BMI 25–30 kg/m^2) and 2831 (79.8%) were identified obese (BMI ≥ 30 kg/m^2). Mean fasting glucose, total cholesterol, triglycerides levels, systolic and diastolic blood pressures were significantly different in high calcium group ($n = 1710$, 48.2%) than low calcium group ($n = 1834$, 51.8%). Fasting glucose, total cholesterol,, HDL-cholesterol, triglycerides, insulin levels, HOMA values, systolic and diastolic blood pressures were positively correlated with calcium levels, not correlated with age, body mass index, waist and hip circumferences.
Conclusion
Our data showed that there was no relation between serum calcium levels and body fat distribution. Altough there was no effect on obesity, different metabolic parameters such as fasting glucose, total cholesterol, triglycerides levels and blood pressures were affected and correlated with serum calcium levels. It should be careful during a slimming program with included high calcium diet in obese or overweight women.

P402

Marginal periodontal pathology in patients with pituitary disorders

Ileana Duncea[1], Carmen Georgescu[1] & Ioana Duncea[2]
[1]Clinic of Endocrinology, University of Medicine and Pharmacy Cluj-Napoca, Cluj-Napoca, Romania; [2]Faculty of Dental Medicine, University of Medicine and Pharmacy Cluj-Napoca, Cluj-Napoca, Romania.

Periodontal changes are induced by hormonal alterations through soft tissue oedema, inflammation, demineralization or abnormal periosteal development of the alveolar bones.
Objectives
The purpose of the present work was to evaluate the frequency, severity and type of periodontal disease in subjects with previously diagnosed and non-treated pituitary disorders.
Patients and methods
Twenty-five subjects (21 women and 4 men) aged 44.7 years old were enrolled in the present study. Of the 25 patients, 10 suffered from clinical non-functional pituitary adenoma, 9 were diagnosed with acromegaly, 3 had a prolactinoma and in 3 a severe pituitary insufficiency was diagnosed. The endocrine disorder was diagnosed based on basal and dynamic hormonal tests, nuclear magnetic resonance image and visual champ evaluation. In addition, all patients were subjected to a thorough dental examination completed by orto-pan-tomography imaging. Following oral hygiene indices were calculated: the Green Vermillon index (OHIS), the Russel periodontal index (PI) and the OMS index of periodontal therapy request (CPITN).
Results
All subjects presented different forms of chronic periodontal pathology. Dystrophic periodontal disease was the most prevalent form, followed by superficial chronic periodontal disease. Severe periodontal disease including marked gingival retraction and periodontal pockets with purulent secretion was diagnosed in three patients. Patients had a mean OHIS of 3.54 suggesting an unsatisfactory oral hygiene; correspondingly, 12% of patients were advised to improve the oral hygiene, 72% needed professional dental care and antibacterial therapy. In 16%, surgical periodontal therapy was advised.
Conclusions
All 25 patients with pituitary diseases had periodontal pathology suggesting that this endocrine pathology may represent a risk factor for periodontal disease. Prevention and therapy of periodontal changes in these patients need careful oral personal hygiene and regular professional dental care.

P403

Tumor induced osteomalacia caused by haemangioma of the acetabular surface

Habib Bilen[1], Gungor Akcay[1], Pinar Polat[2], Mufide Akcay[3] & Omer Yildirim[4]
[1]Ataturk University, School of Medicine, Department of Internal Medicine, Division of Endocrinology, Erzurum, Turkey; [2]Ataturk University, School of Medicine, Department of Radiology, Erzurum, Turkey; [3]Ataturk University, School of Medicine, Department of General Surgery, Erzurum, Turkey; [4]Ataturk University, School of Medicine, Department of Orthopaedic Surgery, Erzurum, Turkey.

Objective
Tumor induced osteomalacia (TIO) belongs to the group of hypophosphatemic osteomalacias and is induced by a tumor. It is not common disease.
Case report
We describe a 34-year-old caucasian man who presented with a 5 year history of diffuse bone pain and muscle weakness. Laboratory investigations showed normal calcium level, low phosphate level between 1.01 mg/dl, and 1.6 mg/dl (reference range: 2.6–5.5 mg/dl), raised alkaline phosphatase of 238 and 885 IU/l (reference range: 30–120 IU/l) and high urinary phosphate level. Intact parathyroid hormone was within normal range (50.61 pg/ml; normal reference range 15–65 pg/ml). The plasma concentration of 1–25(OH) 2D was at the lower limit of the normal range (20 pg/ml; reference range 20–30 pg/ml). The tubular reabsorbtion of phosphate (TRP) was 65% (normal range 85–95%). Chest radiograph showed decreased bone mineralization and multiple fractured ribs. Conventional radiographs also showed fractures at the femoral necks bilaterally. There was no etiology of hypophosphatemia. The clinical, biological and radiological findings were compatible with osteomalacia, possibly related to the tumor. The patient was then further evaluated by magnetic resonance imaging (MRI), which showed marked intensity changes at the vertebrae corpuses due to osteoporosis, decreased signal intensities at the femoral necks due to fractures bilaterally, multiple transverse fractures at the proximal and distal metaphysis of tibias bilaterally. However this MRI images didn't show possible finding which associated tumor.

But on pelvic MRI, we detected a hypointense lesion at the superior and posterior surface of the acetabulum measuring 13 mm in diameter. An excision of the mass was performed and histological diagnosis of hemangioma was established. Upon removal of the tumor, laboratory data returned to a normal range within one month.

P404

Fracture risk in diabetic patients

Anna Gusova[1], Maria Pavlova[1], Aleksey Alekseenko[2], Galina Melnitchenko[1], Leonid Silin[1], Natalia Zaytseva[1] & Vyacheslav Pronin[1]
[1]Sechenov Moscow Medical Academy, Moscow, Russia; [2]Local trauma clinic N56, Moscow, Russia.

Diabetes mellitus (DM) is accompanied with a variety of metabolic changes in different systems including bone. In several previous studies it was shown that DM type 1 is associated with a decreased mineral density, whereas the data regarding DM type 2 are still controversial.

In the present study we examined the risk of different bone fractures in diabetic patients visiting local trauma clinic during one year (total area population 50.500). The incidence of fractures in general population was 1.9%, whereas in diabetic population it was twice higher (4.4%; $\chi^2=27.4$; $P<0.001$). Fracture of distal forearm was the most prevalent type of fractures in diabetic patients (32.5%), followed by fractures of the phalanges (27%), proximal humerus (15%) and tibial bone (12.5%). Fractures of distal forearm and humeral fractures were less prevalent in a general population (20.2% and 12.8%, correspondingly), compared to diabetic group, although the any statistical significant difference was found only for fracture of distal forearm ($\chi^2=2.8$; $0.05<P<0.10$). The incidence of fractures in other locations did not differ between two groups.

In conclusion, our data indicate that patients with diabetes have an increased total fracture risk, mainly due to higher incidence of the fractures of distal forearm. There is no difference in risk of fractures of other locations in diabetic patients compared to general population.

P405

Predictors of bone mineral density in women with primary hyperparathyroidism

Laura Gianotti, Francesco Tassone, Anna Pia, Paola Razzore, Flora Cesario, Giampaolo Magro & Giorgio Borretta
S. Croce & Carle Hospital, Division of Endocrinology & Metabolism, Cuneo, Italy.

Aim
Osteoporosis is common in postmenopausal women and in primary hyperparathyroidism (PHPT). PHPT is more prevalent in postmenopausal women. Aim of the present study was to investigate possible predictors of bone mineral density (BMD) in women with PHPT.
Methods
166 consecutive women with PHPT [age: 59.5±13.5 years; Asymptomatic/-Symptomatic: 84/82; premenopausal/postmenopausal: 31/135; BMI: 25.6±4.8 kg/m^2; PTH: 234.2±287.3 pg/ml; Calcium: 11.2±1.2 mg/dl] were studied. Serum levels of calcium, phosphate, intact parathyroid hormone (PTH), 25 hydroxy-vitamin D (25OHD3), creatinine and creatinine clearance (Ccr) were analyzed and bone densitometry was performed at lumbar spine, hip and forearm.
Results
In univariate analysis, age and menopausal status were negatively related with BMD and T-score at any site. BMI was positively associated with BMD and T-score at femur. PTH levels were negatively associated with T-score and BMD at forearm and lumbar spine, whereas ionized calcium at all the three sites. 25OHD3 was positively associated with BMD at lumbar spine and forearm. Ccr was positively associated with BMD and T-score at all the three sites. In multivariate regression analysis, menopausal status resulted an independent predictor of T-score at any site (forearm: ß=−0.31, $P<0.00001$; femur: ß=−0.21, $P<0.006$; lumbar: ß=−0.17, $P=0.025$), while PTH was an independent predictor of T-score at forearm (ß=−0.33, $P=0.010$) and lumbar spine (ß=−0.30, $P=0.037$). Ionized calcium also independently associated with forearm T-score (ß=−0.23, $P=0.0025$) while Ccr with T-score at forearm (ß=0.15, $P=0.035$, respectively) and femur (ß=0.24, $P=0.0016$).
Conclusions
In women with PHPT, menopausal status represents one of the most important predictors of bone mass. However, other factors related to the disease such as PTH, calcium levels or renal function, can each other influence independently bone mass, mainly at cortical level.

P406

Carotid intima media thickness and bone turnover in type 2 diabetic patients

Seda Sancak[1], Dilek Yavuz[1], Ahmet Toprak[2], Oguzhan Deyneli[1], Ozlem Tarcin[1], Dilek Yazici[1], Hasan Aydin[1] & Sema Akalin[1]
[1]Marmara University Medical School Section of Endocrinology and Metabolism, Istanbul, Turkey; [2]Marmara University Medical School Depatment of Internal Medicine, Istanbul, Turkey.

Recent studies indicate that atherosclerotic process is associated with bone metabolism. The aim of this study was to evaluate carotid intima media thickness (CIMT) and its association with bone mineral density and bone turnover markers as well as inflammation markers in type 2 diabetic male patients.
Material-method
184 type 2 diabetic males (56±8 y) and 85 non-diabetic control subjects (52±7 y) were recruited. Bone mineral density was measured by dual X-ray absorptiometry at lumbar spine and proximal femoral areas. Carotid intima media thickness was evaluated by Doppler ultrasound. Serum osteocalcin, CTX, intact parathyroid hormone (iPTH), hsCRP and HbA1c were measured.
Results are shown in table 1

	Diabetic subjects	Control subjects	*P*
Serum calcium (mg/dl)	9.91±0.4	9.8±05	0.03
Serum phosphorus (mg/dl)	3.59±0.57	3.39±0.52	0.02
Serum PTH (pg/mL)	49.7±24	53±22	Ns
Osteocalcin (ng/ml)	6.8±3.4	9.5±4	0.0001
CTX (ng/ml)	0.27±0.16	0.38±0.1	0.004
hsCRP (mg/L)	3.66±4	2.11±1	0.01
HbA1c (%)	7.02±1.7	5.1±0.32	0.0001
Fasting blood glucose (mg/dl)	151±65	92.62±7.9	0.0001
Density of Proximal Femur (gr/cm^2)	0.91±0.1	0.93±0.1	0.4
CIMT (mm)	0.70±0.16	0.61±0.23	0.008

There was a negative correlation between bone mineral content of femur neck and CIMT in diabetic patients ($r=-0$, 22 $P=0.008$).
Conclusion
Atherosclerosis and bone mineral density (BMD) may be related through similar or common pathophysiological mechanisms in type 2 diabetics. Low-grade inflammation may be one of the pathologic mechanisms that depressed bone turnover in diabetic patients.

P407

Primary hyperparathyroidism is associated with an increased risk of vertebral fracture assessed by morphometric x-ray absorptiometry

Giuseppe Viccica[1], Edda Vignali[1], Daniele Diacinti[2], Romano Del Fiacco[2], Tamara Giacomelli[1], Luisella Cianferotti[1], Filomena Cetani[1], Antonietta Picone[1], Chiara Banti[1], Aldo Pinchera[1] & Claudio Marcocci[1]
[1]University of Pisa, Pisa, Italy; [2]University La Sapienza, Roma, Italy.

Primary hyperparathyroidism (PHPT) is a frequent cause of secondary osteoporosis, but its role about the fracture is still controversial. We evaluated 157 consecutive postmenopausal patients with PHPT compared with two control subjects (C), each one matched for age and month-since-menopause (MSM). We measured ionized calcium (Ca^{2+}), parathyroid hormone (PTH), 25-OH-vitamin D (25-OH vit D), osteocalcin (OC), bone alkaline phosphatase (B-ALP) and serum and urinary cross-laps (S-CTX and U-CTX, respectively). Bone mineral density (BMD) were measured at spine (anterior-posterior, L1-L4) (BMD-V), femur (neck and total) (BMD-N and BMD-T, respectively) and radius (1/3 distal)

(BMD-R) by dual energy X-ray absorptiometry (DXA) technique using a QDR-4500 (Hologic, Inc., Bedford, MA, USA). We also acquired lateral scan of the spine from T_4 to L_4 using the DXA machine. Morphometric X-ray absorptiometry (MXA) was performed by a trained operator on the lateral DXA images, using the software supplied by the manufacturer. Reduction of anterior, middle or posterior vertebral height were classified as mild (20–25%), moderate (25–40%) or severe (>40%) vertebral fracture, according to visual semiquantitative Genant's method. No difference was found between PHPT and C groups in age, weight, height, body mass index (BMI) and MSM. The prevalence of vertebral fracture was higher in PHPT (26.7%, $n=42$) than C (5.4%; $n=17$) ($P<0.0001$) [odds ratio (OD) between PHPT and C was 6.38 (CI = 1.66–14.31)]. BMD-N and BMD-R of PHPT fractured patients were significantly lower than unfractured ones ($P<0.002$ and $P<0.0005$, respectively). In the PHPT group, no difference was found in Ca^{2+}, PTH, 25-OH vit D, OC, B-ALP, S-CTX and U-CTX between fractued and unfractured patients. In conclusion, BMD reduction observed in PHPT patients might account for the increased prevalence of vertebral fracture, but other factors may be involved in bone quality and fracture risk.

P408

Abstract unavailable

P409

Bone mineral density and bone markers in patients on long-term suppressive levothyroxine therapy for differentiated thyroid carcinoma

F. Mouton[1], M. D'Herbomez[2], C. Do Cao[1], F. Tison-Muchery[2], X. Marchandise[2] & J.-L. Wemeau[1]
[1]Clinique d'Enocrinologie Marc Linquette, CHRU Lille, Lille, France; [2]Service de Médecine Nuclaire, Hopital Roger Salengro, CHRU Lille, Lille, France.

Treatment with high doses of thyroid hormone was associated with higher risk of osteoporosis.

Aim of the study

To evaluate the predictive value of serum bone remodelling markers, and osteodensity in patients treated for differentiated thyroid carcinoma (DTC).

Methods

In a prospective longitudinal study, 156 determinations of osteocalcine (OC) as a marker of bone formation, and of C-telopeptide of type-1 collagene (CTX and ICTP) as markers of bone resorption were performed in 103 patients {20 men (median age 50 years), 83 females (median age 56 years – 58% with age >50 years)} treated with suppressive levothyroxine therapy for DTC. Bone mineral density (BMD) of the hip was measured by dual X-ray absorptiometry (DXA) and lateral DXA pictures of the lumbar and thoracic vertebrae were performed ($n=16$ for 13 patients). The mean of follow-up was 9 years (range 0.5–36 years).

Results

All OC results, except three, were in the normal range. Thirsty one ICTP and 36 CTX levels were increased (together 13% of the evaluations). A positive significant correlation was found between the ICTP concentrations and the duration of the follow up ($n=146$, $r=0.15$, $0.02<P<0.05$). BMD and resorption markers were concordant for 69% of the evaluations (7 with both normal, 4 with increased resorption markers and decreased BMD). For the discordant results, BMD were low in ostepenia for 4 patients with resorption markers in normal range, one isolated high ICTP concentration has been found.

Conclusion

1) Only the resorption markers are increased in patients on long term LT4 therapy for DTC 2) prevalence of high CTX and ITP is the same for men and females > 50 years (26%), lower (18%) for women <50 years 3) bone resorption markers could be used for screening patients at risk of osteopenia, when treated with suppressive levothyroxine therapy for DTC.

P410

Bone mineral density in end-stage chronic kidney disease patients

Natalia Karlovich[1], Tatiana Mokhort[1], Natalia Vasiljeva[2], Kirill Komissarov[3], Victor Gromyko[3] & Valery Pilotovich[3]
[1]Belarussian State Medical University, Minsk, Belarus; [2]State Rehabilitation Center, Minsk, Belarus; [3]State Nephrology Center, Minsk, Belarus.

The usefulness of bone mineral density (BMD) and bone turnover markers measurements to assess the renal osteodystrophy in patients with chronic kidney disease, stage 5 (CKD5) are not well determined.

The aim was to analyze BMD, serum levels of parathyroid hormone (PTH) and bone turnover markers in dialysis patients. We examined 45 patients (20 f,25 m; age 45.1 ± 10.8 yrs; age at dialysis onset 40.3 ± 12.3 yrs; dialysis duration 5.0 ± 4.0 yrs). BMD of the lumbar spine (LS), femoral neck (FN) were estimated by DEXA (Lunar). Serum PTH, osteocalcin (OC), C-terminal telopeptide of type I collagen (beta-CTx), alkaline phosphotase (ALP), calcium and phosphates were measured.

Median levels of PTH, OC, beta-CTx were significantly higher, than normal values (688.2 pg/ml; 321.7 pg/ml; 1.66 pg/ml, respectively). We found significant correlation of PTH level and age ($r=-0.51$), age at dialysis onset ($r=-0.57$), serum OC ($r=0.54$), beta-CTx ($r=0.72$) and ALP ($r=0.65$). Median BMD, T- and Z-scores in LS (1.15 g/sm^2; −0.40;0.07) and FN (0.94 g/sm^2; −0.62; −0.27) were normal. Osteopenia and osteoporosis were diagnosed in 20(44.4%) and 5 pts (11.1%), respectively. Comparison of subgroups with low and normal BMD didn't revealed significant differences in age, age at dialysis onset, dialysis duration, BMI, levels of PTH and bone turnover markers. CaxPO4-product was higher in patients with normal BMD 7.24 ± 1.98 vs 5.32 ± 1.73 in ones with low BMD ($P=0.025$). In LS Z-score correlated with PTH ($r=-0.48$; $P=0.011$), BMD – with CaxPO4-product ($r=0.51$; $P=0.038$). In FN we found significant correlation of BMD, Z-score and PTH ($r=-0.54$; −0.56); Z-score and age, age at dialysis onset ($r=0.34$;0.31) and serum Ca ($r=0.40$).

We can assume that low BMD is highly prevalent in CKD5 and associated with high PTH, younger age, and younger age at dialysis onset. Serum OC, beta-CTx, ALP positively correlates with PTH, but similar in patients with different BMD. High CaxPO4-product is well known as an important predictor of cardiovascular morbidity and mortality, but seems to preserve bone loss.

P411

Vitamin D receptor gene Bsm1 and Fok1 polymorphisms and indices of bone mass and bone turnover in healty young Turkish men and women

Dilek Gogas Yavuz[1], Meral Yuksel[2], Ozlem Tarcin[1], Dilek Yazici[1], Ahu Telli[3], Seda Sancak[1], Oguzhan Deyneli[1], Hasan Aydin[1], Goncagul Haklar[3] & Sema Akalin[1]
[1]Marmara University Medical School Section of Endocrinology and Metabolism, Istanbul, Turkey; [2]Marmara University Vocasionla school of health, Istanbul, Turkey; [3]Marmara University Medical School, Department of Biochemistry, Istanbul, Turkey.

Aim

Peak bone mass is a major determinant of osteoporosis risk in later life. It is under strong genetic control; In the present study, we investigated the relationship between polymorphisms in the gene encoding the vitamin D receptor (VDR) (FokI and Bsm 1) and bone mineral density (BMD), bone mineral content (BMC), and markers of bone turnover in 106 young Turkish women (19–23 yrs) and 100 man (19–23 yrs). Methods: BMD and BMC were measured by dual-energy X-ray absorptiometry (Lunar). Serum osteocalcin, C-teleopeptide (Ctx) and iPTH, calcium, phosphor and serum 25(OH)D levels were measured. Physical activity, dietary calcium and coffee consumption were assessed by questionnaire. Muscle strength was measured with hand dynamometer. PCR-RFLP methods were used for genotyping.

Results

Calcium, phosphor, PTH and 25(OH)D levels (43 ± 20 ng/ml) were in normal range not different between man and women. BMD (lomber and femur area), muscle strength, calcium intake (680 mg/d vs 571 mg/d in women $P=0.001$), serum osteocalcin and CTx levels were significantly higher in man compared to women.

Frequencies of FF, Ff and ff genotypes were 44.3%, 47.1% and 8.4% in women, and 41.8%, 52.3% and 4.6% in man. Frequencies for BB, Bb were 15%, 52% and 33% in women, and 9.3%, 60.4% and 30.2% in man. Frequency distribution for Bsm and Fok polimorfisms were not different between man and women. Femur BMC was significantly low in "bb" genotype in women ($P=0.01$). Femur and lomber (L1-4) BMC were low in "ff" genotype in women. Serum calcium levels were found to be lower in ff genotype in women. Bone turnover markers were similar among genotypes both man and women.

Conclusion

VDR gene may influence on attainment and maintenance of peak bone mass. "bb" and "ff" genotypes may have a effect on bone metabolism during accumulation of peak bone mass in women.

P412

Parathyroid sonography in patients with normocalcemic primary hyperparathyroidism

Avraham Ishay[1], Leonid Chervinsky[2] & Rafael Luboshitzky[1]
[1]Endocrine Institute, Haemek Medical Center, Afula, Israel; [2]Department of Radiology, Haemek medical Center, Afula, Israel.

Background
Primary hyperparathyroidism (PHPT) is nowadays an asymptomatic disease characterized by mild hypercalcemia and elevated parathormone (PTH) levels. A non-typical form of the disease distinguished by high PTH levels, normal serum calcium concentrations, and no evidence of secondary hyperparathyroidism was recently identified. The data about parathyroid imaging findings in the normocalcemic type of the disease are lacking. Ultrasonography (US) is the most convenient imaging modality for localization of parathyroid adenoma. The purpose of our study was to investigate whether normocalcemic patients harbor abnormal parathyroid glands on high-resolution ultrasonography.
Methods
We studied 14 patients (aged 53.2±10.3 years) with normocalcemic primary hyperparathyroidism. High-resolution ultrasonography was performed to locate parathyroid adenomas. Ten patients with positive sonography underwent a parathyroid ^{99}technetium sestamibi scintigraphy (MIBI).
The following variables were measured: serum total calcium, PTH, creatinine, phosphate, alkaline phosphatase, 25 hydroxyvitamin D and 1.25 dihydroxyvitamin D. A 24-hour urine collection was obtained for assessment of calcium and creatinine excretion rates. Corrected serum calcium level was used as an indirect assessment of ionized calcium.
The local Institutional Review Board approved the study, and all patients gave informed consent.
Results
All patients had high PTH levels (112±33.1 pg/ml), normal corrected serum calcium (9.6±0.3 mg/dl) and 25 hydroxyvitamin D (27.5±5.3 ng/ml) levels and normal creatinine clearance (97±18.6 ml/mn). Ten out of 14 patients (71%) exhibited a total of 12 single or double typical parathyroid adenomas on sonography. Sestamibi imaging correctly localized 8 of them.
Conclusion
The high prevalence of parathyroid adenomas on sonography indicates that normocalcemic primary hyperparathyroidism is characterized by the same morphologic derangement as the hypercalcemic form of the disease. Thus, NPHP is probably an early manifestation of PHPT.

P413

Diagnostic role of GNAS1 mutation screening in patients with pseudohypoparathyroidism

Miriam Ladsous[1], Virginie Vlaeminck-Guillem[1], Anne-Sophie Balavoine[1], Marie-Laure Kottler[2] & Jean-Louis Wémeau[1]
[1]Clinique Linquette-CHRU, Lille, France; [2]CHU Hôpital Clémenceau, Caen, France.

Pseudohypoparathyroidism (PHP) defines a group of disorders characterized by resistance to PTH. They are classified in type Ia, Ib, Ic and type II according to their clinical and biological characteristics. PHP-Ia is caused by heterozygous mutations in the *GNAS1* gene, encoding the alpha subunit of protein Gs. The aim of our study was to describe the diagnostic role of *GNAS1* mutation screening in a large group of patients, and to define the intrafamilial transmission pattern and parental imprinting profile.

Fourteen patients were studied. Eleven patients, from 5 unrelated families, had PHP-Ia, associating Albright's Hereditary Osteodystrophy (AHO), a decreased erythrocyte Gs-alpha protein activity, and other associated hormonal resistances. Two had PHP-Ib, with isolated PTH resistance, normal Gs-alpha activity and absent AHO. One patient had probable PHP-Ic, exhibiting AHO but normal Gs-alpha activity. *GNAS1* mutations were identified in all the patients with PHP-Ia. Six different mutations, not previously described, were observed. In 4 families, mutations were transmitted by mothers. In one family, the mutation was *de novo*. In one family, affected patients had 2 heterozygous *GNAS1* mutations, both located on the maternal allele. The 3 studied transmitting mothers had pseudopseudohypoparathyroidism, a condition associating AHO, decreased Gs-alpha activity but normal hormonal profile. We identified the familial *GNAS1* mutation in an asymptomatic boy whose father had typical PHP-Ia. Finally, isolated subcutaneous calcifications were identified in 2 related subjects who did not have the familial *GNAS1* mutation. We did not identify *GNAS1* mutations in PHP-Ib and PHP-Ic subjects.

In conclusion, our study confirms 1) the usefulness of *GNAS1* mutation screening in ascertaining PHP-Ia diagnostic, 2) the previously described maternal transmission of PHP-Ia, consistent with paternal imprinting of *GNAS1* gene, 3) the need for mutation screening in PHP-Ia related subjects to identify mutation carriers and provide an appropriate genetic counselling.

P414

CYP3A7*1C polymorphism, serum dehydroepiandrosterone sulphate level and bone mineral density in postmenopausal women

Gábor Speer, Krisztián Bácsi, János P. Kósa & Péter Lakatos
Semmelweis University 1st. Department of Medicine, Budapest, Hungary.

Objective
The CYP3A7 enzyme metabolizes some steroid hormones including dehydroepiandrosterone sulphate (DHEAS). Its expression silenced after birth. Previous study has shown that in case of CYP3A7*1C polymorphism, CYP3A7 enzyme activity persisted a higher level, resulting lower levels of DHEAS in men. The age-related decline of serum DHEAS levels is believed to contribute to osteoporosis. We hypothesized that CYP3A7*1C contribute bone loss through decreased level of serum DHEAS in postmenopausal women.
Patients and methods
319 postmenopausal women were admitted to study and divided into two subgroups: 217 women with osteoporosis and 102 aged-matched women, without osteoporosis. The CYP3A7*1C polymorphism was genotyped. Serum DHEAS levels and bone mineral density (BMD) were measured.
Results
Homozygous CYP3A7*1C carriers had significantly lower BMD at lumbar spine than that of wild type (T-score with CYP3A7*1C mutant type: −3.27±1.02, T-score with wild type: −1.35±1.53, $P=0.041$), after a correction of age and DHEAS levels. We did not find significant association between CYP3A7*1C variant and serum DHEAS level in postmenopausal women. Serum DHEAS levels correlated positively with BMD at both lumbar spine ($P<0.005$) and at femoral neck ($P<0.005$) in the whole study population.
Conclusion
We have shown the CYP3A7*1C may be associated with decreased bone mass at the lumbar spine independently of serum DHEAS concentrations. This finding and the lack of association between CYP3A7*1C polymorphism and serum DHEAS level in women support the hypothesis that this genetic variation might lead to reduced bone mass through other CYP3A7 hormonal substrates, than DHEAS.

P415

Evaluation of diastolic function and its relationship with carotis intima media thickness and endothelial function in asymtomatic hyperparathyroid patients

Dilek Yazici[1], Ozlem Tarcin[1], Beste Ozben[2], Ahmet Toprak[2], Oguzhan Deyneli[1], Seda Sancak[1], Dilek Yavuz[1] & Sema Akalin[1]
[1]Marmara University Medical School, Section of Endocrinology and Metabolism, Istanbul, Turkey; [2]Marmara University Medical School, Department of Cardiology, Istanbul, Turkey.

Symptomatic hyperparathyroid patients are under risk of increased cardiovascular mortality, associated with left ventricular hypertrophy, diastolic dysfunction and accelerated atherosclerosis. Data on asymptomatic hyperparathyroid patients are conflicting. This study aimed to determine diastolic dysfunction and its association with carotis intima media thickness (CIMT) and flow-mediated vasodilation (FMD) in a group of asymptomatic hyperparathyroid patients.

Twenty six patients with asymptomatic hyperparathyroidism (HP) (23.4±3.9 y; F/M:17/9) and 25 healthy controls (24.4±3.6 y; K/E:18/7) were recruited. Left ventricular mass index (LVMI), isovolumetric relaxation time (IVRT), early (E) and late (A) atrial peak filling velocity were measured by conventional and Doppler echocardiography. Tissue Doppler imaging, a method with better results in determining diastolic dysfunction, was used to determine mitral annular early (E') and late (A') peak diastolic filling rate. FMD and CIMT were determined by Doppler echocardiography. The study was approved by the local Ethical Committee.

	ASYMTOMATIC HP ($n=26$)	CONTROL ($n=25$)	P
Calcium (mg/dL)	9.72±0.41	9.69±0.76	NS
Phophorus (mg/dL)	3.98±0.66	3.90±0.40	NS
PTH (pg/mL)	91.53±24.50	42.98±10.69	$P<0.0001$
FMD (%)	9.62±3.74	9.52±3.13	NS
CIMT (mm)	0.46±0.05	0.47±0.04	NS

LVED, LVMI, IVRT, E/A, E'/A' and E/E' ratios were comparable between groups. PTH was weakly correlated with CIMT ($r=-0.26$; $P=0.23$), but not with echocardiographic parameters and FMD.

Diastolic dysfunction was not observed in asymptomatic hyperparathyroid patients. It is evident from this preliminary data that cardiac manifestations do not start at this stage of disease, but further studies with larger groups are needed to confirm this finding.

P416

Cinacalcet (Mimpara®, Amgen) is more effective than bisphosphonates at controlling hypercalcemia in patients with parathyroid carcinoma: a case study

Waldemar Misiorowski
Endocrinology Clinic, Medical Centre for Postgraduate Education, Warsaw, Poland.

Parathyroid carcinoma is an uncommon cause of PTH-dependent hypercalcemia. The clinical features of parathyroid carcinoma are due primarily to the effects of excessive secretion of PTH. Thus, signs and symptoms of hypercalcemia often dominate the clinical picture. The therapeutic goal at this point is to control the hypercalcemia. We describe two cases of parathyroid carcinoma, effectively treated with calcimimetic Cinacalcet (Mimpara®), the first of a new class of compounds with activity at the calcium-sensing receptor: 55-years old women with parathyroid carcinoma, and with persistent hypercalcemia after four consecutive surgical attempts with wide excision of the involved area, and 53–yr-old man presented with diffuse lytic changes in the bones and a tumor in mediastinum (eventually diagnosed as parathyroid carcinoma). In both cases severe hypercalcemia (ranged 15–17 mg/dL) and high levels of intact PTH (1176 pg/mL and 546 pg/mL respectively) had been found. Symptomatic treatment: hydratation with iv sodium chloride and iv pamidronate and zolendronante had been installed, however without effects, and eventually cinacalcet, 60–90 mg/day, orally, has been used to treat. After first week of the treatment, in both cases calcium and PTH significantly decreased (to 10.8–11.3 mg/dL and 332–113 pg/mL respectively).Cinacalcet appears to have been more effective at controlling hypercalcemia than bisphosphonates in patients with parathyroid carcinoma.

P417

Is there any relationship between the BsmI polymorphism in the vitamin D receptor gene and the occurrence of glucocorticoid-induced osteoporosis in asthmatic patients with long-term glucocorticoid treatment?

Urszula Tworowska[1], Bozena Bidzinska-Speichert[1], Andrzej Obojski[2], Marek Demissie[1], Ryszard Slezak[3], Agnieszka Lenarcik[1] & Andrzej Milewicz[1]
[1]Departament of Endocrinology and Diabetology, Medical University of Wroclaw, Wroclaw, Poland; [2]Departament of Internal Medicine and Allergology, Medical University of Wroclaw, Wroclaw, Poland; [3]Departament of Genetics, Medical University of Wroclaw, Wroclaw, Poland.

Introduction
Results of many studies indicate that BsmI polymorphism in VDR gene may influence bone tissue metabolism and may be useful in identifying patients at greater risk of glucocorticoid-induced osteoporosis.
Aim of the study
To determine frequency of polymorphic variants of VDR gene (BsmI) and its relationship to phenotypic features characterizing bone status (BMD and metabolic bone turnover).
Material and methods
Following groups were studied: 1. asthmatic patients – no 85; divided into the subgroups: group OS – 38 patients treated with oral steroids: 27 women and 11 men (47.8 ± 10.7 years, 74 ± 13.8 kg), group IS – 34 patients treated with inhaled steroids: 29 women and 5 men (45.4 ± 11.0 years, 73.7 ± 13.9 kg), group NS – 13 patients treated with other drugs than glucocorticoids: 9 women and 4 men (38.8 ± 15.1 years, 66.7 ± 17.9 kg), 2. control group – 31 healthy volunteers, 17 women and 14 men (39.8 ± 9.8 years, 75.1 ± 16.1 kg). Serum levels of PTH, VD3, osteocalcin, ICTP, Ca and phosphates were measured. VDR gene genotypes were determined using PCR-RFLP method. BMD was measured using DXA method.
Results
Genotype bb was found in 34.3%, BB in 8.8%, and bB in 56.9%. Allelic frequency for allele B was 37.2%, and b – 62.8%. There were no significant differences regarding BMD, biochemical and hormonal parameters between any of genotypes.
Conclusions
The data suggest that the VDR genotypes do not seem to be useful for identifying patients at greater risk of glucocorticoid-induced osteoporosis, however it awaits to be confirmed by a population-based study.

P418

The relationship between the increased body mass index and the bone fracture prevalence in postmenopausal pollen allergic women

Viktoria Ferencz[1], Szilvia Meszaros[1], Emoke Csupor[2], Edit Toth[3], Katalin Bors[4], Andras Falus[5] & Csaba Horvath[1]
[1]1st Department of Internal Medicine, Faculty of Medicine, Semmelweis University, Budapest, Hungary; [2]The Health Service, Budavar Local Authorities, Budapest, Hungary; [3]Department of Reumatology, Flor Ferenc Country Hospital, Kerepestarcsa, Hungary; [4]The Health Sevice, Ferencvaros Local Authorities, Budapest, Hungary; [5]Department of Genetics, Cell- and Immunobiology, Faculty of Medicine, Semmelweis University, Budapest, Hungary.

Our aim was to investigate whether pollen-allergy can affect fractures in postmenopausal women. A total of 125 postmenopausal pollen-allergic women (mean age 61.26 yr) were split into four groups: treated neither with H1 histamine receptor (H1R) antagonist nor with inhaled corticosteroid ($n=43$), treated only with H1R antagonist ($n=53$), treated both with H1R antagonist and inhaled corticosteroid ($n=17$), treated only with inhaled corticosteroid ($n=12$) for at least 5 years, seasonally. One hundred non-allergic postmenopausal subjects matched for age, body mass index (BMI) and age at menopause served as controls. Overweight and obesity ($25\ kg/m^2 \leq BMI$) were common among allergic women (76%). Untreated allergic had almost triple the rate of prevalent low-energy fractures (distal forearm, hip and clinical vertebral fractures: 34.9%) compared to non-allergic women (13%, chi^2 $P=0.003$). Bone fracture occurred more often in H1R-only treated patients (30.19%) than in controls (chi^2 $P=0.01$), however, clinical vertebral or hip fractures developed neither in those treated only with H1R antagonist nor in those who received both H1R antagonist and inhaled corticosteroid. Bone fractures were more frequent among patients with inhaled steroid treatment than among patients with a combined treatment of inhaled steroid and antihistamine (50% vs. 29.4%). BMI predicted prevalent fractures at 1.278 (95% CI, 1.047 to 1.559, $P=0.016$) for $1\ kg/m^2$ increase among untreated allergic patients. In conclusion we found a high prevalence of low-energy fractures among pollen-allergic postmenopausal women, which was associated with obesity. It is possible that the H1R antagonists compensate for the negative effect of pollen-allergy and the adverse effect of inhaled corticosteroid treatment on bone fracture risk.

P419

Decreased bone resorption in H1 histamine receptor antagonist treated allergic children

Szilvia Meszaros[1], Viktoria Ferencz[1], Agnes Bojszko[2], Eva Pallinger[3], Andras Falus[3], Eva Hosszu[4] & Csaba Horvath[1]
[1]1st Department of Internal Medicine, Faculty of Medicine, Semmelweis University, Budapest, Hungary; [2]Heim Pal Paediatric Hospital, Budapest, Hungary; [3]Department of Genetics, Cell- and Immunobiology, Faculty of Medicine, Semmelweis University, Budapest, Hungary; [4]2nd Department of Paediatric, Faculty of Medicine, Semmelweis University, Budapest, Hungary.

Histamine receptor antagonists seem to have effect on bone metabolism according to previous studies. We investigated the bone turnover in allergic children who were treated with H1 hisztamin receptor (H1R) antagonists.

The biochemical bone turnover markers [β-CrossLapps (β-CTx), osteocalcin (OCN), β-CTx/OCN ratio], parathyroid hormone (PTH) and the 25-OH-vitamin D_3 were determined in 37 H1R antagonist treated multiplex allergic children and

in 21 age and gender matched healthy children. The intracytoplasmatic histidine decarboxylase (HDC), hisztamin, and surface H1 and H2 receptors expression were assessed by flow cytometry on peripheral leukocytes. The distribution of lymphocyte subpopulation were also determined.

The serum OCN, PTH and 25-OH-vitamin D_3 levels did not differ between the healthy and the allergic groups. However, the β-CTx was lower in the H1R antagonists treated allergic children (1090.82 ± 80.25 pg/ml) in comparison with controls (1456.58 ± 95.81 pg/ml; $P=0.006$). The β-CTx/OCN ratio was found to be lower in the H1R antagonists treated allergic patients than in the controls (9.24 ± 0.608 vs 12.65 ± 0.53; $P=0.001$). β-CTx serum level correlated with OCN in the controls ($r=0.845$, $P<0.001$) and in the H1R antagonist treated allergic, too ($r=0.519$, $P=0.005$). Higher HDC expression and H1 receptor down regulation was found in allergic children. The CD3+/CD16-56+T cells were in higher rate in children of control group.

Decreased bone resorption was found among H1 receptor antagonist treated allergic children, which is indicated by serum markers. Therefore, bone turnover is shifted toward bone formation in the H1R antagonist treated allergic subjects.

P420

Changes of bone metabolism at the onset of puberty

Violetta Csákváry[1], Erzsébet Toldy[2], Tamás Puskás[3], Gábor L. Kovács[4] & György Oroszlán[1]
[1]Markusovszky Teaching Hospital, Children's Department, Szombathely, Hungary; [2]Department of Practical Diagnostics of Institute of Diagnostics and Management, University of Pécs, Szombathely, Hungary; [3]Markusovszky Teaching Hospital, Radiological Department, Szombathely, Hungary; [4]Institute of Laboratory Medicine, University of Pécs, Pécs, Hungary.

Object
Adolescence is the period during which the greatest accrual of bone mineral occurs. During puberty, changes of bone metabolism primarily depend on maturity. Diagnosis and therapy of childhood bone diseases is difficult due to the lack of reference ranges of metabolic bone markers. Our aim was to establish the reference values of bone markers in primary school students (mean age: 13.2 ± 1.2 years; 65 girls, 56 boys).
Methods
The children were divided into two groups: prepubertal (boys:22, girls:38) and pubertal (boys:34, girls:27). This classification was based on the Tanner stage and levels of serum sexual steroids (testosteron, estradiol). Physical activity, dietary habits, calcium intake, consumption of soft drinks and body mass index (BMI) was established. Bone mineral density (BMD), bone mineral content (BMC), vertebral Z-score (DEXA Medical Systems Prodigy), and serum biochemical markers (osteocalcin: OC; beta-crosslaps: βCL; procollagen type I N-terminal propeptid: P1NP) were measured by an electrochemiluminescence immunoassay system (ECLIA, Elecsys 2010, Roche). The data were analysed in terms of sexual maturation by one way ANOVA.
Results
The Tanner stage (3.14 ± 0.78) and BMD (0.99 ± 0.14) values of girls were significantly higher than those in boys (Tanner stage: 2.75 ± 0.61, BMD:0.87 ± 0.12). A significant ($P<0.001$) positive correlation ($r=0.4$–0.5) was observed between the Tanner stage and the parameters of mineral density (BMD, BMC, Z-score). Significantly ($P<0.001$) higher OC (190 ± 66 vs. 139 ± 61 ng/ml) P1NP (838 ± 280 vs 569 ± 360 ng/ml), βCL (2.03 ± 0.65 vs. 1.50 ± 0.60 ng/ml) values were measured in boys than in girls. Boys not consuming soft-drinks regularly exhibited significantly higher ($P<0.05$) prepubertal Z-score values ($+0.28 \pm 0.77$) that regular soft-drink-consumer boys (-0.72 ± 1.02). iPTH levels in soft drink-consuming prepubertal girls (47.7 ± 13.6) were significantly higher ($P<0,01$) than in the non-consuming prepubertal girls (32.8 ± 9.4 ng/ml).
Conclusion
The results call the attention on the significance of appropriate reference ranges. It is advisable that boys and girls are evaluated separately with the sexual maturity taken into consideration. The assessment of dietary habits strongly suggests insufficient spontaneous calcium intake among children.

P421

Hypophosphatemic rickets and mutations in the PHEX gene

Frédérique Faivre-Defrance, Benoit Soudan, Sophie Marcelli-Tourvieille, Jean-Louis Wémeau & Marie-Christine Vantyghem
Lille University Hospital, Lille, France.

Hypophosphatemic rickets is associated with mutations in the PHEX gene leaving phosphaturic peptides such as FGF23 uncleaved, enabling them to exert their phosphaturic potential in the kidney. Other forms are caused by mutations in the proteolytic processing site of FGF23 itself, while in tumour-induced Hp, overproduction of FGF23 causes the processing capacity to be exceeded, resulting in phosphaturic Hp. The aim of this work was to assess blood FGF23 levels in Hp patients and in normophosphatemic controls. Methods: 17 patients suffering from chronic Hp without HPT were compared to an age-matched control group of 18 patients. Blood levels of calcium, phosphate, PTH, 25-OH vitamin D (Nichols Diagnostics) and FGF 23 (ELISA Immunotopics) were determined. Results: FGF23 levels were higher in Hp: 46.3 ± 614.9 vs. controls: 20.3 ± 1.6 pg/ml, $P<0.05$, in regard of phosphate levels of 20.2 ± 0.7 (Hp) vs 34.5 ± 1.2 mg/l (controls). Vitamin D and calcium levels were normal and similar in both groups. PTH levels were higher in Hp: 70.5 ± 15.5 vs. controls: 31.5 ± 2.6 pg/l; $P<0.05$. FGF 23 correlated neither to phosphate nor vitamin D, nor calcium levels in the whole population and in Hp and control groups.
Conclusion
The lack of correlation between FGF23 levels and Hp suggests an heterogeneity of hypophosphatemic patients despite their higher FGF23 levels than controls. New genes regulating FGF 23 such as the recently discovered DMP1 could explain this heterogeneity (Nat Genet 2006 Nov).

P422

Changes of serum bone marker concentrations after effective therapy of patients with Cushing's syndrome

Miklós Tóth[1], Judit Toke[1], Dóra Lippai[1], Márta Sereg[1], Ágnes Szappanos[1], László Füto[2], Ibolya Varga[1], Nikolette Szücs[1], Róbert Kiss[1], Edit Gláz[1] & Károly Rácz[1]
[1]Semmelweis University, 2nd Department of Medicine, Budapest, Hungary; [2]Makhot Ferenc Hospital, Department of Medicine, Eger, Hungary.

Introduction
The most important feature of bone metabolism in patients with Cushing's syndrome is the uncoupling of osteoblast and osteoclast activity resulting in suppressed bone formation.
Objective
The aim of the present study was to investigate the altered bone turnover in patients with various forms of Cushing's syndrome in the active phase of the disease as well as after successful normalization of cortisol overproduction.
Patients and methods
This retrospective study included 63 patients with Cushing's syndrome (38 Cushing's disease, 6 ectopic ACTH syndrome, 19 ACTH-independent adrenal disease).The patients were monitored over a period of up to 48 months after treatment or up to the recurrence of their disease. Patients with known metabolic bone disease, or with medication affecting bone mineral content were excluded. 148 blood samples were evaluated (67 samples from active and 81 samples from inactive phase of Cushing's syndrome).Serum osteocalcin (OC) and type I collagen breakdown products (beta-CrossLaps, β-CL) were measured with standard test kits. SPSS v13.0 software package was used for statistical analysis.
Results
OC concentration which was suppressed in the active phase of the disease (mean, 12.1 ± 8.0 ng/ml) increased to 38.0 ± 26.0 ng/ml within the first month after the effective therapy, reached the maxiumum level after 6 months (52.3 ± 33.6 ng/ml) and became normal after the second year. There were no significant changes in β-CL concentrations. Using ROC analysis, 17.2 ng/ml serum OC concentration was found as the best cutoff value in differentiating between active and inactive phase of bone disease related to Cushing's syndrome. The sensitivity and specificity of OC at this concentration were 87.1% and 82.1%, respectively.
Conclusion
Our results indicate that the suppressed serum OC concentration increases rapidly and elevates above the normal range after treatment of Cushing's syndrome. Markers of bone turnover are normal after the second year of the cure of Cushing's syndrome.

P423

Percutaneous ethanol injection therapy in patients with primary and secondary hyperparathyroidism

Spyros Stratigis, Kostas Stylianou, Evangelia Mamalaki, Eleftheria vardaki, Nikolaos Tzenakis, Eirini Katsipi, Paraskevi Tzoutzouraki & Eugenios Dafnis
University Hospital of Crete, Iraklio, Crete, Greece.

Introduction
Recent years PEIT has been introduced as an alternative to parathyroidectomy. We evaluated the results of PEIT in patients with primary (pHPT) or secondary hyperparathyroidism (sHPT).
Patients and methods
18 patients (6M/12F) with pHPT and 20 patients (7M/13F) with sHPT underwent PEIT between 2001 and 2005 and had a mean follow up of 24.3±9 and 27±10 months respectively. The PTGs were identified and blood supply to the gland was examined by power Doppler ultrasonography pre and post infusion. 95% ethanol was injected at a volume 85% of the PTG volume. pHTP patients underwent a total of 51 ethanol infusions. sHPT patients underwent a total of 76 infusions in 30 adenomas The volume of the PTGs, serum iPTH, calcium, phosphate, albumin and alkaline phosphatase were measured at the beginning and after each infusion. The patients were divided to responders and non responders based on the normalization of iPTH levels at.
Results
In the pHPT group, 11 patients (61.1%) normalized iPTH levels, 5 (27.8%) had a significant (>50%) reduction of iPTH levels and 2 (11.1%) had a modest response (<50% reduction of PTH) and were referred for surgery. In all patients calcium levels decreased significantly (10.96±0.84 mg/dl to 9.81±0.6)($P<0.001$). Phosphorus increased from 2.52±0.38 mg/dl to 2.96±0.5 mg/dl ($P=0.05$).
In the sHPT group PTH decreased significantly (1280.9±447 pg/ml to 770.5±465, $P<0.001$) in all patients; however it was normal in only 3 patients (15%). Phosphorus decreased from 5.57±0.47 mg/dl to 4.93±0.42 mg/dl ($P=0.03$).
Conclusions
PEIT is a safe and easy to perform technique for the treatment of HPT. In patients with pHPT may be a considerable alternative to surgical PTx with a curative rate of 61% in our series. In patients with sHPT appears a significant adjunct to medical therapy since it reduced iPTH levels by 42%.

P424

Juvenile osteoporosis in untreated GH-deficient patient – is treatment with GH replacement indicated? A clinical case report

Catarina Coelho, Catarina Saraiva, Isabel Manita, Andreia Velosa, Dolores Passos, Maria Carlos, Luisa Raimundo & Jorge Portugal
Hospital Garcia De Orta EPE, Almada, Portugal.

Treatment for osteoporosis in children/adolescents is extremely important not only to improve bone quality but also because, if left untreated, could lead to severe and precocious loss of bone mass. Studies in growth hormone (GH) deficient adults, in turn, have shown that treatment with GH produce bone mass gain and improve the occurrence of both bone formation and reabsorption.

The authors present the following case report of a 16 year old Caucasian female with congenital blindness. Suspected of having short stature at the age of 14 she was referred to the endocrinology department for further examination. Eutocic delivery at the gestational age of 39 weaks, A.I 8/9, W=2850 gr, L=47 cm; PC=35 cm. Breast-fead during the first 3 months. Food diversification from the 4th month, without intolerance. Growth retardation detected at the age of 2 (−3 sds) and delayed psycho-motor development. Puberty arousal at 12, with menarche at 14. oligoamenorrhea since then. Physical examination: bilateral blindness, W=25.7 Kg (−3 sds), H=128 cm (−3 sds); BMI- 15.6 Kg/m2. Bone age exam showed closed cartilage. Laboratory findings revealed: IGF1<20 ng/mL (163–972); GH< 0.1 ng/mL, TSH 4.3 mU/L (0.1–4.0); PRL 9.8; urine density - 1014; CRH test - basal/pick - ACTH 16.6/51 pg/mL and cortisol 10.6/22.8 ug/dL; LHRH test – basal/pick – LH 9.8/89 UI/L and FSH 9.2/20.4 UI/L). The MRI showed hypophysis and pituitary stalk hypoplasia with ectopic location of the posterior lobe; along with bone malformation of the cranial – vertebral gynglymus. The osteodensitometry of the lombar spine revealed severe osteoporosis (Z score of −4.3). Etthynylestradiol 15 mcg/ gestodene 60 mcg and alendronate 70 mg/weekly were started. Reevaluation of bone density after one year showed stable density. Although in Portugal GH treatment is not available for use in adults we ask…

Should this patient be further considered for GH treatment?

P425

Biomarkers of hypercoagulability and inflammation in primary hyperparathyroidism

Elena Chertok- Shacham[1], Avraham Ishay[1], Idit Lavi[2], Naomi Lanir[3] & Rafael Luboshitzky[1]
[1]Endocrine Institute, Haemek Medical Center, Afula, Israel; [2]Department of Epidemiology and Community Medicine, Carmel Medical Center, Haifa, Israel; [3]Department of Hematology, Hematology Laboratory, Rambam Medical Center, Haifa, Israel.

Background
The association between primary hyperparathyroidism (PHPT) and cardiovascular disease (CVD) morbidity and mortality is well known in symptomatic PHPT patients. Atherosclerosis is considered nowadays as an inflammatory process. Elevated serum levels of acute phase proteins, C-reactive protein (CRP) and the proinflammatory cytokines tumor necrosis factor alpha (TNF α), Interleukin-6 (IL-6), as well as insulin resistance, indicating chronic subclinical inflammation, have been associated with cardiovascular disease. The aim of this study was to evaluate CVD- related biomarkers of hypercoagulability and inflammation in PHPT patients.
Methods
Thirty-five PHPT patients (aged 57.5±10.8 years) without known CVD were evaluated. Results were compared with those obtained in 25 weight and gender matched controls of similar age. According to disease severity, patients were subdivided into two groups, symptomatic and asymptomatic hyperparathyroidism (SPHP and APHP, respectively).Local Helsinki committee approved the study, and all participants gave their informed written consent. Plasma levels of plasminogen activator inhibitor 1 (PAI-1), fibrinogen, d-dimers, interleukin 6 (IL-6), C-reactive protein (CRP), white blood cells (WBC) were determined in all participants.
Results
PAI-1 was significantly higher in symptomatic PHPT patients (41.4 mg/ml±20) versus APHP and control groups (25.0±12.8 and 32.5 mg/ml±13.0, respectively; $P=0.009$). Levels of fibrinogen, d-dimers, IL-6, CRP and leukocytes were similar in PHPT and controls. Across all subjects PAI-1 was significantly correlated with PTH levels ($r=8.44$; $P=0.005$). After multivariate regression analysis, a significant correlation between Il-6 and PTH was maintained ($r=0.43$, $P=0.008$). No significant correlations were found between PTH or calcium levels and values of fibrinogen, d-dimers, CRP, leukocytes.
Conclusions
Our results suggest that PAI-1 as a marker of hypercoagulability is increased in symptomatic PHPT patients. Elevated plasma levels of PAI-1 in PHPT and the significant correlation with PTH levels, suggest that hypercoagulability mechanisms may be operating in the development of CVD in these patients.

P426

Dehydroepiandrosterone and bone mineral density in elderly women

Lucyna Papierska, Michal Rabijewski, Agnieszka Wiktorowicz-Dudek & Wojciech Zgliczynski
Center For Postgraduate Midical Education, Warsaw, Poland.

Dehydroepiandrosterone (DHEA) and its sulphate (DHEAS) are weak adrenal androgens, which may exert anabolic effect on bone tissue.

We have measured serum DHEAS levels and bone mineral density in lumbar spine and femoral neck in 131 healthy, agile, postmenopausal women aged 59–89. There were no diseases which aggravate bone loss and no hormonal replacement therapy in medical history of participants.

Women were divided into groups:

1 according to DHEAS concentrations:
a. With extremely low (<500 ng/ml) versus
b. moderate-low (>500 ng/ml) serum DHEAS concentrations
2. according to BMD:
a. "Low lumbar spine BMD", with T-score L2/L4 <−2 versus
b. T-score L2/L4 >−2.0

In 76 women with very low serum DHEAS (DHEAS = 258 ± 89 ng/ml) femoral neck BMD was significantly lower than in 55 women with moderate-low serum DHEAS (T-score = −1.15 ± 0.51 vs. −0,89 ± 0,6 $P < 0.05$). There was no significant difference in L2/L4 BMD (T-score = −0.68 ± 1.17 vs. T-score = −0.45 ± 1.38 ns).

In 30 women with low lumbar BMD (Tscore = −2.71 ± 0.44) serum DHEAS was significantly lower than in other women (432 ± 89 ng/ml vs. 498 ± 92 ng/ml $P < 0.05$).

There was also significant difference between femoral neck BMD in these groups (T-score = −1.56 ± 0.61 vs. T-score = 0.84 ± 0.69 $P < 0.05$)

We have concluded, that women with low DHEAS concentrations have lower femoral neck BMD and women with low lumbar and femoral neck BMD have lower DHEAS concentrations. These findings confirm possible role of adrenal androgens in maintenance bone mass in elderly women.

P427

Bone mineral density and calcium deficiencies in adult patients with celiac disease

Jadwiga Szymczak[1], Anna Bohdanowicz-Pawlak[1], Ewa Waszczuk[2] & Andrzej Milewicz[1]
[1]Wroclaw Medical University, Department of Endocrinology and Diabetology, Wroclaw, Poland; [2]Wroclaw Medical University, Department of Gastroenterology and Hepatology, Wroclaw, Poland.

Background/Aims
Calcium and vitamin D malabsorption in celiac disease predispose to skeletal demineralization. The aim of this study was to evaluate the prevalence of bone mineral density (BMD) and calcium deficiencies in adult patients with CD and assess whether a gluten-free diet is sufficiently effective for BMD restoration or whether calcium and vitamin D should be applied.
Methods
BMD and biochemical parameters of bone and mineral metabolism were measured in 35 adult CD patients receiving (19) or not receiving (16) a gluten-free diet (GFD) and in 36 controls. Then the CD patients were treated with a GFD and calcium (1.0 g/day) plus alfacalcidol (0.25–1 μg/day) for one year.
Results
Reduced BMD was diagnosed in 57–77% of the patients. Mean calcemia, calciuria, and 25(OH) Vitamin D were lower, but serum PTH and bone-turnover markers (ALP, osteocalcin, ICTP) were significantly higher in CD patients than in controls. In the patients on the diet (GFD(+)), BMD was higher than in GFD(−) patients, but lower than in controls. Biochemical parameters were normal in GFD(+) patients except for diminished calciuria. Mean BMD after one year of treatment significantly increased ($P < 0.05$), mostly in the lumbar spine (mean: 7.3%), but decreased in five patients who did not strictly adhere to the GFD.
Conclusions
Impaired calcium and vitamin D intestinal absorption and low BMD are very common in adult CD patients. Gluten avoidance increased BMD, although the values still remained markedly lower in several patients. Because of chronic calcium deficiency despite GFD, we propose calcium and vitamin D supplementation in most adult CD patients.
The Local Ethical Committee approved the study.

P428

Implication of magnesium in calcium metabolism – a case report

Carmen Moldovanu, Carmen Vulpoi, Roxana Dinu, Elena Livadariu, Lidia Andriescu & Eusebie Zbranca
University of Medicine and Pharmacy "Gr.T.Popa", Iasi, Romania.

Magnesium (Mg), the second most abundant intracellular cation of the human body, plays a crucial role in nerve and muscle function. Although a frequent electrolyte abnormality, hypomagnesaemia is one of the most underdiagnosed one, symptoms being present only when Mg levels decrease bellow 0.5 mmol/l, Among the various causes of Mg deficiency endocrine disorders are neither the most frequent nor the most studied. An exception is the implication of Mg in bone and calcium metabolism. Mg deficiency can interfere with the recovery after parathyroidectomy, or from vitamin D deficiency. We present the evolution of postsurgical parathyroidism in the case of a 43 years old woman who has suffered near-total thyroidectomy for Graves' disease. She developed overt signs of tetany, with very low calcium values (1.6 mmol/l) and hyperphosphoremia (2.3 mmol/l). She received high calcium doses (3–4 g/day) associated with vitamin D but the improvement was only temporary and Ca values remained low. Although Mg values were only to the inferior limit of the normal (0.65 mmol/l) we have associated oral sustained preparations (300 mg of mg/day). The Mg supplementation helped to improve patient's state, biologically (Ca = 2.10 g/l) and clinically. The etiology of hypocalcaemia in the setting of hypomagnesaemia is multi-factorial. Hypomagnesaemia has a suppressive effect on PTH secretion and induces PTH resistance by interfering with G protein activation, but in the case of PTH deficiency, the main feature seems to be vitamin D resistance. The correlation between low Mg and low vitamin D levels is not clearly established. Since our patient associated osteoporosis (T score −3.6), dietary calcium supplementation is also necessary to improve bone turnover. Although calcium remains the star of bone remodeling, Mg have also an important contribution. Concomitant Mg intake will prevent the Ca/Mg imbalance and improve bone mineralization.

P429

Abstract unavailable

Clinical case reports – presented on Monday

P430

The effect of surgical cure of acromegaly on glycemic control in an elderly female patient suffering from type 2 diabetes – a case report

Koviljka Milenkovic Vulovic & Snezana Polovina
General Hospital, Cacak, United States.

Introduction
Insulin resistance occurs in 80% of patients with acromegaly. This report illustrates the case of a female patient with unsatisfactory control of type 2 diabetes and a beneficial effect on glycaemic control after the somatotropic pituitary adenoma surgery.
Case report
A 76-year-old female patient with diabetes diagnosed more than twenty years ago was treated with oral hypoglycaemic agents for a long time. In the past ten years she has been taking insulin and has had extremely poor glycaemic control for a long time. She presented with an average daily level of blood glucose 11.2 mmol/l (measured by the device for self-monitoring of blood glucose before and two hours after the main meals) and HbA_{1c} 9%, while taking 62 units of insulin as a total daily dose. On that occasion the body mass index (BMI) was 23.8 kg/m^2, since the patient weighed 61 kg and was 1.6 m tall.
The patient had slightly visible signs of acromegaly. Therefore she underwent IGF-1 tests which showed high levels on two occasions, 380 and 369 μg/l (standard levels being 59–177 μg/l for the patient's age). An MRI scans showed sellar and infra-sellar macroadenoma and the patient underwent a transphenoidal surgery. Two months postoperatively the IGF-1 test showed 94.5 μg/l, the average daily level of blood glucose was 7.6 mmol/l, HbA_{1c} 7.2%, and the daily dose of insulin was 16 units.
Conclusion
This case confirms the significance of an analytical approach to each patient with unsatisfactory glycemic control. The significant reduction of the daily dose of insulin after the somatotropic pituitary adenoma surgery as well as attaining satisfactory glycemic control proves that growth hormone significantly affects insulin resistance.

P431

Endocrine function in a 48,XXYY adult

Séverine Dubois[1], Fréderic Illouz[1], Sandrine Laboureau[1], Catherine Draunet[1], Vincent Rohmer[1] & Agnès Guichet[2]
[1]Pôle d'Endocrinologie, Diabètes et Maladies Métaboliques, Centre Hospitalier et Universitaire, Angers, France; [2]Service de génétique, Centre Hospitalier et Universitaire, Angers, France.

Within the group of gonosomal aneuploidy, the 47,XXY Klinefelter syndrome is a well-known chromosomal anomaly with a clearly delineated phenotype. Since the 48,XXYY polysomy is rather rare and associated with hypogenitalism, it has often been considered as a variant of the Klinefelter syndrome. Nevertheless, several differences have been reported, in particular the greater severity and prevalence of mental retardation and psychiatric illness in patients with a 48,XXYY syndrome. Although the 48,XXYY is now considered to be a distinct clinical and genetic entity, there is very little data available in the literature, especially about adults. Moreover, endocrine studies are rarely performed.

To our knowledge, this is the first report of a case of an adult with the 48,XXYY syndrome concomitant with type 2 diabetes. The diabetes is probably related to a metabolic syndrome associated with the truncular obesity, a common feature in this X/Y polysomy. The physiopathology of abdominal obesity in the 48,XXYY syndrome is unknown.

Endocrine assays in our patient showed normal pituitary function in spite of hypergonadotrophic hypogonadism. The endocrine findings suggest dysfunction of the Leydig as well as the Sertoli cells, probably explained by the lengthy duration of the disorder. Other adult cases will be required to confirm these anomalies since very few accurate endocrine studies on the 48,XXYY syndrome have been published so far. We make a literature review.

Borgaonkar *et al.* reviewed the published data on the height of the 53 patients and they concluded that 48,XXYY boys are taller from an earlier age, compared to the general population. Our patient reached only his genetic target height and GH level was normal. Bertelloni *et al.* reported a central precocious puberty in the 48,XXYY syndrome. We have no indication of this pathology in our case.

P432

Simultaneous occurrence of multicentric medullary and papillary thyroid cancer: a case report

Gianlorenzo Dionigi[1], Patrizia Castano[1], Valentina Bertolini[2], Maria Laura Tanda[3] & Luigi Bartalena[3]
[1]Department of Surgical Sciences, University of Insubria, Varese, Italy; [2]Anatomic Pathology Unit, Department of Human Morphology, University of Insubria, Varese, Italy; [3]Department of Clinical Medicine, University of Insubria, Varese, Italy.

Background
Papillary thyroid cancer is a well-differentiated neoplasm and is the most common, accounting for 65–85% of all thyroid cancer. On the other hand, medullary carcinoma represents only 3–12%. The concurrence of distinct medullary and papillary carcinoma within the same thyroid has been sporadically described.
Case presentation
We report a rare case of simultaneous sporadic both multicentric medullary and papillary thyroid cancer with lymph node metastases in a 65 years old man patient. He presented with a one-month history of solitary right lobe thyroid node and watery diarrhea. He was biochemically euthyroid. Basal serum calcitonin levels was high. Diagnosis of medullary carcinoma was confirmed by positive aspirate immunohistochemical staining for calcitonin and negative thyroglobulin staining. Pheochromocytoma was excluded before operation. Patient was screened for the presence of the specific ret mutations. After total thyroidectomy and dissection of central lymph nodes, histopathological definitive examination of the speciment revealed medullary carcinoma in right lobe (4 cm), two distinct nodules of medullary (0.4 cm) and papillary (0.5 cm, with follicular components) carcinoma in the isthmus, papillary microcarcinoma (0.5 cm) in the left lobe and lymph node metastases of medullary cancer. All tumors were clearly separated from each other, representing the pure entity of each type. The postoperative course was uneventfull. Six months after operation he has no signs of progression of the tumour.
Conclusion
Medullary carcinoma derives from parafollicular cells or C cells of the thyroid. C cells have a neuroendocrine origin, being derived from ectodermal neural crest precursors. Papillary carcinoma derives from the follicular cells. This patient developed distinct histologic type tumors in separate lobes of the thyroid. This is the first report in which different synchronous both multicentric tumors type have been identified in one case. Although the simultaneous occurrence of papillary and medullary carcinoma may have been a simple coincidence, a common oncogenic stimulus may have been involved.

P433

Simultaneous bilateral transperitoneal laparoscopic adrenalectomy (SBTLA)

Ferenc Juhasz, Zsolt Kanyari, Ferenc Gyory & Géza Lukacs
University of Debrecen, Surgical Department, Debrecen, Hungary.

Background and Purpose
Laparoscopic adrenalectomy has become the preferred surgical approach to manage adrenal disorders. Bilateral adrenalectomy is performed for diseases that are unresponsive to medical management and, frequently, for neoplastic disease. The aim of this study was to review our experience with laparoscopic bilateral adrenalectomy and to evaluate its safety, efficacy, and outcomes.
Patients and Methods
Between May 1999 and May 2005, four male and four female patients with a mean age of 37 years (range 24–55 years) presented for bilateral adrenalectomy (pheochromocytoma [$N=4$], Cushing's disease [$N=2$], malignant neuroendocrine tumor [$N=1$] and incidentaloma [$N=1$]. All procedures were performed using a simultan bilateral transperitoneal approach (SBTLA).
Results
Laparoscopic bilateral transperitoneal adrenalectomy was completed simultaneously in eight patients, while in one case the operation was converted due to the neuroendocrine carcinoma localised just behind the confluence of the right renal vein and I.V.C. One tripple tumor was operated by the staged procedure because there was no agreement on a one stage (simultan) operation between the chest surgeon consultant and us. The mean operative time was 189 minutes (range 165–240 minutes), and the mean estimated blood loss was 76 mL (range 55–90 mL). There were no postoperative complications. All patients have been treated postoperatively with daily hydrocortisone and fludrocortisone replacement. After a mean follow-up of 33 months (range 2–45 months), all of the eight patients are alive.
Conclusion
Simultan bilateral transperitoneal laparoscopic adrenalectomy is a safe and effective procedure. Patients are discharged postoperatively in a relatively short time with few complications. Appropriate steroid replacement (if its necessary) and close follow-up allows these patients to return to their regular life style. The meticulous adrenal preserving technic of the LA makes possible to avoid unnecessary hormone supplementation.

P434

Amyloid goitre: report of a case

Gianlorenzo Dionigi[1], Maurizio Salvadore[2], Maria Laura Tanda[3], Eliana Piantanida[3] & Francesca Villa[1]
[1]Department of Surgical Sciences, University of Insubria, Varese, Italy; [2]Department of Surgical Pathology, Anatomia Patologica, Ospedale di Circolo-Universita dell'Insubria, Varese, Italy; [3]Department of Clinical Medicine, University of Insubria, Varese, Italy.

Background
Amyloidosis is an important etiological factor of end stage renal disease. Apart from major target organs as cardiovascular, respiratory and gastrointestinal

system, endocrine organs can also be involved. Amyloid goitre was described for the first time by Beckmann in 1858. Approximately 200 cases of amyloid goiter have been reported in English literature.
Case presentation
The patient is a 67-year-old woman. The patient referred in 1989 the presence of a thyroid nodule of the left lobe investigated by scintigraphy and fine needle aspiration cytology (compatible with goitre). 3 months prior to her admission, the patient noticed a progressive enlargement in the anterior region of the neck associated with dyspnea dysphagia and hoarsness. Preoperative ultrasound showed an enlargement thyroid with US stimated gland volume of 105 mL, a 3 cm nodule in the left lobe and micronodularity in the right lobe. Chest X-ray revealed a deviation of the trachea. She was biochemically euthyroid. Because of the obstructive symptoms the patient underwent thyroidectomy. Histologic examination confirmed diffuse amyloid deposition surrounding thyroid follicles. Moreover, a nodular pattern of amyloid deposition was seen resulting in compression and distortion of the follicular architecture. Confirmation of amyloid was made by the presence of congophilia and apple-green birefringence under polarized-light microscopy. No Immunoreactivity was seen with calcitonin or thyroglobulin. One year after primary surgery, the patient was admitted to the Nephrology Department because of acute renal failure.
Conclusion
Amyloid goitre as the initial manifestation of systemic amyloidosis is an exceedingly rare condition associated with clinically apparent enlargement of the thyroid gland due to massive amyloid infiltration. We describe the clinical and pathological features of amyloid goitre and the difficulties in making a pre-operative diagnosis. In this case, amyloid goitre had no significant influence on thyroid function even when extensive parenchyma replacement was present. A plan of management for this rare thyroid condition must be suggested.

P435

Autoimmune polyglandular syndrome type I associated with motor focal epilepsy – a case report

Alina Maria Suta & Carmen Burloiu
Clinical Hospital Al Obregia, Bucharest, Romania.

Autoimmune polyglandular syndrome type I is a very rare disorder. We present the case of a six-year-old girl admitted to our hospital in September 1999 for recurring seizures and a history of muscle cramps and carpal spasms. Neurological examination showed congenital partial palsy of cranial nerves III and VI, EEG revealed abnormal electric activity and cerebral CT was normal. Laboratory findings (hypocalcemia-5 mg/dl, hyperphosphatemia-10.3 mg/dl and low serum PTH level-4.72 pg/ml; serum cortisol, electrolytes, TSH – in normal range) sustained the diagnosis of motor focal epilepsy and idiopathic hypoparathyroidism and the child was treated with calcitriol, calcium salts and antiseizure drug (carbamazepine). She was followed up for two years and lost after that.

In May 2005 the patient was hospitalized again for symptoms of adrenal crisis preceded by skin hyperpigmentation. New laboratory findings: blood sugar-40 mg/dl, blood ureea-63.8 mg/dl, hyponatremia-120 mEq/l, hypochloremia-80 mEq/l and hyperkaliemia-10.6 mEq/l; random cortisol level-3.13 μg/dl; hypocalcemia-5.9 mg/dl. This time cerebral CT showed calcification of basal ganglia, frontoparietal cerebral cortex and cerebellum. After emergency treatment of adrenal crisis, the maintenance therapy of chronic primary adrenal insufficiency has been initiated: replacement of glucocorticoids and mineralocorticoids with prednisone, respective fludrocortisone. The therapy with calcitriol and calcium salts has been resumed. After two months the patient presented candidiasis of the mouth with a good answer to therapy with fluconazol.

The patient's mother was diagnosed with Hashimoto's thyroiditis at the age of 37 years, in July 2005.

This is a case of an unusual sequence of development of the three major component of PGA1 (hypoparathyroidism, adrenal insufficiency and chronic mucocutaneous candidiasis). Till now, we didn't find other autoimmune or ectodermal disorders, but there is a neurological pathology unrelated to hypoparathyroidism with special problems of management.

P436

Postpartum autoimmune hypophysitis, autoimmune hyperthyroidism and reversible hepatitis at a patient with partial empty sella

Ionut Repede, Ioana Asiminoaiei, Roxana Acasandrei,
Loredana Ungureanu, Mihai Grigoras & Dumitru Branisteanu
University of Medicine and Pharmacy "Gr.T.Popa", Iasi, Romania.

The postpartum period is accompanied by an increased risk for autoimmune diseases. SN, 29 years of age, developed subsequent to her second pregnancy a polymorphic syndrome, characterized by fatigue, paleness, amenorrhea, agalactia, palpitations, weight loss. Hormonal investigations suggested corticotrophic, somatotrophic (basal morning plasma cortisol – 35 ng/dl, basal GH – 0.1 mIU/l, insulin-induced hypoglycemia test: plasma cortisol – 58 ng/dl, GH – 0.1 mIU/l) gonadotrophic (FSH = 0.3 IU/l, LH = 0.2 IU/l, oestradiol = 22 pg/ml), and prolactinic insufficiency (prolactin = 3.5 ng/dl), but measured high levels of thyroid hormones (fT_4 = 3.4 ng/dl) in the presence of low TSH (0.1 mIU/l), setting the diagnosis of autoimmune postpartum thyroiditis in the clinical, immune (positive antibodies vs TPO) and imagistic (thyroid ultrasound) context. NMR investigation of the pituitary region showed partially empty sella and glandular parenchyma with diffusely reduced contrast. Clinical evolution (the appearance of hypopituitarism in the postpartum period, after uncomplicated labor and associated with other autoimmune pathology) chose the diagnosis of autoimmune postpartum hypophysitis the most probable, and glucocorticoid and oestrogenic substitution were started accordingly. During her admission in our department, the patient complained of nausea and lack of appetite. Liver enzymes were increased (TGO = 97 U/l, TGP = 89 U/l) before the onset of antithyroid therapy, but spontaneously got normalised after one week. Subsequent to the therapy with antithyroid drugs, the patient developed a clinically suggestive episode of transient hypothyroidism with low fT_4 values (0.8 ng/dl), but unaccompanied with a correspondant TSH increase, fact certifying the existence of a thyrotrophic defficiency accompanying the autoimmune hypophysitis. This is the first case of association between reversible hepatitis and multiple endocrine immunopathy. The aetiology of hepatitis, although not proven, might have also been autoimmune. Another rare particularity was the tricky co-existence of hyperthyroidism and pituitary insufficiency.

P437

One case of sellar and suprasellar chordoma

Corina Galesanu, Ioana Asiminoaiei, Elena Saviuc, Tamara Bostaca,
Dragos Negru & Dumitru Branisteanu
University of Medicine and Pharmacy "Gr.T.Popa", Iasi, Romania.

Chordomas are slow growing neoplasms arising from notochordal remnants of the axial skeleton. The second most common site for chordomas, after the sacrococcygeal region, is the base of the skull. We describe one case of sellar and suprasellar chordoma found in a 44 year old female, with tumoral syndrome, bitemporal hemianopsia and secondary amenorrhea. Skull X-ray showed an enlarged sella turcica with destruction of the dorsum and impressive intra- and suprasellar calcifications, reason why a craniopharyngeoma was initially suspected. MRI depicted a voluminous and expansive solid tumor mass, accompanied by destruction of the sellar base and temporal bone on the left side. The lesion was compressing the optical chiasm and the third ventricle on the left side. Hormonal investigations showed corticotroph and somatotroph deficiency (morning plasma cortisol of 45 ng/ml, basal GH of 0.2 ng/ml both insufficiently stimulated by insulin-induced hypoglycemia test – to 56 ng/ml for cortisol and 1.1 ng/ml for GH) as well as thyrotroph (basal TSH of 0.19 mIU/l, stimulated only to 1.66 mIU/l at TRH test – 500 microg iv in the context of low total T_4 – 5.2 mg/l) and gonadotroph deficiency (low basal FSH, of 1.5 mIU/ml, in the context of low plasma oestradiol, of 29 pg/ml). Basal prolactin was moderately increased (79.6 ng/ml) and further stimulated by the TRH test (to 117.4 ng/ml), suggesting pituitary stalk disjunction rather than tumoral secretion. The patient was submitted to transfrontal surgery under intravenous glucocorticoid protection. The anatomopathological investigation set the final diagnosis of chordoma, due to the presence of physalyphorous cells. After surgery the visual field broadened, but the patient developed irreversible diabetes insipidus. The patient was hormonally substituted and submitted two years later to telecobaltotherapy due to the growth of tumor reminiscence and re-narrowing of the visual field. Her evolution eight years after the diagnosis is stable.

P438

Cutaneous modifications suggestive for Cushing's syndrome induced by topical corticoid application

Simona Mogos, Dumitru Branisteanu, Anca Zbranca, Eusebie Zbranca & Voichita Mogos
University of Medicine and Pharmacy "Gr.T.Popa", Iasi, Romania.

Topical application of corticosteroids is frequently used in the therapy of various dermatological diseases due to their antiinflammatory and immunsuppressive effects. Systemic pharmacological levels of glucocorticoids lead, on the other hand, to Cushing syndrome, i.e. significant modifications of intermediary metabolism, body composition, bone mass, haematolymphopoietic system and, last but not least, to skin modifications: purple striae, petechiae, infections. We describe a clinical case of cutaneous changes suggestive for Cushing syndrome of pre-existent axillary striae at an obese male using topic corticoid administration, limited to the surface of application. Although transcutaneous corticoid absorption may lead to overt Cushing syndrome through exceeding the physiological level of plasma glucocorticoids, causing at the same time an inhibition of endogenous corticotroph function, the corticotropic axis of our patient was functioning normally at the moment of the admission (morning plasma cortisol of 11.2 microg/dl, 24 hour urinary cortisol excretion of 76 microg/24 h).The patient equally had normal blood pressure, normal electrolytes, normal blood cell count, absence of osteopenia by DXA-assessed bone mineral density. Abdominal ultrasound investigation showed adrenal glands within normal range and the absence of adrenal or extraadrenal tumors. Skin lesions suggestive for glucocorticoid excess, but unaccompanied by other features of Cushing syndrome, should determine the physician to proceed to a thorough anamnesis. Endogenous or exogenous systemic Cushing syndrome should be nevertheless ruled out.

P439

Clinical presentation of a patient with giant prolactinoma

Ekaterina N. Giniatullina, Larisa K. Dzeranova, Andrey Y. Grigoriev & Alexandr V. Voronzov
Research Centre for Endocrinology, Moscow, Russia.

The aim of said presentation was to direct attention to possibility of misdiagnosis of patients with a giant prolactinomas.

A 36 years-old man was admitted to our Centre with complaints of headaches, decline of left eye vision, absence of erection, accompanied with a decline in libido and galactorrhea. These symptoms began since August 2001. At October 2004 reduction of the vision on both eyes was revealed as well as contraction of vision fields. At the presentation the patient had excess weight, muscle weakness, body hair reduction, along the following with lab data (prolactin level was 48527 mU/l, Testosterone level – 2.1 nmol/l, DHA-S 14 mcmol/l) and data of MRI inspection (macroadenoma with endo-ante-supra-infra-laterocellaric expansion). This led us to suspect the diagnosis of giant prolactinomas, secondary hypogonadism, galactorrhea. The treatment of cabergoline (0.5 mg a week with gradual increase until dosage of 3,5 mg a week was reached) was recommended. ?t control examination at March 2005 the decline in frequency of headaches, vision disturbances, galactorrhea and also the recovery of erection was noted. The PRL level decreased to 990 mU/l. Data of MRI – reduction of the tumor size by 2,3 times was noted. During the period of the treatment the patient's wife become pregnant.

The diagnostics of male prolactinomas is a complicated task, because clinical signs of the disease can vary broadly and thus, by their subjective character, can prevent the timely medical attention. But in presence of a primary medicament treatment the positive dynamic, recovery of reproductive function, reduction of the tumor sizes can be observed in most cases.

P440

Thyrotropin-producing pituitary adenoma discovered because of galactorrhea

M Teresa Herrera[1], Cristina Abreu[1], Marion Hemmersbach-Miller[2], Ricardo Darias[1], Pilar Olvera[1] & Enrique Palacios[1]
[1]Hospital Universitario Nuestra Señora De Candelaria, Santa Cruz De Tenerife, Spain; [2]Hospital San Roque Maspalomas, Maspalomas, Gran Canaria, Spain.

Introduction
Thyrotropin-producing adenomas (TSH-omas) constitute about 1% of pituitary adenomas. TSH-omas are a rare cause of hyperthyroidism. In conjunction with biochemical parameters and dynamic endocrine testing, image evaluation of the pituitary gland and sella turcica is mandatory for establishing a correct diagnosis. TSH-omas are usually large tumors and tend to be invasive. Greater amounts of invasion correlate with incomplete surgical removal of the tumor and, thus, continued hormonal secretion. Therefore, an early diagnosis and a complete surgical removal are essential.
Case report
A 29-year-old female was referred to the endocrinology outpatient unit because of a 5 months history of bilateral galactorrhea and amenorrhea. She also complained about symptoms of hyperthyroidism (13 Kg weight loss in 10 months, palpitations, hand tremors, heat intolerance and nervousness). On physical a grade I goiter was observed. Pituitary hormone levels were determined; abnormal values are shown in table 1 – the rest was normal. In order to rule out the thyroid hormone resistance syndrome, TRH testing and a MRI of the pituitary gland was performed. TRH testing was compatible with a TSH-oma (Basal TSH 7.63 μUI/mL; after 20 minutes 7.99 μUI/mL; after 60 minutes 6.97 μUI/mL). Pituitary MRI showed a macroadenoma.
The patient was started on a long-acting somatostatin analog (Octreotide) and is currently awaiting surgery.

Table 1 Results of hormone determinations

	TSH (μUI/mL)	FT4 (ng/dl)	PRL (ng/mL)	FSH (mUI/mL)	LH (mUI/mL)	17-β-estradiol (pg/mL)
28/07/2006	6.63	2.56	61.52	6.36	3.88	<10
10/07/2006	5.64	2.77	43.74	5.09	3.04	13

Discussion
1- Signs and symptoms of TSH-oma vary and are unspecific. Galactorrhea and amenorrhea are present in 30% of these patients.
2- In case of hyperthyroidism without TSH suppression and abnormal pituitary hormone values, a TSH producing pituitary adenoma should be suspected.

P441

An 8-year-old boy with seizures and hypokalemia due to a paraganglioma

Vassilios Petrou[3], Kolofotis George[3], Eleni Georgouli[2], Asteroula Papathanasiou[3], Tertipi Athanasia[3], Constantinos Stefanidis[1] & Charalambos Hadjiathanasiou[3]
[1]Children's Hospital "P. & A. Kyriacou" Department of Nefrology, Athens, Greece; [2]Children's Hospital "P. & A. Kyriacou" B' Pediatric clinic of Athens University, Athens, Greece; [3]Children's Hospital "P. & A. Kyriacou" Department of Endocrinology, Athens, Greece.

Pheochromocytomas and paragangliomas, tumors originating from the chromaffin cells, are rare in children.

We report an 8-year-old boy who was admitted to the intensive care unit with seizures for which the child had to be intubated, severe hypokalemia (1.8 mEq/l), hyponatremia (127 mEq/l) and fever. Parents reported that several months before admission the boy had nocturnal sweating. Brain MRI revealed areas of increased sign intensity in the parietal lobes bilaterally between the cortex and the subcortical region. Blood thyroid hormone levels were normal. He was initially treated as encephalitis and several boluses of potassium chloride were administered and the serum levels of sodium and potassium returned to normal, without fluid restriction. The child showed remarkable improvement in 48 hours. During hospitalization hypertension was diagnosed (180/95 mmHg) and the child complained for headaches, palpitations, polydipsia, polyouria and nocturnal sweatings. He was treated with combination of dihydralazine, felodipine, enalaprile and propranolole but without blood pressure control. Urinary 24-hour catecholamines (6440microg, normal range 14–108) and normetanephrines (19222microg normal range 88–444) were markedly elevated. Serum levels of renin (49.4 microIU/ml, normal range 3.3–41) and aldosterone (37.7 ng/dl, normal range 3–28) were elevated. Abdomen MRI showed a mass (4×4.5×3 cm) in the left paraspinal area pushing down left kidney. Whole body MIBG I-131 scan was negative. The antihypertensive therapy was modified to phenoxybenzamine followed by propranolole with normalization of

blood pressure. A laparotomy with removal of the retroperitoneal mass was perfomed. The intraoperative course was uneventful. Histologically the mass proved a well-demarcated paraganglioma. No infiltration of nearby structures or other malignant features were noted. Postoperatively, the child was asymptomatic, blood pressure and urinary catecholamines returned to normal. Genetic testing of VHL, SDHB, SDHD and RET genes was recommended.

P442

Neuropsychiatric manifestations in patients of primary hyperparathyroidism and outcome following surgery

Alexandra Raceala, Paul Ivanov, Vasiliu Octavian & Diana Paun
[1]Universitary Endocrinology Hospital "Dr. C.I. Parhon", Bucharest, Romania; [2]Emergency Military Hospital "Dr. Carol Davila", Department of Psychiatry, Bucharest, Romania.

Background
Primary hyperparathyroidism (PHPT) associates many psychiatric symptoms and is therefore important to find out if surgery can alleviates the psychiatric symptoms and improve the quality of patient's life.
Objectives
To study the nature and severity of neuropsychiatric manifestations in patients diagnosed with hyperparathyroidism before and after surgery, as well as to evaluate the correlation of such symptoms with levels of serum calcium.
Methods
During this study we monitored the psychiatric symptoms occurrence and their correlation with serum calcium among 24 patients with primary hyperparathyroidism (group I), using a control group with 20 patients that were surgicaly treated with total thyroidectomy (group II). We assessed these patients using Brief Psychiatric Rating Scale (BPRS), Clinical Global Impression (CGI) and Global Assessment of Functioning (GAF) before surgery and at 1, 8, 12 and 24 weeks after surgery.
Results
The PHTP patients had significantly higher levels of total serum calcium and PTH preoperatively, with biochemical normalization after surgery. The baseline BPRS score were higher in PHTP group, mean score 76.5, before surgery, compared to the control group with a mean score of 51.2. The CGI and GAF scores were also different between groups: 3.4 and 68.4 (group I before surgery), compared to 2.1 and 77.2 (group II). The improvement in neuropsychiatric symptoms was significant at 8 weeks after surgery as reflected in BPRS decreasing to 45.3, while CGI and GAF improved also, to 1.7 and 87.2. No correlation was found between the serum calcium levels and the psychiatric manifestations.
Conclusions
The PHTP associated psychopathology is very complex and symptoms significantly improved by 8 weeks post-parathyroidectomy. The evaluation of surgical interventions over the patients status is useful using clinical psychiatric rating scales but there was recorded no correlation of clinical mental status with serum calcium level.

P443

Hyperthyroidism in molar pregnancy: rapid preoperative preparation by plasmapheresis and complete after evacuation

Adil Dogan Azezli[1], Taner Bayraktaroglu[1], Samet Topuz[2], Sevgi Kalayoglu Besisik[3] & Yusuf Orhan[1]
[1]Istanbul University, Istanbul Faculty of Medicine, Endocrinology and Metabolism, Istanbul, Capa/Fatih, Turkey; [2]Istanbul University, Istanbul Faculty of Medicine, Obstetrics and Gynaecology, Istanbul, Capa/Fatih, Turkey; [3]Istanbul University, Istanbul Faculty of Medicine, Haematology, Istanbul, Capa/Fatih, Turkey.

Human chorionic gonadotropin bears structural homology to pituitary thyrotropin. The extremely elevated levels of human chorionic gonadotropin in patients with molar pregnancy or other trophoblastic diseases can lead to hyperthyroidism. We describe a patient with molar pregnancy who had secondary hyperthyroidism prepared rapidly by plasmapheresis for surgery. After first plasmapheresis the clinical picture improved dramatically. Three subsequent plasmapheresis provided a 75.1% decrease in serum free T3 concentrations and 63.9% free T4 concentrations and recovered after evacuation. This is the first using of the plasmapheresis in rapidly preparation of the patient who had secondary hyperthyroidism due to molar pregnancy.

P444

Finasteride treatment of premature androgenetic alopecia

Michaela Duskova, Luboslav Starka, Martin Hill & Ivana Cermakova
Institute of Endocrinology, Prague, Czech Republic.

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 prostate hyperplasia and prostate carcinoma.
Methods
A group of 26 men (mean age: 31 years, mean BMI 25.58), in which premature hair loss begun before 30 years of age was involved in the present study. In all individuals, their hormonal profile involving total testosterone, androstenedione, dehydroepiandrosterone, epitestosterone, dihydrotestosterone, cortisol, estradiol, SHBG, prolactin, TSH, LH, FSH and index of free testosterone was determined and insulin tolerance test before the treatment with finasterid was carried out. Finasteride in the daily dose of 1 mg was administered for 3 months. After the treatment hormonal profile was determined again. Wilcoxon robust test was used for statistic comparison of pre- and post-treatment results.
Results
The hormonal levels before and after the finasteride treatment were compared. The ratios of dihydrotestosterone/testosterone before and after treatment differed significantly while in the other hormonal levels no significant differences were found. Among 26 men examined and treated 17 subjects described the amelioration of hair quality and the stop of hair loss and no side effects during the treatment period. They were satisfied with treatment asking for the treatment to continue. Eight men have observed no treatment effect after the 3 months of finasteride administration. One man has shown the discrete sign of gynecomastia, and interrupted the treatment. No other side effects have been recorded. The insuline tolerance test before treatment was normal.
Conclusions
Finasteride in dose of 1 mg can present safe eventuality of the androgenetic alopecia control experiencing discrete amelioration of problems with hair loss in prematurely balding men.
The study was supported by grant No. NR/8525 – 5 of the IGA MZCR

P445

Secondary adrenal failure and secondary amenorrhea due to hydromorphone treatment

Karsten Müssig[1], Daniele Knaus-Dittmann[1], Helmut Schmidt[2], Klaus Mörike[3] & Hans-Ulrich Häring[1]
[1]Department of Endocrinology, Metabolism and Pathobiochemistry, University Hospital of Internal Medicine, University of Tübingen, Tübingen, Germany; [2]Pharmazentrum frankfurt/ZAFES, Institute of Clinical Pharmacology, Johann Wolfgang Goethe-University, Frankfurt am Main, Frankfurt am Main, Germany; [3]Division of Clinical Pharmacology, University Hospital Tübingen, Tübingen, Germany.

Objective
Opioids are among the most commonly used symptomatic treatments of somatoform pain disorder. Human and animal studies suggest that chronic exposure to opioids suppresses the hypothalamo-pituitary-adrenal (HPA) and the hypothalamic-pituitary-gonadal axis. We report on a rare case of secondary adrenal failure and secondary amenorrhea due to hydromorphone treatment.
Case report
A 32-year-old female patient presented with fatigue, weakness, orthostatic dysregulation, dizziness, and secondary amenorrhea for three months. The patient's past medical history revealed chronic pain syndrome (DSM-III-R) lasting two years. Four months before presentation, analgesic treatment had been changed to hydromorphone 32 mg BID and up to four times daily hydromorphone 2.6 mg as single dosages by a pain clinic. Decreased basal concentrations of plasma ACTH, serum cortisol, as well as mean 24-h urinary free cortisol excretion, and reduced peak responses of cortisol to ACTH 250 µg, to corticotrophin releasing hormone 100 µg, and during an insulin tolerance test with 0.5 IU insulin per kg body weight were consistent with secondary adrenal insufficiency. Estradiol levels were diminished with luteinizing hormone and follicle-stimulating hormone concentrations within the normal range, indicating secondary amenorrhea due to hypogonadotropic hypogonadism. Magnetic resonance imaging of the pituitary gland revealed no abnormal findings. The patient denied traumatic brain injury as well as skull radiation. After tapering from the benzodiazepine treatment we observed a stable increase to normal levels of the serum and urinary concentrations of cortisol as well as of ACTH, estradiol, FSH, and LH levels. The patient tolerated the treatment conversion very well. At the end of the tapering period she reported a clear improvement in vitality.
Conclusion
Clinicians should be alerted to the, though rare, endocrine side effects of hydromorphone treatment.

P446

Pseudophaeochromocytoma in Parkinson's disease

Karsten Müssig[1], Albrecht Pfäfflin[1], Daniele Knaus-Dittmann[1], Marius Horger[2], Klaus Mörike[3], Hans-Ulrich Häring[1] & Erwin D Schleicher[1]
[1]Division of Endocrinology, Diabetology, Nephrology, Angiology, and Clinical Chemistry, Department of Internal Medicine, University Hospital of Tübingen, Tübingen, Germany; [2]Department of Diagnostic Radiology, University Hospital of Tübingen, Tübingen, Germany; [3]Division of Clinical Pharmacology, University Hospital of Tübingen, Tübingen, Germany.

Objective
Despite combination with peripheral decarboxylase inhibitors significant amounts of L-dopa are peripherally metabolised. In patients with Parkinson's disease (PD) treated with L-dopa and a dopa decarboxylase inhibitor, urinary dopamine concentrations are markedly elevated. We describe here a L-dopa treated PD patient presenting with a clinical and biochemical picture suspicious of phaeochromocytoma.
Case report
A 73-year-old female patient diagnosed with dopamine-secreting phaeochromocytoma was referred to the Department of Internal Medicine for preoperative pharmacological treatment of severe and symptomatic paroxysmal hypertension. Endocrine evaluation of an adrenal mass had revealed markedly increased urinary dopamine levels and urinary epinephrine and norepinephrine levels within the normal range. On admission the patient reported that she had been diagnosed three years ago with PD. Medication comprised L-dopa 100 mg/benserazide 25 mg qid and pramipexole 0.7 mg tid. Endocrine evaluation confirmed markedly elevated urinary dopamine and homovanillic acid levels as well as plasma dopamine levels. Cortisol diurnal rhythm was normal. Plasma aldosterone concentration and plasma renin activity were within the normal range. Iodine-131-meta-iodobenzylguanidine (MIBG) scintigraphy proved negative. L-dopa/benserazide treatment was discontinued for three days and replaced by amantadine 200 mg qd. Twelve hours after discontinuation we observed a normalisation of the elevated urinary and plasma dopamine levels as well as the increased urinary homovanillic acid levels, indicating that increased dopamine levels were not due to phaeochromocytoma but due to PD therapy. Radiological follow-up of the adrenal incidentaloma was advised.
Conclusions
Clinicians should be alerted to increased urinary dopamine levels in patients treated with L-dopa. Unawareness of this association may lead to the misdiagnosis of phaeochromocytoma.

P447

Kallmann syndrome – deletion of the short arm of chromosome 8

Joana Guimarães, Margarida Bastos, Leonor Gomes, Miguel Melo & Manuela Carvalheiro
Hospitais da Universidade de Coimbra, Coimbra, Portugal.

Introduction
Kallmann Syndrome (KS) consists of hypogonadotropic hypogonadism and anosmia, and is 5 fold more prevalent in males. There is a considerable clinical and genetic heterogeneity and a crescent interest in autosomal genes. The FGFR1 gene, located on the short arm of chromossome 8, encodes a glycoprotein fibroblast growth factor receptor and FGFR1 mutations has been identified in 10% of KS patients. The clinical picture include typical KS and associated features.
Case study
A female, 30 years old, with primary amenorrhea, short statue (P5–P10), cleft palate, hyposmia, mental retardation and right hearing loss. Laboratory evaluation showed hypoganodotropic hypogonadism, an GnRH stimulation test showed a probable hypothalamic origin of the hypogonadism (IGF-1, GH, FT3, FT4, TSH and cortisol were normal). The pelvic ultrasonography was normal and MRI showed a lipoma of the III ventricle and agenesis of the corpus callosum. Analysis of G-banded prometaphase chromosomes from lymphocyte cultures showed a deletion on the short arm of chromosome 8: 46,XX,del(8)(p12-pter).
Conclusion
We present a patient with an 8p12-pter deletion, agenesis of the corpus callosum, cleft palate, mental retardation, right hearing loss in association with Kallmann syndrome phenotype. There are rare cases describe in literature with this associations. These findings suggest that autosomal genes are important for KS and we have to pay attention to other features associated with KS phenotype.

P448

Hyperprolactinemia in post-acute phase after severe TBI or SAH is mostly iatrogenic or due to physical stress

Ingo Kilimann[1], Günter Karl Stalla[2] & Friedrich von Rosen[1]
[1]Neurological Hospital Bad Aibling, Bad Aibling, Germany; [2]Max Planck Institute of Psychiatry, Munich, Germany.

Background
Recent studies demonstrated partial or complete hypopituitarism in 30–70% of survivors of traumatic brain injury (TBI) and subarachnoid hemorrhage (SAH). Hyperprolactinemia may indicate damage of the pituitary stalk or the hypothalamus. Physical and psychological stress and a considerable list of medications can also lead to increased prolactin values.
Methods
Prolactin was measured in 103 male and 54 female patients aged 14 to 89 years after severe TBI or SAH in the post-acute or chronic state (mean 4 month after onset) as part of a hormone screening also including cortisol, fT4, testosterone, estradiol und IGF1. Cut-off levels for normal prolactin was 18.0 ng/ml in male and 25.0 ng/ml in female patients. Medication, body temperature, serum glucose and C-reactive protein were registered.
Results
23% of the screened patients had increased levels of prolactin. Significantly more male were found to have hyperprolactinemia (25% of males vs. 8% of females). All patients with hyperprolactinemia had common hyperprolactinemic factors such as infection ($n=16$), hypoglycemia (blood glucose below 70 mg/dl) ($n=2$) or medications known to increase prolactin levels such as dopamin antagonists ($n=29$), central catecholamine depletors ($n=8$), GABA agonists ($n=6$) or opiats ($n=4$).
Hyperprolactinemia was not correlated with deficiency of other hormones.

Conclusion
Hyperprolactinemia in patients after severe TBI or SAH is usually secondary to medication or physical stress and does not indicate damage to the hypothalamus or pituitary gland.

P449

Rapid normalization of highly elevated serum chromogranin A after cessation of proton pump inhibitor therapy

Peter Igaz[1], Katalin Müllner[1], Beáta Hargitai[2], Károly Rácz[1] & Zsolt Tulassay[1]
[1]2nd Department of Medicine, Faculty of Medicine, Semmelweis University, Budapest, Hungary; [2]1st Department of Pathology, Faculty of Medicine, Semmelweis University, Budapest, Hungary.

Introduction
Proton pump inhibitors (PPIs) are widely used for treating various upper gastrointestinal disorders. A well-known side effect of PPI therapy that may cause serious differential diagnostic problems is the elevation of serum chromogranin A (CgA).
Objective
We report a case with highly elevated serum CgA in a patient with bilateral adrenal adenomas that was clearly associated with PPI therapy. Suspension of PPI intake for a few days resulted in the normalization of serum CgA.
Results
The 73-year-old woman with a history of hypertension, gastroesophageal reflux disease was found to have bilateral adrenal incidentalomas revealed by routine abdominal ultrasonography and CT. Detailed endocrinological examination including cortisol rhythm, low dose dexamethason suppression, mineralocorticoid activity, urinary catecholamine excretion did not suggest hormonal activity. ^{131}I-MIBG scintigraphy did not show pathologic isotope accumulation either. MRI indicated adrenal cortex-related adenomes. CgA measured by radioimmunoassay (CIS Bio International) was 7-fold higher than the upper normal value (728 ng/ml v.s. 98.1 ng/ml). No clinical or biochemical signs of pheochromocytoma, other neuroendocrine or carcinoid tumours, or renal insufficiency were observed. As the patient took high doses (2×30 mg) of the PPI lansoprazole, iatrogenic elevation of CgA was suspected. Immunohistochemical analysis of biopsy samples from the gastric mucosa did not indicate enterochromaffin-like (ECL) cell hyperplasia. After replacing lansoprazole with sucralphate, CgA fell rapidly, with levels normalizing within five days (84.6 ng/ml). Following the intake of a single dose of lansoprazole, serum CgA again slightly surpassed the upper normal range (132.4 ng/ml).
Conclusions
This case demonstrates that by suspending PPI therapy for a few days, highly elevated CgA can be normalized. It can thus be suggested that for the correct interpretation of results, the suspension of PPI therapy for 5 days before CgA measurement may be sufficient.

P450

Case of primary bilateral adrenal lymphoma

Timur Britvin, Oleg Bogatyriov, Svetlana Vorontsova, Galina Polyakova, Larisa Gurevich, Irina Kazantseva & Arian Kalinin
M.F. Vladimirsky Moscow Regional Clinical and Research Institute, Moscow, Russia.

Primary bilateral adrenal lymphoma is extremely rare. We report a case of bilateral adrenal lymphoma developing in a 61-year-old woman. The patient presented with weakness, fever, anorexia, nausea, and weight loss. Her vital signs were as follows: body temperature 37.2°C, pulse rate 98 beats per minute, and blood pressure 125/70 mmHg. Examination of head and neck was unremarkable. Lymphadenopathy and skin lesion weren't found. The chest X-ray was normal, without evidence of hilar lymphadenopathy. US and CT-scan revealed bilateral adrenal masses: to the right – $90\times36\times78$ mm, to the left – $70\times35\times70$ mm. Endocrine studies didn't show adrenal insuffiency – the serum cortisol (8AM) was 374 nmol/l (normal range: 180–650), serum aldosterone was 0.4 nmol/l (normal range: 0.14–1.24), and the plasma ACTH (8AM) increased to 13.5 pmol/l (normal range: 2.2–13.2). The level of 24-hour urine epinephrine was 24 nmol (normal range: 11–44), norepinephrine 59 nmol (normal range: 47–236), and free cortisol 108 nmol (normal range: 80–250). Ultrasound-guided needle biopsy was performed at the right adrenal mass. Cytologic examination showed adrenal cortical carcinoma. We performed right adrenalectomy. Microscopically, the tumor was composed of large, markedly atypical cells showing high mitotic activity. Complete substitution of tumor tissue for adrenal gland was noted as well as the tumor spread through capsule and invasion of surrounding fat. Immunohistochemical staining revealed positive reaction of tumor cells with LCA and B-lymphocyte antigen. But the cells were negative for CD30, cytokeratin A1/A3, vimentin, chromogranin A, synaptophysin and antigen of T-lymphocytes that allowed to diagnose large diffuse B-cell lymphoma. The patient refused chemotherapy and died 6 months later.

P451

Familial hypocalciuric hypercalcemia: mutation in the calcium sensing receptor gene

Sandra Paiva[1], Cristina Ribeiro[1], Luisa Barros[1], Leonor Gomes[1], Miguel Melo[1], Joana Guimarães[1], Margarida Venâncio[2], Jorge Saraiva[2] & Manuela Carvalheiro[1]
[1]Serviço de Endocrinologia, Diabetes e Metabolismo dos Hospitais da Universidade de Coimbra, Coimbra, Portugal; [2]Serviço de Genática Médica do Hospital Pediátrico de Coimbra, Coimbra, Portugal.

Familial hypocalciuria hypercalcemia (FHH) is an autossomal dominant condition caused by mutations in the calcium sensing receptor gene. It is characterized by moderate hypercalcemia, with normal or slightly elevated PTH levels and hypocalciuria secondary to the increased calcium reabsorption at the distal tubule level.
We present a case report of a 16 year old patient, who was referred to our department at the age of 14 because of obesity (BMI: 36.9 K/m^2). Initial biochemical evaluation revealed hypercalcemia (11.1 mg/dL – Normal range: 8.4–10.4) and normal albumin levels. These findings prompted further evaluation, with the following results: PTH: 98/92 pg/ml (*N*: 9–72), urine 24 hour calcium levels: 92 mg/24 h (*N*: 100–300) and cervical ultrassonography revealed a small 5 mm nodular structure. One could however not exclude that this was in fact parathyroidal tissue. Cintigraphy with Sestamibi did not show abnormal fixation. Given these results, further study was pursued in 1st degree relatives, and it was found that the father and one of the siblings had slight hypercalcemia and hypocalciuria.
Genetical analysis of the *propositus* undercovered a heterozygous mutation in R648X of CASR gene (located in the long arm of chromossome 3).
This case underscores the relevance of genetical characterization in disturbances of calcium metabolism, in particular in differential diagnosis of hyperparathyroidism and FHH, which is often difficult in light of conventional assessment. Accurate diagnosis is essential for correct therapeutic management, which stresses the need for genetical analysis in current clinical practice.

P452

Structured assessment of neuroendocrine dysfunction following traumatic brain injury and aneurysmal subarachnoid hemorrhage – the interdisciplinary German database

Ilonka Kreitschmann-Andermahr[1], Harald Schneider[2], Michael Buchfelder[3], Bernhard Saller[4], Eberhard Koenig[5], Martina Jordan[1] & Günter Stalla[2]
[1]RWTH Aachen University, Aachen, Germany; [2]Max-Planck-Institute for Psychiatry, Internal Medicine/Endocrinology and Clinical Chemistry, Munich, Germany; [3]Erlangen University, Erlangen, Germany; [4]Endoscience GmbH, Munich, Germany; [5]Neurological Rehabilitation Clinic, Bad Aibling, Germany, ClinSupport GmbH, Erlangen, Germany.

The results of recent studies indicate that traumatic brain injury (TBI) and aneurysmal subarachnoid hemorrhage (SAH) must be considered as frequent causes of long-term disturbances of hypothalamo-pituitary function. Indeed, partial hypopituitarism has been established with a pooled frequency of 33% in TBI and of 48% in SAH survivors. Nevertheless, still little is known about risk factors and clinical characteristics of pituitary impairment after these two types of brain damage.

In order to address these questions on a larger scale, a multi-center, structured data assessment to create a national registry of these patients has been established in 2005. It is coordinated by an endocrinological department in the south of Germany and is financed by an independent investigator grant. At present, 10 active neurosurgical, rehabilitation and endocrinological centers in all of Germany participate in the database. Ethical committee approval has been obtained for the project. Data are collected using a structured, internet-based study sheet, obtaining information on clinical, radiological and hormonal parameters. The database aims to connect clinical information on trauma and presence and type of hypopituitarism. At the first data close, which is due in November 2006 more than 500 patients with TBI ($n=322$) or SAH ($n=178$) have been included of whom clinical data and basal hormone values are available. In 112 TBI patients (34.8%) and 46 SAH patients (25.4%) additional endocrine function testing has been performed. This conference contribution aims to present the scientific results of the first data close and to introduce this epidemiological tool which is open to all disciplines treating patients with brain injury in Germany to the European scientific community. The authors present this database on behalf of all participating centers.

P453

Normal age-dependent values of serum insulin growth factor (IGF)–I: results from a healthy Italian population

V Gasco[1], G Aimaretti[2], M Boschetti[3], G Corneli[2], D Valle[4], M Borsotti[5], A Rossi[4], S Rovere[1], L Fazzuoli[3], D Ferone[3], E Ghigo[1] & F Minuto[3]
[1]Division of Endocrinology and Metabolism, Department of Internal Medicine, University of Turin, Torino, Italy; [2]Endocrinology, Department of Clinical and Experimental Medicine, University of Piemonte Orientale, Novara, Italy; [3]Endocrinology, DISEM, University of Genova, Genova, Italy; [4]Eli Lilly Italia SpA, Sesto Fiorentino, FI, Italy; [5]SOD Safety and Quality, Careggi Hospital, Firenze, Italy.

Serum IGF-I levels were measured in 547 non-hypopituitaric, non acromegalic healthy subjects of both sexes in Italy to develop reference values in relation to age and gender. Participant subjects were stratified in three age classes (25–39, 40–59 and ≥ 60 years) and IGF-I assay was carried out by double-antibody radio immunoassay. The Pearson's correlation coefficient between age and IGF-I values was calculated by sex and pre-defined age ranges. IGF-I levels significantly decreased with age ($P<0.001$, Kruskal-Wallis test) while age was not a significant factor. The median IGF-I levels were 206 ng/ml in the range 25–39 years, 147 ng/ml in the range 40–59 years and 103 ng/ml in the range ≥ 60 years. The Pearson's correlation coefficient confirmed the negative correlation between age and IGF-I levels in the total sample of subjects ($r=-0.529$), with no sex-effect ($r=-0.570$ in males and $r=-0.529$ in females). No correlations were also found in the 25–39 years ($r=-0.036$) and in the 40–59 years range ($r=-0.080$), while in subjects aged >60 years, IGF-I levels tended to further decrease with increased age ($r=0.389$). Ranges of normal values set at the 2.5th–97.5th percentile in the 3 age ranges were 95.6–366.7 ng/ml between 25–39 years, 60.8–297.7 ng/ml between 40–59 years and 34.5–219.8 ng/ml in subjects aged ≥ 60 years. This study may contribute in the development of age-specific reference ranges for IGF-I determination in serum of normal subjects of either sex, irrespective of the used method of assay.

P454

Four cases of propylthiouracil–induced antineutrophil cytoplasmatic antibody-associated autoimmune syndrome

Zsuzsanna Nagy, Laszlo Bajnok, Beata Bodis, Zsuzsanna Keszthelyi, Orsolya Nemes, Karoly Rucz, Beata Ruzsa, Csaba Gyorgy Kiss, Gabor Kumanovics, Gabor Suto, Timea Berki & Emese Mezosi
University of Pecs, Pecs, Hungary.

Drug-induced vasculitis or lupus-like syndromes can complicate the clinical course of PTU-treated patients. The clinical manifestations of four patients treated with PTU for Graves' disease are presented.

A 37-year-old woman was treated with PTU for six years. She had severe thyrotoxicosis, high fever and polyarthralgia. Elevated doses of PTU resulted in normalization of thyroid function, but the fever and arthralgia persisted even after steroid administration. ANA, a-MPO, a-PR3 and a-cardiolipin IgM positivities were detected. The patient underwent thyroidectomy. Eight months after the withdrawal of PTU she was asymptomatic with negative serology.

A 34-year old woman was previously treated with PTU for two years. Four years later hyperthyroidism recurred. After PTU therapy she presented with urticaria vasculitis and thrombocytopenia. A-MPO, a-PR3, a-phosphatidil-serine tests were positive. Skin biopsy showed cutan vasculitis. After radioiodine therapy her symptoms resolved within three months.

A 55-year old woman was treated with PTU for six years. She complained arthralgia and a-MPO positivity was found. PTU treatment was stopped which resulted in the complete resolution of her symptoms.

A 53-year old woman received PTU for four years. After one year of treatment, a necrotising vasculitis was diagnosed with renal and pulmonary involvement. Screening for ANA and a-MPO were positive. She was treated six times with bolus cyclophosphamide and continuous oral prednisolone. The PTU therapy was discontinued recently.

The differential diagnosis between drug-induced and idiopathic vasculitis may be difficult in the individual patient, but failure to recognize the relationship with drug can lead to fatal organ damage. In two-thirds of the patients with PTU-induced autoimmune syndromes the stopping of the drug-therapy alone leads to rapid and complete resolution.

This work was supported by the Hungarian Research Found (OTKA) F037639/2002, F042912/2003.

P455

Difficult management of a thyrotoxic patient with abnormal liver function tests

Shabeeha Memon, Robin Polson & Hisham Maksoud
King's Mill Hospital, Sutton In Ashfield, United Kingdom.

Thyrotoxicosis is associated with abnormal liver function test through a poorly understood mechanism.

We report a case of a 67 years old lady presenting with retrosternal chest pain and weight loss. Examination was unremarkable other than marked spider naevi. The Liver function tests showed raised ALT, GGT and Alkaline Phosphatase. She had profoundly deranged thyroid function tests with raised T3 and T4 with highly suppressed TSH.

Hepatic ultrasound showed an irregular mass. A CT scan of Chest and Abdomen showed Liver malignancy (primary or secondary) with lung metastasis and retrocrural lymphadenopathy. A CT guided biopsy confirming Hepatocellular carcinoma.

She was referred to Oncology for further input and started on treatment with carbimazole.

This lady's liver mass could easily have been overlooked if weight loss was attributed solely to thyrotoxicosis, causing a delay in diagnosis.Treatment for this lady is far more complicated than it appears. She was admitted with neutropenic sepsis secondary to carbimazole even before chemotherapy was commenced, which complicated the management further.

She is not a candidate for Radio- iodine to avoid exposure to healthcare workers in the post radiation phase or surgery because of the progression of the tumour and thyrotoxic state.

She was treated with steroids and a limited course of Lugol's iodine until her white cell count recovered sufficiently to allow introduction of Propylthiouracil.

This case illustrates the importance of carbimazole-induced neutropenia and the need to be vigilant in the management of altered liver function tests with thyrotoxicosis.

P456

Dermatological manifestations of the neuroendocrine cancer: a four cases report of primitive and metastatic Merkerl cell tumor

Francesco Buda, Roberto Colle, Oreste Bergamo, Gordana Svenda & Concetta Buda
Dpt Int Med & Medical Oncology. City of Udine Hospital, Udine, Italy; Dpt Int Med & Medical Oncology. City of Udine Hospital, Udine, Italy; Dpt Int Med & Medical Oncology. City of Udine Hospital, Udine, Italy; Dpt Int Med & Medical Oncology. City of Udine Hospital, Udine, Italy; Dpt of Surgery, Univ of Messina, Messina, Italy; Dpt of Radiation Oncology,Univ of Messina, Messina, Italy.

Merkel cell cancer, is a very rare, malignant, neuroendocrine tumour. of the skin The cause is not known. Is and often aggressive malignancy with high tendency for local recurrence, lymph node involvement and distant metastasis and a poor prognosis and rapid progression. The Merkel cell is located in or near the basal layer of the epidermis and is closely associated with terminal axons. The aims in this study we report four cases of Merkel cell tumor of the skin(1 primary and 3 metastatic).The primary carcinoma occurred as multiple dermal nodules on the right arm showing a fast growth and spreading to regional lymph nodes.In the metastatic cases the primary tumor was often ulcerated and local regional metastatis were massive.The main diagnostic role of electron microscopic studies of the primary lesion and the importance of the immunohistochemistry are validated. Superficial lesions were easily detected by fine needle aspiration biopsy and histological examination of surgical excisions.The Surgical of primary tumor were followed by a high incidence of local recurrence and distal metastasis(3 /4 pts);median DSF was 10 months.A correct surgical treatment of primary lesions,independent of site, may influence the rate of local regional invasion.For this reason a close follow-up is advisable, including the seric control of NSE levels because of the good correlation of this enzyme to disease outcone.Since the role oh the complementary therapies has not been completelyestablished, adjuvant therapy may be reserved for high risk pts(young aged,with high L.I.,with lymphatic and /or haematic involvemen-t.As standardized protocols in Merkel cell tumour are lacking, AA. Suggest that the primary treatment consider a wide surgical excision of the primary lesion and regional lymph nodes followed bay local regional radiotherapy. Metastatic cases are treatated with chemotherapeutic regimens used for oat cell carcinoma of the lung because of the close morphobiological similarity exsting between these two tumors.

P457

Retrospective analysis of diagnostic and treatment outcomes of primary aldosteronism

Nikolette Szücs[1], Edit Gláz[1], Ibolya Varga[1], Miklós Tóth[1], Róbert Kiss[1], Attila Patócs[1], Ferenc Perner[2], János Horányi[3], Ferenc Molnár[4], László Fütö[4] & Károly Rácz[1]
[1]Semmelweis University, 2nd. Department of Medicine, Budapest, Hungary; [2]Semmelweis University, Department of Transplantation and Surgery, Budapest, Hungary; [3]Semmelweis University, 1st Department of Surgery, Budapest, Hungary; [4]Markhot Ferenc Hospital, Ist Department of Medicine, Eger, Hungary.

The authors retrospectively analyzed the efficacy of diagnostic procedures and the outcome of treatment by the analysis of data of 187 patients with primary aldosteronism (PA) examined between 1958 and 2004 at the 2nd Department of Medicine of Semmelweis University. Aldosterone-producing adenoma (APA) was detected in 135 patients, whereas idiopathic hyperaldosteronism (IHA) was found in 46 patients. Other subtypes of PA included 5 patients with unilateral primary adrenocortical hyperplasia and one patient with adrenocortical carcinoma. Molecular biological studies of the aldosterone-synthase/11 β-hydroxylase gene chimera were carried out in 30 patients but none of them showed the presence of the chimeric gene. When comparing the clinical parameters of patients with APA and IHA, no significant differences were found in the time period between the diagnosis of hypertension and the diagnosis of PA, in blood pressure, or in serum potassium values. Normokalemic PA was found in 7 cases. The ratio of plasma aldosterone concentration (ng/dl) to plasma renin activity (ng/ml/h) was above 20 in all patients with APA and in all but 5 cases with IHA. The postural test combined with furosemide administration differentiated APA patients from those with IHA with a sensitivity of 69% and a specificity of 92%. In cases of adrenocortical adenomas not clearly detectable by radiological imaging techniques and in cases with bilateral adrenocortical adenomas, selective adrenal vein sampling was performed ($n=55$). All but 4 patients with APA underwent adrenalectomy. After surgery serum potassium concentration returned to normal in all patients showing low serum potassium levels before surgery. Also, the moderate to severe preoperative hypertension disappeared or improved after surgery. The relatively low frequency of normokalemic PA and a less frequent occurrence of IHA in this cohort of patients suggests that a significant number of PA cases that are not accompanied with severe hypokalemia may remain undetected in Hungary.

P458

A case with hypercalcemia caused by hyperparathyroidism and multiple myeloma

Banu Avcioglu[1], Taner Bayraktaroglu[1], Ayse Kubat Uzum[1], Sevgi Kalayoglu Besisik[2], Ferihan Aral[1], Nese Colak[1], Fatma Harika Boztepe[1] & Faruk Alagöl[1]
[1]Istanbul University, Istanbul Faculty of Medicine, Endocrinology and Metabolism, Capa/Fatih, Istanbul, Turkey; [2]Istanbul University, Istanbul Faculty of Medicine, Haematology, Capa/Fatih, Istanbul, Turkey.

Aim
Hypercalcemia is particularly complicated with hyperparathroidism or malignancy such as myeloma. There were several cases report abouth primary hyperparathroidism coexistent with benign monoclonal gammopathy or multiple myeloma. We present clinical managament of a patient who have hypercalcemia caused by hyperparathyroidism and multiple myeloma.
Case
Fifthy-two years old a women, she was complaint with weakness by anemia due to ferrum deficiency. During the evaluation, hypercalcemia and monoclonal gammopathy were detected, and she was admitted to the hospital. Hyperparathyroidism was diagnosed by hypercalcemia (12.6 mg/dl), hypophosphatemia (2.5 mg/dl) and increased parathyroid hormone (149 pg/ml) values. Multiple myeloma was diagnosed by serum gamma-globulin component of 3.47 g/dl with a monoclonal gammopathy spike and peripheral plasmacytosis of 7%. Serum and urine immunoelectrophoresis revealed abnormal IgG and kappa arcs. Multiple myeloma was defined by kappa chain and IgG type plasma cell discrasia in bone marrow biopsy. Glucocorticoid suppression decreased serum calcium levels. Parathyroid sonography and scintigraphy showed an adenoma. She was referred previously to surgery before the management of myeloma.
Conclusion
The association between primary hyperparathyroidism and monoclonal gammopathy was discussed in terms of possible pathogenetic mechanisms by several cases report in the literature. Primary hyperparathyroidism should be suspected in patients with hypercalcemia and multiple myeloma. Most suitable management should be done for each clinical condition.

P459

Prostate specific antigen (PSA) in women with menstrual disturbances and mastopathy

Stanislaw Radowicki & Michal Kunicki
Departement of Gynaecological Endocrinology, Medical University, Warsaw, Poland.

The aim of the study was to assess free and total prostate-specific antigen-PSA in serum of women with menstrual ditubances and mastopathy.
Material and methods
We examined 176 patients who were admitted to the Departement of Gynaecological Endocrinology with benign breasts pathology. According to clinical examination and sonographical findings women were didvided into two groups:

- group I: 114 with fibrocystic breast disease. Sonographical findings revealed the presence of cysts <10 mm in diameter.
- group II: 62 women with fibrocystic breast disease, cysts >10 mm in diameter.

The control group – 46 healthy women aged 18–45 years with regular menses and no pathological finding in ultrasonography examination
The menstrual patterns were definied according to presented classification:

- Eumenorrhoea- cycle lengh 21 to 35 days., Polymenorrhoea- cycle <25 days
- Oligomenorrhoea- cycle >32 days, Amenorrhoea secundaria - absence of menstruation for >180 days.

One-way analysis of variance ANOVA was performed and Mann-Whitney test when apopriate. $P<.05$ was considered statistically significant.
The mean free and total PSA concentrations in relation to menstrual disturbances in women with mastopathy. Presented as $x \pm SD$; *=differ significantly ($P<0.05$)

Menstrual pattern	Free PSA concetration(ng/ml) Total PSA concentration		
	Group I x ± SD	Group II x ± SD	Control x ± SD
Eumenorrhoea	0.18 ± 0.46	0.26 ± 0.84	0.13 ± 0.58
	0.55 ± 1.48	0.90 ± 2.84	0.35 ± 0.88
Oligomenorrhoea	0.15 ± 0.42	Undetectable*	–
	0.48 ± 1.23	0.09 ± 0.18	
Polymenorrhoea	0.29 ± 0.80	0.31 ± 0.70	–
	0.95 ± 2.75	0.97 ± 2.14	
Am. Secundaria	1.07 ± 1.51	Undetectable*	–
	3.25 ± 4.60	0.02 ± 0.03	

Conclusions
1. The mean free and total-PSA concentrations did not differ significantly between healthy women and women with mastopathy and regular menstruation
2. Women with cysts < 10 mm (group I) and oligomenorrhoea or amenorrhoea secundaria had significantly higher free PSA concentrations than women with cysts > 10 mm

P460

Selected parameters of lipid metabolism in patients with Turner's syndrome

Beata Wikiera, Ewa Barg & Anna Noczynska
Medical University, Department of Endocrinology and Diabetology for Children and Adolescents, Wroclaw, Poland.

Women with Turner syndrome (TS) more frequently develop cardiovascular disease. Abnormal lipid metabolism is a well known risk factor for ischemic heart disease. Adiponectin as well as cytokines are useful tool in evaluation the fat tissue metabolism.
The aim of the study was to evaluate the relationship between adiponectin, TNF-alpha, IL-6 and lipids in patients with TS.
Patients and method
The study group consisted of 87 girls with TS without clinical signs of thyroid dysfunction or diabetes mellitus. The mean age was 14.05 ± 6.06 (2–25) years. X chromosome monosomy was found in 59%, mosaicism in 30.12%, structural aberration in the rest of the patients. Most of them (54%) received GH treatment, 30% finished treatment prior to the study, 16% didn't start it yet. Height, weight, BMI, BMISDS, adiponectin, TNF-alpha, IL-6, cholesterol, TG, HDL, LDL, Lp(a), insulin, HBA_1c, IGF_1, $IGFBP_3$ were determined.
Results
Thyroid hormones values were within normal ranges in all the patients. Mean concentration of IL-6 was 8.44 ± 14.07 pg/ml, TNF-alpha was 4.92 ± 3.59 pg/ml, adiponectin was 14783.02 ± 7558.25 mg/ml. There was correlation between IL6 and TNF-alpha ($r = 0.33$), but not other examined parameters. Adiponectin correlated inversely with BMISDS ($r = -0.38$) and HBA_1c ($r = -0.39$). Several correlation was found between: insulin and BMISDS ($r = 0.43$), insulin and TG ($r = 0.51$), insulin and IGF_1 ($r = 0.63$), insulin and $IGFBP_3$ ($r = 0.57$).
We compared the group of GH treated patients with girls who finished GH therapy or didn't start it yet. GH treated patients had lower level of IL6 (7.36 vs 9.16 pg/ml) and higher level of adiponectin (15587.27 vs 14241.69 ng/ml). The difference however was not statistically important.
Conclusion
GH therapy seems to reduce IL6 level and probably augment adiponectin concentration and thus can be protective for ischemic heart disease.

P461

Successive gestational hyperandrogenism with maternal virilization and female pseudohermaphroditism

Chi-Huang Chen, Jah-Yao Liu & Gwo-Jang Wu
Department of Obstetrics and Gynecology, Tri-Service General Hospital, National Defense Medical Center, Taipei, Taiwan.

Objective
Successive female pseudohermaphroditism born to gestational hyperandrogenism accompanied by maternal virilization is extremely rare in literature.
Patient(s)
A housewife, age 29, G2P1A1, revealed no hyperandrogenism before pregnancy. She gave her first child birth complicated by maternal virilization and female fetal pseudohermaphroditism due to hyperandrogenemia of bilateral 6-cm ovarian luteoma at age 27. Peak maternal serum testosterone level as high as 11539 ng/dl (normal: 20–86) was evident. Spontaneous regression of ovarian size and hyperandrogenemia during the puerperium revealed the natural course of pregnancy luteomas, not true neoplasms. She returned to regular menstruation without symptoms and signs of hyperandrogenemia the following two years except irreversible deepening voice in the aftermath of high androgen exposure. She conceived her second pregnancy at age 29. Elevation of maternal serum androgen level commenced as early as 5 weeks gestation, followed by rising androgen level that positively corresponds to acne formation and emerging facial hair by increasing gestational age. A 46 XX karyotype was confirmed after chorion villi sampling at 12 weeks gestation. Both parents made a fully informed decision to terminate the pregnancy until 14 2/7 weeks gestation. Maternal testosterone level reached 751 ng/dl while ovarian size is normal at termination.
Result(s)
The abortus revealed apparently clitoral hypertrophy. The patient returns to normal androgen level two weeks later and free from virilization afterward, leaving lowering of her voice.
Conclusion(s)
Placenta may be protective by virtue of its high capacity to convert androgens to estrogen. Conversion of testosterone to oestradiol was inadequate to protect from high maternal testosterone concentration and, undoubtedly, this fetus would have virilised if female in our observation (1). The risk for male fetus is unknown. Expectant management is the treatment option as there are no pharmacological options which are safe in pregnancy. Imprudent surgical intervention should be withheld in this regard.

P462

Tetraploid/diploid mosaicism: case report of a 35-year-old woman

Sophie Schuldiner[1], Aline Artières[1], Jean Chiesa[2], Anne marie Guedj[1], Nathalie Jourdan[1], Jean louis Richard[1] & Michel Rodier[1]
[1]Endocrinology Department, Caremeau Hospital, Nimes, France; [2]Genetic Department, Caremeau Hospital, Nimes, France.

A lot of chromomosomal abnormalities have been described of which some are very unusual. Mosaicism refers to a condition where chromosomal (abnormalities) altered structure or number of chromosomes are present in some but not all cells. Polyploidy is defined as a condition where cells contain more than two homologous sets of chromosome, due to fertilization abnormalities; tetraploidy rarely allows birth of a living child but accounts for 6% of spontaneaous abortion. We report the case of a 35-year-old woman suffering from severe obesity treated by bariatric surgery; she complained of dizziness attributed to a reactive hypoglycaemia. She had a complex medical history including idiopathic hyperprolactinemia and spaniomenorrhea treated by cabergoline, hypothyroidism treated with levothyroxine, arterial hypertension associated with hypokalemia, bilateral cataract, right carpal tunnel syndrome, patent ductus arteriosus requiring surgery at the age of 14, removal of nevi, papillary malformation and iris muscle dysfunction. Her weight was 106 kg, her height 151 cm. On examination, she presented with a shortened 4th metarcarpal bone, a moderately ogival palate, a short neck and multiple nevi throughout the body. Biologically, no evidence for reactive hypoglycaemia or hyperalmdosteronism was found. Karyotype was normal (46,XX). After stopping hypnotic treatment (antidepressants) and cabergoline, prolactin level was normal but there was a GH deficiency as evidenced by a low IGF-1 level and a GH peak < 2 mU/L after stimulation. Finally, a mosaicism was found on karyotyping a cutaneous biopsy showing both diploid and tetraploid (28%) cells (46,XX/92,XXXX).
Tetraploidy is caused by a mitotic failure during the early stage of zygotic development. Mosaicism is responsible for a great number of congenital abnormalities and a wide range of mental and growth retardation. Currently, fourteen cases have been reported in the literature: five patients deceased in the early life and the older living patient is 21 years of age.

P463

Genotype-phenotype correlation in Romanian patients with classical forms of 21-hydroxylase deficiency

Anca Zimmermann[1], Paula Grigorescu Sido[2], Camelia Al-Khzouz[2], Tiberiu Nistor[2], Matthias M Weber[1] & Egbert Schulze[3]
[1]University of Mainz, Mainz, Germany; [2]University of Cluj, Cluj, Romania; [3]Molekulargenetisches Labor Gemeinschaftspraxis Raue, Heidelberg, Germany.

Congenital adrenal hyperplasia (CAH) comprises autosomal recessive disorders mainly due to defects in the 21-hydroxylase (CYP21) gene. We aimed to perform a genotype-phenotype analysis in Romanian patients with classical 21-hydroxylase deficiency.
Patients and methods
We included 42 patients (13 males, 29 females, 19 with the salt wasting (SW) form, 29 with the simple virilizing (SV) form. Molecular analysis was performed by direct sequencing of PCR amplified products of the CYP21A2 gene.
Results
Age at diagnosis in SW patients was 23 ± 5 days in females, 30 ± 11 days in males. Female SV patients were diagnosed at 28.5 ± 43 months, males with SV were diagnosed at 8 ± 9.6 years. The most frequent mutation in Romanian patients with 21-hydroxylase deficiency was a splice site mutation in intron 2 (IVS2-13A/C>G) (43.2%), followed by deletions and large conversions and the I172N mutation in exon 4, accounting for 14.9% each, a triple mutation (P30L+ IVS2-13A/C>G+ deletion of 8 bp in exon 3) (13.5%), P30L (6.8%), different double mutations (5.4%) and R356W (1.4%). Genotypes were divided in 3 mutation groups (0, A, B), according to their predicted functional consequences and compared to clinical phenotype. Positive predictive values were 100%, 76.5% and 78.3% for group 0, A and B respectively. Overall genotype-phenotype correlation was 88.1%. In female patients we observed in genotype group 0 only severe virilization (Prader-IV), in group A there was a tendency to severe virilization (5 patients with Prader-IV, 3 with Prader-III and 2 with Prader-II), while in group B all Prader stages were encountered (2 patients with Prader-I, 4 with Prader-II and III, respectively and 6 with Prader-IV).
Conclusions
Genotype-phenotype correlation in our patients with 21-hydroxylase deficiency was high, with an overall value of 88.1%. Severe genotypes resulted in more pronounced clinical virilization, expressed as higher Prader stages.

P464

Cushing's syndrome in paediatric age – casuistic, evolution of investigation tests and treatment options in our institution throughout the last 20 years

Miguel Melo[1], Ana Fagulha[1], Isabel Paiva[1], Joana Guimaraes[1], Carla Baptista[1], Fernando Gomes[2], Francisco Belo[2] & Manuela Carvalheiro[1]
[1]Endocrinology Department, University Hospital of Coimbra, Coimbra, Portugal; [2]Nerosurgery Department, University Hospital of Coimbra, Coimbra, Portugal.

Cushing's syndrome is a rare disorder in children and adolescents. The diagnosis can be a challenge for the clinician, as its principal feature – obesity – is extremely common. We present three cases diagnosed in the last 20 years. The first one was a boy aged 17 that presented in 1984 with central obesity, acne, moon face with plethora, abdominal striae, easy bruising and skin atrophy. The investigations performed consisted in cortisol and ACTH plasma measurements (8/24 hours), low and high dose Dexamethasone Suppression Test (DST), and metyrapone test; the results were consistent with Cushing's disease. A head CT scan did not show evidence of any pituitary lesion. A trans-sphenoidal (TS) surgical exploration was performed with removal of a micronodular lesion; histology confirmed it was a corticotrophinoma. Since then, this patient has been in clinical and biochemical remission. The second case is a girl investigated in 1997 when she was 17 years old for secondary amenorrhea, obesity, hirsutism, acne and purple striae. She had cortisol and ACTH plasma measurements (8/24 hours), low and high dose DST and a CRH test that confirmed the hypercortisolism and were suggestive of a pituitary cause. A pituitary MRI scan showed a probable microadenoma. Before TS removal of the adenoma, she was treated with metyrapone. Six months after surgery she resumed regular menses. A third patient, aged 14, presented with slow growth pattern, obesity, hirsutism, striae and amenorrhea in 2002. The investigation was similar to the second case and a pituitary MRI showed an 8 mm adenoma. After TS surgery, she had biochemical remission. One year after, she had recurrence of the disease and a second surgery was performed. Since then, she has showed consistent remission, resumed regular menses and became pregnant without medical help. None of these patients has hypopituitarism now. These cases illustrate the importance of a timed diagnosis, as it may allow total remission of the disease with preservation of anterior pituitary function, a factor of major importance at this age. We analyze the evolution of investigations and therapeutic options available in our institution.

P465

Thyroidectomy as the last chance treatment for life threatening thyrotoxicosis: a case report

Margaret Gietka-Czernel, Helena Jastrzebska, Wojciech Zgliczynski, Agnieszka Dudek & Marek Szczepkowski
Medical Center of Postgraduate Education, Warsaw/Mazowieckie, Poland.

54-year old woman with Graves' disease was admitted to Endocrinology Department because of severe thyrotoxicosis and antithyroid drugs inolerance. The apathetic form of thyrotoxicosis was diagnosed; she lost 12 kg during 3 months and she had heavy muscle weakness. Previously she demonstrated allergic skin reactions (macular rushes) after both: Methimazole and Propylthiouracil. At admission her TSH was 0.001 mU/l, fT3 24 pg/ml, fT4 37 pmol/l. Lithium, propranolol and glucocorticoids were instituted but within few days she deteriorated and threatening thyroid storm was noted. She was given low doses of Methimazole, iopanoic acid, propranolol and glucocorticoids iv. Both clinical and biochemical performance improved during the next days but hepatitis probably due to Methimazole developed. Methimazole and iopanoic acid were stopped and after etablishing T24 RAIU 50%, 20 mCi 131-I was administered. Subsequently glucocorticoids, lithium and propranolol were continued. She became stable for several days and then deteriorated again. Her fT3 and fT4 were 9,4 pg/ml and 44 pmol/l respectively. She was transferred to Surgical Department and successful bilateral subtotal thyroidectomy was performed. Three days after surgery her fT3 and fT4 were within normal range. Substitution with L-thyroxine was started on the third week and no relapse of thyrotoxicosis has occured so far.
Conclusion
Thyroidectomy should be concidered as a method of treatment for severe life threatening cases of thyrotoxicosis.

P466

Insulinoma and gastrinoma in MEN 1: case report

Margaret Gietka-Czernel, Janusz Krassowski & Wojciech Zgliczynski
Medical Center of Postgraduate Education, Warsaw/Mazowieckie, Poland.

23-year old man was admitted to Endocrinology Department because of hypogonadism and pituitary tumor seen at CT. Diagnosis of prolactinoma was established based on high serum PRL level- 800 ng/ml and therapy with bromocriptine was instituted. On the next year a temporary loss of consciousness related to physical exertion occured. The neurological reasons were excluded and laboratory tests showed hypercalcemia, hypophosphatemia and elevated serum PTH levels. Prolactinoma and hyperparathyroidism made the diagnosis of MEN 1 so the insulinoma as the cause of consciousness loss was taken into account. During fasting test hypoglicemia 36 mg/dl and hyperinsulinemia 40 μU/ml was documented. Therapy with diazoxide was instituted and patient was transferred to Surgical Department. Insulinoma was not found no preoperatively nor during surgical exploration. Distal subtotal pancreatectomy was carried out but hyperinsulinemia persisted. Micoscopical analysis showed multiple pancreatic adenomas up to 0.5 cm in diameter.

On the next year subtotal parathyreoidectomy was established. 5 years later, abdominal pain and nusea occured. During gastro-duodenal endoscopy gastric hyperemia and wide duodenal ulcer was seen. Elevated levels of BAO- 15 mEq/h, MAO- 38 mEq/h and gastrin- 530 pg/ml were relevant to gastrinoma. The patient did not accepted further diagnostic procedures nor possible surgical treatment.

This case shows some different features of insulinoma associated with MEN 1 compared to sporadic insulinoma: 1/ insulinoma in MEN 1 is usually multifocal and surgery might be unsuccessful, 2/ GEP in MEN 1 can be multihormonal so strict clinical and biochemical surveillance is needed.

P467

Multiple endocrine dependent tumours in a dog patient without measurable endocrine consequences

Julianna Thuroczy[1], Aniko Tibold[1] & Lajos Balogh[2]
[1]SzIU, Faculty of Veterinary Science, Budapest, Hungary; [2]FJNCH, National 'FJC' Research Institute for Radiobiology and Radiohygiene, Budapest, Hungary.

Testicular neoplasmas are 5–15 percent of total tumours number in male dogs. Seminomas are the most common type of testicular tumours in dog.

The thyroid tumours are large, unilateral palpable masses in neck region in most of the cases. Although seventy percent of malignant thyroid neoplasmas are carcinomas, 5–20% of them are endocrinologically active which induce the clinical signs of hyperthyroidism.

Seven – twenty one percent of skin tumours are mastocytomas in dog but the incidence of them is higher in spayed female and intact male dogs, which should indicate the testosterone dependency.

Eight years old argentin dog was present at our clinic with clinical signs of alopecia, weight loss and ointment faeces. Plasma biochemical parameters were in reference ranges. The total thyroxin concentration was 30.11 nmol/l which is fit to euthyroid state. An altered density focus in right testis was visualized by the ultrasonographic examination. Neither testosterone nor estrogen serum concentrations were high. The Tc-pertechnetate uptake of left thyroid gland was increased in opposite the visualisation of right thyroid gland was decreased. The left thyroid gland, both testes and a 1 cm diameter nodule in skin were surgically removed.

Seminoma in both testes, follicular compact cell carcinoma and C-cell carcinoma in removed thyroid gland and Grade-II type mastocytoma in skin were histologically established.

The faeces got the normal consistency following the operation. The hair grows finished in sixth week after the operation. The thyroxin concentration after transient decrease reached the 35.48 nmol/l level in four month. Plasma TSH concentration was 0.272 ng/ml.

The combination of three different endocrine tumours with a suspected hormone dependent tumour suggests the relation of their development. I spite of hormone dependent tumours the plasma hormone levels were ambiguous and reached to diagnosis with use of complex diagnostic imaging techniques.

P468

Case report: Adrenal glands and stress: hypercortisolism in the course of urosepsis

Lucyna Papierska, Michal Rabijewski & Anna Kasperlik-Zaluska
Center for Postgraduate Medical Education, Endocrinological Department, Warszawa, Poland.

A 76-year obese women with diabetes mellitus t II, hypertension and urolithiasis was referred to our clinic for reason of high cortisol levels, which were detected in regional hospital, two days after cystoscopy and catheterization of right ureter. Cortisolemia was 35 μg% at 8.00 and 37 μg % at 22.00. Short dexamethasone test didn't cause cortisol suppression (cortisolemia after 1 mg DXM was 27 μg %). After two days of dexamethasone (4×2 mg) blood cortisol level was 17 μg %. Blood samples were taken during the antibiotic therapy.

In our clinic we performed a CT scans of kidneys and adrenal glands. It revealed little tumour of left adrenal gland (size 14 mm and low density). Then, Cushing syndrome of adrenal origin was suspected.

Eight hours after the examination, patient's temperature ran up to 39 °C and symptoms of urosepsis occurred. Cortisol level during this event (at the evening) was >50 μg %, DHEAS was low (348 ng/ml). Surprisingly, ACTH level was very high (323 pg/ml).

After ten days of the treatment with ciprofloxacin, when patient's general condition became good, endocrinological tests were repeated. Cortisol levels were normal, with maintained circadian rhythm (18.7–8.6 μg %), ACTH levels were 16 (8^{00}) and 5 (22^{00}) pg/ml. Dexamethasone caused proper suppression of serum cortisol (1,8 μg % after 1 mg), and MRI revealed little hypophysis, without adenoma. The tests were repeated after three months, results were also normal.

In conclusion

Observed disorders came out of normal physiological reaction of hypothalamo-pituitary-adrenal axis to stress – in described case to serious infection. Little adrenal adenoma might contribute to very brisk cortisol response to high, 'stressed' ACTH levels.

P469

Long-term experience and pharmacogenetic aspects of safety in 101 treatment-years with a long-acting formulation of testosterone undecanoate in substitution therapy of 66 hypogonadal men

Michael Zitzmann & Eberhard Nieschlag
Institute of Reproductive Medicine, Muenster, 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 new feasible modality is the intramuscular injection of the long-acting ester testosterone undecanoate (TU).

Design

Analysis of safety data accumulated during 101 treatment-years in 66 hypogonadal men receiving altogether 510 injections of 1000 mg TU in 10–14-week intervals. 35 men had primary, 27 secondary and 4 late-onset hypogonadism. A minimum of 4 injections was necessary for data entry, maximum duration of individual treatment was 9.5 years. Primary endpoints were PSA levels, prostate size, erythropoeisis, lipoprotein profiles and blood pressure. Putative modulators of safety parameters entering regression models were nadir total testosterone concentrations, age (range: 17–66 years), body mass index and androgen receptor CAG repeat length (range: 15 –29).

Results

The medication was well tolerated. PSA levels did not exceed 2.9 ng/ml. Overall, therapy-induced changes within the normal range of PSA, prostate size and erythropoeisis were more pronounced in men with higher nadir testosterone concentrations and shorter androgen receptor CAG repeats (independently and with high significance respectively). Factors leading to observations of adverse nature (assessments beyond normal limits) such as elevated hematocrit, increased blood pressure and unfavorable lipoprotein constellations were due to obesity and advanced age, but not testosterone levels or receptor properties. The absolute incidence of such events remained below 10% of all assessments respectively.

Conclusion

Intramuscular injections of testosterone undecanoate represent a feasible, safe and well-tolerated modality of androgen substitution in hypogonadal men. Testosterone treatment with this regimen is modulated by the androgen receptor CAG repeat polymorphism. Adverse observations are due to obesity and advanced age, but not testosterone levels *per se*.

P470

Thyrotoxic hypokalemic periodic paralysis in two Caucasian females

Cristina Preda, Letitia Leustean, Carmen Vulpoi, Cristina Cristea, Christina Ungureanu & Eusebie Zbranca
University of Medecine 'Gr.T.Popa', Iasi, Romania.

Hypokalemic periodic paralysis is an uncommon complication of thyrotoxicosis especially in Caucasian women. It is most frequent in east asian and Japanese males and is characterized by recurrent episodes of motor weekness of variable intensity associated with hyperthyroidism. It is usually associated with low plasma potassium levels and is often precipitated by physical activity. This condition is a self limiting disorder that is cured by the treatment of the underlying hyperthyroidism. We report two cases of acute onset weakness followed by paraplegia from periodic paralysis in two Caucasian female patients aged 69, 51 respectively. Both patients presented hypokalemia and thyroid function tests showed hyperthyroidism. Oral potassium and antithyroid drugs (thiocarbamides) resulted in disappearance of symptoms. Thyrotoxic hypokalemic periodic paralysis is often under-recognized. This cases shows that thyrotoxic hypokalemic periodic paralysis is not confined only to east-asian males but also to Caucasian females. The treatment with antithyroid drugs and oral potassium given as soon is possible is successful.

P471

Pseudophaeochrocytoma presenting with catatonia - a novel observation

Bahram Jafar-Mohammadi[1], Thomas Walsh[2], John Barry[1], James Ryan[1], Eleanor Mullan[3], Eugene Cassidy[3], Michael O'Connor[2] & Domhnall O'Halloran[1]
[1]Dept. of Endocrinology,Cork University Hospital, Cork, Ireland; [2]Dept.of Geriatric Medicine, Cork University Hospital, Cork, Ireland; [3]Dept. of Psychiatry, Cork University Hospital, Cork, Ireland.

A 74 year old lady was admitted with an agitated depression. She had been taking Lorazepam and Olanzapine throughout the preceding 6 months. Escitalopram had been introduced 2 months prior to admission and the dose was escalated 3 weeks prior to presentation. The dose of Olanzapine was doubled at the same time.

She was treated with sotalol for atrial fibrillation and she had documented labile hypertension (BP range 77/57–250/118). She had fluctuating levels of consciousness and developed catatonia on day 20 of her admission. 24 hour urinary cathecholamines were reported as:

- Noradrenaline 4100 nmol/24hrs (160–485)
- Adrenaline 854 nmol/24hrs (27–165)
- Dopamine 5486 nmol/24hrs(1300–3000)

The patient was referred to our endocrine service on day 21 of admission. Olanzapine and Escitalopram were stopped and she was commenced on phenoxybenzamine (via NG Tube). Within 24hours her level of consciousness had returned to normal. Her alpha-blockade therapy was escalated until a postural drop in BP was achieved.

A CT body (contrasted), MIBG scan and MRI brain were normal.

The patient has remained clinically well, with no features suggestive of phaeochromocytoma 8 months after presentation. These observations and the normalisation of her urinary cathecholamines and negative radiological investigations support a diagnosis of pseudophaeochromocytoma secondary to either Olanzapine or Escitalopram. Catecholamine levels have remained normal in this patient while off antipsychotic and SSRI therapy. This we believe is the first presentation of pseudopheochromocytoma with catatonia as a dominant feature.

This case illustrates the need for vigilance in making a diagnosis of phaeochromocytoma in patients who are on drugs which alter neurotransmitter metabolism.

Table 1 Urinary Volume and Cathecholamine excretion/24 hours

Day of admission	8	24	25	43	66	Range
Volume	1440	962	514	1745	2320	mls
Noradrenaline	4100	638	537	92	197	160–485
Adrenaline	854	176	101	–	–	1300–3000
Dopamine	5486	1100	1108	710	979	600–1300

P472

Relapse of hyperthyroidism in Graves' disease after long-term drug treatment

Emese Mezosi, Orsolya Nemes, Beata Bodis, Zsuzsanna Nagy, Beata Ruzsa, Karoly Rucz & Laszlo Bajnok
University of Pecs, School of Medicine, Pecs, Hungary.

The optimal treatment of hyperthyroidism in Graves' disease is still an unresolved question. Hyperthyroidism recurs in 50% of patients after discontinuation of antithyroid therapy. In this retrospective study, Graves' patients investigated in the endocrine unit of Pecs University between December 2004 and October 2006 were enrolled (68 women, 22 men, age 47 (15–79) years). Antithyroid drug therapy was applied for a minimum of one year and the treatment was withdrawn for at least 5 months. The duration of antithyroid therapy was much longer than usually recommended, on the average 3,4 years, the median follow up was 20 months. The relapse rate in the group of patients treated over two years (on the average 4,6 years) was even higher (59%) than in the group treated for 1–2 years (50%) ($P=0.008$). Predictors of the relapse were age <40 years at the onset of disease, enlarged thyroid gland, positive TSH-receptor antibody (TRAK) level, other autoimmune disease, endocrine orbitopathy and thiamazole allergy. The relapse rate was lower after block-replace treatment regimen (40% versus 64%, $P<0.001$). Recurrence of hyperthyroidism was more frequent in women (58%) than in men (45%, $P<0.001$). The nodularity of the thyroid gland and the negative TRAK level did not affect the recurrence of thyrotoxicosis. In conclusion, long-term (over two years) treatment of Graves' disease did not decrease the risk for relapse after discontinuation of drug therapy.

Clinical case reports – presented on Tuesday

P473

Bloch-sulzberger syndrome, hypothyroidism and a pituitary incidentaloma: a case report

Marko Stojanovic[1], Dragana Miljic[1], Mirjana Doknic[1], Marina Djurovic[1], Sandra Pekic[1], Milos Nikolic[2] & Vera Popovic[1]
[1]Institute of Endocrinology, Diabetes and Diseases of Metabolism, University Clinical Center of Serbia, Belgrade, Serbia; [2]Institute of Dermatology and Veneral Diseases, University Clinical Center of Serbia, Belgrade, Serbia.

A female patient, 34 years old, was referred to endocrinologist, for an incidentally discovered interstellar mass on MR, mild subclinical hypothyroidism and hyperprolactinemia, and irregular menstrual cycles. She was diagnosed with Bloch-Sulzberger syndrome (BSS) in neonatal age. Epilepsy, her most prominent component of BSS, was well controlled but only with triple anticonvulsant therapy (Valproate, Carbamazepine, Clonazepam). She was obese, clinically euthyroid, and exhibited dermal, ocular and dental signs of the late phase of BSS. Elevated serum lipids and insulin resistance were observed. Mild hypothyroidism, with negative anti-thyroid antibodies was confirmed, with a response in TRH test pointing to primary hypothyroidism accompanied by mild hyperprolactinemia, responsive to TRH. Normal basal gonadotropins with a slow response to LHRH test were observed. Slightly lower IGF-1 was accompanied by a low normal response of GH to GHRH-GHRP-6. The pituitary tumor apparently exhibited no hormonal activity and no mass effects were observed by profile craniography and computerized perimetry. It was thus decided that it currently demanded only surveillance. The mild thyroid, reproductive and metabolic disturbances were attributed to the known side effects of antiepileptics. Lacking the opportunity to exclude the antiepileptic drugs and thus revert their side effects, a decision was made to relieve the subclinical hypothyroidism by levothyroxine replacement. Two months after introducing the replacement therapy, a marked clinical and laboratory improvement was notable.

BSS is a rare, X linked syndrome caused by an inactivating mutation in the NEMO gene. Dermal manifestations are the most prominent, followed by neurological (including epilepsy), ocular, dental and other. It is also associated with a higher tumor incidence. There is a possibility that a pituitary tumor, as observed in our patient, can represent a component of BSS, which was never previously reported.

P474

Extreme obesity as an important obstacle in diagnosing a patient with MEN1

Przemyslaw Witek[1], Wojciech Zgliczynski[1], Piotr Zdunowski[1], Andrzej Cichocki[2] & Jaroslaw B. Cwikla[3]
[1]Department of Endocrinology Centre for Postgraduate Medical Education, Warsaw, Poland; [2]The Marie Sklodowska-Curie Institute of Oncology, Warsaw, Poland; [3]MSWiA Hospital, Warsaw, Poland.

Background
MEN1 is an autosomal dominant inherited syndrome. Primary hyperparathyroidism, tumors of the endocrine pancreas, and of the pituitary, are the characteristic features of the syndrome.
Objective
To present a case of MEN1 in a patient with extreme obesity, causing serious difficulties in diagnostic procedures leading to localization of pancreatic tumors.
Case presentation
22-year-old male with extreme obesity (BMI 59), hypogonadism, gynaecomastia, galactorrhoea and duodenal ulceration, came to our Department before a planned surgery for obesity. Hormonal tests showed elevated levels of PRL (2000 ug/l), low levels of LH/FSH (<0.1 U/l) and of testosterone (0.7 ng/ml) with heighten estradiol (55.6 pg/ml) and elevated levels of S-DHEA (12312 ng/ml). MR examination showed a pituitary macroadenoma, size $45\times32\times25$ mm. Treatment with bromocriptin lowered PRL to 45 ug/l. Further diagnostic confirmed hyperparathyroidism (PTH-115 pg/ml; Ca-10.41 mg/dl) and a left adrenal gland tumor. High levels of gastrin (1340 μU/ml; $N<115$) and of chromogranin A (833 U/l; $N<18$) led to suspicion of gastrinoma. Endosonography showed 4 hypoechogenic foci in the head of pancreas. Octreoscan confirmed a high expression of somatostatin receptors. It was impossible to perform computed tomography because of the extreme obesity. In spite of that subtotal splenopancreatectomy, left side adrenalectomy and subtotal gastrectomy were performed. Histopathological examination confirmed multifocal well differentiated

neuroendocrine carcinoma with a single metastasis to lymphatic node, and a benign adrenal tumor. Postoperative scintigraphy did not show abnormal uptake of radioisotope. The level of gastrin decreased to 113 μU/ml, and of CgA to 81 U/l. Patient is currently treated with IPP and bromocriptin. In case of relapse or liver metastasis radiotherapy will be considered, using radiolabeled somatostatin's analogs.

Summary

Localizing diagnostic and treatment procedures in cases of tumors of the endocrine pancreas as a part of MEN1 remain a significant challenge. In case of the above mentioned patient the decision of surgery was made on the basis of the result of octreoscan and endosonography because the extreme obesity made computed tomography impossible.

P475

Six months physiological DHEA substitution in female adrenal failure: impact on quality of life and sexual parameters.

Jens J Christiansen[1], Mimi Mehlsen[1], Anna Maria Geraldi[2], Claus Gravholt[1], Jens O Jørgensen[1] & Jens S Christiansen[1]
[1]Århus Universityhospital, Århus, Denmark; [2]Rigshospitalet, Copenhagen, Denmark.

Female adrenal failure involves impaired DHEA secretion and very low circulating androgens. To address the impact of a daily physiological substitution dose of capsule DHEA 50 mg on quality of life and sexual parameters, we performed a 6 month trial in a randomised, crossover and placebo controlled design. The trial was approved by the local ethical committee and conducted according to GCP guidelines. Ten patients were enrolled. Seven patients reported seborrhoic side effects in the DHEA treatment period. On this background two patients left the study.

Short Form 36 (SF36) and Female Sexual Function Index (FSFI) were obtained before and after each period. Delta values on physical function (pf), role-physical (rp), bodily pain (bp), general health (gh), vitality (vt), social functioning (sf), role-emotional (re), mental health (mh) were all positive in the DHEA treatment period but failed to reach statistical significance separately. Delta value on FSFI total score was not differently influenced by the treatments (delta placebo -2.1 ± 2.0, delta DHEA -3.2 ± 0.6; $P = 0.598$), neither were subheadings as desire, arousal, lubrication, orgasm, satisfaction and pain. A spousal questionnaire handling 15 questions recorded 15, 67, 7 (positive, neutral, negative observations) after placebo treatment and 32, 53, 5 after DHEA treatment. After both treatment periods, an interview was performed by a clinical psychologist. Topics as knowledge to DHEA and expectations to treatment effects were handled as well as side effects and clinical effects. In summary, this blinded study in a well-motivated group of patients recorded a high frequency of side effects due to DHEA treatment and no significant effects on quality of life or sexual parameters.

P476

Central hypothyrodism and dyslipidemia induced by bexarotene in patients with cutaneous T-cell lymphoma

Carlos Sánchez-Juan, Elena Roche Gamón, Xelo García Fabra, Victor Alegre de Miquel, Raquel Albalat Galera & Juan-Carlos Ferrer García
Universitary General Hospital, Valencia, Valencia, Spain.

Background and aims

Bexarotene is the first retinoid receptor X (RXR)-selective agonist approved for cutaneous T-cell lymphoma in patients resistant to at least one previus systemic treatment. However, it produces often two endocrine-metabolic alterations: central hypothyroidism and dyslipidemia. We assessed, in a group of patients with Mycosis Fungoide or Sezary syndrome treated with bexarotene, the endocrine-metabolic side effects.

Patients and methods

Descriptive and retrospective study of 13 patients (4 women) treated with bexarotene (300 mg/m^2) in the department of Dermatology of our Hospital between 2003 and June of 2006 by Mycosis fungoide or Sezary syndrome. We analyzed the clinical characteristics of the patients, the efficacy of the treatment and the endocrine-metabolic side effects relationated with the drug.

Results

Patients assessed were 59,53 years old (28–79). Median period of treatment was 11,3 months but 4 patients were continuing at the end of the period of the study. 3/13 patients (23,1%) achieved partial remission, 4/13 (30.8%) achieved complete remission, 4/13 (30.8%) were stable and 2/13 (15.5%) progressed. 3/13 cases (23.1%) were treated with oral bexarotene as monotherapy and 10/13 (76.9%) in combination with other active agents (included topic steroids).

The most frequent side effects were hypertriglyceridemia in 13/13 (100%), hypercholesterolemia in 12/13 (92.3%) and central hypothyroidism in 7/13 (53.8%). Thyroid hormone replacement therapy and additional treatment with statin or fenofibrate was used in these cases. In patients who discontinued bexarotene treatment, thyroid function and lipid levels returned to baseline values.

Conclusions

Bexarotene is an effective therapeutic option in patients with cutaneous T-cell lymphoma but usually it produces central hypothyroidism and dyslipidemia which require treatment with levothyroxine and lipid-lowering agents. These frequent alterations must be in mind when bexarotene treatment is prescribed.

P477

Thyroid dysplasia – 30 cases of lingual thyroids

Eusebie Zbranca, Cristina Preda, Voichita Mogos, Letitia Leustean, Carmen Vulpoi, Bogdan Galusca, Valeriu Rusu & Radu Negru
University of Medecine, Iasi, Romania.

Thyroid dysplasia (ectopy, hypoplasia or aplasia) is a common cause of congenital hypothyroidism. Lingual thyroid is a rare embryological aberration caused by failure of migration of the thyroid gland to its normal position in the neck.This retrospective study involved 30 patients with lingual thyroid diagnosed in our Department between 1970–2005. The diagnosis was based on physical examination, evaluation of the mental development (IQ) and following tests:TSH, fT4, ultrasound imaging of the neck and sublingual region and neck scintigraphy. Among the patients with congenital hypothyroidism the incidence of lingual thyroid was 29%. Females (83%)were affected more than males (17%). In our group the age at diagnosis was between 6 months and 35 years. The mental retardation (mild to moderate) was present in 85% of cases. The analysis of physical development reveal growth disturbances in 56% of cases. On the basis of this findings it may be stated that the early diagnosis and treatment are the most important for the normal development of children with lingual thyroid.

P478

Primary hyperparathyroidism during pregnancy – case report

Voichita Mogos, Simona Mogos, Mircea Onofriescu, Elena Cotea, Eugen Tarcoveanu, Niculina Florea & Eusebie Zbranca
University of Medicine, Iasi, Romania.

Until 2002 less than 200 cases of primary hyperparathyroidism identified during pregnancy were reported. We present a case of primary hyperparathyroidism discovered during pregnancy in a 28-year-old woman. The disease was suspected due to a hypercalcemia discovered during a routine blood assessment during pregnancy (calcium = 11 mg/dl, phosphorus = 2.4 mg/dl). The patient was investigated in the Clinic of Endocrinology and the diagnosis of primary hyperparathyroidism was made on biological investigations: calcium = 12.80–15.84 mg/dl, phosphorus = 1.06 mg/dl., alkaline phosphatase = 428 IU/l, urinary hydroxyproline = 118 mg/24 h. Ultrasound neck examination showed a solid formation of $33 \times 18 \times 20$ mm. under the lower pole of the right thyroid lobe outside of thyroid tissue. The gestational age was of 30 weeks. The patient was transferred to the 1st. Clinic of Obstetrics and Gynecology and treated with glucocorticoids on order to mature the lung surphactant of the fetus in case of premature labor induced by surgery. At 32 week of gestation the parathyroid adenoma was removed under local anesthesia and confirmed by pathological examination. After resection of the parathyroid adenoma patient's calcium dropped to 8.5 mg/dl. She gave birth to a healthy newborn at 38 weeks. The patient and her infant were seen after 1 year and both were normal biological parameters. We reported this case because very low incidence of such association and the successful management that prevented the birth of a newborn with severe hypocalcemia due to exposure to hypercalcemia during pregnancy

P479

Pituitary insufficiency after traumatic brain injury in southwest Hungary

Orsolya Nemes, Zsuzsanna Nagy, Beata Bodis, Laszlo Bajnok, Dora Szellar, Endre Czeiter, Andras Buki, Tamas Doczi & Emese Mezosi
University of Pecs, School of Medicine, Pecs, Hungary.

Traumatic brain injury (TBI) often results in long-term pituitary insufficiency. Regular endocrine screening of TBI patients is advised after the acute phase of the treatment period. We monitored pituitary functions in 32 TBI patients (28 men, 4 women). Endocrine tests were performed from 3 to 36 months after the brain injury. Thyroid functions, cortisol and ACTH levels, prolactin, sex hormone concentrations, GH/IGF1 axis and posterior pituitary function were evaluated. Additional stimulatory tests were done if data indicated pituitary hypofunction: insulin/arginine/glucagone/TRH tests. Mean age of the patients was 35.1 years (men: 35, women: 36). Endocrine abnormalities developed in 37.5% of the patients, 75% of these in one axis and 25% in two axes. Three patients had hyperprolactinemia. Normal endocrine functions were detected in 62.5% of TBI patients. GH deficiency was the most frequently found abnormality in TBI patients (9 cases-28.1%), central hypogonadism was diagnosed in 4 patients (12.5%), and central hypadrenia in 2 (6.25%). Central hypothyroidism and diabetes insipidus were not present in our studied patient group. In conclusion, approximately one third of monitored TBI patients had pituitary dysfunction during follow-up. The majority of these cases displayed single axis disturbance, with GH deficiency representing the leading abnormality. Systematic endocrine follow-up of TBI patients should be extended in Hungary.

P480

Bartter syndrome – a case of secondary hyperaldosteronism

Isabel Manita, Andreia Veloza, Catarina Coelho, Catarina Saraiva, Dolores Passos, Maria Cordeiro, Luísa Raimundo & Jorge Portugal
Hospital Garcia de Orta, Almada, Portugal.

Introduction
Bartter syndrome represents a set of closely related autosomal recessive renal tubular disorders characterised by hypokalemia, hypochloremia, metabolic alkalosis and hyperreninemia with normal blood pressure. The underlying abnormality results in excessive urinary losses of sodium, chloride and potassium. Bartter syndrome is classified into 3 main clinical variants: neonatal Bartter syndrome, classic syndrome and Gitelman syndrome.
Case report
We present a 19 year-old male caucasian, the only child of a consanguinuos marriage, referred for severe hypokalemia detected during investigation of anemia (spherocytosis). Data concerning pregnancy, delivery and early childhood is not available. There is a history of nocturnal enuresis that lasted until 12 years of age, and of persistent polyuria and polydipsia. Growth and pubertal development were normal. Symptoms such as parestesias, fatigue and spasms were absent.
Laboratorial tests revealed hypokalemia alkalosis, normomagnesiemia, hypercalciuria and hyperaldosteronism. Renal ultrasound did not show alterations. We are waiting for the opportunity to order genetic testing. Other causes of hypokaliemia were excluded such as surreptitious diuretic and laxative abuse, persistent vomiting and diarrhoea.
On the ground of clinical appearance and biochemical data, the Bartter syndrome in classic variant was diagnosed. Good therapeutic effect was achieved using spironolacton, indomethacin and potassium supplementation.
Conclusion
Bartter syndrome is a rare autosomal recessive disorder. Recent molecular diagnosis has revealed that Bartter syndrome results from mutation in 5 distinct genes that affect the function of ion channels of the distal nephron segments. The literature confirms a lack of correlation of genotype and phenotype in this disease. In this case the authors emphasize the unusual late and asymptomatic presentation.

P481

A Wellbeing patch induced Adrenal crisis

Rajagopalan Sriraman, Mary Armitage & Tristan Richardson
Royal Bournemouth Hospital, Bournemouth, BH7 7DW, United Kingdom.

A 29-year-old lady with known Addison's disease and hypothyroidism was admitted with a history of increasing lethargy and dizziness for 2 weeks. At the time of admission she was on (and compliant with) Hydrocortisone 20 mg twice daily, Fludrocortisone 100 mcg once daily and Thyroxine 150 mcg once daily. On the day of admission her BP was 128/92 mmHg with no postural drop. Her electrolytes were normal, however an early morning cortisol measured 28 mmol/l. She was treated with IV Hydrocortisone for 24 hrs following which she was changed to oral Hydrocortisone. She was discharged after 3 days on Hydrocortisone 10, 10, 5 mg and Fludrocortisone 100 mcg once daily.

Unfortunately she was readmitted 7 days later. Her symptoms included postural dizziness and pins and needles over her face. During this admission her blood pressure was 136/97 mmHg lying and 118/97 mmHg sitting. Her electrolytes were again normal. She was treated with IV Hydrocortisone for the first 24 hrs and Endocrinology review requested. On further questioning, it was noted that the only change in her medication within the last few weeks was use of 'Wellbeing Detox Patches'. She denied any previous Addisonian crisis and had been very well controlled previously on oral steroids. On stopping the patch, her steroid replacement has since been unproblematic
Discussion
Detox patches contain multiple natural ingredients (up to 15 different 'natural' products). They are sold on the pretext that they 'cleanse' the body of harmful by-products. Others are said to stimulate acupuncture points through action of wood/bamboo vinegar, far infrared (a form of safe radiated energy) or minus ion emissions (formed naturally).
Herbal medicines may contain several enzyme inducers that metabolise cortisol leading to hypocortisolaemia and crisis. Subjects on steroid replacement should be warned about the usage of over-the-counter medicines even those thought to be 'natural remedies'.

P482

Antidepressants and elevated catecholamines

Rajagopalan Sriraman, David Cavan & Tristan Richardson
Royal Bournemouth Hospital, Bournemouth, BH7 7DW, United Kingdom.

Urinary catecholamine assessment is one of the screening tests for phaeochromocytoma but false positives results can occur. The pretest probability for phaeochromocytoma is 0.5% (1 in 200 patients tested) in the presence of hypertension and suggestive symptoms. We present two cases of elevated urinary catecholamines in hypertensive subjects treated with serotonin and noradrenaline re-uptake inhibitors (SNRI).
Case1
A 27 year old male presented with palpitations, tremor, sweating, myalgia, nausea and fatigue. His past medical history included acute depression for which he took venlafaxine and then sertraline 50 mg/day. His BP fluctuated between 170/105 and 115/55 mmHg. General examination and investigations including thyroid function tests were normal. Three urinary catecholamine collections were mildly elevated (24 hr adrenaline 107, 105, 38 nmol/d (normal $<$100 nmol/d), dopamine 3796, 3584, 3048 nmol/d (normal $<$3000 nmol/d)). Further investigations excluded phaeochromacytoma.
Case 2
A 43 year old male with type 2 diabetes, anxiety and depression presented with palpitations, sweats and hypertension (BP 180/106). His other problems included lithium-induced thyroid abnormalities and sleep apnoea. In addition to bendrofluazide, felodipine, metformin, and lithium, he was taking venlafaxine 150 mg/day. His thyroid function was normal, but urinary catecholamines were mildly elevated (24 hr noradrenaline output 680, 806 nmol/d (normal $<$500 nmol/d), dopamine output 4811, 3821 nmol/d (normal $<$3000 nmol/d)). There was no further evidence of phaeochromocytoma radiologically.
Discussion
Medications may cause raised catecholamines and result in false positive tests for pheochromocytoma. Tricyclic antidepressants and phenoxybenzamine have been most commonly implicated, accounting for 40% of medication-associated false positive results. We present two cases where small rises in catcholamines have occurred in patients taking SNRIs, which could be consistent with their mode of action. Clinicians should be aware of this possible effect when assessing patients, particularly with a background of depression.

P483

Myasthenia gravis and autoimmune Addison's disease in a patient with thymoma

Mesut Seker, Hulya Iliksu Gozu, Berkant Sonmez, Fatih Yavuz, Taflan Salepci, Ekrem Orbay, Mehmet Sargin, Haluk Sargin, Mahmut Gumus, Ulku Turk Boru, Mustafa Yaylaci & Ali Yayla
Dr. Lutfi Kirdar Kartal Education Hospital, Istanbul, Turkey.

The association of thymoma with myasthenia gravis is well known, however association of these two syndromes (Thymoma + Myasthenia gravis) with Addison's disease is very rare. In here we report myasthenia gravis and autoimmune Addison's disease in a patient with thymoma.

A 32-year-old man was admitted to our hospital with symptoms of weakness, anorexia, nausea, vomiting, pigmentation of skin and mucous membranes for 2 years. He had undergone to the operation because of thymoma 17 months before to admission.

On physical examination, generalized pigmentation, especially in oral mucosa, and tongue, was observed. Except ptozis in the right eye, neurologic examination was normal. Unexplained pigmentation and other symptoms suggested possibility of diagnosis of adrenal insufficiency. He was diagnosed as Addison's disease on the basis of the findings of a high plasma ACTH level; >185 pmol/L (normal; <125 pmol/L), low plasma cortisol level; 1.85 ug/dl (normal; 5–25 ug/dl). ACTH stimulation test revealed that cortisol levels were not stimulated upon stimulation by ACTH (Basal ACTH level: 2.97 ug/dl, stimulated ACTH level: 2.84 ug/dl. Anticorticosurrenal antibodies was measured as 640 (N: <5). Thyroid stimulating hormone (TSH), free thyroxine (FT4) were normal. Anti TSH reseptor antibody was measured as 3.00 U/L (normal; 0.00–10.00 U/L).

He had complaint of ptozis in the right eye for 2 years. Skull radiographs and orbita MRI were normal. Although electromyography and edrophonium test were negative; myasthenia gravis was diagnosed on the basis of findings of a high titer of acetylcholin receptors levels (2.4 nmol/l; normal: 0.00–0.50 nmol/l). Prednisolon (7.5 mg/day) and prostigmine (180 mg/day) tablets have been started. Symptoms and signs were improving by this treatment.

In here we report another example of this rare syndrome in which myasthenia gravis, autoimmune Addison's disease and thymoma ocurred together.

P484

Severe hyperandrogenism during the entire course of pregnancy does not cause virilization of a female infant born

Rita Bertalan[1], Zita Halász[1], László Csabay[1], János Rigó Jr[1], Sándor Németh[2], Anna Blázovics[1], Judit Toke[1], Belema Boyle[1] & Károly Rácz[1]
[1]Semmelweis University, Budapest, Hungary; [2]G. Richter Ltd, Budapest, Hungary.

Objevtives
Maternal hyperandrogenism occurs rarely during pregnancy as the consequense of maternal ovarian or adrenal disorders, or placental aromatase deficiency.
Case
A 33-year-old pregnant women was referred because of high serum testosterone (240 ng/dl; normal, 20–60 ng/dl) measured at the 7th week of pregnancy. At presentation she had symptoms of moderate hyperandrogenism, which slightly increased until delivery. Abdominal and pelvic ultrasound exams showed no evidence for adrenal or ovarian masses. Serum hormone measurements indicated severe hyperandrogenism and marked increases of serum estradiol levels during the whole tenure of pregnancy. Serum hCG and SHBG levels were normal. The patient refused fetal karyotype exam. Fetal ultrasound indicated normal female external genitalia.

Mother's hormone levels during gestation	13th week	17th week	28th week	35th week	Postpartum 12 hours
Testosterone ng/dl	458	664	607	590	808
Estradiol pg/ml	3139	11073	28973	33733	609

At 39 weeks of pregnancy she delivered a girl with normal female external genitalia. Umbilical cord hormone levels were normal, except a modest increase of testosterone (94 ng/dl). At the age of six weeks the baby's androgen concentrations were normal and bone age was not accelerated. One week after delivery androgen levels of the mother decreased markedly, but they remained slightly above the upper limit of normal. Placental aromatase activity, measured by conversion of testosterone to estradiol in microsomal preparations was normal.
Conclusion
This case clearly shows that severe hyperandrogenism detected as early as 7 weeks of pregnancy and persisting until delivery presumably due to hyperreactio luteinalis does not necessarily cause virilization of a female fetus. The marked difference in maternal and umbilical blood testosterone levels, together with the largely increased maternal estradiol suggest that placental aromatase activity plays a key role in preventing fetal androgen excess.

P485

Regression of metastatic gastric carcinoid associated with atrophic gastritis and after octreotid treatment

Peter Gergics[1], Gabriella Dabasi[2], Eva Csoregh[1], Zsuzsa Jakab[1], Peter Nagy[3], Beatrix Sarman[1], Peter Pusztai[1], Mark Juhasz[1], Peter Reismann[1], Nikolette Szucs[1], Ibolya Varga[1], Miklos Toth[1], Karoly Racz[1] & Zsolt Tulassay[1]
[1]2nd Department of Medicine, Semmelweis University, Budapest, Hungary; [2]Isotope Laboratory, Department of Transplantation and Surgery, Semmelweis University, Budapest, Hungary; [3]1st Department of Pathology and Experimental Cancer Research, Semmelweis University, Budapest, Hungary.

A 57-year-old female patient was admitted for evaluation of multiple focal liver lesions diagnosed with abdominal ultrasound and CT. Her medical history included severe rheumatoid arthritis and pernicious anaemia treated with vitamin B12. Gastroscopic examination revealed numerous small polypoid lesions within the stomach, and histology of tissue samples obtained by biopsy showed carcinoid associated with atrophic gastritis. Although the patient had no symptoms of carcinoid syndrome, 24 hour urinary excretion of 5-hydroxyindole acetic acid (5-HIAA) was elevated and serum chromogranin A (CgA) was three times higher than the upper limit of the reference range. Octreoscan showed focal radionuclide accumulation corresponding to the stomach and the liver. Because of the severe rheumatoid arthritis surgical therapy was not considered. After 7 months of octreotide LAR treatment abdominal ultrasound and CT showed a complete remission of liver lesions and repeat octreoscan failed to show pathologic radionuclide accumulation. Repeat gastroscopy was also negative and biopsy revealed chronic atrophic gastritis and a scattered pattern of chromogranin-positive cell-nests. In accordance with regression of the carcinoid tumor, urinary 5-HIAA excretion and serum CgA levels returned to normal.

Although somatostatin analogues have been shown to induce regression of gastric carcinoid tumors associated with pernicious anemia-related hypergastrinemia, a complete regression of liver metastases after somatostatin-analogue treatment has rarely been documented. In addition, our case demonstrates not only the efficacy of octreotide for treatment of metastatic gastric carcinoid but also the importance of octreotide treatment in cases without carcinoid syndrome.

P486

Persistent fever after surgical removal of a craniopharyngioma: diagnosis pitfalls and therapeutic difficulties

Raluca Alexandra Trifanescu[1], Monica Livia Gheorghiu[1], Irina Ogrezean[2], Vasile Ciubotaru[2], Daniela Preotescu[3], Rudolf Fahlbusch[4] & Mihail Coculescu[1]
[1]Department of Endocrinology, 'Carol Davila' University of Medicine and Pharmacy, Bucharest, Romania; [2]'Bagdasar Arseni' Neurosurgery Hospital, Bucharest, Romania; [3]'Matei Bals' Institute of Infectious Diseases, Bucharest, Romania; [4]Endocrine Neurosurgery Department, International Neuroscience Institute, Hannover, Germany.

Background
Thermoregulatory disorders after neurosurgery of craniopharingyomas were seldom reported.
Aim
To present the difficulties of etiologic diagnosis and treatment of a persistent febrile syndrome in a patient with surgically removed craniopharyngioma.
Patient and methods
A 34 years old man with a giant craniopharyngioma situated in the basal-anterior part of the third ventricle is reported. Anterior pituitary hormones were measured by fluoroimmunoassay. MRI, CT, X-rays were used for imaging. Cultures from various biologic fluids were performed.

Results
The patient underwent two successive transfrontal neurosurgical interventions. Postsurgery, diabetes insipidus and panhypopituitarism occurred. Substitutive hormonal therapy was introduced. After the second operation, the patient presented fever (up to 39 °C), abdominal pains, hypodypsia with hypernatremia and hyperphagia. Suspected colitis was excluded by colonoscopy. Thereafter, the patient developed a left inferior pneumonia complicated with minimal pleuresia; the bronchial aspirate identified Klebsiella pneumoniae and the patient received antibiotics according to the antibiogram. The pneumonic and pleural opacities on X-rays and on CT scan resumed, but the fever persisted. No inflammation markers were noticed: normal C reactive protein (0.52 mg/dL) and fibrinogen (391 mg/dL) levels, negative procalcitonine. Repeated hemocultures and cerebrospinal fluid cultures were negative. The urocultures and the cultures from the ventriculo-subcutaneous shunts were also negative. The fever persisted despite intensive, wide spectrum antibiotherapy, combined tuberculostatic therapy or high doses of corticosteroids. Excluding the infection, we conclude that the fever had central origin.
Conclusion
Hypothalamic thermoregulatory dysfunction with fever should be considered in patients with surgically removed craniopharyngiomas of the third ventricle.

P487

Study of aldosterone secretion in patients with essential hypertension using a modified suppression test

Aggeliki Gouli, Ioannis Androulakis, Athina Markou, Despoina Ragkou, Konstantina Dimitriou, Alexandra Stathopoulou, Tilemachos Anagnostou, Georgia Kondyli & George Piaditis
General Hospital of Athens 'G. Gennimatas', Department of Endocrinology & Diabetes Center, Athens, Greece.

Background
The renin-angiotensin system is important for blood pressure control. Screening and diagnostic tests used so far to diagnose patients with aldosterone related hypertension does not take into consideration the stimulating ACTH effect on aldosterone secretion.
Objective
To assess the role of aldosterone in essential hypertension using a modified suppression test, performed under suppressed ACTH levels.
Subjects and methods
117 hypertensive patients with essential hypertension and 34 age and sex matched normotensive controls were studied. A modified fludrocortisone suppression test (FST) under suppressed ACTH levels was performed to all participants (fludrocortisone 0.4 mg daily in 4 divided doses for 4 days and overnight dexamethasone suppression with 1 mg on day 4). Basic biochemical parameters, ACTH, plasma aldosterone and plasma active renin were measured at 08.00 am on day 1 and 5. Median value of aldosterone to renin ratio (ARR) +2 standard deviations in the control group after the test was used to define normal cut-off.
Results
Basal aldosterone, renin, ARR, K^+, and urine 24-hour K^+ did not differ between the two groups. Post-test aldosterone and ARR were significantly higher in hypertensives compared to controls (47.79 ± 3.97 (mean $\pm$ S.E.M) vs 132.2 ± 11.18 pmol/L, $P < 0.0001$ and 23.4 ± 3.25 vs 55.54 ± 7.53 pmol/L/pg/ml, $P < 0.0001$). Baseline K^+ levels were inversely correlated to post-test aldosterone and ARR only in the hypertensive group ($r = -0.21$, $P < 0.05$ and $r = -0.24$, $P < 0.01$ respectively). A significant proportion of hypertensives (29.05%) failed to suppress aldosterone levels to normal range after the test.
Conclusions
A modified FST revealed that a high percentage (29.05%) of patients who were thought to have essential hypertension, have autonomy of aldosterone secretion. This observation could possibly explain the cause of the low renin levels of the 25% of patients with essential hypertension reported in literature.

P488

Abstract unavailable

P489

GH-secreting adenomas may disappear with long-acting somatostatin analoque (octreotide-LAR) treatment

Senay Arikan[1], Deniz Gokalp[1], Alpaslan Tuzcu[1], Mithat Bahceci[1] & Hatice Akay[2]
[1]Dicle University School of Medicine Department of Endocrinology, Diyarbakir, Turkey; [2]Dicle University School of Medicine Department of Radiology, Diyarbakir, Turkey.

Background and aim
Somatostatin analogues such as octreotide acetate were used in acromegalic patients as primary or secondary treatment. In this study we aimed to report completely disappearing of adenoma and clinical cure in 3 acromegalic cases that rejected surgical treatment.
Case1
E.A (62 years old, female) She reported enlargement of his hands, deepening of his voice and increases in shoe size. MRI revealed a macro adenoma which was spread to cavernous sinus (20×15 mm). She has been treated ocreotide-LAR 20 mg/per month for 24 month. Adenoma size gradually became small and completely disappeared after 24 months.
Case 2
S.S (46 years old, male) Magnetic resonance imaging (MRI) revealed a 12 mm pituitary macro adenoma. He has been treated with octreotide-LAR 20 mg/per month for 25 months. After Eight months octreotide LAR treatment adenoma disappeared. Octreotide-LAR treatment was continued because risk of enlargement of adenoma
Case3
B.U (44 years old, male) Magnetic resonance imaging (MRI) revealed a 9 mm pituitary adenoma. He had treated with short acting octreotide analogue for 6 month (octreotide 100 μ three times a day) then he has treated with octreotide-LAR 20–30 mg/month for 36 months. With this treatment pituitary adenoma of the patient completely disappeared in MRI of pituitary gland.

Age and sex matched serum IGF-1 levels decreased to normal range in case 1. IGF-1 levels of case 2 and case 3 decreased but not achieved to normal range. Growth hormone levels of the patients with the treatment achieved normal range in case 2. Growth hormone levels during oral glucose tolerance test decreased in case 1 and case 3 but not achieve normal range. Biochemical data were shown in the table.
Conclusions
1- Octreotide treatment decreased IGF-1and GH hormone levels in acromegalic patients.
2- Adenomas may completely disappeared with octreotide-LAR treatment
3- Octreotide-LAR treatment may be used in selected patients instead of surgical treatment.

P490

Adrenal rest tumours in 11-β hydroxylase deficiency

Tabinda Dugal[1] & Ken Laji[2]
[1]St Georges Hospital, London, United Kingdom; [2]St Richards Hospital, West Sussex, United Kingdom.

Adrenal rest tumours are well described in 21-hydroxylase deficiencies. However there are few reports in literature of rest tumours in 11-β hydroxylase (11- β OH) deficiency. We report a case with an established diagnosis of 11- β OH deficiency with non-compliance to steroid treatment and endocrine follow-up

He presented to Urology with haemospermia. He was found to have scrotal swellings. Ultrasound confirmed bilateral testicular tumours. CAT scan showed small para-aortic lymph nodes and one below the renal hilum. He was presumed to have bilateral testicular tumours with congenital adrenal hyperplasia (CAH). He had Oncology review with sperm banking for prospective orchidectomy.An endocrine referral for his CAH was sought.Scans were re-examined. The blood flow was found to be intralesional. An alternative diagnosis of adrenal rest was made. He had raised Androstenedione and 17-OH progesterone His blood pressure was also elevated. All these features are consistant with non-compliance of treatment with steroids. He was meant to be on Prednisolone 5 mg B.D. Compliance issues were discussed and the risks mainly infertility and complications of elevated blood pressure reiterated.

Adrenals and gonads both originate from the urogenital ridge and adrenal rest tissue can be found in the gonads. CAH has an incidence of 1:10,000 and 27–30% of them have adrenal rest (Vanzull etal 1992). 2/3 of these are salt losing. 18% not previously diagnosed. 83% are bilateral and palpable (up to 10 cm). With adequate replacement tumour shrinkage occurs in >30% (Stickelbroek etal). Compliance with treatment prevents occurrence (Srikanth MS etal). However adenomatous transformation can occur. Diagnosis is by imaging with ultrasound and MRI.

We suggest that sufficient replacement from the start should be ensured. Regular screening with ultrasound of the male CAH. Fertility issues should be discussed. Azoospermia patients may need screening for CAH.

P491

The possible role of genetics in severity of thyrotoxicosis

Nosheen Iqbal & George Thomson
Sherwood Forest Hospitals NHS Trust, Sutton in Ashfield, United Kingdom.

We report the cases of two identical twins with Graves' disease which proved very difficult to control and followed very similar stormy course. Twin A was a 20 years old female of 12 weeks gestation when she was referred to endocrine clinic with history of weight loss, palpitations and tremor. Her thyroid function tests revealed TSH <0.08 mU/L (0.03–4.30), FT4 82.5 pmol/L (12–22 pmol/L) and FT3 44.4 pmol/L (2.8–7.1) with positive thyroid receptor antibody. Twin B presented when she was aged 21 years with similar complaints. Her Thyroid Function Tests revealed TSH <0.08 mU/L (0.03–4.40), FT4 73.7 pmol/L (12–22), and FT3 38.4 pmol/L (2.8–7.1). On clinical examination they both had evidence of small goitre, tremor and tachycardia with significant thyroid eye disease. Due to the severity of their disease it was difficult to treat them medically as they did not respond to the maximal doses of antithyroid drugs. Radioablation was also not an option due to high risk of thyroid storm in view of incomplete response to high dose antithyroid drugs. Therefore after adequate pharmacological preparation (with Lugol's iodine and propylthiouracil) Twin A was referred for subtotal thyroidectomy and Twin B had intrapartum thyroidectomy at 24-weeks gestation recently. Biochemical euthyroid status was achieved in both the twins within 4-days post-operatively, and they are currently on thyroid replacement therapy. This is a rare presentation of identical twins presenting at around the same age with marked thyrotoxicosis and ophthalmic involvement in both siblings. Their disease course and severity was almost identical. This could be a serendipity, but raises the issue of the need for screening for thyroid disorders in siblings of those with known thyroid disease, particularly females and more so in identical twins. It also raises the interesting possibility that disease course and severity may have significant genetic determinants.

P492

The challenge of managing thionamide induced agranulocytosis in a patient with Graves' disease

Nosheen Iqbal, A Dissanayake & George Thomson
Sherwood Forest Hospital NHS Trust, Sutton in Ashfield, United Kingdom.

We report a 44 year old lady with a history of weight loss, anxiety and 'prominent eyes'. She was clinically and biochemically thyrotoxic (FT4: 158.1 pmol/L [12–22], FT3:56.5 pmol/L [2.8–7.], TSH: <0.08 mU/L [0.30–4.30]). She was commenced on carbimazole and propranolol. Failure to attend regular clinic appointments or comply with drug therapy over the next few years resulted in huge swings of her thyroid status from severe thyrotoxicosis to profound hypothyroidism (TSH: >100 mU/L). After several years lost to follow up she was admitted to hospital with severe neutropaenia (WCC: 2.9×10^9/L [$4–11\times10^9$/L], Neutrophil: 0.22×10^9/L [$2–7.5\times10^9$/l]) secondary to carbimazole, which was stopped. Treated with antibiotics, anti-fungals and G-CSF her cell count improved gradually. However she remained unwell and in persistent thyrotoxicosis (FT3: 13.3 pmol/L, TSH: <0.08 mU/L).

Due to issues around compliance she was kept hospitalised while on Lugol's iodine to render her euthyroid before more definitive treatment with subtotal thyroidectomy. Her blood results started improving and she was discharged home with elective thyroidectomy planned after a fortnight. Due to worsening of thyrotoxicosis again, she was re-admitted and her surgery was postponed. Her medical treatment continued but unfortunately she exhibited the phenomenon of 'iodine escape' and her thyroid function tests continued to deteriorate posing her at high risk of perioperative thyroid storm. After thorough consideration of all treatment options she was started on low dose Propylthiouracil and dexamethasone in addition to Lugol's iodine. Her thyroid function tests showed progressive improvement with a stable cell count rise until 5 days prior to surgery when she developed agranulocytosis. Her Propylthiouracil was therefore discontinued. She underwent subtotal thyroidectomy under antibiotic cover and made an uneventful recovery. Our case illustrated that although thyrotoxicosis is a common condition its treatment can remain a challenge. All treatment options of thyrotoxicosis has its own risks and benefits and therefore treatment should be tailored to patient specific considerations.

P493

Five-year treatment experience with metformin in polycystic ovary syndrome

Gyula Petrányi[1] & Maria Zaoura[2]
[1]Private practice, Limassol, Cyprus; [2]General Hospital, Nicosia, Cyprus.

In 2002, we introduced metformin as an alternative treatment option of the anti-androgenic contraceptive pill in polycystic ovary syndrome (PCOS). The analysis of our observations is presented here.

170 women (age 14–45 y) were diagnosed with PCOS according to the Rotterdam criteria. 88% had acne, 68% hirsutism, 46% irregular menstrual cycles, 39% BMI over 25 kg/m^2, 13.5% had apple-type obesity and 4.7% acanthosis nigricans. 104/170 patients were offered metformin 500 mg tablets three times daily who did not want to take the anti-androgenic contraceptive pill. Body mass index, waist-to-hip ratio, Global Acne Score, Ferriman-Gallwey score and the regularity of menstrual periods were registered every three months.

12 patients had transient vertigo, diarrhoea or abdominal discomfort at the beginning of the treatment; four patients discontinued metformin because of them. A 3 to 42 month follow-up period of 47 patients on metformin could be evaluated. Irregular menstrual cycles of 13/24 patients became regular within three months of treatment. Six women became pregnant during the 1st–17th months on metformin, two continued metformin throughout and delivered healthy babies. One of them who suffered from pre-eclampsia during all of her previous pregnancies remained symptom-free throughout this pregnancy. The Global Acne Score diminished from 20.0 ± 12.9 to 6.3 ± 7.1, and the Ferriman-Gallwey score from 16.9 ± 8.3 to 10.5 ± 6.8 in 15 patients during the first 12 months of treatment.

The direct comparison of these results cannot be made to those who opted for contraception because of the different indication of treatment, furthermore the metformin group contained more severe cases in many respects (obesity, acne and hirsutism). Despite this, metformin treatment resulted in favourable improvement of the symptoms in patients with PCOS and seems to be suitable for long-term use.

P494

Hand-Foot-Uterus syndrome in a patient with secondary amenorrhea: a rare case

Neslihan Kurtulmus[1], Kivanc Cefle[2], Sukru Ozturk[2], Sukru Palanduz[2], Solmaz Deniz[3] & Mahmut Remzi Savas[3]
[1]Vakif Gureba Educational Hospital, Department of Endocrinology, Istanbul, Turkey; [2]Istanbul University, Meducal Faculty of Istanbul University, Department of Genetic, Istanbul, Turkey; [3]Vakif Gureba Educational Hospital, Department of Radiology, Istanbul, Turkey.

Introduction

Hand-Foot-Uterus Syndrome (HFUS) is a rare genetic condition. It is characterized by abnormalities of the hand, foot, reproductive tract, and urinary tract. There are also wrist- and ankle-bone fusions, very small feet, short great toes, urinary-tract abnormalities, duplications of the reproductive tract in women, urethral openings on the underside of the penis in men, and curved penis. The genetic associations of HFUS is not fully understood. It seems that the most cases of HFUS is caused by a mutation in HOXA13, but other genes may be involved.

Case

We present a 27-years-old woman who had a history of secondary amenorrhea for several years. On physical examination, her secondary sexual chacteristics were normal, but she had strabismus and small feet and hands, as well as clinodactyly. We referred her to Genetic Department. X rays of the hands and feet, and imaging of reproductive tract were performed. On x-rays, clinodactyly, traphesium/scaphoid fusion and fusions of other bones in hands, and shortened thumbs were detected. On ultrasonography and MRI, There were bicornate uterus and bilateral ovarium hypoplasia of reproductive tract. Serum Luteinizing hormone and Follicule stimulating hormone were elaveted to 50.5 IU/L and 114 IU/L, respectively and estradiol level was low to 20 ng/ml. Other pituitary hormones and laboratory findings were within normal ranges. She had a normal karyotype(46XX). MRI of pituitary gland was normal. After above-mentioned physical and radiological examination, diagnosis of HFUS was obtained. In addition to these, she had a secondary amenorrhea along with HFUS.

Conclusion

It is very often observed the case of amonerrhea at endocrinologic clinics. The ethiological reasons are generally similar and caused by over or pituitary disorders. However as we present in our case that the amonerrhea could accompany to other syndrome. To our best knowledge that this is the first case of HFUS associated to amonerrhea.

P495

Neonatal ventricular septal defect and late diagnosis of Turner syndrome

Serafino Lio & Marino Cibin
Dpt Internal Medicine 3, Oderzo-TV, Italy.

The high morbidity and mortality rate of women with Turner syndrome (TS) is primarily a results of the cardiovascular complications and so it is necessary an accurate and precocious diagnosis of this disease. Congenital cardiac anomalies, whose causes remains unknown, are common in TS (21–40%), in particular among patients with 45 X; between these the ventricular septal defect (VSD) is very rare (in a recent review, 3/1092 cases) (Gravholt 2004) and so in neonatal with VSD may not suppose the presence of TS.

We describe a female with TS (45 X, dic.(Y,15)(q12;p11.2) and VSD. Pt is a 17-year-old Caucasian female who first presented to endocrine evaluation for no pubertal development. The patient, born at term of normal pregnancy, at 7-months-old is operated of VSD. Clinically is present short stature (<third percentile) and cubitus valgus. Endocrine function show an hypergonadotropic hypogonadism. The chromosomal analysis showed 45 X and the presence of dicentric chromosome (Y,15)(q 12;p11.2) and so the patient it has been submitted to prophylactic laparoscopic excision of the gonads for risk of gonadoblastoma. Moreover, a hormone replacement therapy has been begun with induction of puberty.

In summary, this is a patient with mosaic TS with VSD; it is important remember that the VSD is rare but possible in TS and so suggested in these patients for precociously treated each problem of this syndrome.

P496

Interleukin-6-producing pheochromocytoma presenting with fever of unknown origin

Ozer Taranoglu[1], Sema Yarman[1], Esma Altun[2], Taner Bayraktaroglu[1], Meral Mert[1] & Refik Tanakol[1]
[1]Istanbul University, Istanbul Faculty of Medicine, Department of Internal Medicine, Division of Endocrinology and Metabolism, Istanbul, Turkey; [2]Ministry of Health, Istanbul Research and Training Hospital, Department of Internal Medicine, Istanbul, Turkey.

Pheochromocytomas are tumors capable of producing catecholamines and a variety of biologically active resulting in unusual clinical manifestations. We report the case of 18-year-old female with pheochromocytoma exhibiting fever of unknown origin. She had experienced continuous fever (ranging between 37.1–41 °C) and chills for previous several weeks. Antipyretics had been ineffective in lowering the body temperature and she was referred to our hospital when an adrenal incidentaloma of 5.5 cm diameter was detected during evaluation for fever. At the time, specific and nonspecific blood and urine cultures yielded in no pathogenic agents with negative viral serological markers. On admission, physical and laboratory examinations revealed normotension, a fever of 38.7 °C, markedly elevated sedimentation rate and CRP level, anemia, thrombocytosis, anemia with high ferritin levels and elevated levels of urinary norepinephrine and normetanephrine. A diagnosis of pheochromocytoma was made and the fever resolved promptly after beginning treatment with adrenergic blockers. Serum interleukin-6 level was measured to be 12.5 (normal; <3.0) pg/ml before adrenergic blockade was started. Additional measurement of 9.9 pg/ml was obtained in the second month of the treatment. She was sent to operation where complete resection of the tumor was achieved. It is suggested that the elevation of interleukin-6 might play an important role in clinical and biochemical inflammatory response. To our knowledge, our paper represents a rare case of interleukin-6 secreting normotensive pheochromocytoma associated with clinical markers of inflammation. Pheochromocytoma should be considered in the differential diagnosis of unexplained fever even for normotensive patients.

P497

Neonatal severe hyperparathyroidism associated with a novel de novo heterozygous R551K inactivating mutation and a heterozygous A986S polymorphism of the calcium-sensing receptor gene

Judit Toke[1], Gábor Czirják[2], Attila Patócs[1], Balázs Enyedi[2], Péter Gergics[1], Violetta Csákváry[3], Péter Enyedi[2] & Miklós Tóth[1]
[1]Semmelweis University, 2nd Department of Medicine, Budapest, Hungary; [2]Semmelweis University, Department of Physiology, Budapest, Hungary; [3]Markusovszky Teaching Hospital of Vas County, Department of Pediatrics, Szombathely, Hungary.

Objectives
Neonatal severe hyperparathyroidism (NSHPT) is induced by inactivating mutations of human calcium-sensing receptor (CaSR). We report the case of a now 11 year-old boy with NSHPT. We characterize a novel inactivating mutation with the results of some functional analyses.
Case
In the neonatal age the patient presented the clinical syndrome of NSHPT. At the age of 6 years, persisting hypercalcemia without clinical symptoms was documented, and the patient remained completely symptom-free without parathyroid surgery until his present age of 11 years.
Methods
The entire coding region of the CaSR gene of the patient, and exons 6 and 7 from his family members were sequenced. Functional investigation was performed in HEK-293 cells, transiently transfected with wild type and mutant CaSR plasmid constructs.
Results
Sequence analysis revealed a novel de novo heterozygous mutation at codon 551 (AGG→AAG) predicting a change of arginine to lysine (R551K) and a known heterozygous polymorphism (A986S) on the same allele, which was inherited from the father. We demonstrated that the novel R551K mutation significantly reduced the calcium sensitivity of CaSR (EC50: from 3.38 ± 0.62 to 6.10 ± 0.83 mmol/l) which was not alleviated by the simultaneous presence of A986S polymorphism.
Conclusion
We present the fourth NSHPT case induced by a novel de novo heterozygous inactivating mutation (R551K) of the CaSR gene. The disease gradually reverted to a symptomless, benign condition resembling familial hypocalciuric hypercalcemia without any surgical intervention.

P498

A case of paraganglioma of *glomus caroticum* with lung metastasis

Ferhan Mantar[1], Meral Mert[2], Ayse Kubat Uzum[2], Ferihan Aral[2] & Nese Colak Ozbey[2]
[1]Okmeydani Training Hospital, Department of Internal Medicine, Endocrinology and Metabolism, Istanbul, Turkey; [2]Istanbul University, Istanbul Medical Faculty, Department of Internal Medicine, Division of Endocrinology and Metabolism, Istanbul, Turkey.

Introduction
We report a case of paraganglioma of glomus caroticum with lung metastases treated with 150 mCi 131I-MIBG.
Case
A 25-year-old woman was referred to our department for cachexia. She had low BMI (18) normotension, mild normochromic normocytic anemia with a mass under left mandibula. Radiologic imaging of neck revealed that $24 \times 36 \times 45$ mm diameter mass surrounding of carotis externa and interna at the level of bifurcation. She underwent surgical operation. Pathological examination revealed that the tumor was paraganglioma with index of Ki-67 2–3%. Further endocrine evaluation showed increased urinary normetanephrine (607 microgram/dl (normal: 88–444) and dopamine 405 microgram/dl (normal: 65–400). Radiological scan of thorax, abdomen and cervical region were performed for evaluation of metastases. Bilateral,small lung nodules were shown in thorax CT. 131I-MIBG scintigraphy was positive only on the right side of neck and octreotide scan was negative. Lung biopsy was performed for pathological confirmation of metastases. Pathological examination revealed that the lung tumors were paraganglioma with index of Ki-67 2–3%. 131I-MIBG therapy was performed with 150 mCi. Post-therapeutic MIBG scan was showing no uptake in the lung. No further elevation of urinary cathecolamine metabolites was observed during follow-up. Mass size and clinical findings were stable.
Conclusion
Paragangliomas are very rare tumours in the head and neck but should be considered in the differential diagnosis of neck masses. As these tumours can form part of a familial syndrome, long-term follow-up is necessary.

P499

Intracranial giant sarcoma in an acromegalic patient after radiotherapy

Ferhan Mantar[1], Meral Mert[2], Laika Karabulut[1], Yunus Aydin[3] & Murat Musluman[3]
[1]Okmeydani Training Hospital, Department of Internal Medicine, Endocrinology and Metabolism, Istanbul, Turkey; [2]Istanbul University, Istanbul Medical Faculty, Department of Internal Medicine, Division of Endocrinology and Metabolism, Istanbul, Turkey; [3]Sisli Etfal Training Hospital, Department of Neurosurgery, Istanbul, Turkey.

Introduction
Primary pituitary sarcoma causally unrelated to radiotherapy, but an increased risk of second brain tumors continues beyond 20 and 30 year after treatment.

We report an acromegalic patient with intrasellar giant adenoma invased suprasellar and nasal cavity during follow-up after radiotherapy.
Case
A 39-year-old woman had transsphenoidal and transcranial operation in 2002 and 2003 respectively. She had also conventional external beam radiotherapy after surgery. Thereafter, she treated with octreotide LAR 30 mg/month at the same time. Cabergoline had added one year later because of resistance of the therapy. She applied to outpatient clinic with severe headache and nevralgia on her face. Huge sellar mass invased suprasellar region and cavrnous sinus was found in sellar MRI. Sellar mass was bigger three times compared to previous MRI scan which was performed only three months ago. Growth hormone and IGF-I levels were markedly elevated. She underwent hypophyseal surgery immediately. Pathological examination and immunohistochemical stains revealed undifferentiated pleomorphic sarcoma. Though the surgery was performed, sellar mass regrew through the nasal cavity, clinical signs were progressively worsen and she died within two months.
Conclusion
In an acromegalic patient, persistently raised levels of growth hormone may associated with increased risk of second brain tumors in patients with pituitary adenoma treated with surgery and radiotherapy. An increased risk of second brain tumors usually appear more than 5 years after radiotherapy, in our case sarcomateous transformation was seen only two years later. It might be related either radiotherapy or high levels of GH or both.

P500

Short stature and neurofibromatosis type 1 – issues of diagnosis

Letitia Leustean, Cristina Hurjui, Cristina Preda, Carmen Vulpoi, Liliana Moisii & Eusebie Zbranca
University of Medecine and Pharmacy "Gr. T. Popa", Iasi, Rwanda.

Neurofibromatosis type 1 (NF1) is an inherited disorder characterized by formation of neurofibromas in the skin, brain and other parts of the body, in association with skin pigment changes. It is well known that this condition may be a risk factor for short stature with growth hormone deficiency (GHD) in children, due to suprasellar lesions. We present the case report of a 9-year-old boy admitted in our Service for short stature (-2DS). Physical examination revealed 'café-au-lait' spots, underarm and inguinal freckles (the same as his father and great father). No neurofibromas were found. The ophthalmologic exam was normal: no evidence of Lisch nodules or optic glioma. Psychological evaluation was also normal (IQ = 105). The serum GH levels were low (1.7 ng/dl), with no response to exercise test (1.3 ng/dl) and with inappropriate response to arginine provocative test (2.3 ng/dl). The serum levels of IGF-1 were low (25 ng/dl). Magnetic resonance imaging demonstrated no intrasellar mass lesion, but foci involving the cerebellum, globus pallidus and cerebral pedunculy. The final diagnosis was pituitary dwarfism and NF1, but with no connection between the two. The presence of GHD in short children with NF1 independent of organic, pituitary change is frequently quoted in the literature, the latest studies suggesting that NF1 could represent a novel etiology for GHD.

P501

Paraneoplastic Cushing's syndrome presenting as psychosis – case report

Cristina Spiroiu, Aurelian Emil Ranetti, Ana- Maria Mihai, Adina Mazilu & Nicolae Diaconu
Central Military Emergency Hospital, Bucharest, Romania.

We present the case of a 51 years old woman, nonsmoker, without relevant past medical history, who presented with acute psychotic state starting the third day of treatment with prednisone 30 mg indicated for allergy. She had also arterial hypertension and a significant and progressive loss of proximal muscle strength in her legs. The initial evaluation showed hyperglycemia, metabolic alkalosis and severe hypokalemia. Basal plasma cortisol was high (>90 μg/dl) and did not suppress after high dose of dexamethasone. Abdominal computed tomography revealed bilateral adrenal hyperplasia; thoracic computed tomography showed a lung mass, which proved to be a small cell lung carcinoma at fiberoptic bronchoscopy with brushing and cytology exam. The psychotic state resolved in a couple of days; despite intensive oral and intravenous potassium supplement, high doses of spironolactone and aminoglutethimide, the serum level of potassium increase but did not normalize. Combination chemotherapy did not improve the patient's condition. She died a month later of severe lung infection.

P502

Endocrine tumour registry – tools for endocrine epidemiology

Corin Badiu[1] & Sorin Sava[2]
[1]Institute of Endocrinology, Bucharest, Romania; [2]Infoworld, Bucharest, Romania.

Endocrine tumour registry is a web-based system which is divided in several categories of endocrine tumours: pituitary adenomas, thyroid cancer, parathyroid tumours, adrenal and other types.

The program is intended to give epidemiological data concerning the prevalence of each type, age and sex distribution, therapy and basic results of it. The centres involved are the medical universities and expertise centres in Romania, centres in which there are enough resources to diagnose, treat and monitories treatment of various endocrine tumours.

The data entered are personal patients ID's, tumour type, extension and complications, type of treatment and its results as tumour dimensions, and endocrine tumour markers. Thyroid cancer registry and pituitary tumour registry are subdivisions of the system.

From each centre, 2 persons dedicated to enter data in the system are designated by the system administrator, which will be located in the Institute of Endocrinology in Bucharest. The access to the site is web secured. The network started with 10 centres and will be developed afterwards using the already existent resources. An import software filter for this site was developed, which will allow dynamic recording of cases from an institutional database (in the last 5 years) towards the registry. This hardware and software infrastructure is the base of future epidemiological public health surveys in this thematic area.

P503

Tumour induced osteomalacia – a phosphaturic mesenchymal tumour

Polyxeni Bompoti, Grigoris Effraimidis, Kalliopi Bilariki, Alkmini Nikou & NC Thalassinos
Department of Endocrinology, Diabetes and Metabolism, Evangelismos General Hospital, Athens, Greece.

A case of a 29-year old woman presented with a 6-year history of bone pain located in the lower spine and gradually extended to the spinal skeleton and the lower extremities, worsening by activity. The progressive symptoms and the established weakness finally led to patient's complete disability. The investigation revealed low serum phosphorus and elevated 24 h urinary phosphate excretion, normal serum calcium and 24 h urine calcium excretion, normal to normal-high PTH and elevated serum alkaline phosphatase, particularly the bone isoenzyme. Calcidiol levels were normal and calcitriol values were low. Iliac bone biopsy showed osteomalacia. Renal phosphate wasting can occur in disorders of vitamin D metabolism, in the Fanconi syndrome or in primary phosphaturic syndromes, which can be inherited or acquired, either as idiopathic disorders or in association with mesenchymal tumors (tumor-induced osteomalacia TIO). TIO is more likely to be the diagnosis for this patient based on symptoms and the above findings (osteomalacia, acquired hypophosphatemia, renal phosphate wasting, inappropriately low plasma calcitriol concentration, negative family history). The major diagnostic challenge was the identification of the primary tumor. The scintigraphy using indium-111 labeled octreotide was negative. The total body CT scan showed a soft tissue mass, extensive osteolysis of the ala and the body of the left ilium and extension to the ipsilateral limbus of the acetabulum. FGF23, a potential phosphaturic hormone which has been implicated in TIO, was highly elevated in our patient (1625 RU/l normal values <100). She was treated with calcitriol 3 μg/day, phosphate 3 gr/day and calcium 1500 mg/day until the removal of the causative tumor, with substantial improvement. The surgical resection of the tumor took place at the Royal National Orthopeadic Hospital, Stanmore-Middlesex. The histology demonstrated a phosphaturic mesenchymal tumour without a high-grade component. The excision of the tumor led to reversal of the biochemical and the clinical abnormalities. Unfortunately, FGF23 levels were not measured postoperatively.

P504

Unusual onset of Graves' disease – case report

Cristina Cristea[1], Adriana Ciornohuz[2] & Eusebie Zbranca[1]
[1]University of Medecine "Gr.T.Popa", Iasi, Romania; [2]Medical Cennter "Praxis", Iasi, Romania.

Graves' thyrotoxicosis frequently occurs after delivery through immune rebound mechanism. A 34 years old patient, in postpartum period was referred to rheumatologist for gradually gait impairment. Examination showed only weakness of pelvic girdle muscles which required an extensive differential diagnosis including: neurological diseases and inflammatory/metabolic/toxic myopathies. Routine lab tests were unremarkable except low cholesterol (128 mg/dl) and slightly increase of total alkaline phosphatase (ALP). Immunological and inflammatory tests were negative and muscle enzymes were within normal range. Three month later the patient had significant weight loss, persistent muscular symptoms and bright-eyed stare. On examination performed by the endocrinologist Graves' disease was considered and confirmed by abnormal levels of TSH, FT4 and TRAb. The patient was treated with antithyroid drugs. After eight weeks muscular strength became nearly normal. FT4 was normal (14.4 pmol/l), but ALP level increased up to 3 times normal. Serum calcium and phosphorus were normal and so were the liver tests. Elevated ALP and osteocalcin levels were included in an accelerated bone turnover, which characterized hyperthyroidism.

Discussion

In women diagnosed with Graves' disease during the ages of 20 to 35 years, 66% have a postpartum onset. The diagnosis is often quite simple, but it can be challenging when extrathyroidal manifestations occur early in the course of disease.

P505

Growth hormone replacement therapy and metabolic parameters in adult-onset GH-deficiency: long-term effects.

Claudia Giavoli, Emanuele Ferrante, Silvia Bergamaschi, Ronchi Cristina L, Doanadio Francesca, Lania Andrea, Spada Anna & Beck-Peccoz Paolo
Department of Medical Sciences, University of Milan, Endocrine and Metabolic Unit, Fondazione Ospedale Maggiore Policlinico IRCCS, Milan, Italy.

Aim of this study was to evaluate the impact of rhGH treatment on glucose and lipid metabolism in in 26 patients (17M and 9F, age 47.0 ± 11.1 years) with adult onset GH deficiency. Metabolic parameters (fasting glucose and insulin, glycated haemoglobin, lipid profile, body composition, OGTT) and indices of insulin resistance (IR) and sensitivity (IS), i.e. homeostasis model assessment (HOMA-IR and derived ISI-HOMA), quantitative insulin check index (QUICKI), ISI-composite, insulinogenic index (IGI) and area under the curve (AUC) for glucose and insulin derived from OGTT, were evaluated at baseline, after 1 ($n=26$) and 3 years ($n=15$) of rhGH therapy (GH dose: 0.3 ± 0.2 mg/day). At baseline, all patients had low IGF-I levels, high BMI and percent of body fat. Two out of 26 patients had impaired glucose tolerance (IGT). After 1 year, IGF-I normalization, BF% reduction and lean mass increase occurred ($P<0.005$) and persisted after 3-years treatment. Fasting insulin, glycated haemoglobin, total cholesterol, triglycerides, HOMA-IR, QUICKI, ISI-HOMA, AUC for insulin, IGI and ISI-composite did not differ after 1 and 3 years from baseline, while glucose and LDL-cholesterol levels had a transient increase and reduction after 1 year, respectively. After 3 years HDL-cholesterol levels increased ($P<0.05$) and basal insulin secretion (HOMA-B%) decreased ($P<0.05$). AUC for glucose significantly increased after 1 and 3 years of treatment ($P<0.02$). One patients progressed to diabetes after 1 year, while 5 showed IGT after 3 years. In conclusion, rhGH therapy improves body composition and lipid profile, but causes a small transient increase in fasting glucose. Since deterioration of glucose tolerance, as indicated by increase in AUC for glucose and development of IGT, a strict monitoring of glucose metabolism during long-term GH replacement therapy should be performed.

P506

Conventional glucocorticoid replacement therapy in patients with Addison's disease: effects on metabolic and bone parameters

Marcella Balbo, Roberta Giordano, Lorenza Bonelli, Rita Berardelli, Andreea Picu, Andrea Benso, Angela Bertagna, Ezio Ghigo & Emanuela Arvat
Div Endocrinology And Metabolism, Dpt Internal Medicine, University Of Turin, Turin, Italy.

In primary adrenal insufficiency hydrocortisone or cortisone are commonly used at doses of 30–37.5 mg/day as replacement therapy, though recent studies showed that cortisol normal production is about 5.7 mg/m^2, equivalent to 20 mg/day of hydrocortisone, suggesting that supraphysiological doses are used. In 19 Addison's disease patients (8 M, 11 F, 23–71 yr) under conventional glucocorticoid replacement therapy (37.5 mg cortisone/day) with low DHEAS levels, BMI, fasting glucose and insulin, glucose response to OGTT, cholesterol, triglycerides (TG), homocysteine, calcium, phosphate, PTH, 25OH-vitaminD, bone formation and resorption markers as well as intima-media thickness (IMT) by eco-doppler ultrasonography, bone mineral density (BMD) by a DEXA and vertebral morphology by spinal radiograph were measured. Mean BMI was in the upper range of normal, though higher than 25.0 kg/m^2 in 8 patients; mean fasting glucose, insulin, HOMA as well as glucose response to OGTT were normal, though HOMA were high in 5 patients; mean lipid profile was in the normal range; none of the patients had low HDL levels, whereas LDL and TG were higher than normal in 3 patients. Homocysteine was normal, though high in 5 patients. IMT was below 0.9 mm in all patients. Decreased mean BMD was found (T score < -1.0), while osteoporosis (< -2.5) was present in 2 eugonadal men and 3 postmenopausal women, vertebral fractures were found in 1 osteopenic and 1 osteoporotic patient. Mean calcium, phosphate, PTH, 25OH-vitaminD and osteocalcin were in the normal range, whereas urinary cross-laps were higher than normal. In conclusion, our preliminary results suggest that conventional glucocorticoid replacement therapy, associated with low DHEAS levels do not have a significant impact on glico-lipidic metabolism in patients with primary hypocortisolism, even in presence of slight overweight. On the other hand, increased risk of bone loss and vertebral fractures is confirmed in these patients.

P507

Gastric electrical stimulation in patients with severe diabetes mellitus associated gastroparesis – a cost benefit analysis

Mark J Hannon[1], Obada Yousif[2], Sean Dineen[3], Christopher J Thompson[4], Domhnaill J O'Halloran[1] & Eamonn MM Quigley[1]
[1]Cork University Hospital, Cork, Ireland; [2]Wexford General Hospital, Wexford, Ireland; [3]University College Hospital Galway, Galway, Ireland; [4]Beaumont Hospital Dublin, Dublin, Ireland.

Introduction

The management of diabetic gastroparesis resistant to medical therapy is very difficult – the most severely affected patients often spend many days as hospital inpatients with intractable nausea and vomiting and consequent dehydration, leading to a marked reduction in quality of life. Recently, gastric pacing (also known as gastric electrical stimulation (GES)) has been tried in these patients as a means of correcting the physiological deficit. It has shown promise in some international trials although patient numbers are still quite small. It has seen use in four patients in Ireland. Here we outline our experiences with these patients.

Methods

The records of all four patients with gastric pacemakers inserted were reviewed. The number of days spent as an inpatient by each patient before and after pacemaker insertion was calculated. From these figures, a cost benefit analysis was performed to see if the commencement of GES led to a reduction in the costs incurred due to inpatient admission for gastroparesis. The costs were calculated using 2004 bed day costs for Cork University Hospital from the Irish Health Service Executive (costings department).

Results

The bed cost for the inpatient stays of all four patients in the twelve months preceding pacemaker insertion was €306,399. The corresponding extrapolated figure for the year following pacemaker insertion was €322,543. There was no HbA1c change following GES.

Conclusion

Severe diabetic gastroparesis leads to recurrent patient admissions and places a large cost burden on the Irish healthcare system. However, the cost benefits of GES are as yet unproven in Ireland. There is very little data available worldwide which convincingly shows a cost benefit with GES, although some studies have shown a subjective improvement in patients' symptoms. Therefore, more research is needed on this contentious area.

P508

Levels of serum and salivary cortisol during low dose ACTH test in young adult-onset diabetes mellitus Type 1 patients

Katerina Simunkova, Karel Vondra, Richard Hampl, Martin Hill, Hana Kvasnickova, Jana Vrbikova & Luboslav Starka
Institute of Endocrinology, Prague, Czech Republic.

Detailed information on adrenal function in autoimmune Type 1 diabetes with onset in adults is still lacking. This work aimed at gathering own data on adrenal response to low-dose (1 μg) ACTH in blood and saliva.

Twenty-three diabetics were investigated; age 44 ± 10 yrs (mean ± S.D.), age at diagnosis 28.5 ± 10 yr, disease duration 15 ± 8 yr, BMI 24.5 ± 2.7 kg/cm^2, HbA1c 7.2 ± 1.2%.

The control group had 16 healthy subjects; age 27 ± 6 yr, BMI 21.7 ± 2.3 kg/cm^2. Neither group showed any clinical signs of adrenal disorders.

The study was approved by the Ethical Committee.

Adrenal reserve was tested by low dose ACTH test. Fasting blood and saliva were collected between 8–9 a.m. Blood and salivary cortisol were determined at times 0, 20, 30, 40, 60 min, ACTH and adrenal autoantibodies at 0′ only.

Maximum stimulated value in serum above 500 nmol/l was reached in 15 out of 23 patients (65.2%), normal-responders, NR. This cut off value was not reached in 8 patients (34.8%), low-reponders, LR. The results were compared with the control group (C).

NR: Basal and stimulated serum cortisol levels did not differ significantly from those in controls, while salivary cortisol in this subgroup was significantly lower at 20th min and 30th min, $P < 0.05$.

LR: Both basal and stimulated serum cortisol, as well as salivary cortisol were significantly lower than C, $P < 0.001$ for all times.

LR did not differ from NR in either average insulin doses, or HbA1c or basal ACTH value. Adrenal cortex autoantibodies were negative in all subjects.

In conclusion, surprisingly, in 34.8% of young adults-onset with diabetes mellitus Type 1 without signs of adrenal autoimmunity, in 1 μg ACTH test serum cortisol levels corresponding to subclinical hypocorticalism were found. Investigation of salivary cortisol brought additional information, which should be further evaluated.

The study was supported by grant IGAMZCR No.NR/9154-3.

P509

Long-term pegvisomant treatment in acromegaly

Valentina Gasco, Alessandra Mainolfi, Damiano De Giorgio, Ezio Ghigo & Silvia Grottoli
University of Turin, Division of Endocrinology, Turin, Italy.

In acromegalic patients not suitable for first-line surgical treatment, pharmacotherapy is a valuable choice. Depot somatostatin analogs (SSA) represent efficacious and well-tolerated drugs; however, they normalize hormonal parameters in no more than 65–75%. Pegvisomant (PEGA), a GH receptor antagonist, has been shown to normalize IGF-I levels in more than 90% of patients. We report our experience in 13 acromegalic patients (7 M, 6 F; age: 50.2 ± 3.9 yrs; 7 macroadenomas, 3 microadenomas and 3 empty sella) treated for 3–44 months (mean 28.8 ± 3.7 month) with PEGA (5–25 mg/day, mean 15.8 ± 1.6 mg/day) alone (n. 8) or combined with SSA (octreotide 10–30 mg/month). Diabetes mellitus or IGT was present in 5 patients. IGF-I and IGFBP-3 levels, glucose metabolism, clinical picture, MRI and safety parameters were monitored. Basal IGF-I and BP-3 levels were 858.3 ± 90.4 μg/l and 6.2 ± 0.4 μg/ml, respectively. During PEGA IGF-I normalized (222.4 ± 20.6 μg/l, $P < 0.005$) in 12/13 patients within 12 months with a mean PEGA dose of 15.8 ± 1.6 mg/day. Also IGFBP-3 markedly decreased (3.8 ± 0.3 μg/ml, $P < 0.005$). Morning glucose levels decreased from 104.2 ± 6.3 mg/dl to 92.6 ± 6.2 mg/dl ($P < 0.05$) but HbA1c didn't change (5.7 ± 0.2% vs 5.9 ± 0.3%) even when only diabetic and IGT patients were considered (7.1 ± 0.9% vs 6.8 ± 0.4%). All patients improved clinical picture and acromegalic signs and symptoms. No change occurred at pituitary MRI imaging in any patient. One patient had slight and transient increase in transaminases. One female patient complained abdominal lipodistrophy in the injection site. Thus, PEGA normalizes IGF-I in almost all patients, improves the clinical picture and also glucose levels, in front of good safety profile.

P510

The assesment of life quality satisfaction in women with Turner's syndrome

Beata Wikiera, Elzbieta Szpich, Ewa Barg & Anna Noczynska
Medical University, Department of Endocrinology and Diabetology for Children and Adolescents, Wroclaw, Poland.

Quality life satisfaction is important for personal resources analysis and perspectives for coping with ilness.

The aim of the study was to present a psychological portrait of a woman with Turner's syndrome (TS) and assessing perspectives for increased well-being of such patients. The study concerns psychological aspects of TS women's own assessment of their health and illness.

The area of interest was:

- TS patients' own health assessment
- life quality satisfaction experienced by the above mentioned patients
- the level of Optimism Available in each patient as an important element of natural resources.

Patients and methods

26 women with TS aged 18–25 participated in the study. All the patients have experienced many years of treatment and coping with their illness.

The evaluation was based on medical files analysis, an individual patient – doctor and patient – psychologist conversations. The information was gathered in the form of structured interview containing questions concerning health – illness aspects, current life and family situation and life aims of the women analysed. SWLS – Quality of Life Assessment Test and LOT-R Life Orientation Test were used to assess the level of optimism.

Results

The backgrounds of the patients tested varied. In general, the assessment of their own health condition was positive. Establishing a family was placed as No 1. life aim. Life contentment was high. Average results on AWLS scale were 48%. High results on AWLS scale were 44%. As concerns Optimism Available, 52% of the patients described their optimism level of medium, 28% as high and 20% as low. Optimistic patients seem more effective in coping with stress, which means a potentially better adjustment to changing life situations.

Conclusions

The behaviour and suffering levels in patients with TS are closely related to their natural resources. Proper specialist care and general social support may greatly facilitate such patients' natural resources.

P511

Pheochromocytoma in pediatric age

Amaya Fernández-Argüelles García[1], Cristóbal Morales Portillo[1], Cristina Campos Martín[1], Rafael Espino[2] & Luis López-Canti[2]
[1]Universitary Hospital Virgen Macarena, Seville, Spain; [2]Universitary Hospital Ntra. Sra. de Valme, Seville, Spain.

Introduction

Pheocrhomocytomas are rare tumors, principally benigns, and with high risk of morbimortality because of secretion of big amountos of catecholamines. They are an unfrequently cause of arterial hypertension in pediatric age but physicians must remember it because they can be diagnosed, treated and cured in a proper way.

Objectives

To evalue the cases reports of pediatric pheochromocytoma found in our area, to analyze the differences in diagnosis, pronostics and treatment if we compare with adult age.

Material and method

Demographic, analitical, morphological and histological characteristics are analized in the three cases of pheochromocytomas found in our area in last fifteen years. A bilateral pheochromocytoma with asynchronous presentation is exposed.

Results

The average age was 12,5 years. The both children were male. Clinical presentation was arterial hypertension (66%), tonicoclonic seizures (33%), and atypical symptons as hypoglycemias, arterial hypotension, tremors and malnutrition (weight < p3). The catecholamines determination in 24 hours tinkles, abdomen TC, I123 gammagraphy were the way to diagnose these tumors. Before surgery a α and β block was required. Histological study confirmed the benignancy of three tumors.

Conclusions

-Atypical symptons in presentation, extraadrenal and bilateral tumors, are more frequent in children than in adult age.

-Malignant pediatric pheochromocytomes are very unusual.

-Physicians shoud practise a genetic study to these children, because of the high association with herediatary syndromes as Von Hippel-Lindau disease.

P512

Ectopic localization of the pituitary bright spot in a patient with idiopathic central diabetes inspidus

Nur Kebapci[1], Erhan Erdogan[2] & Baki Adapinar[2]
[1]Eskisehir Osmangazi University School of Medicine, Department of Endocrinology and Metabolism, ESKISEHIR, Turkey; [2]Eskisehir Osmangazi University, School of Medicine, Department of Radiology, ESKISEHIR, Turkey.

We report the MRI findings of an unusual case of posterior pituitary ectopia (PPE) in a young female patient with idiopathic diabetes inspidus (DI). She was 29 years- old and presented with polydipsia (7–8 lt/day), and polyuria (7–8 lt/day) that had been present for about 5 months. She had regular menstrual cycles. She didn't have any history of significant medical illness or any history of head trauma. An 8-hour fluid deprivation test followed by desmopressin (DDAVP, 0.03 μg/kg SC) was performed in which the results were consistent with pituitary DI. She had complete correction of her thirst after DDAVP treatment was started (10 μg, bid,) and her water intake was limited to 3 L/d and urinary output decreased to 2.6 L/d. We evaluated the patient with dynamic pituitary MRI to see whether she had any problems in the hypothalamo-pituitary axis. Her pituitary MRI showed a normal appearing adenohypophysis without any space occupying lesions, the infundibulum was in the midline and of normal thickness. The pituitary bright spot was not observed at its normal location within the sella, instead we observed two discrete foci of hyperintensity at the median eminence of hypotalamus. Insulin hypoglycemia test revealed increased cortisol (>20 μg/dl) and growth hormone (>20 ng/ml) responses. Chest radiographs were normal. Analyses of lymphocyte subgroups for Sarcoidosis were in normal range. C-ANCA was negative for Wegener's granulomatosis. Control MRI 6 months later revealed exactly the same findings as the initial MRI. This case is one of the few cases in the literature since it is a case of PPE with preserved anterior pituitary functions and without any space occupying lesion in the sella and traumatic or infiltrative lesion of the infundibulum.

P513

Diabetes insipidus due to pituitary metastasis of breast cancer

Ayse Kubat Uzum, Ilkay Kartal, Meral Mert & Ferihan Aral
Istanbul University, Istanbul Faculty of Medicine, Deparment of Internal Medicine, Division of Endocrinology and Metabolism, Istanbul, Turkey.

Introduction
We have reported a case of breast carcinoma complicated by diabetes insipidus due to pituitary metastasis.
Case
A 47 years old woman had been referred our clinic with the symptoms of polyuria, polydypsia, weight loss, and fatigue. She had a diagnosis of breast carcinoma for six years, underwent radically mastectomy, chemotherapy and radiotherapy, subsequently. Vertebral metastasis was detected and local radiotherapy was performed six months before admission. Symptoms of polyuria, polydypsia began in the first years of the disease and got worse over time. Her skin turgor was reduced and her mouth was completely dry. She had 11L urinary output and oral intake in a day. Laboratory findings on admission were as follows: serum sodium: 144 mmol/L (135–146 mmol/L), potassium: 4,9 mmol/L (3.5–5.1 mg/dl) and chloride: 100 mmol/L (95–107 mmol/L), serum creatinine: 0.4 mg/dL (0.7–1.4 mg/dl). Free T4: 17.7 pmol/L, TSH: 1.23 mIU/L, LH: 3,2, FSH: 1,9, estradiol: 23 mIU/L, Prolactin: 0,6 ng/ml, cortisol: 21.3 microg/dl. According to urinalysis, the density of the urine was 1000. Urinary and plasma osmolality were 101 and 324 mOsm/L, respectively. Her gonadotropine levels were not compatible with menoposis. On the day after admission, dDAVP 0,1 mg/day was administered orally. The urinary output decreased to 3 L/day and the oral intake was 4 L/day. Magnetic resonance imaging of sella revealed a huge mass filling sella turcica, arising from suprasellar cistern, surrounding cavernous sinus and compressing to optic chiasm and infindubulum. The mass was compatible with breast cancer metastasis to hypophysis, and radiotherapy was performed. Three months after irradiation, panhypopituitarism had developed. She is still alive under full replacement therapy.
Conclusion
Extension of breast cancer to the pituitary gland is a rare and late complication. Although life expectancy is limited in advanced breast cancer, hormonal insufficiency should be corrected to increase the life quality.

P514

Multicystic dysplastic kidney – a potential accelerant of complications in type I diabetes mellitus

A Hillick, B Callaghan, EH El Tayeb, LA O'Shea & AG Stack
Department of Reanl Medicine, LGH, Co. Donegal, Ireland.

A Multicystic dysplastic kidney (MCDK) is a congenital, renal, cystic transformation usually diagnosed perinatally with 1:1000-4,000 incidence[1]. The natural history of MCDK is disputed with involution[1], enlargement and development of hypertension[2], infection and malignant transformation reported in the literature. We describe the incidental detection of An occult MCDK was detected in a 25-year-old chef who presented with a 4 month history of diarrhoeal episodes and left flank discomfort. He had noted a sensation of fullness in the flank for a number of years but had not sought medical investigation. It had increased in size and discomfort with the onset of diarrhoeal episodes. He had a 13 year history of Type I Diabetes Mellitus Medications included Novorapid 8iu/10iu/8iu, glargine 22iu nocte and lisinopril 2 mg daily. Blood pressure was 160/103 mmHg, and bilateral pre-proliferative retinopathy with neovascularisation. Abdominal palpation revealed a large left flank mass, confirmed on CT Abdomen and a non-functioning left kidney on DMSA scan. Laboratory studies revealed striking polycythaemia (Hb 21 g/dL), elevated erythropoietin level 36 mIU/ml (normal range 6–25), HbA1c 12.2%, diabetic proteinuria (0.16 grams/24 hours) and glomerular hyperfiltration GFR 130 mls/min/1.73 m^2. Preoperatively laser treatment and repeated venesection was required to manage worsening diabetic retinopathy and secondary polycythaemia. Following nephrectomy, stabilisation of retinopathy, normalization of haemoglobin and an improvement in hypertension control was observed. This case strengthens the arguement[2] for removal of all MCDKs in childhood to prevent complications in adulthood.

1. Rabelo EAS et al. Conservative management of multicystic dysplastic kidney: clinical course and ultrasound outcome. *Jornal de Pediatria* 2005 **81** 400–404.
2. Webb NJA *et al.* Unilateral multicystic dysplastic kidney: the case for nephrectomy. *Archives of Diseases of Childhood* 1997 **76** 31–34.

P515

MEN-1 phenotype without detectible MEN-1 mutation

Ivana Bozic, Katarina Mirkovic, Djuro Macut, Bojana Popovic, Tatjana Isailovic, Milan Petakov, Sanja Ognjanovic & Svetozar Damjanovic
Institute for endocrinology, diabetes and metabolic diseases, Department for endocrine oncology, Belgrade, Serbia.

We describe a 52-year-old woman, with acromegaly, clival chondroid chordoma, meningioma and lung carcinoid. There was no family history of MEN-1. She was diagnosed as acromegaly in 2000. Radiological evaluation (MRI) revealed pituitary tumor, however, another infiltration of scull base was detected which invaded sphenoidal and ethmoidal sinuses, lamina cribroza and bilateral orbit walls. Pituitary tumor was completely removed and the reduction of extra-sellar mass was performed. Hystopathological and immunohistochemical analysis confirmed somatotroph pituitary adenoma and chondroid chordoma. After surgery, she almost normalized IGF-I levels (288 ng/mL) while GH remained unsuppressible during oGTT. In 2001 the second surgery was performed, para- and infra-sellar mass was reduced and pathohystology confirmed diagnosis of chondroid chordoma. In 2004 irradiation therapy gave no results regarding regression of scull base tumor, but IGF-I (113 ng/mL) and GH suppressibility normalized one year later. Atypycal bronchial carcinoid from the left lung was extirpated the same year and meningeoma arising from the falx cerebri was detected on MRI. Until now, the residual chordoma showed no further progression. On ^{111}Indium-labelled octreotid scintigraphy performed after lung operation, only meningeoma was detected. Even six years after the initial diagnosis there are no sings of primary hyperparathyroidism.

Possible mechanisms explaining MEN 1 phenotype with negative genetic result: the patient might have sporadic MEN 1 syndrome caused by double independent somatic events or to have germline mosaicism that has to be confirmed by genetic analyses of various tissues. Additionaly we have to exclude large deletion in MEN 1 gene.

P516

Composite medullary and papillary tumor with mixed lymph node metastases

Tatjana Isailovic, Milan Petakov, Djuro Macut, Bojana Popovic, Ivana Bozic, Sanja Ognjanovic, Jelena Nikolic, Jovana Vignjevic, Katarina Mirkovic & Svetozar Damjanovic
Institute of Endocrinology, diabetes and metabolic diseases, Belgrade, Serbia.

A 34 year-old female patient was admitted to the hospital because of a large nodule in the left thyroid lobe and elevated calcitonine level. A large encapsulated tumor was found and total thyroidectomy with left neck dissection was performed. Pathohistology revealed medullary and papillary carcinoma separate from each other in tumor tissue but mixed in regional lymph nodes. Papillary component was dominant in thyroid tissue but not in lymph nodes. Both calcitonine and thyroglobulin plasma levels were elevated after the surgery witch suggested distant metastases. I^{131} scintigrafy showed focal accumulation in the left side of the neck, thoracal vertebrae and diffuse accumulation in the ribs. DMSA and I^{131}MIBG scintigraphy revealed pathologic focuses in the left thyroid

lobe region. None of these focuses was confirmed by MRI. The octreoscan was negative. Genetic analysis of RET protooncogene was negative. The patient was treated with radiotherapy.
Conclusion
Synchronous occurrence of medullary and papillary carcinoma of the thyroid gland is very rare and a few cases were described in the literature. Concurrency of two distinct cell lines may suggest that they have a common stem cell origin or possible activation of a common tumorigenic pathway. However, synchronous coincidental genetic event cannot be excluded.

P517

Management of endocrine syndrome in a patient with plurihormonal neuroendocrine tumour

Bojana Popovic, Djuro Macut, Ivana Bozic, Tatjana Isailovic, Milan Petakov, Sanja Ognjanovic, Katarina Mirkovic, Jovana Vignjevic & Svetozar Damjanovic
Institute of endocrinology, diabetes and metabolic diseases, Belgrade, Serbia.

Neuroendocrine tumours (NETs) have a unique ability to produce and secrete a variety of biogen amines and peptide hormones. They arise from multipotential stem cells, which differentiate during tumorigenesis into specific cell lines. Some of these tumours are functional, producing characteristic clinical syndromes. We present a female patient incidentally discovered to have diffuse liver metastases of neuroendocrine tumour of unknown primary origin. She was free of symptoms at initial presentation, and pathohistological analysis after liver biopsy revealed the tumour to be well differentiated (Ki-67 – 4.5%) with somatostatin positive in more than 70% of tumour cells. Fasting glycemia was normal, but results of oral glucose tolerance test were in favor of diabetes. Thorough examination including the octreoscan did not reveal the site of primary tumour. Expression of somatostatin receptors was intensive in metastases. Three months later, she reported episodes of night sweats, tremulousness, tachycardia and amnesia. Hypoglycemia was recorded during first hour of fasting, with extremely high levels of insulin and C-peptide. Further imunohistological investigation of tumor bioptate revealed positivety for proinsulin in 30%, and insulin in less than 0.2% of tumour cells. After the initiation of diazoxide the glycemic control was improved but only after the initiation of combined therapy with short-acting somatostatin analogue she managed to have euglycemia during the whole day. In an attempt to control both endocrinological syndrome and tumour growth, the patient underwent hemoembolisation. Clinical syndromes caused by plurihormonal secretion make therapeutical treatment difficult, especially in cases of cosecretion of physiological antagonists.

P518

Brown tumor in hyperparathyroidism – clinical case

Zelija Velija-Asimi & B Helijc
University Clinical Centre of Sarajevo, Bosnia and Herzegovina.

A 66 years old woman on orthopedic clinic, was resection of tibias tumor in both legs because was suspicion of primary bone neoplasm or metastases (X-ray showed osteolytic lesion). Histology was: osteitis fibrosa cystica - Brown tumor. After resection the patient was referred to the endocrinologist because of persistently high calcemia (3.3 mmol/l). Blood tests showed normal CRP but elevated alkaline phosphatase of 173 U/l. Phosphate was low at 0.75 mmol/l (0.81–1.58). Parathyroid hormone (PTH) was elevated at 1450 pg/ml (10–65 pg/ml). Renal function was normal. CT scans of chest and abdomen was normal. But echosonography of parathyroid gland showed tumor structure size 4 cm of lower parathyroid gland in the right side. This tumor resected. The histological examination confirmed the parathyroid carcinoma. Post parathyroidectomy her calcium, phosphate, alkaline phosphates and PTH are in normal limits. When X-ray shows an osteolytic lesion, PTH and calcemia should be performed to exclude the primary hyperparathyroidism.

Neuroendocrine and pituitary behaviour – presented on Monday

P519

Investigation of early atherosclerotic changes in acromegalic patients

Ilkay Kartal, Huseyin Oflaz, Burak Pamukcu, Ferihan Aral, Nese Ozbey & Faruk Alagol
Istanbul University Istanbul Medical Faculty, Istanbul, Turkey.

Background
Functional and morphological changes of endothelium are risk factors for mortality attributed to atherosclerosis. Studies investigating early atherosclerotic alterations and the effect of the treatment of acromegaly on these alterations gave conflicting results.
Objective
Surrogate markers of early atheosclerotic changes, i.e. brachial artery flow-mediated dilation (FMD) and carotid artery intima-media-thickness (IMT) in active and inactive acromegalic patients are compared with control subjects matched to patients for age, sex, cardiovascular risk factors in order to find out the direct effects of GH/IGF-1 excess.
Methods
In 14 active acromegalics and their 14 matched controls, 14 inactive acromegalics and their 14 matched controls, carotid artery IMT and FMD of brachial artery were measured. Inactive acromegalics were in remission for at least 1 year.
Results
Active acromegalics had higher IMT than matched controls and inactive acromegalics (0.85 ± 0.20 mm, 0.64 ± 1.77 mm, 0.66 ± 0.20 mm respectively; $P<0.005$, $P<0.05$) and IMT of inactive acromegalics was not different from their matched controls (0.61 ± 0.12 mm). FMD was significantly lower in active acromegalics than in matched controls and inactive acromegalics (2.910 ± 2.00 mm, 6.5 ± 2.81 mm, 5.68 ± 2.9 mm respectively; $P<0.005$, $P<0.05$). FMD of inactive acromegalics was not significantly different from their matched controls (7.96 ± 3.12 mm). A significant inverse relationship was found between GH and FMD in active acromegalics ($r=-0.659$; $P=0.010$).
Conclusion
In active acromegalics, early atherosclerotic changes are not only attributed to the high prevalence of risk factors, but also to the abnormal GH secretion itself.

P520

Food preference, central serotoninergic activity, depression and insulin resistance in obese and lean healthy men: a pilot study

Ludmila Brunerova[1], Jana Potockova[1], Jiri Horacek[2] & Michal Andel[1]
[1]Diabetologic Centre, II. Department of Internal Medicine, Faculty Hospital Kralovske Vinohrady and 3rd Faculty of Medicine, Charles University, Prague, Czech Republic, [2]Psychiatric Centre Prague, 3rd Faculty of Medicine, Charles University, Prague, Czech Republic.

Objective
Higher anxiety/depression scores can be connected with food preference of carbohydrates, with increased insulin secretion and thus can lead to insulin resistance. Three hypotheses were postulated: 1. more depressive (still in the normal range) subjects do not differ in the central serotoninergic activity from those less depressive. 2. the former do not differ in food preference and 3. in insulin resistance from the latter.
Methods
healthy men, 30–55 years, 9 lean (44.5 ± 7.7 years, BMI 22.8 ± 1.8 kg/m^2) and 8 obese (45.3 ± 6.0 years, /ns/ BMI 30.5 ± 4.0 kg/m^2 /$P=0.0005$/) were involved in the study, which was approved by the local Ethical Comittee. The study protocol included filling in the SCL-90 questionnaire (for excluding psychopathology), self-assessing questionnaires for depression and anxiety (SAS, SDA), carbo-hydrate craving questionnaire (CCQ), 3-days diet records, 4-hours hyperinsuli-nemic euglycemic clamp on two insulin levels (1mU/kg/min and 10mU/kg/min) with calculating the metabolic clearance of glucose: MCR1, MCR2 and citaloprame challange test with 0.3 mg/kg citaloprame infusion followed by plasma sampling for prolactine at –30, −5,0,15,30,45,60,90,120,150 minutes and calculation of area under the curve for prolactine (AUC/PRL). ANOVA, Kruskall-Walis test and linear regression were used for statistical analyses.
Results
No correlations were found between AUC/PRL and SAS/SDA scores. Positive correlations were determined between SAS and SDA scores and % of carbohydrates in diet records ($r=0.74$; $P<0.01$, resp. $r=0.75$; $P<0.01$) and between depression/anxiety scores (SCL-90) and CCQ score (0.53; $P<0.01$, resp.0.54; $P<0.01$). We have not observed any relationships between central serotoninergic activity respectively SAS/SDA scores and MCR1/ MCR2.
Conclucions
We have proved that the subjects with higher depression/anxiety scores prefer more carbohydrates in the food. However, we have not observed any relationships between insulin resistance and depressive/anxiety scores or the central serotoninergic activity.
The study was supported by VZ MSM 0021620814.

P521

Somatic, body composition and psychological particularities in a group of untreated adult pituitary dwarves

Ioan Gotca, Dumitru Branisteanu, Letitia Leustean, Eusebie Zbranca & Voichita Mogos
University of Mediciine and Pharmacy "Gr.T.Popa", Iasi, Romania.

Aside its growth promoting effects, growth hormone (GH) displays other actions upon carbohydrate, lipid and protein metabolisms, and possibly also direct central nervous system effects. Fourteen adult pituitary dwarves (mean height of 132.3 ± 8 cm, mean age of 30.7 ± 9.6 y), 5 women and 9 men, never having received rGH therapy, were investigated. Body composition (BC) was assessed by bioelectrical impedance, and bone mineral density (BMD) was evaluated by quantitative ultrasound. Patients were submitted to psychological tests and examined by a psychologist and psychiatrist.

BC of GH-deficient adult dwarves was significantly modified: a reduced percentage of water (45.7 ± 13.6% compared to 69.4 ± 15% water in a BMI- and age-matched group with normal adult height) and an increased fat percentage (48.3 ± 12.9% compared to 25.2 ± 9.4% in normal-sized BMI-matched healthy controls, $P < 0.05$). BMD was decreased in the group of pituitary dwarves, with a mean T score of −1.45 ± 0.8 (in the range of osteopenia). When psychologically assessed, certain pituitary dwarves scored poorly at family and society adaptation (10 and 12 patients, respectively), whereas all but one patient had mild to profound self-esteem disturbances. Ten patients were resistant to refractory at any external help. Two dwarves had a high Beck depression score, three had suicidal thoughts and one had a suicidal attempt in her history. A strong correlation between the patients' IQ and their quality of life estimated with the Guilford-Zimmerman score was observed ($R^2 = 0.764$). Non-treated, childhood-onset GH deficiency leads therefore not only to dwarfism, but also to alterations in body composition and energy output. Modifications in their body image may have significant impact upon the adaptation of pituitary dwarves in the society, and on their quality of life. Their adaptation is dependent to a great extent of their mental capacity as well as of the degree of tolerance from the family and society.

P522

L-thyroid hormone enhancement of antidepressant treatment in major depressive episode

Paul Ivanov[1], Alexandra Raceala[2], Octavian Vasiliu[1] & Diana Paun[2]
[1]Emergency Military Hospital Dr. Carol Davila Department of Psychiatry, Bucharest, Romania; [2]Universitary Endocrinology Hospital Dr. C.I. Parhon, Bucharest, Romania.

Objective
To determine the impact of combined antidepressant drugs and LT4 enhancers in treatment of patients with Major Depressive Disorder.
Method
We conducted a randomized, placebo-controlled trial to determine whether LT4 supplementation had any augmentation effect on selective serotonin reuptake inhibitors (SSRIs). The study involved 70 patients with major depressive disorder; patients with hypothyroidism were excluded. Of the participants, 38 were assigned to receive LT4, and 32 received placebo. All of the patients received SSRI – paroxetine (50%), sertraline (28%) and fluoxetine (22%). A total of 66 patients completed the three month study. We made weekly psychological evaluations using clinical scale HAM-D (Hamilton Depression Scale). Thyroid data, consisting of values for thyroid-stimulating hormone TSH and LT4, measured by radioimmunoassay were collected before and after treatment.
Results
A decrease in HAM-D score was observed in both groups, with a medium improvement of 12.3 points and a significant difference in favour of LT4 group. In the LT4 group, 30 patients (83.3%) responded to treatment compared with 21 patients (70%) in the placebo group. The onset of antidepressant effect was earlier in the LT4 group with an average response in 2 weeks. Those in the group receiving LT4 supplements had lower levels of LT4 and TSH after the study when compared to baseline. Final TSH values correlated strongly with response to treatment as measured by change in HAM-D scores.
Conclusion
Supplements of levothyronine (LT4) enhance the antidepressant effects of SSRIs. LT4 is efficacious as an enhancer of antidepressant therapy. Low TSH values correlated with greater improvement in depressive symptoms.

P523

Evaluation of cognitive functions by using P300 auditory event related potentials (ERPs) in amateur kickboxers: a preliminary study

Fatih Tanriverdi[1], Hande Yapislar[2], Cem Suer[2], Ismail Kocyigit[1], Kursad Unluhizarci[1] & Fahrettin Kelestimur[1]
[1]Erciyes University Medical School Department of Endocrinology, Kayseri, Turkey; [2]Erciyes University Medical School Department of Physiology, Kayseri, Turkey.

Objective
Impaired cognitive function has been demonstrated in adults with GH deficiency (GHD) by using different neuropsychological tests. P300 ERP application is a well established neurophysiological approach in the assessment of cognitive function. Kickboxing is a novel cause of hypopituiatarism due to sports related traumatic brain injury (TBI) and isolated GHD is the most common problem (1).

The present preliminary study was therefore designed to investigate the effects of sports related head trauma induced GHD on cognitive function by using P300 ERPs.
Methods
The study comprised 15 amateur kickboxers (13 male, 2 female), with a mean age of 30.0 ± 5.9 yr. GHD was diagnosed in 6 kickboxers by using two stimulation tests (GHGH + GHRP-6 and glucagon). 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
The mean P300 latencies (at all electrode sites) of the kickboxers with GHD were prolonged when compared with those of GH normal kickboxers. However the difference did not reach to a significant level. There was a significant negative correlation between IGF-I levels and latencies at Fz electrode site (r: −0.530, P: 0.04).
Conclusions
P300 latency is related to stimulus evaluation time and prolonged P300 latencies suggest an impaired cognitive function in GH deficient kickboxers. The differences did not reach to a significant level due to a small sample size. This is an objective electrophysiological evidence for cognitive dysfunction in GHD and further data with high number of kickboxers are warranted.

P524

Changes in hypothalamo-pituitary-testicular axis sensitivity in aging male

Svetlana Vujovic, Milos Stojanovic, Miomira Ivovic, Branko Barac, Milina Tancic Gajic, Marija Barac, Ljiljana Marina & Milka Drezgic
Institute of Endocrinology, Belgrade, Serbia.

Involutive hypoandria (late onset hypogonadism) is characterized by decline in serum testosterone and increase of gonadotropins. Changes of hypothalamo-pituitary-testicular axis sensitivity are influenced by primary testicular changes and altered neuroendocrine regulation during aging. To evaluate age-related changes in gonadotroph and Leydig cell sensitivity two groups were formed: 1) 35 men, 51.8 ± 3.2 years old, BMI = 28.2 ± 3.1 kg/m^2; 2) 32 men, 63.2 ± 6.8 years old, BMI = 27.2 ± 3.1 kg/m^2. Blood samples for FSH, LH, prolactin, estradiol, testosterone, SHBG were taken at 8 am. LHRH test was then performed (100 microg LHRH i.v., FSH and LH were taken before, 20 and 60 min later). Next three days HCG test was done (Pregnyl amp. a 5000 i.j./day, testosterone, estradiol and SHBG were detected before and after test). Hormone analyses were done by RIA. Statistics: Spearman, Mann-Whitney test, area under the curve-AUC. Neither increase of LH (4.5 ± 3.1 vs. 5.8 ± 3.5 IU/L, $P > 0.05$) nor decrease of testosterone (19.7 ± 7.6 vs. 14.8 ± 6.9 nM/L, $P > 0.05$) reached significant difference. The maximal LH response in 20 minutes (17.6 ± 13.2 vs.27.0 ± 11.8 IU/L, $P = 0.03$) and LH AUC (962.5 ± 738.2 vs. 1428.5 ± 658 IU/L/min) were higher in older men. Higher sensitivity of Leydig cell testosterone response was observed in older group (19.2 to 33.1 vs. 14.2 to 31 nM/L, $P = 0.0021$). Negative correlation was found between testosterone and BMI ($P = 0.02$). Conclusion: Older men show significantly increased gonadotrophin release due to amplified secretory burst mass, diminished gonadal hormone negative feedback or primary alterations in hypothalamo-pituitary unit with aging. Leydig cell sensitivity is preserved during aging. Secondary testicular failure in aging male is due in part to decreased GnRH gene expression rather than to decreased pituitary responsiveness to LHRH.

P525

Glutamatergic neurons and synaptic contacts between glutamatergic axon terminals and chemically identified nerve cells in the rat hypothalamic suprachiasmatic nucleus

József Kiss[1], Zsolt Csaba[2], Ágnes Csáki[3] & Béla Halász[4]
[1]Hungarian Academy of Sciences and semmelweis University, Neuroendocrine Research Laboratory, Budapest, Hungary; [2]Department of Human Morphology and Developmental Biology, Semmelweis University, Budapest, Hungary; [3]Department of Human Morphology and Developmental Biology, Semmelweis University, Budapest, Hungary; [4]Hungarian Academy of Sciences and semmelweis University, Neuroendocrine Research Laboratory, Budapest, Hungary.

The hypothalamic suprachiasmatic nucleus (SCN) is the key-structure of the control of circadian rhythms. Several observations support the view that glutamate is the primary transmitter of the retinal projection to this cell group. The glutamatergic innervation of the nucleus is not limited to this projection, it is much more extended. The aim of our investigations was (1) to examine whether are glutamatergic neurons existing in the SCN and (2) to get information about the relationship between glutamatergic axon terminals and vasoactive intestinal polypeptide (VIP)-, GABA- and arginine-vasopressin (AVP)-containing neurons. Vesicular glutamate transporter type 2 (VGluT2) was used as marker of the glutamatergic elements. Single and double label immunocytochemistry was applied and the brain sections were examined by confocal laser scanning microscopy and under the electron microscope. We detected VGluT2 immunoreactive neurons in the SCN and observed VGluT2 axon terminals in synaptic contact with GABA, VIP, AVP and with VGluT2-positive perikarya or dendrites. The morphology of the contacts indicated asymmetric type synapses. Our observations provide the first neuromorphological evidence for the view that glutamatergic neurons exist in the SCN and further they demonstrate for the first time terminations of glutamatergic boutons on prominent cell groups of the SCN. The findings are in line with the view that the intranuclear organization of the circadian clock is extremely complex.

This work was supported by the National Scientific Research Fund (OTKA T-042516 to B.H., OTKA T-049455 to K.J.), the Ministry of Health (ETT 020/2006), National Research Program (NKFP 1A002-04 8/6/04) and the Hungarian Academy of Sciences.

P526

Laterality in the supraspinal innervation of the adrenal gland: a dual-virus tracer study

Ida Tóth[1], Ory Wiesel[1], Dorina Tóth[2], Zsolt Boldogkoi[3], Kamill Bálint[3] & Ida Gerendai[1]
[1]Neuroendocrine Research Laboratory, Hungarian Academy of Sciences and Semmelweis University, Department of Human Morphology and Developmental Biology, Budapest, Hungary; [2]Department of Physiology, Albert Szent-Györgyi Medical University, Szeged, Hungary; [3]Department of Biology, Albert Szent-Györgyi Medical University, Szeged, Hungary.

Recent studies using the viral transneuronal tracing technique allowed to reveal brain neurons synaptically connected with the adrenal gland, via specific infections within functionally related chains of neurons. The aim of the present study was to investigate whether cerebral neurons involved in the innervation of the adrenal gland exhibit asymmetry or not. In order to label simultaneously the supraspinal neurons connected with the right- and left-sided adrenal glands, dual-virus tracer was applied in young female rats. Two genetically almost identical, however, histochemically distinguishable, newly constructed Bartha strains of pseudorabies virus (PRV) were used, each expressing a unique marker antigen in infected host cells (green fluorescent protein: BDG-strain or ß-galactosidase, BDL-strain). The virus suspensions were injected into the left- or right-sided adrenal, then the spinal cord and brain were investigated by immunofluorescent staining of the marker genes. In the brain stem the nucleus of the solitari tract, dorsal vagal, ambigus, parapyramidal nuclei, caudal raphe nuclei and the ventrolateral areas were labeled, mainly by monolabeled neurons. In addition, neurons of the A5, lateral hypothalamus, paraventricular (PVN), periventricular, arcuate nuclei were infected. In PVN many neurons were double-labeled. Viral infection of the above cell groups projecting to the left adrenal was more prominent compared to that of the right gland. Data suggest predominance in supraspinal innervation of the left adrenal gland.

Supported by grants from the National Research Found (OTKA T-046624), the Ministry of Health (ETT 448/2006) and the Hungarian Academy of Sciences.

P527

Prolonged orocecal transit time and small intestinal bacterial overgrowth in acromegalic patients

Eugenia Resmini[1], Andrea Parodi[2], Vincenzo Savarino[2], Alfredo Greco[2], Alberto Rebora[1], Francesco Minuto[1] & Diego Ferone[1]
[1]University of Genoa, Department of Endocrinology, Genoa, Italy; [2]University of Genoa, Department of gastroenterology, Genoa, Italy.

Gastrointestinal abnormalities in acromegaly include dolichomegacolon and increased prevalence of colonic polyps. No data are available on the small intestine. The aims of this study were to investigate orocecal transit time (OCTT) and the presence of small intestinal bacterial overgrowth (SIBO) in acromegaly. 41 acromegalic patients and 30 controls entered the study. Acromegalics were classified according to whether they were on medical treatment with somatostatin analogs (SSA): "treated" and "untreated" and according to clinical control: "controlled", "uncontrolled" and "partially controlled". Acromegalics and controls were submitted to a 10 g lactulose hydrogen (H_2) breath test (LH-BT) in order to determine the OCTT and presence of SIBO.

There is an increased prevalence of SIBO in acromegalics comparing to controls ($P=0.000$). OCTT was significantly slower in acromegalics comparing to controls ($P=0.000$).

Nine treated and 9 untreated acromegalics were positive for SIBO, without a statistical significant difference. Six controlled, 9 partially controlled and 3 uncontrolled acromegalics were positive for SIBO, without a statistical significant difference.There was a significantly lower OCTT in treated compared with untreated patients ($P=0.02$) and between these two groups and controls ($P=0.00$). There was no statistically significant difference for OCTT between controlled and uncontrolled acromegalics.

These data demonstrate for the first time that SIBO occurs more frequently in acromegalics than in controls, and medical therapy with SSA does not influence the presence of SIBO. OCTT is significantly delayed in acromegalics both in treated and in untreated ones and this suggests that acromegaly determines *per se* impairment of intestinal motility. Clinical control does not influence the OCTT, suggesting that this may be an irreversible complication. The slower OCTT may represent a risk factor for the development of SIBO. These alterations might be related to the occurrence of an autonomic intestinal disorder, as we have previously demonstrated for cardiac autonomic activity in acromegaly.

P528

Adulthood follow-up of a large number of patients with congenital morphological alteration of anterior and/or posterior pituitary lobes

Danilo Ribichini[1], Cecilia Ragazzini[1], Stefano Zucchini[2], Piero Pirazzoli[2], Alessandro Cicognani[2], Valentina Vicennati[1], Uberto Pagotto[1] & Renato Pasquali[1]
[1]Unità Operativa di Endocrinologia, Bologna, Italy; [2]Clinica Pediatrica, Bologna, Italy.

Congenital morphological alteration of anterior and/or posterior pituitary lobes is a rare disease often associated with isolated or multiple hormonal deficiency. In this study we re-evaluated 24 adult patients, 20 males and 4 females, (23–46 years), in whom Nuclear Magnetic Resonance (NMR) showed congenital morphological alteration of the gland. Twenty out of 24 patients presented with an association of hypoplasic adenohypophysis and ectopic neurohypophysis, while 4 presented with hypoplasic adenohypophysis and undetectable neurohypophysis as by NMR. All patients are currently under hormonal replacement therapy. Fourteen out of 24 patients were panhypopituitaric (58.3%); 3 presented with multiple GH, LH-FSH, TSH deficit (12.5%); 2 with GH and combined LH-FSH deficit (8.3%); 2 with isolated GH deficit (8.3%); 1 with combined GH, ACTH, TSH deficit (4.2%); 1 with multiple GH, LH-FSH, AVP deficit (4.2%); and 1 with isolated AVP deficit (4.2%). Anthropometric parameters showed that 11 patients are overweight (BMI 25–29.3), and 4 obese (BMI 31.5–34.6). Biochemical evaluation showed that 9 patients were hypercolesterolemic (37.5%), 7 were hypertrygliceridemic (29.2%), and 4 patients presented with low levels of HDL-colesterol (16.7%); other metabolic or biochemical parameters were not significantly altered. Epilepsy has been recently observed in 2 of these patients presumably associated with a novel radiological findings of areas of subependymal heterotopia. In conclusion, the association of hypoplasic adenohypophysis and ectopic or undetectable neurohypophysis are congenital conditions frequently associated with single or multiple hormonal deficits. The etiopathogenesis of these conditions is still unknown, and our preliminary genetic observation seem to exclude that HESX1 or PROP1 mutations play a role in these congenital malformations (11 out of 24 patients were tested and no mutations were found for both genes). Moreover, periodic long-life radiological monitoring of the brain is necessary to detect areas of subependymal heterotopia in order to pharmacologically prevent epilepsy.

P529

Non-NMDA glutamate receptor antagonist injected into the mesencephalic dorsal raphe nucleus inhibits the suckling-induced prolactin release and administered into the lateral cerebral ventricle interferes with the diurnal fluctuations of plasma prolactin of male rats

Ibolya Bodnár, Zsuzsanna Bánky & Béla Halász
Hungarian Academy of Sciences and Semmelweis University, Neuroendocrine Research Laboratory, Budapest, Hungary.

We examined the functional significance of the glutamatergic innervation of the dorsal raphe nucleus (DR) in the mediation of the suckling stimulus inducing prolactin release. A non-NMDA (6-cyano-7-nitroquinoxaline-2,3-dione disodium, CNQX) or an NMDA glutamate receptor antagonist (dizocilpine hydrogen malate, MK-801) was injected into the DR of freely moving lactating rats at the end of 4 h separation. The litters were then returned and blood samples for prolactin were taken at different time points. In addition, we studied the effect of the non-NMDA receptor antagonist on the diurnal fluctuations of plasma prolactin and corticosterone. Adult male rats received by means of ALZET minipump CNQX (0.5 or 10 pM/µl/h) into the lateral cerebral ventricle for 72 hrs before and during blood sampling. CNQX, when injected into the DR in higher dose, inhibited the suckling-induced prolactin release. After MK-801 administration the prolactin response was significantly diminished. There were no diurnal fluctuations in plasma prolactin concentrations and only attenuated changes in corticosterone levels of rats treated with CNQX compared to controls getting physiological saline into the lateral ventricle. The findings suggest that (1) the glutamatergic innervation of the dorsal raphe nucleus is involved in the mediation of the neural signal of the suckling stimulus inducing prolactin release and (2) glutamatergic innervation of brain structures participating in the control of diurnal fluctuations of plasma prolactin and corticosterone concentration contributes to the maintenance of the circadian rhythm of these hormones.

This work was supported by the National Scientific Research Fund (OTKA T-042516 to B.H.), the Ministry of Health (ETT 020/2006 to B.H.) and the Hungarian Academy of Sciences.

P530

The expression of the neuroprotective factor seladin-1 is up-regulated by thyroid hormones in human neuronal precursor cells, but not in mature neurons

Susanna Benvenuti[1], Paola Luciani[1], Ilaria Cellai[1], Cristiana Deledda[1], Riccardo Saccardi[2], Serena Urbani[2], Gabriella B Vannelli[3], Fabio Francini[4], Roberta Squecco[4], Mario Serio[1], Aldo Pinchera[5] & Alessandro Peri[1]
[1]Endocrine Unit, Dept of Clinical Physiopathology, University of Forence, Florence, Italy; [2]Department of Haematology, Careggi Hospital, Florence, Italy; [3]Dept of Anatomy, Histology and Forensic Medicine, University of Florence, Florence, Italy; [4]Department of Physiological Sciences, University of Florence, Florence, Italy; [5]Department of Endocrinology and Metabolism, Section of Endocrinology, University of Florence, Florence, Italy.

Thyroid hormones (TH) play a fundamental role during brain development by modulating the expression of different genes involved in neuronal differentiation, proliferation, migration, myelinization, and synapse formation. *Seladin-1* (for SELective Alzheimer's Disease INdicator-1) is a recently identified anti-apoptotic gene, which has been found to be down-regulated in brain regions affected by Alzheimer's disease (AD). We hypothesized that seladin-1 might be a novel mediator of the effects of TH in the developing brain. Thus, in the present study we determined whether TH modulate the expression of seladin-1 in human neuronal precursors and/or in differentiated cells. Two different cell models were used: fetal human neuroepithelial cells (FNC) isolated previously from fetal olfactory epithelium; and human mesenchymal stem cells (hMSC), isolated from bone marrow, which have a demonstrated ability to differentiate into neurons. In our hands, hMSC were differentiated into neurons (hMSC-n), following previously established protocols. The neuronal phenotype was confirmed by the positivity for the specific markers nestin, glypican 4, necdin, neurofilament subunit L, neurofilament subunit M, neurite outgrowth-promoting protein, choline acetyltransferase, neuronal nuclei. Electrophysiological evaluation revealed the presence of inward Na and Ca currents typical of neuronal cells. In basal conditions, the amount of seladin-1 was significantly higher in undifferentiated cells than in mature neurons, as assessed by real-time RT-PCR. T3 and T4 (1 nM) significantly increased the amount of seladin-1 mRNA in both FNC (140% and 66% increase, respectively) and hMSC (61% and 16% increase, respectively), but not in hMSC-n. The amount of the protein, evaluated by Western blotting, changed accordingly. This is the first demonstration that TH stimulate the expression of seladin-1 in human neuronal precursors, but not in terminally differentiated neurons. These results suggest that this neuroprotective factor may play a prevalent role during brain development, together with other well-known TH-dependent factors.

P531

Long-term evaluation of hypotalamic-pituitary-adrenal (HPA) axis in acromegalic patients during somatostatin analogs therapy and after successful surgery

Cristina L Ronchi, Erica Rizzo, Emanuele Ferrante, Claudia Giavoli, Andrea G Lania, Paolo Beck-Peccoz & Anna Spada
Unit of Endocrinology, Dep. Medical Sciences, Fondazione IRCCS Ospedale Maggiore Policlinico, Mangiagalli e Regina Elena, Milano, Italy.

Long-term effects of trans-naso-sphenoidal surgery (TNS) and long-acting somatostatin analogs (SSTa) on hypotalamic-pituitary-adrenal (HPA) function have been poorly investigated. Aim of the study was to evaluate over time the integrity of HPA axis in acromegalic patients with baseline preserved adrenal function and treated with one or both available treatments. We selected 23 patients (15F & 8M, age ($\pm$S.D.) = 46.8 $\pm$ 13.7 yrs) with normal (n = 19) or subnormal HPA axis not requiring replacement therapy (n = 4). In particular, 15 patients well responsive to chronic SSTa therapy (11 previously operated and 4 de novo) were investigated before and during treatment (median = 63 months), while 8 patients cured by TNS were studied 2–3 months after surgery and during follow-up (median = 100 months). HPA function was studied by morning circulating cortisol and ACTH levels, urinary free cortisol (UFC) and cortisol response to low-doses short Synacthen test (LDSST, 1 mcg). The cut-off for a normal function was a cortisol peak $>$ 500 nmol/liter while a peak between 450 and 500 indicated a partial hypoadrenalism. All patients were studied for serum GH and IGF-I, basal thyroid and gonadal function and MRI. Basal cortisol, ACTH and UFC levels did not significantly change over time and remained in the normal range. Considering the cortisol peak after LDSST, 3 patients with subnormal function at baseline developed overt hypoadrenalism (peak $<$ 450 nmol/liter), 7 with normal adrenal function developed partial (n = 4) or overt hypoadrenalism (n = 3), while HPA function remained unchanged in 13. No significant correlations between HPA axis deterioration and GH/IGF-I levels, type of treatment, SSTa formulation, occurrence of other pituitary deficiencies, presence of secondary empty sella, changes in tumor or residual volume were observed. In conclusion, the HPA axis integrity must be carefully monitored over the time in all acromegalic patients, independently from the type of treatment, and not limited to patients undergoing radiotherapy.

P532

Growth hormone deficiency and recombinant hGH (rhGH) replacement in children with idiopathic isolated GH deficiency: effects on the hypothalamus-pituitary-adrenal axis

Silvia Bergamaschi[1], Claudia Giavoli[1], Emanuele Ferrante[1], Roberto Rusconi[2], Andrea G Lania[1], Anna Spada[1] & Paolo Beck-Peccoz[1]
[1]Endocrine-metabolic Unit, Department of Medical Sciences, University of Milan, Fondazione Ospedale Maggiore Policlinico, Mangiagalli e Regina Elena IRCCS, Milan, Italy; [2]Pediatric Unit, University of Milan, Fondazione Ospedale Maggiore Policlinico, Mangiagalli e Regina Elena IRCCS, Milan, Italy.

Cortisol and cortisone are interconverted by type 1 and type 2 11ßhydroxysteroid dehydrogenase (11ßHSD) isoenzymes. The type 1 isoenzyme is a widely expressed reductase that converts cortisone to cortisol regulating glucocorticoid tissue exposure. Its activity is inhibited by GH and IGF-I, being increased in GH deficiency (GHD) and decreased in acromegaly. In our experience rhGH therapy unmasked a central hypoadrenal state in adults with organic GHD, likely by normalizing 11ßHSD1 activity and reducing cortisone to cortisol conversion.

Aim of this study was to evaluate the hypothalamus-pituitary-adrenal (HPA) axis in 9 children (5M and 4F, mean age 12.0 $\pm$ 1.1 (S.E.) yrs, mean height SDS -2.1 ± 0.4) with idiopathic isolated GHD. Measurements were performed at baseline and on rhGH therapy (mean duration: 12 $\pm$ 3 months, mean dose: 0.03 $\pm$ 0.01 mg/kg bw/day). HPA function was assessed by basal serum cortisol levels and after 1 mcg ACTH test (n = 4 patients) or insulin tolerance test (ITT, n = 5 patients). Central hypoadrenalism was excluded for both tests by the presence of either a peak of cortisol $>$ 500 nmol/L or a cortisol absolute delta $>$ 200 nmol/L. Serum IGF-I levels normalized on rhGH. Mean basal serum cortisol levels on rhGH, though showing a slight decrease, did not significantly differ from those recorded at baseline (215 $\pm$ 28 *vs* 256 $\pm$ 52 nmol/L, respectively, P = NS). The serum cortisol peak either after 1

mcg ACTH and after ITT was the same on rhGH therapy and at baseline (515±126 *vs* 574±131 nmol/L, respectively, P=NS). Plasma ACTH levels did not vary significantly. In conclusion, according to the diagnostic criteria, no child became central hypoadrenal on rhGH, contrary to what observed in adult patients with organic GHD and multiple pituitary deficits. This finding further supports the view that only in patients with organic multiple pituitary hormone deficiency GH deficiency may mask the presence of a hidden central hypoadrenalism.

P533

Effects of glucocorticoid replacement on bone mass in women after long-term remission of Cushing's syndrome

Maria-Jose Barahona, Nuria Sucunza, Pilar Lluch, Jose-Manuel Fedrnandez-Real, Wifredo Ricart, Teresa Puig, Ana M Wäger, Caroline van Elzelingen, Jordi Farrerons & Susan M Webb
Endocrinology, Hospital Sant Pau, UAB, Barcelona, Spain; Internal Medicine, Hospital Sant Pau, UAB, Barcelona, Spain; Epidemiology, Hospital Sant Pau, UAB, Barcelona, Spain; Endocrinology, Hospital J Trueta, Girona, Spain.

High dose and long-term glucocorticoid (GC) therapy reduce bone mass and negatively affect the metabolic profile. Patients in remission after successful treatment of Cushing's syndrome (CS) often present hypoadrenalism and require long-term GC replacement.
Objective
To evaluate the effect of GC therapy on bone and metabolic parameters in women after long-term remission of CS. Materials and methods: Thirty-two women (mean age: 50±14 years) with cured CS were enrolled. Mean time of cure was 11±6 years. Twenty-three patients had pituitary and 9 adrenal tumours. Bone mineral density (BMD) and body composition was measured by dual-energy x-ray absorptiometry scanning (DEXA). Anthropometric and laboratory parameters were measured (lipid profile, adiponectin, glucose, insulin, serum calcium, alkaline phosphate, fibrinogen, IGF-I and free T4). Duration of GC treatment, GC dose, and duration of hypercortisolism (including duration of CS symptoms pre-diagnosis and from diagnosis until cure) were calculated. Results were compared with those of 25 age-matched control women. Results: Duration of GC treatment, GC dose and duration of hypercortisolism were negatively correlated with bone mineral content (BMC) and BMD, and positively with fibrinogen. After multiple linear regression analysis, duration of GC treatment (P=0.003) and current age (P=0.019) were significantly related to BMC; only duration of GC treatment was related to BMD (P=0.002); whereas duration of hypercortisolism was significantly related to fibrinogen (P=0.004) and insulin (P=0.015). Daily GC dose was related to adiponectin (P=0.012). Patients treated longest with GC therapy (>24 months) had less BMC (P=0.002) and BMD (P=0.001) than those treated for <24 months and controls.
Conclusions
'Replacement' therapy with GC in women in remission after successful therapy for CS who are adrenal insufficient, is correlated with a reduction in bone mass and adiponectin. Thus, GC should be prescribed in the lowest dose and shortest time possible.
Supported by FIS 05/0448and CIBER 06 07/044.

P534

Factors influencing the rhythmic secretion of melatonin in human

Roxana Rosca[1], Olga Ianas[2], Dana Manda[2], Suzana Vladoiu[2], Carmina Busu[2], Dana Dinu[2], Carmen Chioasca[3] & Camelia Procopiuc[2]
[1]"Carol Davila" University of Medicine and Farmacy, Bucharest, Romania; [2]"C.I. Parhon" Institute of Endocrinology, Bucharest, Romania; [3]Neuropsychiatry Hospital, Buzau, Romania.

Objective
The present study examined the influence of light and dark, seasonal differences, electromagnetic fields, adrenergic and GABA-ergic control, age on melatonin secretion and also which factors affect rhythmicity of melatonin secretion, both in health and disease.

Data were combined from a series of studies conducted between 1997 and 2006. Hormones were temporally assayed by immunometric assays.

The results showed that melatonin is secreted in a circadian pattern with high levels during the night and low ones in the day time and constant from day to day in the same subject when the individual behavior and environment remain relatively unchanged but there is very large variability in amplitude among subjects. In a structured environment there are changes in melatonin production in seasonal photoperiod. In short photoperiod seasons the melatonin circadian profile amplitude is the highest while in long photoperiod seasons it is diminished. Occupational exposure to extremely low frequency magnetic fields altered profiles of melatonin secretion in electric power station workers.

Inhibition of the beta-adrenergic receptor by beta-blockers accounts for approximately 80% of the nightly increase in melatonin production. Benzodiazepine receptors have been found to modulate melatonin production.

The amplitude of circadian rhythm of melatonin decreases with age. Changes in melatonin secretion in puberty development and menstrual cycle and also in disorders of hypothalamic-pituitary-gonadal axis suggest that melatonin by its circadian secretion regulates the temporal organization of HPG axis. Results related to epileptic disorders in children showed disturbances of melatonin circadian rhythm.
Conclusion
The results showed that melatonin is a chronobiotic hormone regulated by light with the ability to modulate various bodily functions using hormones and restore the balance when disorders of circadian regulation occur.
Acknowledgements
The study was done under research project CEEX 100/2006.

P535

Neurotropic profiles of androgens - mechanisms and targets

Franziska Goetz[1], Vladimir K Patchev[2], Wolfgang Rohde[1] & Alexandre V Patchev[1]
[1]Institute of Experimental Endocrinology, Charité - University Medicine Berlin, Berlin, Germany; [2]Schering AG/Jenapharm, Jena, Germany.

Identification of pure neural androgenic effects is difficult due to 1) regional distribution of androgen receptors (AR) in the CNS; 2) cross-talk between molecular pathways of steroid hormone signalling, and 3) chemical nature and biotransformation of androgens in the CNS. Testosterone is transformed in the CNS by 5α-reductase and aromatase to the pure AR-agonist dihydrotestosterone (DHT) and the estrogen receptor-agonist estradiol, respectively. Decreased sexual activity is a symptom of hypogonadism, whereas anxiety and poor control of pituitary-adrenal responsiveness to stress are hallmarks of affective disorders (e.g. major depression). Age-related androgen deficiency has been associated with affective disorders, and androgens have been sporadically used as treatment. Three androgens with different pharmacological profiles were investigated in rats to elucidate whether 1) biotransformation to estrogens and 2) pronounced anabolic properties differentially contribute to behavioural and neuroendocrine actions. We used the aromatizable and 5α-reducible testosterone and the non-aromatizable dihydrotestosterone as well as the synthetic steroid anadrol (oxymetholone), a 5α-reduced androgen with pronounced anabolic properties. By chronic administration in castrated rats only testosterone was able to fully restore mounting activity to the level seen in intact rats, the non-aromatizable AR-agonist DHT showed merely a trend towards induction of sexual behaviour, while anadrol failed to induce male sexual activity. Anadrol displayed significant anxiolytic effects, whereas testosterone was effective only at higher doses; DHT failed to produce anxiolysis. Stress-induced cortico-sterone secretion was suppressed in all treatment groups, but most pronounced under testosterone. The results of this comparative examination of pure AR-agonists (DHT), aromatizable androgens (testosterone) and androgenic-anabolic steroids (anadrol) indicate differential neurotropic profiles and, consecutively, applicability to defined neurological symptoms (e.g. sexual dysfunction, anxiety or inadequate responsiveness to emotional stress).

P536

Age-related changes of circadian rhythmicity: relationship with melatonin.

Dana Manda, Olga Ianas, Suzana Vladoiu, Carmina Busu & Roxana Rosca
"C.I. Parhon" Institute of Endocrinology, Bucharest, Romania.

The suprachiasmatic nucleus (SCN) is the 'master clock' of the mammalian brain. It coordinates the peripheral clock in body, including the pineal clock that receives SCN input via a multisynaptic noradrenergic pathway. Melatonin is exclusively involved in signaling the 'time of day', 'time of year' to all tissues and is thus considered to be the chronological pacemaker or 'zeitgeber'.
Objective
To determine the chronology of age-related changes in melatonin secretion and relationship with gonadotropin and cortisol levels.
Subjects and methods
Data were combined from a series of studies conducted between 1997 and 2006. A total of 60 healty subjects, aged 3 to 70 years, without sleep complaints or histories of endocrine psychiatric disorders were enrolled. Twenty-four hour

profiles of urine aMT6 s, cortisol and gonadotropins were assayed by cosinor analysis.
Results
The circadian patterns of melatonin secretion exhibited a significant decline around pubescence; in younger adults there was no significant change or sex-differences. Correlations between melatonin secretion and gonadotropins showed a positive correlation at the onset of puberty and negative one in both premenopausal women (at ovulation) and men (<60 y). In menopausal women there was a very large variability in chronobiological parameters associated with an increase in gonadotropin excretion, LH and FSH. An age-related decline in melatonin was found after 55–60 years of age. Whereas circadian rhythms persisted, they were associated with earlier timing acrophases and blunted amplitudes. Cortisol secretion exhibited significant circadian rhythm but with a surprisingly long time log; the acrophase occurred across the 24 h.
Conclusion
Aging influences both the amplitude and phase of circadian rhythmicity and melatonin could be an index of circadian rhythm function.
Acknowledgements
This study was done under research project CEEX nr. 100/2006

P537

Idiopathic isolated GH deficiency (IGHD) and combined pituitary hormone deficiency (CPHD) in Italy: genetic screening and clinical correlates

Ginevra Corneli[1], Daniela Vivenza[1], Simona Mellone[2], Luigi Tiradani[2], Yari Carlomagno[2], Michela Godi[2], Simonetta Bellone[1], Gianluca Aimaretti[3], Mara Giordano[2], Patricia Momigliano-Richiardi[2] & Gianni Bona[1]
[1]Unit of Paediatrics, Department of Medical Sciences, University of Piemonte Orientale "Amedeo Avogadro", Novara, Italy; [2]Laboratory of Human Genetics, Department of Medical Sciences, University of Piemonte Orientale "Amedeo Avogadro", Novara, Italy; [3]Endocrinology, Department of Clinical and Experimental Medicine, University of Piemonte Orientale "Amedeo Avogadro", Novara, Italy.

Mutations in genes encoding pituitary-specific factors have been identified in patients with idiopathic isolated GH deficiency (IGHD) or combined pituitary hormone deficiency (CPHD), with or without neuro-morphological abnormalities. We screened 205 IGHD (M/F:131/74; 183 sporadic and 22 belonging to 12 families) for mutations in *GH1*, *GHRH-R, HESX1* and 129 CPHD, (M/F:75/54; 118 sporadic and 13 belonging to 9 families) for mutations in *PIT1*, *PROP1*, *LHX4* and *HESX1*. We considered as familial cases both patients with family history of the disease and those with consanguineous parents. All the CPHD patients had GH deficiency. All IGHD were diagnosed during childhood. Among CPHD patients 82 were diagnosed in childhood, 14 during adolescence and 33 in adulthood. Neuroradiological abnormalities at MRI scan were found in 26.8% of IGHD and 65.1% of CPHD. Mutations were detected in the *GH1* gene in two IGHD familial cases (a homozygous tandem duplication within exon 2 and a heterozygous IVSdel+56–77) and in two CPHD familial cases, one in *PIT1* (IVS2+3insA heterozygote) and one in *PROP1* (R73C/R73H compound heterozygote). Among sporadic cases likely causal mutations were identified in one IGHD in *HESX1* (IVS2+3G→A heterozygote) and in three CPHD, of which two in *PROP1* (296delGA and 150delA, both in homozygosis) and one in *HESX1* (Q6H heterozygote). No mutations were found in the *LHX4* gene. Thus, we found mutations in 4 out of 21 families (19%) and 4 out of 301 sporadic cases (1,3%). In four further sporadic cases sequence variations were detected (one V10G in *GHRH-R* and three V129I in *HESX1*) but there is still no evidence of their pathogenic role. In conclusion, most causal mutations in the genes analysed in this study were found in familial cases. Thus, the inclusion criteria for the genetic analysis, at least for sporadic patients, should be better clarified, prior to offering genetic testing.

P538

Increased intraovarian levels of noradrenaline and NGF precede the follicular changes in the rat ovary at the end of reproductive period

Alfonso H Paredes, Eric Acuña, Romina Fornes & Hernan E Lara
University of Chile, Santiago, Metropolitan, Chile.

Reports in the literature have demonstrated an increased number of nerve fibers and the presence of a follicle development similar to polycystic ovary during perimenopausal ovary in women. Since differentiation, proliferation and growth of nerves depends of nerve growth factor (NGF), changes in the content of nerves fiber could be preceded by increases in NGF and p75 neurotrophin receptor (p75NTR). Our purpose was to evaluate the changes in noradrenaline (NA) at the celiac ganglion at the ovary and plasma levels through the establishment of the anovulatory condition associated with age. We also measured NGF and p75NTR mRNA, in relation to the changes in ovarian morphology. We used Spraque-Dawley rat between at 6 and 16 month old. The NA was determined by HPLC, NGF proteins by ELISA and NGF and p75NTR mRNA by real time PCR. The results show that plasma NA content decreased gradually with age, while in the ovary NA content increased at ageing. NA content in celiac ganglion only decreased after 12 month old,. The local changes of NA are accompanied by an intraovarian increase of NGF mRNA. Nevertheless, the content of p75NTR not changed. The ovarian morphological analysis shows after rat 12 month old present an increased number of type III, luteinized as cystic follicles. In conclusion, the increase of cystic and type III follicles in the rat aging, after 12 month old and at the end of reproductive function are preceded by a local increase of intraovaric neurotrophin and nerves sympathetic activity.

P539

Incorporation and release of ^{3}H-norepinephrine by granulosa cells: Novel functionality for endocrine cells

Monika Greiner & Hernán E Lara
University of Chile, Santiago, Metropolitan Area, Chile.

Ovarian function in mammals is regulated by gonadotropins and by sympathetic nervous system. Norepinephrine (NE) is one of the major neurotransmitters present in the fibres innervating the gonad and regulates follicular development and ovarian steroids release. Surgical section of the sympathetic fibres partially decreased the release of NE as compared with non-denervated rats. The remnant release capability supposes the existence of an intraovarian compartment able to incorporate and release NE independent of the sympathetic innervation. To study one of these compartments, we used fresh isolated rat granulosa cells and observe that they incorporate and release ^{3}HNE in response to a depolarizing stimulus. These cells are immunoreactive for the dopamine transporter (DAT), and cocaine, a selective inhibitor of DAT, blocks the norepinephrine incorporation. In contrast to granulosa cells, luteal cells presented a weak immunoreactivity to DAT and a diminished capability for incorporate and release norepinephrine. This data provide information for a role of granulosa cells in the control of intraovarian norepinephrine homeostasis and possibly to the ovarian function.
Supported by: Fondecyt 1050765, PG 63/2004

P540

Treatment of Cushing's disease by transsphenoidal pituitary micro-surgery: prognosis factors and long-term follow-up

Svetlana Arapova, Evgeniy Marova, Galina Kolesnikova, Andrey Grigorev & Anastasia Lapshina
National Research Center for Endocrinology Russian Academy of Medical Sciences, Moscow, Russia.

In our study we included retrospective analyses of 101 patients (mean age 35 years, 85 women and 16 men) with Cushing's disease (CD), who underwent transsphenoidal surgery (TSS) treatment. CD is based on clinical suspicion, hormonal research of cortisol (F), ACTH, 24-hous urine F, results of dexamethasone suppression tests low (1 mg) dose (LDDST) and high (8 mg) dose (HDDST) and MR-imaging (MRI). Before the operation all patients have high F, ACTH, negative LDDST and positive HDDST, abnormal responses to tests desmopressin (DDAVP), insulin and pituitary adenomas on MRI (76% - microadenoma and 24% - macroadenjma). Post-operative pituitary and adrenal functions were assessed after 5–10 days (serum F - post F), then every year. 74% of patients had adrenal deficiency after TSS. The results of serum post F, circadian rhythm F, ACTH, LDDST, desmopressin, and insulin tests were the criteria to define cure or remission. 82% of patients had clinical and biochemical remission over 6 month, 84% over 12 month after TSS.

75% of the patients have prolonged remission during long-term follow-up (in average 8,6 years).

Recurrent (R) in 12.4% of patients initially deemed to be remission, at a mean of 69 months. After 12 months the patients with R had post F >50 nmol/l, evaluation ACTH and F after DDAVP, but normal test LDDST.
Conclusion
Results of the study confirm the facts that the predictive value for long-term remission CD are: postoperative 09.00 h serum cortisol values $<$ 50 nmol/l; normal 24-hous urine F; normal circadian rhythm F, ACTH; normal LDDST, negative test with (DDAVP), normal response F and ACTH to insulin test over 12 month after TSS.

P541

Ketoconazole before transsphenoidal surgery in Cushing's disease patiens as a good alternative to glucocorticoids perioperatory treatment

E Venegas[1], A Soto[1], M Vazquez[1], R Guerrero[1], A Pumar[1], JM Montero[2], N Garcia[1], MA Mangas[1], A Leon[3] & A Leal-Cerro[1]
[1]Departament of Endocrinology and Nutrition, Hospital Universitario Virgen del Rocío, Sevilla, Spain; [2]Departament of Neurosurgery, Hospital Universitario Virgen del Rocío, Sevilla, Spain; [3]Departament of Laboratory, Hospital Universitario Virgen del Rocío, Sevilla, Spain.

Cushing's disease is a debilitating endocrinopathy characterized by excessive cortisol levels in the blood which may be produced from tumours of the pituitary gland. The only way to achieve long term cure of Cushing's disease is by Transphenoidal removal of the adenoma. ketoconazole, inhibit steroid (cortisol) production in the adrenal glands.
The use of glucocorticids treatment before and after hypophisectomy is a classic management in the perioperative Cushing disease patients.
Aim
To assess if ketoconazole treatment previous to pituitary surgery could free the plasma cortisol postsurgical determination from any interference from steroid substitute treatment without clinical risks for patients. To evaluate in how many patients we can avoid systematic substitutive treatment.
Method
We have treated 38 Cushing's disease patients with ketoconazole (400–800 mg/d) during 3–6 weeks before the pituitary surgery and we have evaluated the plasmatic cortisol levels immediately after the surgery. Neither intraoperatory nor immediately postoperatory glucocorticoids were administrated until hypocortisolims were diagnosticated.
Results
In 9 of 38 patients (23.68%) substitutive treatment was not necessary. 26 of 38 patients needs glucocorticoids treatment: 11 in the 3–7 days after the surgery, (2 of them with symptomatic hypocortisolims), and 13 about 30 days after the surgery. In 12 cases (31.58%) the substitutive treatment was iniciated because of laboratory hypocortisolims and in 14 cases (36.8%) the treatment was started because of clinical suspicious of hypocortisolims.
Conclusions
The treatment with Ketoconazole before pituitary surgery can allow us the measure of plasmatic cortisol postoperatory without the interference of de substitutive treatment in a security way, and in some patients we can avoid systematic substitutive treatment.

P542

Analysis of three different tests in the diagnosis of growth hormone deficit (GHD) in patients with severe cerebral trauma

A Soto[1], E Venegas[1], A Caro[1], R Gurrero[1], MD Rincon[2], S Garcia[2], A Pumar[1], A Leon[3], JM Flores[2], F Murillo[2] & A Leal-Cerro[1]
[1]Department of Endocrinology, HH.UU Virgen del Rocío, Sevilla, Spain; [2]Department of Critical Care, HH.UU Virgen del Rocío, Sevilla, Spain; [3]Department of Laboratory, HH.UU Virgen del Rocío, Sevilla, Spain.

Introduction
Patients with severe cerebral Trauma are a risk population for developing hipopituytarism. Diagnosis of GHD need to study pituitary gland reserve with a stimulation test. Insulin Tolerance test (ITT) is the best standard test, although has important contraindication in these patients and its realization is not always a possible. We evaluate the diagnostic capacity of two alternative test (Glucagon Test an GHRH-GHRP6 test) and compared them with ITT.
Material and methods
In 52 adult patients with severe cerebral trauma (Glasgow < 8) occurred at least 12 month before study, we perform three consecutive test, with a minimum period of 72 hours among each other, in the following order: (1) Glucagon test. Glucagon 1 mg s.c with extractions at 90, 120, 150, 180, 210 and 240 minutes to determinate GH and glucose. (2) GHRH-GHRP-6 test. GHRH 100 mg and GHRP-6 100 mg, bolus i.v, and extractions in 0, 30, 60, 90 and 120 minutes to determinate GH. (3) ITT. Regular insulin 0.05–0.15 UI/Kg, bolus i.v and extractions in 0, 30, 60 minutes to determinate GH and Glucose, to get glucose level <40 mg/dl. In 10 patients ITT could not be done because of contraindications. GH values used for diagnosis of GHD are those published in de literature for each of these test. (Glucagon test, <3 mg/dl, GHRH-GHRP-6 <15 mg/dl and ITT < 3 mg/ml).
Results
15/47 (31.91%) patients were diagnosticated of GHD with ITT, 8/58 (13.57%) patients were diagnosticated of GHD with GHRH-GHRP-6 test and 23/46 (41.07%) patients were diagnosticated of GHD with Glucagon test. Glucagon test sensitivity and specificity was 73.3%, Positive Predictive Value was 57.8% and Negative Predictive Value 84.61%, when we compared with ITT. GHRH-GHRP-6 test sensitivity was 40% and specificity was 100%, Positive Predictive Value was 100% and Negative Predictive Value 82.92%, when we compared with ITT.
Conclusions
Variability among three test is important. Glucagon test is not a good test since it has neither good sensitivity nor specificity. GHRP-GHRP-6 test is very specific, and may be valuable as confirmation test.

P543

Meningiomas in patients diagnosed with acromegaly: the report of two cases

Malgorzata Trofimiuk[1], Dariusz Adamek[2], Ryszard Czepko[3], Grzegorz Sokolowski[1], Agata Baldys-Waligorska[1] & Bohdan Huszno[1]
[1]Chair and Department of Endocrinology, Collegium Medicum, Jagiellonian University, Krakow, Poland; [2]Neuropathology Department, Collegium Medicum, Jagiellonian University, Krakow, Poland; [3]Neurosurgery Department, Collegium Medicum, Jagiellonian University, Krakow, Poland.

Introduction
Only several cases of co-existing meningiomas and pituitary tumours secreting growth hormone (GH) have been described so far in patients not treated previously with irradiation.
Aim
The aim of the study was to describe two cases of co-occurrence of acromegaly and meningioma and to discuss their relationship.
Case reports
Case 1. 52-year old female complained of visual disturbances. She was diagnosed with pituitary microadenoma secreting GH, and subsequently underwent successful transsphenoidal surgery. MRI performed after surgery revealed the presence of the second tumour invading right optic nerve canal. She was re-operated, meningioma was confirmed on histopathological examination. After the surgery her visual field has improved.
Case 2. 26-year old female was admitted to the hospital due to rapidly progressing apathy and extremities paralysis. Head CT showed the giant tumour of parasellar region invading neighbouring central nervous system structures. She was operated by transcranial approach). The histopathological assessment showed fibrous meningioma. After second transsphenoidal surgery, the GH-secreting pituitary tumour was confirmed. Although the operation did not removed the whole tumour, the patient improved substantially. One year later she was re-operated because of high levels of GH not controlled by somatostatin analogue injections. Unfortunately, the surgery did not normalised GH levels. The patient declined irradiation.
Conclusions
Co-existence of meningiomas and acromegaly may result from pro-proliferative action of high levels of GH and/or IGF-1 on central nervous system tumours expressing growth hormone and insulin-like growth factors receptors, although most of the clinical observations argue against the close relationship between increased IGF-1 levels and development of meningiomas in humans.

P544

Topoisomerase II alpha expression in pituitary tumours – preliminary results

Malgorzata Trofimiuk[1], Dariusz Adamek[2], Ryszard Czepko[3], Grzegorz Sokolowski[1] & Bohdan Huszno[1]
[1]Chair and Department of Endocrinology, Collegium Medicum Jagiellonian University, Krakow, Poland; [2]Neuropathology Department Collegium Medicum Jagiellonian University, Krakow, Poland; [3]Neurosurgery Department Collegium Medicum Jagiellonian University, Krakow, Poland.

Introduction
Topoisomerase II alpha is regarded as the important marker of cellular proliferation. Pituitary tumours are usually benign, but some of them are characterized by rapid growth, high recurrence rate and local invasiveness. Proliferation marker Ki-67 most commonly used in pituitary tumours is not entirely reliable as the indicator of the aggressive growth of the lesion. Topoisomerase II alpha expression assessment may be a valuable tool for identification such pituitary neoplasms.
Aims
The aim of the study was to assess topoisomerase II alpha expression in pituitary tumours as a factor influencing tumour behaviour.
Material and methods
The study included 24 subjects (15 males and 9 females aged 24–79 years, mean age 53 years) who had underwent surgery due to pituitary tumour. The tissue

samples were stained immunohistochemically for ACTH, FSH, LH, GH, PRL, TSH and topoisomerase II alpha. Topoisomerase index (IT) was assessed as a number of positive-stained nuclei per 100 tumour cells.
Results
The IT in studied subjects varied from 0 to 93 (median value – 0.8; males – 0.2; females – 0.8). The highest IT value was observed in the case of pituitary germinoma. Among the patients diagnosed with pituitary adenoma, the highest expression of topoisomerase was noted in GH positive- (IT value of 1.35) and ACTH positive tumours (IT of 0.8). The lowest IT values were noted in adenomas co-expressing LH/FSH and PRL/GH (IT of 0.3 and 0.1, respectively). Only in 8% of all studied tumours no expression of topoisomerase was found. The IT in larger tumours invading neighbouring structures was higher but the difference did not reach the statistical significance.
Conclusion
Topoisomerase II alpha seems to be useful marker for assessment of proliferation activity of pituitary tumours, particularly in case of rapidly growing tumours such as germinal neoplasms or metastases. We have presented preliminary results.

P545

Is there an endocrine explanation for persistent neuropsychological disabilities long after traumatic brain injury (TBI)?

Edwige Yollin[1], Odile Kozlowski[2], Benoît Soudan[3], Michèle D'Herbomez[4], Pierre Fontaine[1], Marc Rousseaux[4] & Christine Cortet-Rudelli[1]
[1]Department of Endocrinology and Diabetology, Clinique Marc Linquette, Centre Hospitalier Régional Universitaire, Lille, Nord, France; [2]Department of Reeducation, Hôpital Swynghedaw, Centre Hospitalier Régional Universitaire, Lille, Nord, France; [3]Laboratory of biochemistry, Centre Hospitalier Régional Universitaire, Lille, Nord, France; [4]Laboratory of nuclear medicine, Centre Hospitalier Régional Universitaire, Lille, Nord, France.

The aims of this study were to determine the prevalence of pituitary dysfunction in patients keeping neuropsychological disabilities long after TBI (at least 1 year), to research predictive factors and to evaluate consequences of endocrine abnormalities on metabolism and quality of life in these patients.
We studied 50 patients (42 men, mean age 36, range 20–59 years, mean BMI 25, range 17–42 kg/m^2) who had survived severe ($n=38$), moderate ($n=2$) or mild TBI ($n=10$) at a mean of 59 months (range 13–247) post event. 52% had moderate, 32% had severe disability (GOS score: 2 or 3 respectively), 30% had anosognosia.
No patient showed posterior pituitary dysfunction, hyperprolactinemia or gonadotropin deficiency. Six patients (12%) showed TSH deficiency. Ten patients (20%) had partial ACTH deficiency (diagnosed by ITT or metyrapone test). Severe GH deficiency was diagnosed in 44.5% (glucagon stimulation test confirmed by ITT or arginine + GHRH test) and was isolated in 40% of cases. GHD patients had significantly higher BMI, triglycerid, fasting and postprandial insulin plasma levels than no-GHD patients, but mean QoL-AGHDA or NHP questionnaires scores were not significantly different in the 2 groups even among non-anosognosic patients. Totally 46% of the patients showed at least one anterior pituitary deficiency requiring a substitutive treatment (multiple and isolated hormone deficiency in 24% and 22% respectively). Hypopituitarism was not related to GCS score, initial CT scan lesions, GOS score, self-sufficiency (EBIS scale score) or resumption of work.
The high risk for anterior pituitary deficiency in patients with persistent neuropsychological disabilities long after TBI justify a pituitary exploration in all of them, with reference tests, even long after the TBI. Evaluation of quality of life must be adjusted to TBI patients, with specialised neuropsychological testing and multidisciplinary collaboration.

P546

ACTH and cortisol responses to ghrelin and DDAVP in patients with Cushing's disease (CD)

Dragana Miljic, Mirjana Doknic, Sandra Pekic, Marina Djurovic, Zorana Penezic, Svetozar Damjanovic, Djuro Macut, Mirjana Sumarac-Dumanovic, Bojana Popovic, Mohammad Ghatei & Vera Popovic
Institute of Endocrinology, Belgrade, United States; Imperial College, London, United Kingdom.

Aim of this study was to investigate head to head ACTH and cortisol responses to DDAVP and ghrelin in patients with CD. Study was approved by the local ethics committee and informed consent obtained. Nine patients with CD were submitted to ghrelin 1 μg/kg and DDAVP 10 μg bolus iv administration on two separate occasions. Blood was sampled at 0.15, 30, 45, 60, 90 min. for ACTH (ELISA) and cortisol (RIA, Cis, Bio International, France) measurements.
Ghrelin induced significant ACTH (65.3 ± 54.7 vs 188.6 ± 128.8 pg/ml; $P<0.05$) and cortisol responses (642.5 ± 357.2 vs 856.0 ± 447.4 nmol/l; $P=0.05$) in our patients. After DDAVP there was also a significant increase in ACTH (53.5 ± 49.3 vs 227.6 ± 359.2 pg/ml; $P=0.05$) and cortisol levels (444.5 ± 249.2 vs 658.8 ± 369.6 nmol/l; $P<0.05$). When compared peak ACTH and cortisol values after both tests were not statistically different. Integrated ACTH (pg/ml.min) (ghrelin:11 677.7 ± 7 253.6 vs DDAVP: 12 470.4 ± 16 911.2) and cortisol (nmol/l.min) secretion (ghrelin: 81 810.6 ± 49 437.6 vs DDAVP: 64 677.0 ± 38 399.9) were not significantly different after two tests.
Although limited by size our study shows that ghrelin compared to DDAVP induces similar cortisol and ACTH responses. The mechanisms of ghrelin action on ACTH and cortisol secretion in patients with CD merit further investigation.

P547

Effect of treatment with somatostatin analogue on glucose homeostasis in patients with acromegaly

Nikos Gavalas, Andrea Sabo, Lida Papastathopoulou, Efi Botoula & Marinella Tzanela
Evangelismos Hospital, Athens, Greece.

Long acting somatostatin analogues (SMS) are extensively used as second and even first line treatment of acromegaly. Except of the inhibition of GH secretion, somatostatin is a potent inhibitor of insulin secretion from the pancreatic b- cells. As defects of glucose homeostasis are very common in acromegaly, we decided to examine the effect of the control of GH hypersecretion with SMS on glucose metabolism.
We study 44 acromegalic patients divided in 3 groups. Patients of group I ($n=18$) were evaluated at the time of diagnosis and before any therapeutic intervention, while patients of groups II and III were evaluated after control of their disease (indicated by normal IGF-I values for age and sex and GH levels <1 μg/L during OGTT, Consensus 2000) either by transphenoidal surgery, alone or followed by pituitary irradiation, (group II, $n=16$) or by somatostatin analogue administration (group III, $n=10$).
Insulin levels were significantly lower in groups II and III compared to group I (7.5 ± 0.6 and 5.2 ± 0.8 vs 15.7 ± 2.7 μIU/ml, $P<0.05$) with a parallel drop of insulin resistance (as estimated by HOMA-IR) from 4.9 ± 0.9 in group I to 1.8 ± 0.2 and 1.4 ± 0.2 ($P<0.05$) in groups II and III respectively. Insulin secretion (as estimated by HOMA-β) was statistically lower in group III than in group I and II (42.4 ± 6.97 vs 117.2 ± 18.8 and 85.6 ± 7.49 respectively, $P<0.05$). These alterations led to lower mean glucose levels in group II compared group I (99 ± 4.9 vs 120.9 ± 8.3 mg/dl, $P<0.05$) but not in group III (108.4 ± 3.1 mg/dl). The incidence of Diabetes Mellitus dropped from 50% in group I to 12% in group II and 10% in group III, while that of Impaired Glucose Tolerance from 33% in group I to 18.7% in group II but to 30% in group III.
In conclusion, despite of treatment modality, successful control of acromegaly reduces the incidence of Diabetes Mellitus. However, control of GH hypersecretion with SMS treatment seems to be less effective to fully reverse the impaired glucose tolerance, probably due to inhibition of insulin secretion by SMS.

P548

Effects of combined treatment with cabergoline and somatostatin analogues (SAA) on GH and IGF-I levels and tumor volume in patients with acromegaly not fully responsive to SAA

Renata S Auriemma, Mariano Galdiero, Maria Cristina De Martino, Monica De Leo, Gaetano Lombardi, Annamaria Colao & Rosario Pivonello
Department of Molecular and Clinical Endocrinology and Oncology, "Federico II" University, Naples, Italy.

Dopamine agonists have been used as first or adjunctive therapy for acromegaly for many years, but relatively few studies have assessed the efficacy of a newer agonist, cabergoline (CAB) alone or in combination with somatostatin analogues (SSA). The aim of this study was to evaluate the efficacy of combined treatment with SSA *plus* CAB in patients with acromegaly and resistance to SSA, defined as lack of normalization of IGF-I levels after long-term (>1 year) and high dose (30 mg/month) treatment with SSA. Twelve patients (8 men and 4 women, age 32–70 years) with active acromegaly after unsuccessful surgery entered the study: 10 patients had been treated with octreotide LAR and 2 with lanreotide; 7 had a pituitary

macroadenoma, 2 a microadenoma and 3 an empty sella. None of the patients had hyperprolactinemia. CAB was added at the initial dose of 1 mg/week for 1 month, then increased to 3.5 mg/week. After long-term SSA treatment, no significant difference in GH ($P=0.56$) and IGF-I ($P=0.08$) levels was found, whereas tumor volume was significantly reduced ($P=0.014$) as compared to baseline. After 6-month treatment with SAA *plus* CAB, both GH ($P=0.004$) and IGF-I ($P=0.005$) levels as well as tumor volume ($P=0.014$) were significantly decreased compared to baseline. Moreover, GH ($P=0.02$) and IGF-I ($P=0.002$) levels, as well as tumor volume ($P=0.014$), measured after SAA *plus* CAB treatment were also significantly lower than those measured after SSA treatment alone. The addition of CAB to SSA induced a percent GH, IGF-I and tumor volume decrease of $46\pm41\%$, $24\pm23\%$ and $17\pm37\%$ respectively. After six months of combined treatment, six patients (50%) showed a normalization of GH and IGF-I levels. In conclusion, combined treatment with SAA *plus* CAB can be effective in inducing IGF-I normalization in acromegalic patients resistant to SSA and deserves an important role as alternative treatment in the therapeutic algorithm of acromegaly.

P549

Macroprolactin: the clinically and diagnostically importance

Larisa K Dzeranova, Ekaterina N Giniatullina, Nikolai P Goncharov, Anna D Dobracheva & Galina S Kolesnikova
Research Centre for Endocrinology, Moscow, Russia.

Recently, the phenomenon of macroprolactinaemia has manifested itself into a great interest for physicians. This problem forces both physicians and patient to waste sizeable resources, and can lead to iatrogenic and unjustified emotional stress and further material losses. At the same time the problem of differential diagnostics of pseudoprolactinomas and true prolactinomas remains challenging. The purpose of the present study was to determine the clinic-analytical repercussion of the presence of maPRL in female patients with hyperprolactinaemia.

The patients and methods

321 patients with hyperprolactinaemia (PRL level was more than 700 mU/l) were examined (36 male and 285 female). The age mediana was 29 ± 3 years. The quantitative estimation of biologically active monomeric PRL was conducted. A polyethylene glycol (PEG) precipitation test (Delfia System) was used to detect the presence of maPRL in all consecutive samples with prolactin levels >700 mU\L). A recovery $<60\%$ was taking as indicating of maPRL.

The results

maPRL was found in 57 (18%) of 321 patients with total PRL >700 mU\l; all other 264 patients (82%) had maPRL below 60%. Mediana of PRL level in the group with macroprolactinaemia was – 1167 mU/l (700–1635); the mediana of maPRL-997 mU/l (700–1295). The most frequent reason for the initial PRL request was menstrual disturbance (36.8% patients). As for clinical presentations, Galactorrhea was noted in 19.2% cases; the headaches -in 38.5% patients, the increasing of the mass of the body in 24.5% of cases. The microadenomas were revealed in 38.7% events, and macroadenomas – in 4.5% cases.

The conclusion

Macroprolactinaemia is a frequent condition. The estimation of PRL fractions is an important problem and nessesary for diagnostic mistakes elimination, to avoid the unnecessary diagnostic procedures, to the needless medical treatment or surgery prevention. The determination of maPRL in routine studies is recommended.

P550

OASIS: observational study on the international clinical practice for the treatment of recently diagnosed acromegalic patients

Lesly Portocarrero[1], Güngör Akçay[2], Hakim Bouterfa[3], Davide Carvalho[4], Yona Greenman[5], Feng Gu[6], Mirtha A Guitelman[7], Sungwoon Kim[8], Christine Kriesche[9], Moises Mercado[10], Chiung-Chi Shen[11], Antonio Silva[3], Cesar Varela[12], Henri Wallaschofski[13] & Susan Webb[14]
[1]National Institute of Neurology and Neurosurgery, Department of Endocrinology, Mexico City, Mexico; [2]Ataturk University Hospital, Erzurum, Turkey; [3]Novartis Pharma AG, Basel, Switzerland; [4]Hospital de São João, Porto, Portugal; [5]Tel Aviv Medical Center, Tel Aviv, Israel; [6]Peking Union Medical College Hospital, Beijing, China; [7]Department of Endocrinology, Hospital Carlos G. Durand, Buenos Aires, Argentina; [8]Kyung Hee University, Seoul, Korea, South; [9]Chemnitz, Germany; [10]Endocrinology Service, Hospital de Especialidades, Centro Medico Nacional Siglo XXI, IMSS, Mexico City, Mexico; [11]Taichung, Taiwan; [12]Hospital Ramón y Cajal, Madrid, Spain; [13]University of Greifswald, Greifswald, Germany; [14]Hospital Sant Pau, Barcelona, Spain.

Background

There is significant interest in how the use of different treatment regimens (e.g. surgery, medical therapy) impacts the clinical course of Acromegaly. This study has been designed to better understand clinical decision making in the context of various treatment options.

Methods

OASIS is an international, observational study in recently diagnosed acromegalic patients. Ethical committee approval was obtained where applicable. Observations include biochemical control, acromegaly symptoms, tumor volume, safety and tolerability. Patient data are collected under normal practice conditions over a 12 months period.

Results

As of November 2006, 380 patients are enrolled from 103 centres in 21 countries. Baseline characteristics of the first 133 patients with available data are reported here. About half of the patients are female (56%), the majority (82%) are Caucasian, and the mean age is 48 years. Most patients (70%) have a diagnosis of macroadenoma. At baseline, 61% of patients had a planed treatment with Sandostatin LAR alone or combined with surgery. 39% of patients received other treatment options (e.g. surgery alone, radiotherapy or non- Sandostatin LAR medical therapy). The most common starting dose for Sandostatin LAR was 20 mg (74% of the patients treated with Sandostatin LAR). At baseline median levels of GH were 8.8 ng/mL in 58 patients treated with Sandostatin LAR (alone or in combination) and 12.8 ng/mL in 46 patients treated with other therapies. IGF-1 levels were 626 ng/mL in 56 patients with Sandostatin LAR and 713 ng/mL for patients with other therapies. At first quarter follow-up data were available for 35 patients with GH and for 27 patients with IGF-1 levels. The median values of GH showed a 40% decrease in the Sandostatin LAR group and 70% decrease in patients with other therapies. Similarly, IGF-I decreased by 22% and 40%, respectively.

Conclusions

These first data show a large proportion of patients treated with Sandostatin LAR as first treatment option. Observation of the treatment practice over the complete course of the study will provide a more complete picture of the treatment choice for these patients.

P551

Comparison of basal ghrelin and leptin serum levels and after an oral glucose tolerance test in active and inactive acromegalic patients

Josefine Roemmler, Bärbel Otto, Birgit Steffin, Martin Bidlingmaier & Jochen Schopohl
Medizinische Klinik – Innenstadt, Munich, Germany.

Leptin and ghrelin are correlated to acute and chronic nutritional status. Elevated BMI and fat mass as well as food intake increase leptin levels whereas ghrelin levels are reduced. Ghrelin stimulates growth hormone (GH) secretion. The influence of GH on ghrelin is unclear. Since GH reduces fat mass and is dependent on nutritional status we performed this prospective cross sectional study in order to investigate any interaction between GH, ghrelin and leptin levels in active and inactive acromegalic patients (pat).

We measured glucose, insulin, ghrelin, leptin and GH concentration during a 3 h oral glucose tolerance test (OGTT) and IGF-I in 36 acromegalic patientsients (19f/17m, median age 53.3y (20-75)). 29/36 patients underwent surgery. At time point of evaluation none of the patients had received radiotherapy or any medication for acromegaly. Concentration of GH and IGF-I were determined by a single laboratory using the same immunoassay (Nichols Advantage, San Clemente, CA). Active disease was defined as IGF-I above the upper limit of age- and sex-adjusted normal.

11 patients had active acromegaly, 25 were inactive. BMI was not significantly different between active and inactive patients. Baseline ghrelin levels were significantly reduced in active compared to inactive patients ($P<0.01$), baseline leptin levels were only slightly reduced (p=n.s.). Basal leptin was positively correlated to baseline blood glucose in active patients ($P<0.01$) and to BMI in inactive patients ($P<0.05$). During OGTT ghrelin and leptin significantly decreased (active: $P<0.01$; inactive: $P<0.001$). The ghrelin decline was significantly higher in inactive patients ($P<0.05$).

In active acromegalic patients the ghrelin regulation by nutritional status and food intake is reduced which could be due to a negative feedback of GH and IGF-I on ghrelin secretion. The tendency of lower leptin levels in active acromegalic pat might be caused by lipolytic effect of elevated GH levels.

P552

Determinants of the acromegalic cardiomyopathy: a prospective, controlled study in 205 patients

Annamaria Colao[1], Rosario Pivonello[1], Renata S. Auriemma[1], Mariano Galdiero[1], Letizia Spinelli[2], Maurizio Galderisi[3] & Gaetano Lombardi[1]
[1]Department of Molecular and Clinical Endocrinology and Oncology, section of Endocrinology, University "Federico II" of Naples, Naples, Italy; [2]Department of Internal Medicine I, Section of Cardiology; University "Federico II" of Naples, Naples, Italy; [3]Department of Experimental Medicine, Section of Cardiology, II University of Naples, Italy, Naples, Italy.

The most frequent cause of death in acromegaly is cardiomyopathy. To evaluate determinants of the acromegalic cardiomyopathy we performed an analytical, observational, open, prospective, controlled study in 205 patients with active acromegaly (108 women and 97 men) and 205 non-acromegalic subjects sex- and age-matched with the patients. We determined the prevalence of Left Ventricular (LV) hypertrophy (LVH), diastolic and systolic dysfunction, by echocardiography- measured LV mass index (LVMi) early-to-late mitral flow velocity (E/A) and LV ejection fraction (LVEF). The role of age, estimated disease duration, BMI, GH and IGF-I levels, systolic and diastolic blood pressure, lipid profile and glucose tolerance was investigated. Compared to sex- and age-matched controls, the patients had lower BMI, E/A, LVEF, HDL-cholesterol levels and higher LVMi, total and LDL-cholesterol, triglycerides, glucose and insulin levels, HOMA-R and HOMA-β. The relative risk to develop mild [Odds ratio (OR) = 2.2 (1.3–3.8) $P = 0.002$] or severe hypertension [OR = 3.2 (1.7–6.0), $P < 0.0001$], arrhythmias [OR = 3.7 (1.1–5.6), $P = 0.017$], impaired glucose tolerance [OR = 2.6 (1.5–4.6), $P = 0.0002$], diabetes [OR = 2.1 (1.2–3.8), $P = 0.006$], LVH [OR = 11.5 (7.1–19.0), $P < 0.0001$], diastolic [OR = 5.4 (3.2–9.2), $P < 0.0001$], and systolic dysfunction [OR = 6.3 (3.1–13.8), $P < 0.0001$], was higher in acromegaly. Disease duration and systolic blood pressure level was the most important predictor of LVH ($t = 2.4$, $P = 0.02$) and systolic dysfunction ($t = -2.8$, $P = 0.006$) while diastolic dysfunction was predicted by patient's age ($t = -3.3$, $P = 0.001$). The patients were divided into three groups based on disease duration: short ($\leq$60 months), intermediate (60–144 months; 75 percentile) and long ($>$144 months). Patients with long estimated disease duration had a relative risk to present LVH 9.9 times, diastolic dysfunction 4.8 times and all cardiac complications 3 times higher than patients with shorter estimated disease duration. In conclusion the prevalence of different features of cardiomyopathy is 5.4–11.5 times higher in the acromegalic than in the non-acromegalic population. The major determinant of cardiomyopathy is disease duration.

P553

Growing incidence of idiopathic isolated secondary adrenal insufficiency.

Anna Kasperlik-Zaluska[1], Barbara Czarnocka[1], Lucyna Papierska[1], Sophie Bensing[2], Anna Hulting[2] & Patricia Crock[3]
[1]Centre for Postgraduate Medical Education, Warsaw, Poland; [2]Karolinska Institutet, Stockholm, Sweden; [3]University of Newcastle, Newcastle, Australia.

Objective
The origin of idiopathic isolated secondary adrenal insufficiency (IISAI) is uncertain, however autoimmunity seems to be the most probable cause. Within last eight years the initial number of about 100 such cases, increased in our registry by 250%. We searched for features of autoimmune diseases in our group of patients to prove autoimmune etiology in a majority of these patients.
Materials and methods
The material consisted of 260 patients with IISAI (female/male ratio 10.8, age 17–78 years). The diagnosis was based on clinical characteristics and hormonal (especially cortisol and ACTH) examinations, including $^{1\text{-}24}$ACTH stimulating test. Methods: clinical examination, hormonal investigations (TSH, LH, FSH, PRL, fT_4), immunological studies (routine antithyroid autoantibodies + pituitary autoantibodies by an immunoblotting assay with human pituitary cytosol as autoantigen, in 65 patients), imaging methods (MRI of the pituitary – in a part of patients).
Results
Autoimmune disorders were diagnosed in 181 patients (70%), the most frequently thyroid diseases (especially hypothyroidism), vitiligo and premature ovarian failure. The thyroid autoantibodies were detected in 65% of the patients, while pituitary autoantibodies in 34% of the patients under study (immunoreactivity to a 49-kDa and to a novel 36-kDa pituitary autoantigen). Partially empty sella was the most frequent finding in MRI.
Conclusions
1/ The incidence of the diagnosed idiopathic isolated secondary adrenal insufficiency is growing in last years, probably mainly due to a better detectability of disease. 2/ Association of autoimmune disorders with IISAI in 70% of the patients suggests autoimmune origin of pituitary disease, confirmed by the presence of pituitary autoantibodies in 34% of the patients under study.

P554

The role of fibrinogen and CRP in cardiovascular risk in patients with acromegaly

Marcin Kaluzny & Marek Bolanowski
Dept. of Endocrinology, Diabetology and Isotope Therapy, Medical University, Wroclaw, Poland.

Patients with acromegaly have 2–3 fold increased mortality from cardiovascular diseases. It is associated with elevated growth hormone (GH) levels. Alterations of acute phase proteins, observed in patients with acromegaly, could lead to increased cardiovascular mortality. There are limited data on influences of GH excess on acute phase reactants.
The aim of the study was to evaluate selected acute phase proteins levels: fibrinogen and C-reactive protein (CRP) in patients with acromegaly.
Seventy-seven patients were divided into two groups: active acromegaly (AA, $n = 56$) and controlled acromegaly (CA, $n = 21$) according to minimal GH level during an oral glucose tolerance test and IGF-1 levels. Twenty sex matched healthy subjects were controls. The following parameters were measured: fibrinogen, CRP, fasting glucose, insulin, total cholesterol, LDL and HDL cholesterol, triglycerides and BMI.
Comparison of all groups using Mann-Whitney U testing revealed statistically significant: higher LDL cholesterol and insulin levels and lower CRP levels and BMI values in AA than CA groups ($P < 0.04$, 0.02, 0.01 and 0.03, respectively); higher fibrinogen, triglycerides, glucose levels and BMI values in AA group than controls ($P < 0.000001$, 0.002, 0.01 and 0.001, respectively); higher CRP, fibrinogen, triglycerides levels and BMI values in CA group than controls ($P <$ 0.01, 0.002, 0.04 and 0.001, respectively).
Fibrinogen levels in all patients with acromegaly were significantly higher than in healthy subjects irrespective of disease status. CRP levels were significantly and paradoxically lower in patients with active acromegaly than in patients with well controlled disease and did not explain increased cardiovascular mortality in acromegaly. The role of CRP levels as a cardiovascular risk factor in the mortality of patients with uncontrolled acromegaly ought to be better explained in future studies.

P555

Evaluation of insulin sensitivity with euglycemic hyperinsulinemic clamp technique in non-obese patients with microprolactinoma

Alpaslan Tuzcu, Serkan Yalaki, Senay Arikan, Deniz Gokalp & Mithat Bahceci
Dicle University Department of Endocrinology, Diyarbakir, Turkmenistan.

Background and aim
Hyperprolactinemia may associate with insulin resistance. This fact has been determined by many studies with methods which show insulin sensitivity. In this study we aimed to search insulin resistance with golden standard euglycemic hyperinsulinemic clamp technique on hyperprolactinemic patients.
Subjects and methods
This study was performed in Endocrinology Department of Dicle University. Sixteen patients with microprolactinoma (mean age: 32.06 ± 11.60 year and BMI: 24.43 ± 3.23 kg/m^2) 12 healthy subjects (mean age: 31.25 ± 9.40 year and BMI:24.33 ± 3.42 kg/m^2) were included to the study. Fasting glucose, insulin levels and lipid parameters were measured in both groups. HOMA-B and HOMA-IR values of groups were calculated. Euglycemic hyperinsulinemic clamp technique was performed to the both group and M value of the groups was defined. Mann-Whitney U and Chi-Square tests were used in statistically analysis.
Result
Age, BMI, total cholesterol, triglycerides, LDL-cholesterol, HDL-Cholesterol and fasting glucose levels of the groups were not show statistically difference. Basal insulin level of hyperprolactinemic patients were higher than control group (6.85 ± 4.68; 3.66 ± 0.88 pU/ml respectively; $P < 0.05$). Mean HOMA-IR and HOMA-B values of patients were higher than control group (1.49 ± 1.30, 0.78 ± 0.27 respectively; $P < 0.05$) (136.28 ± 72.53, 64.77 ± 23.31 respectively, $P < 0.05$). Insulin resistance was determined on 5 patients by euglycemic

hyperinsulinemic clamp technique (M<4). M values of the patients were statistically lower than control group (5.64±2.36, 7.05±1.62 kg/mg/m^2 respectively, $P<0.05$).
Conclusions
1- Hyperprolactinemia is associated with an insulin-resistant state
2- Insulin resistance of hyperprolactinemic patients is not associated with obesity.

P556

Abstract unavailable

P557

Comparative analysis of reactivity of macroprolactin in first and second-generation prolactin assays

Erzsébet Rigó[1], Erzsébet Toldy[2], Zoltán Löcsei[1], Erzsébet Toldy[4], István Szabolcs[3] & Gábor L. Kovács[5]
[1]1st.Department of Medicine Markusovszky Teaching Hospital of County Vas, Szombathely, Hungary; [2]Central Laboratory Markusovszky Teaching Hospital of County Vas, Szombathely, Hungary; [3]Department of Endocrinology National Medical Centre, Budapest, Hungary; [4]Department of Practial Diagnostics of Institute of Diagnostics and Management University of Pécs, Szombathely, Hungary; [5]Institute of Laboratory Medicine, University of Pécs, Pécs, Hungary.

Issue
Macroprolactin (MPRL) is a high molecular mass complex of prolactin with minimal bioactivity in vivo that may be the cause of elevated serum prolactin (PRL) as determined by immunoassay. Unrecognised macroprolactinemia can lead to misdiagnosis and mistreatment. The frequency of MPRL is highly dependent on the affinity of the antibody used in the assay.
The aim of this study
Was to compare the frequency and quantity of MPRL measured by a first and second-generation prolactin assay.
Methods
109 sera sent for PRL estimation were analysed: PRL was measured both in the native sera and after PEG-precipitation by a first and second-generation electrochemiluminscence immunoassay (ECLMA1 and ECLMA2, Elecsys 2010, Roche).
Results
The mean PRL concentration was lower if measured by ECLMA2 (961±687 versus 1419±1079 IU/l, $P<0.001$). The rate of elevated PRL was 59% by ECLMA1 and 51% by ECLMA2 respectively. The mean recovery following PEG-precipitation was not different (89±23 and 89±15), but the rate of macroprolactinemia defined (as less than 40% recovery) was 10-times more often by ECLMA1 ($N=19$) than ECLMA2 ($N=2$). Normalisation of elevated PRL levels after PEG precipitation occurred in 10% ($N=11$) and 12% ($N=12$), respectively of the sera with not difference according to the method used and typically in cases with slight hyperprolactinemia.
Conclusion
The affinity of the second-generation ECLMA2 assay to MPRL seems to be less than that of the first generation assay. Approximately 8% less cases of macroprolactinemia are to be expected by the novel assay even is the normal "cut off" level of PRL is decreased from 700 to 500 IU/l. The dramatic decrease of cases with less than 40% recovery raises the proposition that instead of %-recovery the normalisation of the PRL concentration following PEG precipitation should be used to define cases with macroprolactiemia.

P558

Cabergoline treatment in Cushing's disease: effect on hypertension, glucose intolerance and dyslipidemia.

Rosario Pivonello[1], Maria Cristina De Martino[1], Antongiulio Faggiano[1], Monica De Leo[1], Gaetano Lombardi[1], Leo J Hofland[2], Steven WJ Lamberts[2] & Annamaria Colao[1]
[1]Department of Molecular and Clinical Endocrinology and Oncology, "Federico II" University, Naples, Italy; [2]Department of Internal Medicine, Erasmus Medical Center, Rotterdam, Netherlands.

Cabergoline has been recently demonstrated to normalize cortisol secretion in more than one third of patients with Cushing's disease (CD). The aim of this study was to evaluate short-term (3-months) and long-term (12-24 months) effects of cabergoline treatment on the main systemic complications of CD, including hypertension, glucose intolerance and dyslipidemia. Twenty patients with CD unsuccessfully treated by neurosurgery entered the study. Cabergoline was admistered at the initial dose of 1 mg/week and a maximal dose of 7 mg/week. At 3-months follow-up, 15 (75%) patients were responsive whereas 5 (25%) were resistant to cabergoline treatment. Systolic and diastolic blood pressure, serum glucose and insulin levels, HOMA index, and serum cholesterol levels significantly decreased in parallel with the normalization of cortisol secretion. A significant improvement of blood pressure and a slight improvement in glucose tolerance and cholesterol levels was found both in responsive and resistant patients. Cabergoline treatment was continued in the 15 responsive patients, although treatment escape was observed in 5 patients, so that the long-term study was performed in 10 patients, who was followed-up for 12-24 months. During long-term treatment, urinary cortisol levels remained within the normal range. Serum glucose and insulin levels, HOMA index and serum cholesterol levels further decreased. At the last follow-up, the prevalence of hypertension decreased from 50% to 0%, glucose intolerance from 62.5% to 30%, and dyslipidemia from 33.3% to 0%. In conclusion, the results of the current study confirmed that cabergoline treatment is effective in controlling cortisol secretion for at least 1-2 years in more than one third of patients with CD, and demonstrated that it is able to improve hypertension, glucose intolerance and dyslipidemia in patients responsive and, partially, also in patients resistant to the treatment. Therefore, cabergoline is confirmed to be a useful treatment option in patients with CD unsuccessfully treated by neurosurgery.

P559

Development and validation of a questionnaire to evaluate health-related quality of life in patients with Cushing's syndrome

Susan M Webb[1], Xavier Badia[2], Maria- Jose Barahona[1], Annamaria Colao[3], Christian J Strasburger[4], Antoine Tabarin[5], MO van Aken[6], Rosario Pivonello[3], Gunther Stalla[7], O Chabre[8], Steven WJ Lamberts[6] & Joan E Glusman[9]
[1]Hospital S Pau, UAB, Barcelona, Spain; [2]IMS, Barcelona, Spain; [3]University Federico II, Naples, Italy; [4]Charité Campus Mitte, Berlin, Germany; [5]Hôpital Haut Lévêque-CHU, Bordeaux, France; [6]Erasmus Medical Center, Rotterdam, Netherlands; [7]Max-Planck-Institut für Psychiatrie, Munich, Germany; [8]CHU Hopital Nord, Grenoble, France; [9]Novartis, New Jersey, United States.

Chronic exposure to hypercortisolism has a significant impact on patient's health and Health-Related Quality of Life (HRQoL), as demonstrated with generic questionnaires. Objective: Develop and validate a disease-generated questionnaire to evaluate HRQoL in patients with Cushing's syndrome-CS- (Cushing QoL). Methods: After a literature review, interviews with expert endocrinologists and 10 patients identified HRQoL domains and clinical aspects of the disease; an analysis of the content allowed a qualitative reduction of items and design of version-1 (V-1) of the questionnaire, which was administered to 5 Spanish patients to detect and correct comprehension problems (cognitive debriefing); this allowed the obtaining of the V-2 version, the items of which were scored by 10 endocrinologists in terms of importance and frequency to select the most relevant ones and design the V-3 questionnaire, which was translated into 16 languages. This questionnaire was presented to 125 patients in an observational, international, multi-center, cross-sectional study, including 14 investigators from Spain, France, Germany, The Netherlands and Italy; the generic SF-36 questionnaire and a question on self-perceived general health status, as well as clinical and hormonal data were also collected. Results: 107 were pituitary-dependent and 18 adrenal- dependent CS; 83% were females, median age 45 yrs; 34% were currently hypercortisolemic and 38% adrenal insufficient. CushingQoL was feasible (94% of patients fully responded to the questionnaire in 4 minutes), reliable (Crohnbach's alpha =0.87) and valid (factorial analysis demonstrated unidimensionality and Rasch analysis lead to a final version with 12 items). A significant ($P<0.001$) correlation was observed between CushingQoL score and patients self-perceived general health status and dimensions of SF-36 (Pearson correlation coefficient >0.597). Patients with hypercortisolemia (56±22 vs 48±20, P=0.043) and increased UFC (56±19 vs 46±23, P=0.009) scored worse than those without.
Conclusion
Conclusion: CushingQoL is useful to evaluate HRQoL in patients with CS and correlates with clinical parameters.

P560

Differential expression of genes related to aggressiveness in non-functioning pituitary adenomas

Maria Asuncion Martinez-Brocca[1], Carmen Saez[2], Alfonso Soto[1], Carolina Castilla[2] & Miguel Angel Japon[2]
[1]Department of Endocrinology. Hospitales Universitarios Virgen del Rocio, Seville, Spain; [2]Department of Pathology. Hospitales Universitarios Virgen del Rocio, Seville, Spain.

Prediction of the biological behavior in non-functioning pituitary adenomas (NFPA) according to morphological criteria is highly inaccurate. Reliable prognostic molecular markers could be useful in providing guidance in NFPA post-surgical follow-up.
Aim
To identify differentially expressed genes between aggressive and non-aggressive NFPAs and to assess their prognostic value.
Methods
Samples analyzed were selected from a series of 60 NFPAs consecutively resected in our institution between 1998 and 2005 and kept frozen at $-80\,^{\circ}C$. Criteria for aggressive NFPA were invasion of surrounding structures or central nervous system at diagnosis (Hardy III/IV), recurrence and/or regrowth of post-surgical remnants. cDNA from pooled aggressive and non-aggressive NFPAs samples were labelled and hybridized on cDNA arrays (Superarray Bioscience), containing 192 genes related to invasiveness and angiogenesis, and normalized expression for each gene was calculated. Overexpression of selected genes was individually assessed by RT-PCR and its association to clinical parameters of agressiveness was analyzed.
Results
61.6% adenomas were classified as aggressive, and 38.4% as non-aggressive NFPAs. The expression of a subset of genes was 1.5 to 3.9 fold higher in aggressive NFPAs; among them, growth factors and their receptors (KGF, HGF, PDGFa, TGFb1, TGFb3, FGFR2, FGFR3), chemokines (CXCL1, CXCL4), metalloproteases (Meth1, MMP9) and other proteins related to cellular adhesion and migration, such as osteopontin and cadherin-5, were identified. By RT-PCR, cadherin-5 was found to be expressed in 100% of aggressive-NFPAs but only in 8.7% of non-aggressive NFPAs. Moreover, a trend toward a higher expression of osteopontin in NFPAs invading cavernous sinus was found. Differences in CXCL4 expression were not individually detected.
Conclusions
cDNA arrays are useful to identify differentially expressed genes in NFPAs with discordant clinical behavior. Cadherin-5 and osteopontin are potential markers of aggressiveness in NFPAs, a fact that might be related to a pro-angiogenic and pro-invasive state.

P561

Effects of CST-8, a synthetic cortistatin analogue, in humans

Andrea Benso[1], Flavia Prodam[1], Elena Gramaglia[1], Fabrizio Riganti[1], Barbara Lucatello[1], Aart Jan van der Lely[2], Romano Deghenghi[1], Giampiero Muccioli[3], Ezio Ghigo[1] & Fabio Broglio[1]
[1]Div Endocrinology and Metabolic Diseases, Dpt Internal Medicine, University of Turin, Turin, Italy; [2]Div Endocrinology, Dpt Internal Medicine, Erasmus University Rotterdam, Rotterdam, Netherlands; [3]Dpt Anatomy, Pharmacology and Forensic Medicine, University of Turin, Turin, Italy.

Cortistatin (CST), a neuropeptide with high structural homology with somatostatin (SS), binds all SS receptor (SS-R) subtypes but, unlike SS, also shows high binding affinity to ghrelin (GRLN) receptor (GRLN-R). In humans CST exerts the same endocrine activities of SS, suggesting that the activation of the SS-R might mask the potential interaction with the GRLN system.
CST-8, a synthetic CST-analogue devoid of any binding affinity to SS-R but capable to bind the GRLN-R, has been reported able to exert antagonistic actions on GRLN actions either *in vitro* or *in vivo* in animals. We studied the effects of CST-8 (2.0 μg/kg iv as a bolus or 2.0 μg/kg/h iv as infusion) on both spontaneous and GRLN- or hexarelin (HEX) (1.0 μg/kg iv as bolus)-stimulated GH, PRL, ACTH and cortisol secretion in 6 normal volunteers. The effect of CST-8 iv infusion at 4.0 μg/kg/h on the GH response to GRLN was also studied in 3 subjects. The study was approved by an independent Ethical Committee.
During saline, spontaneous ACTH and cortisol decrease was observed while no change occurred in GH and PRL levels. GRLN and HEX increased ($P<0.05$) GH, PRL, ACTH and cortisol levels. CST-8 administered either as bolus or as continuous infusion did not modify both spontaneous and GRLN- or HEX-stimulated GH, PRL, ACTH and cortisol secretion. The GH response to GRLN was unchanged even under exposure to the highest CST-8 dose.
In conclusion, CST-8 seems devoid of any modulatory action on either spontaneous or GRLN-stimulated somatotroph, lactotroph and corticotroph secretion. Thus, CST-8 seems an inactive peptide in humans, at least in term of modulation of pituitary hormone secretion.

P562

Prevalence of anterior pituitary dysfunction in patients following traumatic brain injury (TBI) in a German multi-centre screening program

Christian Berg[1], Alexandra Oeffner[4], Petra Maria Schumm-Draeger[4], Georg Brabant[2], B Gerbert[3], Matthias Weber[5], Klaus Mann[1] & Burkhard Herrmann[6]
[1]University of Duisburg-Essen, Clinic of Endocrinology, Essen, Germany; [2]University of Hannover, Hannover, Germany; [3]University of Dresden, Dresden, Germany; [4]University of Munich, Klinikum Bogenhausen, Munich, Germany; [5]University of Mainz, Mainz, Germany; [6]Institute of Cardio-Diabetes, Technology-Centre, Bochum, Germany.

Recent data suggest that hypopituitarism is a common complication of TBI. Prevalence differs between 10–40% and is based on different diagnostic tests and criteria. Hence, under field conditions TBI-mediated hypopituitarism may be less frequent than previously thought. We determined the prevalence of anterior pituitary dysfunction in a multi-centre screening program across five German endocrine centres in patients rehabilitating from TBI (GCS <13).
Patients & methods
246 patients (43 ± 14 yrs; 133 males, 12 ± 8 months after TBI) underwent baseline endocrine testing with central assessment of TSH, free T4, prolactin, LH, FSH, testosterone (m), estradiol (f), cortisol and IGF-I. If IGF-I was <-1 SDS GHRH + arginine or insulin tolerance test was performed. GHD was defined according to BMI-dependent cut-off values for GH response to GHRH + arginine of <4.2, <8.0 and <11.5 ng/ml in obese, overweight and lean subjects, respectively, and <3 microg/L in ITT. Hypocortisolism was defined when basal cortisol was <200 nmol/l and confirmed by ITT.
Results
In TBI patients some degree of impaired pituitary function was shown in 21% ($n=52/246$). Total, multiple and isolated deficits were present in 1%, 2% and 18% respectively. 19% ($n=46$) had an IGF-I of <-1 SDS. In 4% ($n=9$) GHD was confirmed. IGF-I did not correlate with BMI, gender or time after injury, but with age ($P=0.03$). 9% ($n=23$) had hypogonadism (total testosterone <9.5 nmol/L /low estradiol and low gonadotropins). Total testosterone levels did not correlate with BMI or age. 10.7% ($n=35$) had mild hyperprolactinemia. 4% ($n=11$) had hypocortisolism and 1% ($n=3$) had confirmed ACTH-deficiency. 12% ($n=29$) had TSH-deficiency.
Conclusion
In summary, in this large series carried out on an unselected group of TBI survivors we could not confirm a high prevalence of anterior pituitary dysfunction. Only every fifth patient with low IGF-I had confirmed GHD according to strict criteria and based on BMI-dependent cut-off values for GHRH + arginine testing. Hence IGF-I is a poor predictor for GHD in TBI.

Neuroendocrine and pituitary behaviour – presented on Tuesday

P563

Distribution of type 1 cannabinoid receptor (CB1) immunoreactive axons in the mouse hypothalamus

Gábor Wittmann, Levente Deli, Imre Kalló, Erik Hrabovszky, Masahiko Watanabe, Zsolt Liposits & Csaba Fekete
Department of Endocrine Neurobiology, Institute of Experimental Medicine, Hungarian Academy of Sciences, Budapest, Hungary; Department of Anatomy, Hokkaido University School of Medicine, Sapporo, Japan; Department of Neuroscience, Faculty of Information Technology, Pázmány Péter Catholic University, Budapest, Hungary; Tupper Research Institute and Department of Medicine, Division of Endocrinology, Diabetes, Metabolism and Molecular Medicine, New England Medical Center, Boston, MA, United States.

Type 1 cannabinoid receptor (CB1) is the principal receptor for endocannabinoids in the brain which mainly occurs in preterminal/terminal axons and mediates

retrograde neuronal signaling mechanisms. A large body of physiological and electrophysiological evidence indicates the critical role of CB1 in the regulation of hypothalamic functions. Conversely, the distribution of CB1-containing axons in the hypothalamus is essentially unknown. Therefore, we have analyzed the distribution and the ultrastructural characteristics of the CB1-immunoreactive (IR) axons in the mouse hypothalamus using an antiserum against the C-terminal 31 amino acids of the mouse CB1. We found that CB1-IR axons innervated densely the majority of hypothalamic nuclei, except for the suprachiasmatic and lateral mamillary nuclei where only scattered CB1-IR fibers occurred. CB1-IR innervation of the arcuate, ventromedial, dorsomedial and paraventricular nuclei and the external zone of the median eminence corroborated the important role of CB1 in the regulation of energy homeostasis and neuroendocrine functions. Ultrastructural studies to characterize the phenotype of CB1-IR fibers established that most CB1-immunoreactivity appeared in the preterminal and terminal portions of axons. The CB1-IR boutons formed axo-spinous, axo-dendritic and axo-somatic synapses. Analysis of labeled synapses in the paraventricular, supraoptic and arcuate nuclei detected approximately equal numbers of symmetric and asymmetric specializations.

In conclusion, the study revealed the dense and differential CB1-IR innervation of most hypothalamic nuclei and the median eminence of the mouse brain. At ultrastructural level, CB1-IR axons established communication with hypothalamic neurons via symmetric and asymmetric synapses indicating the occurrence of retrograde signaling by endocannabinoids in hypothalamic neuronal networks.

P564

Immunohistochemistry of pure growth hormone-containing and mixed growth hormone/prolactin-containing pituitary adenomas

Evgeniya Marova, Petr Yushkov, Natalja Molitvoslovova & Elena Luljeva
National Endocrinology Research Centre, Moscow, Russia.

Transsphenoidal surgery is the most efficient primary treatment for acromegaly. However, some patients do not meet remission criteria after operation. Mixed growth hormone (GH)/prolactin (PRL)-secreting pituitary adenomas are known to predict poor surgical outcome. The aim of our study was to evaluate immunohistochemical markers in pure GH-and mixed GH/PRL-containing tumors and to investigate their prognostic value. In our study we included 39 acromegalic patients, who underwent transsphenoidal surgery as primary treatment. We used immunohistochemical staining of removed adenomas for PRL to evaluate hormonal content of adenomas' cells; for proliferation marker (Ki-67), angiogenesis index (CD31) and marker for malignancy potential (galectin-3) to assess the biological tumor behavior. In addition to immunostaining of removed pituitary adenomas we evaluated clinical, hormonal and radiological data based on magnetic resonance imaging (MRI). Immunohistochemistry showed mixed GH/PRL-containing adenomas in 9 patients (23%), whereas pure GH-secreting adenomas in 30 cases (77%). Ki-67 was present in all mixed adenomas, but not in pure GH-secreting tumors. Galectin-3 was positive in 2 GH/PRL-cosecreting tumors (22%) and 9 pure GH adenomas (30%). CD31 was found in 3 mixed tumors (33%) and 13 pure GH adenomas (43%). In patients with GH/PRL co-secreting tumors MRI-predictors of unsuccessful surgical outcome were present: large size ($P=0.0007$, under Mann-Whitney's test) and intracavernous extension of adenomas ($P=0.0262$, under two-tailed Fisher's exact test). In addition, there were no cases of remission in patients with mixed GH/PRL-containing tumors. In conclusion, evaluation of immunohistochemical predictors of removed adenomas in combination with immunostaining for PRL in acromegalic patients gives the additional information which can determine surgical outcome and postoperative adjunctive therapy for such patients.

P565

The effects of pasireotide (SOM230) on glucose metabolism and growth hormone (GH) nadir during oral glucose tolerance test (OGTT) in 12 patients with acromegaly from a Phase II study

Stephan Petersenn[1], Joan Glusman[2], Nicole Unger[1] & Klaus Mann[1]
[1]University of Essen, Essen, Germany; [2]Novartis Pharmaceuticals Corporation, East Hanover, United States.

Introduction
Pasireotide (SOM230) is a novel multi-ligand somatostatin analogue with high binding affinity for four of the five somatostatin receptor subtypes ($sst_{1,2,3}$ and sst_5). A randomized study of 59 patients showed that pasireotide effectively controls GH and IGF-I levels in patients with acromegaly and reduces pituitary tumor size. The impact of pasireotide on GH levels during glucose suppression and glucose metabolism in 12 patients enrolled in the study is reported.
Methods
Patients in this study had GH levels >5 μg/L, elevated IGF-I and lack of suppression of GH to <1 μg/L post-OGTT. After treatment with octreotide 100 μg sc tid for 28 days, patients received pasireotide 200, 400 and 600 μg sc bid in random order for 28 days each. Glucose and GH levels were measured during OGTT in 12 patients prior to treatment, after octreotide treatment and after each pasireotide treatment phase.
Results
During glucose suppression, 4 of the 12 patients had a similar GH nadir ($<10\%$ difference) after pasireotide (-71.0%) or octreotide (-72.3%) treatment, and 8 patients had a stronger GH suppression with pasireotide (-75.1%) than with octreotide (-22.8%). Under fasting conditions prior to therapy, 7 patients had normal glucose tolerance (NGT), 2 patients had impaired glucose tolerance (IGT), and 3 patients had diabetes mellitus (DM). At the last assessment during treatment with pasireotide, 9 patients remained in the same category, 1 patient improved, and 2 patients had increased glucose levels. Similar results were seen for glucose metabolism 120 minutes post-OGTT.
Conclusions
Pasireotide suppressed GH levels during OGTT to a similar extent (4/12 patients) or greater extent (8/12 patients) than octreotide, indicating that it may be effective in patients with octreotide-resistant acromegaly. Furthermore, using stringent criteria, the majority of patients did not demonstrate relevant changes in glucose metabolism by the end of the pasireotide treatment period.

P566

Cerebrospinal fluid (CSF)/serum albumin ratio shows no alteration of the blood-brain barrier in patients with pituitary adenomas and high CSF levels of pituitary hormones

Monica Livia Gheorghiu[1], Mariana Purice[2], Catrinel Rusu[3], Simona Galoiu[1], Ruxandra Tanasescu[1], Dan Niculescu[1] & Mihai Coculescu[1]
[1]'C. Davila' University of Medicine & Pharmacy, Bucharest, Romania; [2]'C.I.Parhon' Institute of Endocrinology, Bucharest, Romania; [3]'N. Kretzulescu' Medical Center of the Internal Affairs Ministry, Bucharest, Romania.

Some patients with pituitary adenomas show high CSF levels and/or high CSF/serum ratio for peptidic pituitary hormones (PR), potentially due to a blood-brain barrier (BBB) damage. We evaluated albumin.CSF/serum ratio (AR) in patients with pituitary adenomas and elevated PR, as an accurate index for a BBB damage.
Patients and methods
We evaluated 10 controls (21–79 years, 6M /4 F) before undergoing abdominal or peripheral surgery and 52 patients with pituitary adenomas (PA) (17–79 years, 25 M/27F, 16 before and 36 after pituitary surgery), with the approval of the local Ethical Committee. Anterior pituitary hormones and albumin were measured in simultaneously sampled serum and CSF by rapid fluoroimmunoassay and nephelometry, respectively. AR >0.007 was considered abnormal.
Results
In PA, median albumin in serum (4625 ± 1134 mg/dl) and CSF (24.7 ± 37.7 mg/dl) was not statistically different from controls ($3710+710$ mg/dl and 20.2 ± 8.2 mg/dl, respectively). In 1/7 (14%) controls and 9/52 (17%) PA, AR was >0.007 (NS).
PR >1 for at least one pituitary hormone was found in significantly more patients with tumors in contact with BBB (suprasellar extension + neuroophtalmic syndrome or intracavernous sinus invasion), either before pituitary surgery ($10/21=47\%$) or after surgery ($9/16=56\%$), compared with only 1/15 (6%) in PA without contact with BBB before surgery ($P=0.001$). Albumin CSF, serum and R were not statistically different between contact and non-contact tumors or in patients with PR >1 compared to those with PR <1.
Conclusion
CSF/serum albumin evaluation shows that there is no alteration of the CSF flow rate in patients with pituitary adenomas and increased CSF/serum ratio for the anterior pituitary hormones, compared to controls. It is tempting to believe that the increased hormonal level in CSF is due to the tumor secretion.

P567

Medial cerebral artery occlusion 25 years after cranial radiation therapy in acromegaly

Pietro Maffei[1], Chiara Martini[1], Martina Barban[1], Marta Carli[1], Renzo Manara[2], Massimo Scanarini[3], Renzo Mazzarotto[4], Giorgio Meneghetti[5], Carla Menegazzo[1], Roberto Mioni[1], Eugenio De Carlo[1], Roberto Vettor[1] & Nicola Sicolo[1]
[1]Department of Medical and Surgical Sciences, Padua, Italy; [2]Department of Neuroradiology, Padua, Italy; [3]Department of Neurosurgery, Padua, Italy; [4]Department of Radiotherapy, Padua, Italy; [5]Department of Neurology, Padua, Italy.

Introduction
Epidemiological studies indicate that patients with acromegaly have increased mortality rates for cardiovascular and cerebrovascular disease (CVD). Potentials risk factors for increased CVD include hypopituitarism and cranial radiation therapy (CRT).
Case Report
A 43-year-old acromegalic woman was admitted because of worsening headache, left lower facial numbness and left arm weakness. Two weeks later she developed a central palsy of the left 7th cranial nerve. Past medical history was remarkable for gigantism-acromegaly which was diagnosed at age 17. At that time a pituitary macroadenoma was partially removed by transsphenoidal surgery and few months later she underwent conventional radiation therapy (two-fields, 50 Gy, 25 sessions). The patient's current medications were estroprogestin (since age 18), L-thyroxin (since age 39) and cortisone (since age 42). GH deficiency was also diagnosed (IGF-I 70 ug/L). Finally, a prothrombin mutation (G20210A) was discovered in family members including the patient.
Results
On examination there were a left lower facial palsy and bilateral cutaneous brownish of temporal regions. Laboratory investigations were uneventful for homocysteine, glucose and lipid metabolism. Thyroid and adrenal glands were regularly replaced. Intensive general (EKG; BP values; echocardiogram; 24-h Holter EKG; chest X-ray; abdominal US) and neurological study (carotid US; transcranial US; EEG; CT; MRI; MR-angiography; CT-angiography) disclosed several ischemic lesions of right parietal lobe, lenticular nucleus, anterior limb of internal capsule and occlusion of right medial cerebral artery near its origin. The diameter of right carotid artery was also reduced. Post-radiotherapy brain damage was visible by MRI. Atrial septal defect was excluded. The patient was treated with aspirin plus low dose heparin s.c. and neurological disturbances relieved completely.
Discussion
Life-long follow-up of acromegalic patients receiving CRT is essential so that early diagnosis of radiation-induced vascular damage can be made. In this particular context, treatment and monitoring of cerebrovascular thrombosis remain almost empiric.

P568

The hypothalamic-pituitary-adrenal axis in premenopausal females with psoriatic arthritis

Miroslav Vlcek[1], Jozef Rovensky[2], Adela Penesova[1], Zofia Radikova[1], Helena Raffayova[2] & Richard Imrich[1]
[1]Institute of Experimental Endocrinology, Slovak Academy of Sciences, Bratislava, Slovakia; [2]National Institute of Rheumatic Diseases, Piestany, Slovakia.

Objective
Changes of hypothalamic-pituitary-adrenal (HPA) axis activity, in particular decreased production of adrenal androgens, have been observed in patients with rheumatic diseases. However, data on adrenal steroid status in patients with psoriatic arthritis (PsA) are scarce. The present study was aimed at evaluation of HPA status in context of chronic inflammation in glucocorticoid-naïve premenopausal females with PsA.
Subjects and methods
Concentrations of ACTH, cortisol, androstenedione (ASD), 17OH-progesterone (17OHP), dehydroepiandrosterone (DHEA), and DHEA-sulphate (DHEAS) were analyzed before and during insulin-induced hypoglycaemia in 16 female premenopausal patients (age 40.1 ± 1.4 y, BMI 23.5 ± 1.1 kg/m^2) with PsA and in 11 age and BMI matched healthy women. Basal levels of IL-1alpha, IL-1beta, IL-6, TNF alpha and CRP were measured in all studied subjects as well. The study was approved by local ethical committee.
Results
The disease activity of PsA patients was low. No significant differences in levels of IL-1alpha, IL-1beta, TNF alpha or CRP were found between patients and controls. IL-6 levels were higher in PsA compared to controls ($P=0.045$). Basal levels and response to stimulation of ACTH and cortisol did not differ between the study groups. PsA patients had lower basal levels of ASD (2.79 ± 0.24 nmol/l vs. 4.89 ± 0.87 nmol/l; $P=0.013$) and DHEAS (2.42 ± 0.32 μmol/l vs. 3.79 ± 0.63 μmol/l; $P=0.044$) and levels of DHEA tended to be lower (13.2 ± 1.9 nmol/l vs. 20.4 ± 3.5 nmol/l; $P=0.065$). During stimulation PsA patients had significantly lower response of 17OHP and ASD when compared to controls ($P=0.046$, $P=0.004$ respectively). We did not find any significant correlation between basal levels of steroid hormones and cytokines.
Conclusions
The results suggest a shift in production of adrenal steroids from adrenal androgens towards production of cortisol in patients with PsA. Whether or not the observed changes in production of adrenal androgens are secondary due to ongoing inflammatory process remains to be elucidated.

P569

Cortisol and dexamethasone exert different negative feedback action in humans

Roberta Giordano, Rita Berardelli, Andreea Picu, Marcella Balbo, Lorenza Bonelli, Anna Barberis, Ezio Ghigo & Emanuela Arvat
Division of Endocrinology and Metabolism, Department of Internal Medicine, University of Turin, Turin, Italy.

HPA response to glucocorticoids (GCs) feedback is usually tested by dexamethasone (DEX), a synthetic GC; it poorly crosses BBB and preferentially activates pituitaric glucocorticoid receptor (GR), with a binding potency to GR 7 fold higher and an anti-inflammatory potency about 35 fold higher than cortisol. Cortisol, which easily penetrates into CNS, could better evaluate the GC feedback by acting also at supra-pituitary level. We studied the effects of 150 min infusion of hydrocortisone (HC: 15, 30 or 60 μg/kg/h) or DEX (0.4, 0.8, 1.6 or 2.1, 4.2, 8.5 μg/kg/h, covering either HC:DEX 1:35 or 1:7) on ACTH and cortisol levels in 9 normal subjects who underwent also a testing session with placebo. The study had been approved by an independent Ethical Committee. During placebo, ACTH and cortisol levels showed progressive decrease ($P<0.05$). The different doses of HC induced dose-dependent cortisol increases ($P<0.05$) coupled with dose-dependent ACTH decreases ($P<0.05$). 0.4, 0.8 and 1.6 μg/kg/h DEX doses did not modify cortisol levels; 0.8 and 1.6 but not 0.4 μg/kg/h DEX doses induced a dose-dependent ACTH decrease ($P<0.05$). Conversely, 2.1, 4.2 and 8.5 μg/kg/h DEX doses inhibited cortisol levels in dose-dependent manner ($P<0.05$) and induced more marked ACTH decrease ($P<0.05$). In conclusion, based on the potency of binding to GR, similar doses of hydrocortisone and dexamethasone are needed to reduce ACTH levels. Conversely, taking into account the anti-inflammatory potency, doses of dexamethasone higher than hydrocortisone are needed to inhibit ACTH secretion. These latter findings are likely to reflect different sites where natural and synthetic GCs exert their feedback action, i.e. mainly the CNS for hydrocortisone and the pituitary for dexamethasone. It is suggested that the HPA sensitivity to the feedback action of GCs in various pathophysiological conditions would better be evaluated by using natural GCs.

P570

The role of vasopressin in the hypothalamo-pituitary-adrenal axis regulation during the perinatal period: paradoxical corticosterone elevation without an ACTH rise

Dóra Zelena, István Barna, Zsuzsa Mergl, Ágnes Domokos, Katalin Csabai & Gábor B Makara
Hungarian Academy of Sciences, Institute of Experimental Medicine, Budapest, Hungary.

Early life events have special importance in the development and may affect the lifetime vulnerability to diseases. For correct interpretation of the long-term consequences it is crucial to understand the immediate effects. The role of vasopressin in hypothalamo-pituitary-adrenal axis regulation as well as in stress-related affective disorders is important therefore we addressed the question if the lack of this hormone will modify the perinatal stress reactivity.
Vasopressin producing (di/+) and deficient (di/di) Brattleboro rat pups were used. The separation of the 9-day-old pups from their mother for 24 h resulted in a remarkable corticosterone elevation in both genotypes without an ACTH increase in di/di rats. As the time-course of ACTH and corticosterone can be different we examined the 1-4-12-24 h separation period, too, with similar result (no ACTH elevation at any time point in di/di rats parallel with a remarkable corticosterone increase). Altered sensitivity of the adrenal gland might also explain the findings,

so we examined adrenal secretion *in vivo* with exogenous ACTH administration, but failed to find a significant difference between the genotypes. Tenth postnatal day is in the middle of the stress hyporesponsive period so we examined earlier (4–5 day old) and later (20 day old) postnatal phases too. After 24 h separation the ACTH levels did not change in di/di, but increased in di/+ pups with the highest rise at 10 days old, although corticosterone was significantly higher in both genotype at each time-point.

We can conclude that the role of vasopressin is an important factor in ACTH-secretion regulation during the postnatal period. However in the absence of ACTH other secretagogues may become important in the regulation of the adrenal gland secretion. The marked corticosterone elevation in the absence of ACTH rise is possibly not due to the different time-course of the two hormones or an altered sensitivity of the gland and it is present during the whole postnatal period up to 20 day.

P571

Riluzole treatment does not affect growth hormone (GH) secretion in amyotrophic lateral sclerosis

Lisa Linda Morselli[1], Paolo Bongioanni[2], Maura Genovesi[1], Rosaria Licitra[2], Bruno Rossi[2], Luigi Murri[2], Maurizio Gasperi[1] & Enio Martino[1]
[1]Department of Endocrinology, Pisa, Italy; [2]Department of Neurosciences, Pisa, Italy.

Amyotrophic lateral sclerosis (ALS), the most common motor neurone disorder in human adults, presents is characterized by selective and progressive degeneration of upper and lower motor neurones in central nervous system. GH secretion, evaluated by GHRH + arginine test, has been recently reported to be impaired in about 70% of untreated ALS patients. The currently available drug for ALS treatment is riluzole, a compound acting through inhibition of glutamate relase, post-synaptic receptor activation and voltage sensitive channel inhibition.

The aim of the present study was to evaluate whether riluzole administration can interfere with GH secretion and the diagnosis of adult GH deficiency. Ten patients (6 M, 4 F, mean age 59 ± 11 years) were studied. GHRH + arginine test was performed before and 1–3 months after starting riluzole treatment (100 mg/die). Blood samples for GH were collected at baseline and 30 and 60 minutes. Two patients showed severe (peak GH < 9 ng/ml), 5 patients mild (9 < peak GH < 16 ng/ml) GH deficiency and 2 patients had a normal GH response (peak GH > 16 ng/ml). Mean peak GH levels were similar before and during riluzole treatment (13.4 ± 10 vs 14.2 ± 10.1 ng/ml; P = NS). No significant correlation was observed between peak GH concentrations and age, BMI, disease duration, severity or clinical form. In conclusion, the present data confirm, in a new series of ALS patients, that GH secretion is impaired in these patients and indicate that riluzole treatment does not interfere with GH secretion. Therefore adult GH deficiency can also be diagnosed during riluzole therapy.

P572

Impairment of GH secretion by ghrelin stimulation test in primary hyperparathyroidism (PHP)

Elisabetta Cecconi[1], Clara Giovannetti[1], Luca Manetti[1], Valentina Raffaelli[1], Massimo Procopio[2], Ezio Ghigo[2], Maurizio Gasperi[1] & Enio Martino[1]
[1]Department of Endocrinology, Pisa, Italy; [2]Division of Endocrinology, Turin, Italy.

Pituitary GH secretion is regulated by the interplay of at least two hypothalamic hormones, GH-releasing hormone (GHRH) and somatostatin, through their interaction with specific cell surface receptors on the anterior pituitary somatotrophs. A third type of receptor, the growth hormone secretagogue receptor, called GHS receptor type 1a (GHSR1a), was identified in the pituitary and the hypothalamus. Ghrelin is an acylated peptide produced predominantly by stomach and a natural ligand of the GHS-R1a. In HEK-293 cells expressing the GHS-R1a, ghrelin induces a biphasic cytosolic calcium elevation. We recently reported that untreated PHP patients have an impaired GH secretion, as demonstrated by a blunted GH response to maximal stimulation with GHRH + Arginine test. The aim of the present study was to evaluate effects on GH secretion induced by ghrelin in PHP. Eleven patients (2 male/9 female, age range 41–67 yrs, mean 54 yrs, BMI 26.6 ± 3.4) with PHP were studied. The control group consisted of 35 normal age- and sex-matched subjects (12 male/23 female, age range 23–78 yrs, mean 59 yrs, BMI 26.3 ± 3.1). Patients and controls were submitted on two separate days to ghrelin administration (1 μg/Kg iv) and to GHRH + arginine test.

Serum GH secretion was reduced (GH response to GHRH + arg test: 9.54 ± 3.1 μg/liter) in 7 patients (64%) and normal (38.57 ± 10.5 μg/liter) in the remaining 4 (36%); in the control group no GHD was found (peak GH 38.0 ± 3.5 μg/liter, P < 0.001).

The mean peak GH response to ghrelin in PHP was significantly lower than in normals (17.99 ± 8.3 vs. 84.0 ± 36 μg/L, P < 0.001) in accordance to the values obtained by GHRH + arginine test.

In conclusion, this study confirms the impaired GH secretion to GHRH + Arg stimulation in PHP patients and represents the first demonstration that ghrelin administration unveils GH deficiency in PHP.

P573

The influence of cabergoline treatment on seminal fluid

Ekaterina N Giniatullina, Larisa K Dzeranova, Ludmila Y Rozhinskaya & Anna D Dobracheva
Research Center for Endocrinology, Moscow, Russia.

This study evaluated the effects of the long treatment with cabergoline on seminal fluid parameters and sexual and gonadal function in hyperprolactinemic males. Eleven males with macroprolactinoma were treated with cabergoline at a dose of 1.5–2.5 mg a week for 6 months. All the patients suffered from libido impairment, reduced sexual potency, six had infertility. In three patients provocative bilateral galactorrhea was found.

Seminal fluid analysis, functional seminal tests, prolactin and testosterone concentrations and cerebrum magnetic resonance imaging were assessed before and after 6 months of cabergoline treatment. Baseline prolactin was 11530.7 ± 222.6 mU/l. Baseline testosterone was 6.25 ± 0.2 nmol/l. Before treatment, all patients had a low sperm count with oligoasthenospermia, reduced motility and rapid progression with an abnormal morphology and decreased viability, and a low number of erections.

After 6 months, serum PRL level was significantly reduced 682 ± 16.6 mU/l (P < 0.005). Testosterone level significantly increased to 19.8 ± 0.04 nmol/L (P < 0.002). After 6 months, a significant increase of sperm volume, number, total motility, rapid progression and normal morphology was recorded in patients treated with cabergoline. An increase in the number of erections during the first 3 months of treatment was noted. The number of erections was normalized after 6 months of treatment in all patients. Positive dynamics of the tumors volume was noted at 9 patients (81.2%) - adenoma has reduced. No dynamics observed in 2 men (18.8%). The bilateral galactorrhea in all three patients was not found.

The treatment with cabergoline normalized prolactin and testosterone levels, improving gonadal and sexual function and fertility in hyperprolactinaemic males and can be successfully used as primary therapy in men with large macroprolactinomas.

P574

Fractionated stereotactic conformal radiotherapy for skull base benign tumours: an endocrinological follow-up

Giuseppe Minniti, Daphne Traish, Sue Ashley, Adam Gonsalves & Michael Brada
Neuro-oncology Unit1, The Institute of Cancer Research and the Royal Marsden NHS Foundation Trust, Downs Road, Sutton, Surrey SM2 5PT, UK, and Fulham Road, London SW3 6JJ, United Kingdom.

Background
Stereotactic radiotherapy techniques have been recently employed in the control of skull base tumours, such as pituitary edenomas, craniopharyngiomas and meningiomas.
Purpose
To assess the long-term endocrinological effect of fractionated stereotactic conformal radiotherapy (SCRT) in patients with residual and recurrent sellar and parasellar tumours treated at Royal Marsden Hospital.
Patients and methods
245 patients (median age 50 years) with residual or recurrent pituitary adenomas (n = 98), meningiomas (n = 108) and craniopharyngiomas (n = 39) were treated between 1995 and 2004 at The Royal marsden Hospital. 102 patients had partial or complete hypopituitarism before SCRT (69, 29 and 5 patients with pituitary adenomas, craniopharyngiomas and meningiomas), including 44 with a complete and 58 with a partial hypopituitarism. Patients were treated supine and immobilized in a Gill-Thomas-Cosman relocatable frame. High-resolution planning CT scan was fused with magnetic resonance imaging (MRI) scan.

The treatment was delivered by 4–6 noncoplanar conformal fixed fields using a 6-MV linear accelerator to a dose of 45–55 Gy in 25–33 fractions.
Results
At a median follow-up of 38 months (range 3–120) the 5 year actuarial progression free is 98.9%, 93%, 92% and overall survival is 98%, 97% and 100% for adenomas, meningiomas and craniopharyngiomas. The treatment was well tolerated with minimal acute and long-term toxicity. Hypopituitarism was the most common long-term effect and 26%, 42% and 6% of patients with a pituitary adenoma, a craniopharyngioma and a meningioma worsened pituitary function. Hypopituitarism was more common in patients with pre-SCRT pituitary hormone abnormalities.
Conclusion
SCRT is an effective treatment for patients with benign skull base tumours and is associated with low toxicity. Tumour control was equivalent to that seen following conventional radiotherapy and radiosurgery. Longer follow-up is needed to assess a potential reduction in long-term morbidity. Hypopituitarism develops in a significant number of patients requiring a regular follow-up in these patients.

P575

The GH releasing activity of ghrelin is insensitive to the negative growth hormone (GH) autofeedback in humans

Fabrizio Riganti, Flavia Prodam, Elena Gramaglia, Andrea Benso, Barbara Lucatello, Ezio Ghigo & Fabio Broglio
Division of Endocrinology and Metabolism, University of Turin, Turin, Italy.

Growth hormone (GH) secretion is regulated by a complex interplay between GH-releasing hormone (GHRH), somatostatin and several other central and peripheral modulatory signals. Ghrelin has been hypothesized as physiological amplifier of GH pulsatility and acts via mechanisms, at least partially, independent of GHRH and somatostatin. The GH response to GHRH is strongly inhibited by previous administration of recombinant human GH (rhGH), likely as a consequence of a somatostatin-mediated negative GH auto-feedback. The effect of exogenous rhGH on the GH-releasing effect of ghrelin has never been tested so far. In 5 normal young volunteers we studied the acute GH response to ghrelin (2.0 mcg/kg iv at 0 min) during saline or rhGH infusion (4.0 μg/Kg/h i.v. from −180 min to +60 min). Mean GH levels during saline infusion were: 0.7 ± 0.4 mcg/l. The rhGH administration increased mean GH levels to: 22.1 ± 2.3 mcg/l ($P<0.01$). During saline, ghrelin administration induced clear cut increase of GH secretion (Δpeak: 55.0 ± 6.7 mc/l; ΔAUC: 2096.4 ± 193.2 mcg/l/h; $P<0.01$ vs baseline). During rhGH infusion, ghrelin elicited the same potent GH-releasing effect (Δpeak: 92.2 ± 53.4 mcg/l; ΔAUC: 2298.3 ± 684.4 mcg/l/h; $P<0.01$). In conclusion, these results show that the acute rhGH administration does not modify the GH-releasing action of ghrelin. As GH auto-feedback is known to act by a concomitant reduction in the activity of GHRH-secreting neurons and increase of somatostatinergic tone, these data further indicate that the impact of the ghrelin system on somatotroph function is remarkably independent of either GHRH or somatostatin.

P576

Impaired GH secretion in women with HIV-related lipodystrophy

Vincenzo Rochira[1], Lucia Zirilli[1], Bruno Madeo[1], Giovanni Caffagni[1], Alessia Sanguanini[1], Antonio RM Granata[1], Maria Cristina De Santis[2], Gabriella Orlando[3], Nicola Squillace[3], Giovanni Guaraldi[3] & Cesare Carani[1]
[1]Integrated Department of Medicine, Endocrinology, Metabolism and Geriatrics, University of Modena and Reggio Emilia, Modena, Italy; [2]Laboratory of Endocrinology, Policlinico of Modena, Modena, Italy; [3]Department of Medicine and Medical Specialties, Clinics of Infective Diseases, University of Modena and Reggio Emilia, Modena, Italy.

Introduction
Patients with human immunodeficiency virus-1 (HIV-1) infection develop a lipodystrophic syndrome characterised by accumulation of central fat both in visceral and in subcutaneous compartment. In recent studies approximately 20% of male patients with HIV-related lipodystrophy presented an inadequate peak of GH secretion in response to GHRH-arginine testing, which is strongly inversely related to visceral adipose tissue-(VAT).
Aim of the study
To investigate GH secretion in female patients with HIV-related lipodystrophy according to their body composition.
Subjects and methods
We included 35 HIV-infected female patients (mean age 44.6 ± 7.6 S.D.) with lipodystrophy according to the Marrakech scale. We investigated their GH response to standardised GHRH-arginine testing in order to compare it with BMI, VAT and subcutaneous adipose tissue (SAT) evaluated by CT scan. On the basis of current clinical guidelines we considered a severely impaired GH secretion (IGHS) when the GH peak after GHRH-arginine testing was ≤5 μg/L; a mildly IGHS when it was >5 μg/L but <9 μg/L and a normal GH secretion with a peak ≥9 μg/L, according to the degree of obesity together with preliminary data obtained in male HIV-related lipodystrophy.
Results
The 37.5% of our patients had IGHS (12.5% a severe IGHS, 25% the mild form). The average GH peak in the three group and the compared data among them are shown in the table:

IGHS	GH peak	IGF-1	IGFBP3	BMI	VAT cm^2	SAT cm^2	VAT/SAT
Severe	3.2+	112.8+	1682.5±	27.1±	102.3±	154±	0.66±
	1.6	23.5	606.1	6.6	66.7	46	0.32
Mild	6.5+	157+	2149.3±	25.9±	119.8±	307.2±	0.43±
	0.9	67.9	650.2	3.1	70.4	132.8	0.22
Normal	21.5+	183.9+	2144.4±	26.1±	106.7±	215.8±	0.57±
	8.1	84.6	639	2.4	45.1	93.6	0.29

Conclusion
The pituitary GH secretion may be impaired in HIV-positive women. The percentage of subjects with IGHS seems to be higher in HIV-positive women than in men. IGF-1 results lower in IGHS subjects. Furthermore, body composition does not change according to GH-peak status.

P577

Midnight salivary cortisol vs. urinary free cortisol for the diagnosis of Cushing's syndrome

Maria Yaneva, Georgi Kirilov & Sabina Zacharieva
Clinical Center of Endocrinology and Gerontology, Medical University, Sofia, Sofia, Bulgaria.

Introduction
Midnight salivary cortisol measurement (MSC) has been recently introduced as a diagnostic test for hypercortisolism. The aim of our study was to compare the diagnostic value of two methods of screening for Cushnig's syndrome (CS): MSC and 24-h urinary free cortisol (UFC), wildely accepted as a 'gold standard' for this diagnosis.
Patients and methods
Three groups were studied: 30 patients with CS (mean age ± S.D., 39.9 ± 12.8 y, f/m 25/5, BMI 29.5 ± 7.2 kg/m^2), 34 with metabolic syndrome (MS) (41.1 ± 13.6 y, f/m 24/10, 36.5 ± 4.8 kg/m^2) and 40 healthy normal weight controls (37.2 ± 9.3 y, f/m 24/16, 23.4 ± 2.8 kg/m^2). Saliva was sampled at midnight (Salivette, Sarstedt®). Urine was collected over 24 hours at the same day. An electrochemiluminescence immunoassay was used to measure salivary cortisol. UFC was assessed by a radioimmunoassay.
Results
Mean MSC in healthy volunteers, patients with MS and CS was 8.3 ± 3.6, 8.1 ± 4.5 and 33.1 ± 21.7 nmol/l, respectively. Mean UFC was 129.1 ± 72.7, 124.25 ± 106.1 and 773.7 ± 761.7 nmol/d. No significant difference was found between MSC and UFC in healthy controls and MS ($P>0.05$). By contrast, MSC and UFC were significantly higher in patients with CS ($P<0.0001$) as compared to both other groups. The cut-off point of 14.2 nmol/l for MSC yielded a sensitivity of 93.3% and a specificity of 94.2%. The cut-off point of 222 nmol/d for UFC showed a sensitivity of 100% and a specificity of 90%. Analysis of the areas under the curve (AUC) showed no significant difference between MSC and UFC ($P<0.05$, AUCMSC = 0.984 ± 0.01 (0.965–1.000); AUCUFC = 0.975 ± 0.01 (0.948–1.000) (mean ± S.E.M. (confidentail interval of 95%)).
Conclusion
MSC and UFC determination have comparable diagnostic value. They both have reliably high sensitivity and specificity. We recommend the use of MSC as a first-line screening test for CS because of its convenience, escpecially in the ambulatory practice.

P578

Behavioural and biological effects of des-Gln14-ghrelin

Roberta Dochnal, Mónika Mácsai & Gyula Szabó
University of Szeged, Department of Pathophysiology, Szeged, Hungary.

Ghrelin, ligand for the growth hormone secretagogue receptor (GHS-R), was isolated from the stomach. Immunoreactive neurons were observed in the hypothalamic nuclei and the ependymal layer of the third ventricle. Lower amounts are produced in the small intestine, pancreas, liver, kidney, placenta, and pituitary. Receptors have widespread distribution in the body, mainly concentrated in the hypothalamus-pituitary unit.

Ghrelin, a 28-amino acid peptide, has an *n*-octanoyl modification at its third serine residue. This modification is necessary for biologic activity. A second endogenous ghrelin form was discovered which is derived from an alternative splicing of the ghrelin gene. This 27 amino acid peptide is called des-*Gln*14-ghrelin, and has an *n*-octanoyl modification at its third serine residue, identical to ghrelin, except for deletion of one glutamine.

Considerable amount of data has accumulated regarding biological effects of ghrelin 28 but des-*Gln*14-ghrelin was less studied. No experiment investigating behavioral effects of des-*Gln*14-ghrelin has been carried out in mice. Therefore in the present study we aimed to elucidate how des-*Gln*14-ghrelin influences locomotion, anxiety, body temperature, and pain threshold in CFLP mice. The peptide was injected intracerebroventicularly (icv.) and we performed open-field, plus-maze, and tail flick tests.

Our experiments showed that des-*Gln*14-ghrelin increased locomotion and exploratory behavior. The most effective dose was 2 μg/μl, which induced a significant increase in both the vertical and horizontal locomotor activity in the open field test. The increased locomotion was confirmed by the plus maze test also, where the number of entries was increased. In addition, the peptide in higher doses (4 μg/μl) seems to induce anxiolytic effect. Lower doses did not change the anxiety level. Analgesia and body temperature seems to be influenced by des-*Gln*14-ghrelin, but our results were not statistically significant.

This work was supported by ETT (050/2003), NKFP (1/027/2001), OTKA (T043095).

P579

Diagnostic accuracy of bilateral inferior petrosal sinus sampling performed following a combined stimulation with CRH and desmopressin

Stylianos Tsagarakis[1], Dimitra Vassiliadi[1], Ioannis Kaskarelis[2], Ioannis Komninos[1], Emmanouil Souvatzoglou[1] & Nicolaos Thalassinos[1]
[1]Evangelismos Hospital, Department of Endocriology, Diabetes and Metabolism, Athens, Greece; [2]Evangelismos Hospital, Department of Radiology, Athens, Greece.

Although bilateral inferior petrosal sinus sampling (BIPSS) is the most accurate procedure for the differential diagnosis of ACTH-dependent Cushing's syndrome, a false-negative rate of 4–15% has been reported. An even lower sensitivity has been shown in patients with equivocal responses to CRH and/or high-dose dexamethasone suppression test (HDST). In the present study we investigated whether the administration of CRH plus desmopressin (DDAVP) during BIPPS, which is considered to be a more potent stimulus, improves the sensitivity without compromising the specificity of the procedure.

The results in 55 patients, 48 with confirmed Cushing's disease (CD) (36 women, 12 men, mean age 42.4 ± 12.5 years) and 7 with confirmed occult ectopic ACTH syndrome (oEAS) (1 woman, 6 men, mean age 44 ± 20.4 years) that underwent BIPSS using a combined stimulus with CRH plus DDAVP were retrospectively analysed. The sensitivity for a basal IPS/P gradient >2 was 60.4%, with 100% specificity and a diagnostic accuracy of only 65.5%. After stimulation with DDAVP and CRH, 47/48 patients with CD had an IPS/P gradient $>$ 2 but, none of the patients with oEAS, resulting in a sensitivity of 97.9%. The specificity was 100%, diagnostic accuracy 98.18% and the positive and the negative predictive values were 100% and 87.5%, respectively. A subgroup of 19 patients (17 with CD and 2 with oEAS) had contradictory responses to routine tests with CRH and/or HDST; sensitivity, specificity and accuracy of BIPSS in this subgroup were 100%.

In conclusion, the application of a combined stimulation with CRH plus DDAVP may be the preferred stimulus during BIPSS, since it seems to substantially decrease the false negative rate resulting in higher sensitivity but with no loss of specificity.

P580

The empty sella syndrome – particularities of the clinical features depending on etiology

Cristina Ghervan, Cristina Olarescu, Daniel Leucuta & Ileana Duncea
Iuliu Hatieganu University of Medecine and Pharmacy, Cluj-Napoca, Cluj, Romania.

The empty sella syndrome (ESS) is caused by the herniation of the suprarahnoidian space into the sella turcica, generating the compression of the pituitary gland and, in most cases, a remodelling of the sella. The purpose of this study was to evaluate the etiology, the degree of hormonal deficit and the occurrence rate of the signs and symptoms accompanying ESS.

Material and methods

We performed a descriptive, retrospective study by analysing the medical records of the patients admitted to the Endocrinology Clinic, between 1995 and 2005. We identified 49 patients with ESS (39 women and 10 men) with ages between 18 and 68 years, with a mean age of 49.81 ± 10.14. The following parameters were examined: ESS etiology, clinical symptoms, hormonal values, neurological and ophthalmologic evaluation. The following statistical tests were used: Fisher's exact test, the Hi^2 test, the paired t test (student) and the Mann-Whitney U test.

Results

Regarding etiology: 38 patients (77.6%) had primary ESS (pEES) and 11 patients (22.4%) had secondary ESS (sEES). Total hormonal deficit was identified in 3 patients, all with pESS. Gonadal insufficiency was identified in 12 patients (11/1), central hypothyroidism in one patient with sEES and functional hyperprolactinemia in 6 patients (5/1). Diabetes Insipidus was found in 3 patients (2/1). Headaches were present in 43 patients (33/10), psychological disturbances in 20 patients (15/5), visual disturbances in 18 patients (10/8), obesity was present in 29 patients (21/8), and arterial hypertension in 27 patients (21/6).

Conclusions

Primary ESS was more frequent than the secondary form, and was more often accompanied by different degrees of pituitary insufficiency. Headaches, psychological disturbances, hypertension and obesity had high occurrence rates in both categories, while visual disturbances and gonadal insufficiency were more frequent in the patients with secondary ESS. Diabetes insipidus can be (rarely) present in both forms.

P581

Effects of successful transsphenoidal surgery on cardiovascular function in elderly acromegalics

Giuseppe Minniti[1], Carlo Moroni[2], Vincenzo Esposito[1], Rosario Cassone[2], Gianpaolo Cantore[1] & Marie Lise Jaffrain-Rea[3]
[1]Neurological Sciences, IRCCS NEUROMED, Pozzilli (IS), Italy; [2]Internal Medicine, University of Rome "La Sapienza", Rome, Italy; [3]Department of Experimental Medicine, University of L'Aquila, L'Aquila, Italy.

Background

Transsphenoidal surgery (TSS) is able to determine the biochemical remission of acromegaly in 45%–80% of the patients, thereby inducing an improvement of cardiovascular function and glucose metabolism. Only 3–5% of acromegalics are diagnosed over 65-years-old, so few data are available about post-operative cardiovascular and metabolic changes in this group.

Patients and Methods

Fifteen acromegalic patients $\geq$65 years-old who underwent successful TSS were studied. Doppler-echocardiography and 24-hour ambulatory blood pressure monitoring (ABPM) studies were performed immediately before and 6 months after TSS. Calculation of insulin sensitivity was derived from OGTT.

Results

Both left ventricular mass (LVM) and LVM index decreased significantly after surgery ($P=0.0021$ and $P=0.0015$, respectively). Nine out of 13 patients who fulfilled echocardiographic criteria for left ventricular hypertrophy (LVH) before surgery normalized LVMi, whereas LVH persisted in 3 hypertensive patients. Significant post-operative improvement of diastolic function was also observed. 24-h systolic BP (123.5 ± 12.2 *vs* 131.1 ± 15.6 mmHg, $P=0.003$) and diurnal diastolic BP (76.9 ± 7.8 *vs* 81.6 ± 6.3 mmHg, $P=0.04$) decreased after surgery. Three out of the 9 patients who were pre-operatively defined as hypertensive according to ABPM had normal post-operative diurnal BP values. Glucose metabolism improved after surgery, with a significant decrease of fasting ($P<0.05$) and post-load ($P<0.01$) glucose and insulin levels. This was associated with an improvement on insulin sensitivity ($P<0.003$).

Conclusions

Successful TSS is able to induce a significant improvement of cardiac mass and function even in elderly acromegalics, and this is associated with a slight decrease in BP values and improvement of glucose metabolism abnormalities. Long-term studies are necessary to evaluate the effect of biochemical cure on cardiovascular morbidity and mortality in such patients.

P582

Clinicopathologic correlation in cases with macronodular hyperplasia

Ayse Kubat Uzum[1], Meral Mert[1], Yesim Erbil[2], Nese Colak Ozbey[1] & Aral Ferihan[1]
[1]Istanbul University, Istanbul Faculty of Medicine, Department of Internal Medicine, Division of Endocrinology and Metabolism, Istanbul, Turkey; [2]Istanbul University, Istanbul Faculty of Medicine, Department of General Surgery, Istanbul, Turkey.

Introduction
We designed a retrospective study to investigate the clinical presentation, laboratory and pathological findings of 14 patients with ACTH-independent macronodular adrenal hyperplasia.
Materials and methods
Diagnose of Cushing' s syndrome was confirmed by biochemical tests, adrenal hyperplasia was confirmed by pathological examination in all patients.
Results
No supression was observed in overnight, low and high dose dexamethasone supression tests. Thirteen (92.9%) were females. Mean age was 39.71±9.18. ACTH concentrations were 23.20±9.70 (12–40 pg/ml). Two patients (14.3%) were diagnosed incidentally, whereas 12 patients had clinical findings. Two patients had diabetes mellitus (14.3%), eight (57.1%) had hypertension. Patients were found to have dorsocervical fat pad (n:9; 64.3%), central obesity (n:9; 64.3%), striae (*n*:6;42.9%), plethora (*n*:7;50%), amenorrhea (*n*:4;28.6%), acantosis (*n*:4;28.6%), hirsutismus (*n*:2;14.3%) and myopathy (*n*:1;7.1%). One had vertebral fracture during follow-up. One of 14 patients was diagnosed as subclinical cushing syndrome and underwent bilateral adrenalectomy. Seven patients underwent unilateral and seven patients underwent bilateral adrenalectomy. Hypocortisolemia developed in six patients after unilateral adrenalectomy and continued for 12.50±9.29 months. Radiotherapy for hypophysis was performed for four patients (*n*:1;before unilateral adrenalectomy, *n*:3;after adrenalectomy). Nelson syndrome developed in two patients against radiotherapy in 9th and 10th years. Eight (57.14%) of 14 patients had macronodular, five (35.71%) had micronodular, and one (7.14%) had primary pigmentated nodular adrenocortical nodules (PPNAD). Compact and clear cells were the most frequent cells in pathologic examination.
Conclusion
We have concluded that patients who underwent adrenalectomy had ACTH-dependent adrenal hyperplasia at first, by long and continuous stimulation of ACTH, bilateral nodular hyperplasia had developed in adrenal glands. Through years, nodules may became autonomous and partially lose ACTH dependence and secreted cortisol continously. Therefore, treatment should be chosen as unilateral or bilateral adrenalectomy.

P583

The endocrine and behavioural actions of neuromedine S

Miklós Jászberényi[1], Zsolt Bagosi[1], Gyula Szabó[1] & Gyula Telegdy[2]
[1]University of Szeged, Department of Pathophysiology, Szeged, Hungary; [2]Hungarian Academy of Sciences, Neurohumoral Research Group Szeged, Szeged, Hungary.

Since earlier publications revealed a prominent and versatile impact of the neuromedin peptide family on several neuroendocrine processes, in the present experiments we focused on the effects of a recently discovered member of neuromedines, neuromedine S on such phenomena as open-field behaviour and hypothalamic-pituitary-adrenal (HPA) activation. The peptide was administered intracerebroventricularly to freely moving rats and 30 minutes later the aforementioned neuronedocrine parameters were investigated. We also investigated the putative effect of neuromedine S on dopamine and GABA release from rat striatal slices in a superfusion system. Our results disclosed that neromedine S has a profound and dose-dependent action on the HPA system, evoking a threefold increase in plasma corticosterone level in a dose of 1 μg. It also activated grooming in a dose of 0.25 μg. The latter action displayed a bell-shaped dose-response curve. However, the neuropeptide does not influenced neither such open field paradigms as square crossing, rearing and defecation nor has an impact on the release of GABA and dopamine. Our results reinforce the hypothesis that, indeed, neuromedines are important regulators of neuroendocrine processes and shed light on the possible functions of the newly described neuromedine S in the central nervous system. It appears, that centrally administered neuromedine S can stimulate such CRF dependent processes as corticosterone release and grooming. However, further experiments are needed to clarify the exact mediation of these processes.

P584

Acute and long-term pituitary insufficiency in traumatic brain injury: a prospective cohort study

Marianne Klose[1], Michael Kosteljanetz[2], Lars Poulsgaard[2], Jannick Brennum[4], Anders Juul[3] & Ulla Feldt-Rasmussen[1]
[1]Dept. of Endocrinology, Rigshospitalet, Copenhagen, Denmark; [2]Dept. of Neurosurgery, Rigshospitalet, Copenhagen, Denmark; [3]Dept. of Growth and Reproduction, Rigshospitalet, Copenhagen, Denmark; [4]Dept. of Neurosurgery, Glostrup County Hospital, Copenhagen, Denmark.

Objective
To estimate the occurrence of hypopituitarism 12 months following traumatic brain injury (TBI), describe the time course, evaluate the predictive value of early hormonal changes and trauma related parameters, as well as out-come.
Methods Forty-six patients with TBI (mild (GCS:13–15) $n=22$; moderate (GCS:9–13) $n=9$; severe (GCS <9) $n=15$) were included. Patients were tested early post-injury (baseline hormone levels + Synacthen-test), and re-tested at 3 and 12 months post-injury (baseline + post-stimulatory hormone levels performing an insulin tolerance test or if contraindicated an arginineGHRH-test).
Results
In the early post-traumatic phase, pituitary hormone alterations were observed in 34/46 (74%) of TBI patients, primarily affecting the gonadal (31/46) and thyroidal (15/46) axes. These changes were most prevalent in severe TBI. At three months, 6/46 patients failed anterior pituitary testing. Twelve months post-injury, one patient had recovered, whereas one developed GH-deficiency in addition to existing ACTH-deficiency. No patients being sufficient at 3 months developed insufficiency during the 9 months follow-up. All insufficient patients had GH-deficiency (5/46 (11%)), followed by ACTH- (3/46), TSH- (1/46), LH/FSH- (1/46) and ADH-deficiency (1/46). The risk of long-term hypopituitarism was positively related to trauma severity ($P=0.04$; 4=severe TBI; 1=moderate TBI), but unrelated to early hormonal alterations when adjusted for trauma severity ($P>0.1$). Insufficient patients had lower self-evaluated health status ($P=0.05$), and a higher increase in BMI ($P=0.01$) and total cholesterol ($P=0.04$) as opposed to sufficient patients.
Conclusion
Head trauma patients had a high frequency of non-specific early hormonal alterations being non-predictive of long-term posttraumatic hypopituitarism. The prevalence of long-term posttraumatic hypopituitarism is clinically relevant in patients with severe TBI, and these patients should be referred to neuroendocrine evaluation in the stable posttraumatic phase. Clinicians should moreover become aware of potential hypoadrenalism in the initial posttraumatic period, as insufficiencies are most certainly present in some patients already from the eliciting trauma.

P585

Genetic analysis of PROP1 gene in patients with childhood-onset combined pituitary hormone deficiency (CPHD)

Zita Halász[1], Judit Toke[2], Attila Patócs[2], Rita Bertalan[2], Zsófia Tömböl[2], Ágnes Sallai[1], Éva Hosszú[1], Ágota Muzsnai[3], László Kovács[4], János Sólyom[1], György Fekete[1] & Károly Rácz[2]
[1]Semmelweis University, 2nd Department of Pediatrics, Budapest, Hungary; [2]Semmelweis University, 2nd Department of Medicine, Budapest, Hungary; [3]Buda Children's Hospital, Department of Endocrinology, Budapest, Hungary; [4]National Medical Center, Department of Medicine, Division of Endocrinology, Budapest, Hungary.

Introduction
Combined pituitary hormone deficiency (CPHD) may be associated with mutations of genes coding for pituitary transcription factors, of which the PROP1 and Pit1, gene mutations have been most extensively studied. However, there are controversial data about the prevalence of these gene mutations in non-acquired childhood-onset CPHD patients.
Objectives
To examine the prevalence and spectrum of PROP1 and Pit1 gene mutations in CPHD patients a multicenter study was performed.
Patients and methods
Patients were selected on the basis of evidence of childhood-onset growth hormone deficiency combined with at least one other pituitary hormone defect. Twenty-nine sporadic and 6 familial cases (2 affected siblings from 3 families) were examined. Genomic DNA was extracted from peripheral blood leukocytes. Mutational analysis of the coding exons of the PROP1 gene was carried out in all patients. In 14 patients in whom disease-causing mutation of the PROP1 gene was absent, mutational analysis of exon 6 of the Pit1 gene was also performed.
Results
Genetic testing indicated disease-causing mutations of the PROP1 gene in 15 patients (homozygous mutations in exon 2: 296-302delGA in 4 patients, 150delA

in 4 patients, C217T in one patient; homozygous mutations in exon3: F117I in one patient; and compound heterozygous mutations: 150delA/296-302delGA in 3 patients, 150delA/F117I in one patient, R99X/296-302delGA in one patient). No novel PROP1 gene mutation was detected. Mutational analysis of exon 6 of the Pit1 gene did not reveal disease-causing mutation.
Conclusion
With our selection criteria for genetic testing, disease-causing PROP1 gene mutations can be detected in a high proportion of childhood-onset, non-acquired CPHD in the Hungarian population.

P586

The role of G-protein- and β-arrestin dependent signaling mechanisms in the tonic regulation of prolactin secretion

Márk Oláh[1], Zsuzsanna Szepesi[2], Ibolya Bodnár[1], György M. Nagy[1] & Zsolt Tidrenczel[1]
[1]Hungarian Academy of Sciences-Semmelweis University Neuroendocrine Research Group, Budapest, Hungary; [2]Hungarian Academy of Sciences-Eötvös Loránd University of Sciences Neurobiology Research Group, Budapest, Hungary.

It is well known that hypophyseotrophic dopamine (DA) exhibits a tonic inhibitory effect on pituitary lactotrops in vivo. We have previously observed that prolactin (PRL) cells obtained from lactating rats become partially resistant to DA following a brief suckling period compared to non-suckled control female rats. This, so-called "desensitization" (and a parallel appearance of "tolerance") to DA is mediated through by a selective change of protein phosphatase 2A (PP2A) in the pituitary lactotrops. Besides the known G_i-protein-cAMP-PKA pathway, stimulation of D_2-receptor (D_2-R) leads to the activation of the p44/42 extracellular-regulated kinase (ERK1/2) in the pituitary gland. Moreover, an additional signal-transduction pathway has recently been described in case of the striatal D_2-R that is a G-protein independent and β-arrestin dependent mechanism. In this signaling β-arrestin is coupled with PP2A that dephosphorilates, therefore inactivates protein kinase B (Akt). We have investigated the changes in phosphorilation of ERK1/2 and Akt following physiological (suckling) and/or pharmacological (inhibitor of DA biosynthesis and/or D_2-R antagonist) manipulations of the hypophyseotrophic DA system using western-blot technique. Suckling stimulus compared to 4 h separation of lactating rats resulted in higher phosphorilation level of ERK1/2 in the AL as well as in male rats treated with DA biosynthesis inhibitor α-methyl-p-thyrosine (αMPT, 250 mg/kg b.w. ip.). Phospho-ERK1/2 content of the NIL was also higher after αMPT treatment in male rats. Suckling had no effect on Akt phosphorilation, but systematic administration of D_2-R blocker, haloperidol (2.5 mg/kg b.w. ip.) as well as αMPT significantly increased the level of phospho-Akt (Thr308) in both the AL and the NIL in male rats. These observations may help to explain the differences in the regulatory mechanism between male and female rats as well as the development of DA "tolerance" and "dependence" on the tonic regulation of lactotrops in lactating animals.

This work was supported by the National Scientific Research Fund (OTKA T-04337) and the Ministry of Health (ETT 177/2006) to GMN.

P587

Secondary hypothalamic amenorrhea as the initial manifestation of HIV infection

Garyfallia Tzifa, George Kalikakis, Ioannis Zaganas, Martha Spiliotis, Diamantis Kofteridis, Vasiliki Daraki, Valentina Petkova, Paraskevi Tzoutzouraki & Stathis Papavasileiou
University Hospital Of Crete, Iraklion, Crete, Greece.

Hypothalamic hormonal deficiency and anterior pituitary hormonal deficiency is a rare occurrence in patients presenting with HIV infection. We describe a patient with HIV infection who presented with secondary amenorrhea as the initial manifestation.
Case report
A 34-year-old woman with previously regular menses presented with secondary amenorrhea by 9 months. The patient had mild gait instability for 7 months; anorexia and weight loss (10 kg) for the last 4 months was also reported. Pregnancy test was negative. Gonadotropins were at the lower normal limits (FSH: 2.69 m U/ml. LH: 2.18. m U/ml) with low oestrogen values (E_2: 37. 4 pg/ml). Pelvic ultrasound confirmed the lack of oestrogen activity (endometrium 4 mm thick). A GnRH stimulation test showed an adequate response, pointing to the hypothalamic cause for the amenorrhea. The patient underwent a brain MRI that revealed an empty sella turcica, with accompanying multifocal leukoencephalopathy of unknown aetiology.
Due to the MRI findings and development of chorea serological and immunological tests were performed. Serological tests were positive for HIV1, HIV2 and CMV virus. The absolute number of CD4 was 39. The patient was diagnosed with CMV encephalopathy due to HIV infection (Stage C3) and was managed with combined antiviral therapy. The patient showed dramatic improvement in her symptoms. The CD4 number increased (225) and the viral load became undetectable. The hypothalamus – pituitary – gonad axis as well as the menstrual cycle was fully restored.
Conclusion
CMV encephalopathy, secondary to HIV infection may present with hypothalamic amenorrhea as the initial manifestation. Systemic and neurological symptoms and signs follow this setting. Combined antiretroviral and anti-CMV therapy can result in dramatic improvement and restoration of menses.

P588

Hypopituitary patients have an increased prevalence of cardiovascular risk factors

Beata Matuszek[1], Mariusz Kowalczyk[1], Tomasz Zapolski[2] & Andrzej Nowakowski[1]
[1]Department of Endocrinology, Medical University, Lublin, Poland; [2]Department of Cardiology, Medical University, Lublin, Poland.

Introduction
Hypopituitary patients receiving conventional hormone replacement, 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 an 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 the metabolic syndrome markers and cardiovascular risk factors.
Material and methods
The study included 18 patients (13-M and 5-F) within the age range from 21 to 59 years ($x=39$) with multihormonal hypopituitarism which lasted from 1 to 24 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 the metabolic syndrome were evaluated: body mass index (BMI), waist, arterial pressure, insulin resistance ratios, HOMA–IR and QUICKI, lipidogram, fibrinogen, homocysteine, adiponectin and echocardiography. The control group consisted of 12 healthy individuals.
Results
Hypopituitary patients had an obesity value ($P=0.0063$), independently of sex and age, with a higher circumference of waist ($P<0.0001$). Mixed hyperlipidemia was found in 88% of the studied patients, a higher low-density lipoprotein cholesterol ($P=0.001$), and triglyceridemia ($P=0.003$). Serum homocysteine was significantly higher ($P=0.02$) and adiponectin concentration was significantly lower in patients than in controls ($P<0.005$). Furthermore, the patients had a significantly increased left atrium size ($P=0.05$), but no difference was observed for other cardiac measure.
Conclusions
In patients with multihormonal hypopituitarism, it is necessary to apply simultaneous replacement treatment with regard to all hormonal deficiencies, including GH. Close monitoring for premature development of the metabolic syndrome risk factors is important, especially in the young.

P589

The evaluation of ghrelin concentration in patients treated for acromegaly and of ghrelin expression in pituitary somatotropinomas

Magdalena Jaskula[1], Ryszard Wasko[1], Wodzimierz Liebert[2], Elzbieta Poreba[3], Robert Plewa[4], Anna Gozdzicka-Jozefiak[3] & Jerzy Sowinski[1]
[1]Department of Endocrinology, Metabolism and Internal Diseases, University of Medical Sciences, Poznan, Poland; [2]Department of Neurosurgery, University of Medical Sciences, Poznan, Poland; [3]Department of Molecular Virology; Adam Mickiewicz University, Poznan, Poland; [4]Department of Experimental Biology, Adam Mickiewicz University, Poznan, Poland.

Ghrelin has been considered one of the factors that might contribute to the development of pituitary somatotropinoma.
The aim of our study was to assess whether serum concentrations of ghrelin differ in patients with acromegaly treated with surgery or/and long-acting octreotide (LAO) and also to evaluate the presence of ghrelin mRNA in tissues of somatotroph adenomas. The appoval of Ethical Committee to perform the study was obtained.
Materials
Serum ghrelin was measured with the use of radioimmunoassay RIA (Phoenix Pharmaceuticals) in 42 acromegalic patients and in 18 healthy control subjects. Acromegalic patients were divided into groups according to the treatment that had been administered: 1) surgery /−/, LAO/+/ 2) surgery /+/, LAO /+/ 3) surgery /−/, LAO /−/ 4) surgery /+/, LAO /−/. Human pituitary somatotroph adenoma tissues were obtained at transsphenoidal surgery from 3 acromegalic patients with macroadenomas and studied for ghrelin mRNA expression. Before surgery patients received long acting octreotide at doses 20 mg, 30 mg, 30 mg at 30 days intervals. The reverse transcription and real-time PCR were performed according to *Korbonits et al.* method.
Results
The difference between mean ghrelin level in the healthy subjects and acromegalic patients was not statistically significant ($P=0.08$), neither between patients who had and who had not undergone surgery ($P=0.1$). Patients treated with somatostatin analogue (Sandostatin LAR) had serum ghrelin levels significantly lower than patients who had undergone surgery and than healthy subjects (p=0.001). Ghrelin mRNA was not detected in any examined tissues.
Conclusions
Ghrelin concentrations were significantly lower in acromegalic patients who had been receiving long acting somatostatin analogue treatment; the absence of ghrelin mRNA might be due to the treatment with somatostatin analogue administered preoperatively, which could have suppressed the ghrelin gene transcription.

P590

Ghrelin, inhibits AMPK (AMP-dependent protein kinase), a regulator of cell proliferation and metabolism

Chrysanthia Leontiou, Blerina Kola, Paolo Dalino, Nabila Salahuddin, Giulia Franchi, Ashley Grossman & Marta Korbonits
Barts and the London Medical School, Queen Mary University of London, London, United Kingdom.

Background Ghrelin stimulates cell proliferation in a number of tissues including pituitary. AMPK, a heterotrimer kinase enzyme, is an important sensor and regulator of cellular energy balance. We have shown that ghrelin can change AMPK activity in various tissues and this mechanism could play a role in its metabolic effects. AMPK has recently been established to strongly inhibit cell proliferation and tumorigenesis. We therefore hypothesised that ghrelin stimulates cell proliferation *via* inhibition of AMPK activity in the pituitary.
Methods
The GH3 cell line was treated with ghrelin 10^{-6}, 10^{-7} and 10^{-9} M and cells were harvested in lysis buffer at 30 min, 60 min, 90 min, 2 h, 3 h, 6 h and 24 h. The effect of ghrelin on AMPK activity was studied with a kinase assay using γ^{32}P-ATP and with immunoblotting using phosphorylation-specific antibodies for alpha-AMPK.
Results
AMPK activity was significantly decreased in the ghrelin-treated cells compared to the media treated controls at 60 and 90 minutes for the 10^{-6} and 10^{-7} M, but also at the 6 h for the 10^{-9} M. The peak effect was at 60 minutes (control 21.0$\pm$ 0.7 pmol ATP/min/mg protein vs ghrelin 10^{-7} M 4.7$\pm$0.4 pmol ATP/min/mg protein; $P<0.01$). Immunoblotting for pAMPK showed a reduction in pAMPK content at 60 min after 10^{-6}M ghrelin treatment (88% of control).
Conclusion
We propose that in pituitary cells the proliferative effects of ghrelin involve the inhibition of AMPK which could lead to upregulation of the Akt and/or mTOR-S6kinase pathways and downregulation of the p53–p21 pathway, leading to increased protein synthesis and cell cycle progression.

P591

Self-concept in patients with PCOS

Ioan Gotca[1], Cristina Cristea[2], Letitia Leustean[2], Draghici Ioana[2] & Eusebie Zbranca[2]
[1]"Socola" Hospital, Iasi, Romania; [2]Unversity of Medecine " Gr.T.Popa", Iasi, Romania.

Polycystic ovary syndrome (PCOS) is a major source of psychological morbidity and can negatively affect quality of life. The aim of the study was to identify characteristics of self-concept in female patients diagnosed with PCOS ($n=22$, mean age 26+/− 11 years) and comparative analysis with a control group ($n=22$).
Methods
Psychology questionnaire, selection tests based on geometrical figures/ words and also graph logical analysis were applied to all patients.
Results
PCOS was accompanied by a significantly depreciation of self-concept (68%). Global and symptomatic depression was more severe in persons without important masculinity. Manifest masculinity was significantly associated with reduction of global and symptomatic anxiety and hostility (70%). Superior adaptability was seen for subjects with a lower masculinity. For patients diagnosed with PCOS the domains of interest and behaviour indicated right brain laterality. Graph logical analysis revealed for all patients a masculine / mixture script trend. Protection tendency evaluated by geometrical figure tests were more important for these patients. In summary: hormonal changes modify self-concept, psychological pattern and behaviour of patients with PCOS.

P592

Evaluation of hypotalamic-pituitary-gonadal (HPG) axis and GH-IGF-I axis in adult patients with celiac disease

Emanuele Ferrante[1], Claudia Giavoli[1], Silvia Bergamaschi[1], Luca Elli[2], Annamaria De Bellis[3], Cristina L Ronchi[1], Andrea Lania[1], Maria Teresa Bardella[2], Antonio Bellastella[3], Paolo Beck-Peccoz[1] & Anna Spada[1]
[1]Department of Medical Sciences, University of Milan, Endocrine and Metabolic Unit, Fondazione IRCCS Ospedale Maggiore Policlinico, Mangiagalli e Regina Elena, Milan, Italy; [2]Department of Medical Sciences, University of Milan, Fondazione IRCCS Ospedale Maggiore Policlinico, Mangiagalli e Regina Elena, Milan, Italy; [3]Departement of Clinical and Experimental Medicine and Surgery, Chair of Endocrinology, Second University of Naples, Naples, Italy.

Celiac disease is a chronic inflammatory autoimmune disorder often associated with other endocrine autoimmune diseases, such as type I diabetes mellitus, Addison's disease and Hashimoto's thyroiditis. In these patients, LH, FSH and GH secretion has been poorly investigated. Aim of this study is to evaluate anterior pituitary function, and in particular hypotalamic-pituitary-gonadal (HPG) axis and GH-IGF-I axis, in adult patients with treated celiac disease. For this purpouse, 22 celiac patients (15 M, 7 F, mean age: 34 years, range: 19–74 years) were studied by GHRH+arginine test. In male patients (mean age: 30 years, range: 19–47 years), GnRH test was also performed. All patients were evaluated for serum IGF-I, testosterone (M), basal thyroid and adrenal function and antithyroid antibodies. In 20 out of 22 patients, antipituitary antibodies (APA) were also evaluated.
No alterations in basal TSH, FT4, FT3, ACTH, cortisol, LH, FSH and testosterone levels were detected. Three patients (2 F, 1M) resulted positive for antithyroid antibodies. A normal response to GnRH test was detected in all cases. Four out of 22 patients (18.8%) showed an impaired GH secretion after GHRH+arginine test; in particular, four male patients (4/15, 26.7%) showed a GH deficiency (GHD)(1 patient with complete GHD and 3 with partial GHD), while in none female patients an impaired GH response was recorded. IGF-I levels where low in the patient with complete GHD. All patients, including these with complete or partial GHD, resulted negative for APA. No correlation between GHD deficiency and onset of disease was found.
In conclusion, adult celiac patients show an impaired GH secretion in a significant proportion of cases, this alteration seeming to be predominant in males and independent from disease onset. Given the absence of APA, the cause of this pituitary dysfunction is still unclear and requires further elucidations.

P593

The effects of salsolinol on the peripheral sympathetic activity of hypophysectomized, adrenalectomized and medullectomized rats

Dániel Székács, Ibolya Bodnár, György M Nagy & Márton IK Fekete
Semmelweis University and Hungarian Academy of Sciences, Neuroendocrine Research Laboratory, Budapest, Hungary.

Salsolinol (1-methyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline), is a recently identified endogenous prolactin (PRL) releasing factor. Salsolinol (SALS) seems to be a selective and potent stimulator of PRL secretion both *in vivo* and *in vitro*.

1-Methyl dihydroisoquinoline (1MeDIQ) is an antagonist of salsolinol induced prolactin release and causes increase in plasma norepinephrine (NE) level. SALS decreased the peripheral tissue dopamine (DA) level dose dependently, consequently increased the NE/DA ratio, indicating reduced release of newly formed NE from sympathetic terminals. These effects can be antagonized by 1MeDIQ pretreatment. The aim of our study was to investigate the effect of medullectomy (MEDX), adrenalectomy (ADX) and hypophysectomy (HYPOX) on the interaction of SALS and 1MeDIQ on the catecholamine concentration of the selected sympathetically innervated peripheral tissues (spleen, atrium, etc). We used HPLC-EC method for measurement of NE and DA concentrations. In ADX as well as in MEDX rats, SALS was able to reduce DA level and increase the NE/DA ratio that could be prevented by 1MeDIQ pretreatment. Therefore the presence of adrenal gland is not required for the reduction of peripheral sympathetic activity induced by SALS. Investigating the possible role of pituitary hormones on the peripheral sympathetic system, the effect of SALS has been tested in HYPOX rats. We have found that the effect of SALS on peripheral sympathetic terminals is not affected by HYPOX, conseqently pituitary hormones do not play any role in the catecholamine depleting activity of SALS. The possible physiological significance of these observations need further clarifications.

This work was supported by the National Scientific Research Fund (OTKA T-04337) and the Ministry of Health (ETT 177/2006) to GMN.

P594

Cardiovascular risk and hypopituitarism: evaluation of the global cardiovascular absolute risk, using the individual score of the Progetto CUORE of the Istituto Superiore della Sanità

Carolina Di Somma, Maria Cristina Savanelli, Francesca Rota, Gaetano Lombardi & Annamaria Colao
Department of Molecular and Clinical Endocrinology and Oncology Federico II University, Naples, Italy.

Adults with hypopituitarism are known to have reduced life expectancy with a 2-fold higher risk of death for cardiovascular disease compared with controls. In Italy, to identify individuals at high risk for cardiovascular disease, the function of the Progetto CUORE has been identified and the global cardiovascular risk score has been built using data from different Italian cohorts. To assess the global cardiovascular risk score in adult hypopituitaric patients: 108 hypopituitaric GHD patients (m:45, f: 47; 35–69 yrs), 62 hypopituitaric non GHD patients (m:21, f: 41; 35–69 yrs) and 108 matched controls were studied. At study entry, all subjects were tested with GHRH + ARG and serum IGF-1, total cholesterol, HDL-cholesterol; systolic blood pressure (SBP), smoking habit, diabetes and hypertension treatment were assessed in all subjects. The score was calculated using a test on the website www.cuore.iss.it. At baseline, the global cardiovascular risk score, total cholesterol and SBP were higher ($P<0.001$), while HDL cholesterol ($P<0.0001$) GH peak and IGF-I levels were lower in patients than in controls ($P<0.001$). In particular, the global cardiovascular risk score and total-cholesterol ($P<0.05$) were higher, while GH peak and IGF-I levels ($P<0.001$) were lower in GHD patients than in non GHD patients. No significant difference was found in age, SBP, HDL-cholesterol between two patient groups. An inverse correlation was found between the risk score and GH peak and serum IGF-1 both in patients and in controls. In conclusion, a significant impairment of the global cardiovascular risk score was found in hypopituitaric patients who were replaced for the other pituitary hormones except for GH, indicating a high risk for the development of major coronary or cerebrovascular events in the next ten years. However, whether GH replacement can reduce this risk remains to be established.

P595

Traumatic brain injury (TBI) and lipid profile abnormalities: study 12 months after the brain injury

Carolina Di Somma[1], Francesca Rota[1], Silvia Rovere[2], Maria Rosaria Ambrosio[3], Alessandra Fusco[4], Ginevra Corneli[2], Antonio Bianchi[4], Laura De Marinis[4], Ettore Ciro degli Uberti[3], Gaetano Lombardi[1], Ezio Ghigo[2], Annamaria Colao[1] & Gianluca Aimaretti[4]
[1]Department of Molecular and Clinical Endocrinology and Oncology University of Naples Federico II, Naples, Italy; [2]Division of Endocrinology and Metabolism, Department of Internal Medicine, University of Turin, Turin, Italy; [3]Department of Biomedical Sciences and Advanced Therapies Section of Endocrinology, University of Ferrara, Ferrara, Italy;\ [4]Division of Endocrinology Catholic University Rome, Rome, Jamaica.

Aim of this study was to evaluate lipid profile and the severity of GHD, in a large group of TBI patients with or without GH deficiency. We assayed lipid profile (Total-, HDL- Cholesterol, Triglycerides) in 62 TBI subjects 12 months after TBI (41 M, 21 F, 13–81 yrs, BMI: 24.6 ± 0.6 kg/m^2), and in 62 sex-, age- and BMI-matched controls. Based on the GH peak after GHRH + ARG test, patients were stratified as: 1) severe GHD (GH peak $\leq$ 9 μg/l; $n=13$; 20.9%); 2) partial GHD (GH peak in between 9.1–16.5 μg/l; $n=6$; 9.7%); 3) non-GHD (GH peak >16.5 μg/l; $n=43$; 69.3%). IGF-I levels were lower ($P<0.001$) in patients with severe GHD (88.7 ± 11.1 μg/L) than in those with partial GHD, non-GHD and in controls (148.1 ± 33.9, 219.2 ± 10.7, and 251.8 ± 10.8 μg/L, respectively). HDL-cholesterol were lower ($P<0.01$) in patients with severe GHD (44.1 ± 2.7 mg/dL) than in those non-GHD and in controls (54.4 ± 1.3 and 59.3 ± 1.1, respectively), while, no significant differences was found in partial GHD. In patients with severe GHD, total- and HDL-cholesterol ratio (4.9 ± 0.4, $P<0.01$) were higher than in those with partial GHD (4.4 ± 0.2,), non-GHD (3.9 ± 0.2), and controls (2.9 ± 0.1). In addition, partial GHD patients had total- and HDL-cholesterol ratio (4.4 ± 0.2, $P<0.01$) higher than those non-GHD (3.9 ± 0.2), and controls (2.9 ± 0.1). Triglycerides levels were not different among severe GHD, partial GHD and non GHD TBI patients and controls. In all subjects, a significant correlation was found between the GH peak and age ($r=-0.41$; $P<0.01$), BMI ($r=-0.33$; $P<0.05$), IGF-I ($r=0.36$; $P<0.01$), total cholesterol ($r=-0.37$; $P<0.05$), HDL cholesterol ($r=0.36$; $P<0.05$), total- and HDL- cholesterol ratio ($r=-0.47$; $P<0.01$). IGF-I was correlated with age ($r=-0.54$, $P<0.001$), total cholesterol ($r=-0.46$; $P<0.01$), HDL cholesterol ($r=0.39$; $P<0.05$), total- and HDL- cholesterol ratio ($r=-0.51$; $P<0.01$). In conclusion, impairment of lipid profile was evident in TBI patients with severe GHD.

P596

Abstract unavailable

P597

Does concealment of bad news stimulate the HPA or the SAS axis?

Gesthimani Mintziori[1], Efharis Panagopoulou[1], Dorothea Kapoukranidou[2] & Alexios Benos[1]
[1]Department of Hygiene, Medical School, Aristotle University of Thessaloniki, Thessaloniki, Greece; [2]Department of Physiology, Medical School, Aristotle University of Thessaloniki, Thessaloniki, Greece.

According to recent research in the field of Psychoneuroendocrinology each stressor appears to have its own neurochemical signature. The present study examined whether keeping a secret stimulates the HPA or the SAM axis as well as cortisol involvement in lying.

Methods

Sixty seven (N = 67) healthy young male medical students participated in the study. Students were randomly assigned in 3 groups. All students were informed that they were about to have a 5 min consultation with a 26 year-old woman with non-operable brain tumour. They were also given information about prognosis, treatment and side effects. Group A (disclosure group) was instructed to reveal the information about the diagnosis, prognosis, and treatment. Group B (concealment group) was instructed not to reveal the truth concerning the diagnosis, and prognosis, while students in group C (control group) were instructed to conduct a structured interview concerning dietary habits. Mood, cardiovascular reactivity and salivary cortisol was assessed at baseline (T1), 30 minutes later (T2), and immediately after the task (T3). In addition heart rate was assessed during the consultation using a digital signal extraction pulse oximeter.

Results

Compared to the control group, there was a significant increase in anxiety and negative affect in both experimental groups from T1 to T2 that significantly decreased from T2 to T3 to baseline levels only in the concealment group. In the concealment group there was also a significant decrease of heart rate throughout the consultation ($F=5.304$, $P=0.011$). The salivary cortisol significantly changed in all three groups throughout the process ($F=5.557$, $P=0.007$).

Conclusions

Results show that performance anxiety is involved in cortisol secretion. However concealment/ secrecy only results in SAM activation. Further research is needed to ascertain the endocrine proceedings taking place and eventually design a strategic plan on training for handling bad news in medical settings.

P598

Non-dopaminergic neurons expressing individual enzymes of dopamine synthesis in the arcuate nucleus: development and functional significance

Michael Ugrumov
Institute of Developmental Biology RAS, Moscow, Russia.

Although non-dopaminergic neurons expressing individual enzymes of dopamine (DA) synthesis are widely distributed in the brain, their functional significance remains uncertain. This study was aimed to evaluate the development and functional significance of the neurons expressing one of the enzymes of DA synthesis, tyrosine hydroxylase (TH) or aromatic L-amino acid decarboxylase (AADC), in the arcuate nucleus of rats *in vivo* and *in vitro* by using immunocytochemistry, *in situ* hybridization, image analysis, confocal microscopy, high performance liquid chromatography with electrochemical detection and the radioimmunoassay. According to our data:

- The number of so-called monoenzymatic TH-expressing or AADC-expressing neurons highly exceeded that of DA-ergic neurons expressing both enzymes in fetuses and neonates, whereas there was a reverse in adult animals;
- Monoenzymatic TH-neurons and AADC-neurons synthesize DA in cooperation: synthesis of L-DOPA from L-tyrosine in TH-neurons is followed by its release and uptake by the neighbouring AADC-neurons, where L-DOPA is further converted to DA;
- The 6-hydroxydopamine(neurotoxin)-induced degeneration of DA-ergic neurons in the arcuate nucleus and the development of hyperprolactinemia were accompanied by the increase of the number of monoenzymatic neurons and cooperative synthesis of DA that is considered as a compensatory reaction.

Thus, non-dopaminergic neurons expressing individual complementary enzymes of the DA synthetic pathway produce this neurotransmitter in cooperation that is a compensatory reaction to the failure of DA-ergic neurons.

P599

Impact of somatostatin analogs on the heart in acromegaly: a meta-analysis

Patrick Maison[1], Anne- Isabelle Tropeano[1], Isabelle Macquin-Mavier[1], Andrea Giustina[2] & Philippe Chanson[3]
[1]Assistance Publique-Hôpitaux de Paris, Hôpital Henri Mondor, Créteil, France; [2]University of Brescia, Brescia, Italy; [3]Assistance Publique-Hôpitaux de Paris, Hôpital de Bicêtre, Le Kremlin-Bicêtre, France.

Context
Acromegaly can be complicated by cardiomyopathy. Treatment with somatostatin analogs has been shown to improve some cardiac parameters, but most published clinical trials involved few patients and were not randomized or controlled. In addition, their results are rather variable.
Objective
To conduct a meta-analysis aimed at obtaining a more accurate picture of the effect of somatostatin analogs on the heart in patients with acromegaly.
Design
We systematically reviewed all studies of somatostatin analogs in acromegaly. Eighteen studies were identified in three databases. We conducted a combined analysis of the effects of somatostatin analogs by using the overall effect size to evaluate significance and by computing the weighted mean differences with and without treatment to assess the effect size.
Results
Somatostatin analog treatment was associated with significant reductions in the heart rate (−5.8 [2.1] beats/min), the left ventricular mass index (−22.3 [6.7] g/m^2), inter-ventricular septum thickness (−0.3 [0.2] mm), left ventricular posterior wall thickness (−0.8 [0.4] mm) and the ratio of the E-wave and A-wave peak velocities of the mitral flow profile (0.2 [0.1]). It was also associated with improved exercise tolerance (+1.6 [0.4] min]. Trends towards beneficial effects were noted for the left ventricular end-diastolic dimension (−1.5 [2.2] mm) and the left ventricular ejection fraction (3.3 [1.7] %). Overall effect sizes were not significant for blood pressure, left ventricular end-systolic dimension or fractional shortening. Bigger improvements were observed in studies with larger falls in IGF-I and/or GH levels, and in studies of younger patients.
Conclusion
This meta-analysis confirms that somatostatin analog therapy aimed at achieving stringent control of serum GH/IGF-I concentrations in patients with acromegaly is associated with significant positive effects on morphological and functional hemodynamic parameters.

P600

Thyrotropinoma response to somatostatin receptor ligand (SRL) – key feature in preoperative treatment

Wojciech Zgliczynski[1], Piotr Zdunowski[1], Malgorzata Gietka-Czernel[1] & Grzegorz Zielinski[2]
[1]Medical Center Postgraduate Education, Warsaw, Poland, [2]Army's Medical Institute, Warsaw, Poland.

Background
TSH-secreting tumors appears as extremely rare cause of hyperthyroidism. Major clinical feature is preserved TSH level in subjects with apparent thyrotoxicosis. Misdiagnosis of primary thyroid hyper function led to mistreatment with anti-thyroid agents. This worsens disease course and outcome. Neurosurgery success rate is limited by large tumor size and extrasellular expansion. Somatostatin plays key role in regulation of TSH secretion. Tumors in most cases expresses receptors for somatostatin therefore SRL are potent option in TSH-oma treatment.
Aim
Of the study was to determine SRL efficacy in patients before neurosurgical treatment of TSH-oma. Secondary aim was to verify long-period outcome of SRL in cases of neurosurgery failure.
Material
Comprise of 9 patients with secondary thyrotoxicosis, 6 women and 3 men, aged 35 to 69 yrs (mean 49) presenting with pituitary macroadenoma (18 to 45 mm). Before diagnosis was established, 5 out of 9 received antithyroid medication, and in 1 case strumectomia was performed.
Intervention
Somatostatin analogue octreotide long-acting repeatable (LAR) administration 3 months prior to the surgery.
Results
Initially, all patients had abnormal fT4 and alfa-SU levels (mean 38.8 pmol/l SD 11.6 and 6.1 ng/ml SD 6.4, respectively) as well as lack of TSH increase after TRH stimuli (mean rise 15% from basal value, SD 52). 3 months of SRL treatment led to marked TSH and alfa-SU levels decrease (to 1.2 mU/l SD 1.1 and 0.8 ng/ml SD 0.6, resp.), normalization of thyroid hormones (fT4 mean 15.7 pmol/l SD 5.0) and clinical improvement. Patients in euthyroid state were referred to neurosurgery unit. Tanssphenoideal adenomectomy was successful in 8 out of 9, and in this group TRH stimuli performed 3 months after surgery provokes significant TSH response (mean rise 210% SD 310). In one case after unsuccessful surgery euthyroid state is achieved during SRL treatment for 2 years, without noticeable adverse events.
Conclusions
Somatostatin analogue treatment is efficient in TSH-secreting tumors in inhibition of TSH secretion, thyroid hormone normalization, visual field improvement, thyroid volume decrease and neurosurgery success rate. Post-surgery TSH increase during TRH test indicates restored pituitary-thyroid axis. In cases of surgery failure prolonged SRL may be efficient option.

P601

Glucose resistance in acromegaly is reversible during somatostatin analogues treatment

Piotr Zdunowski & Wojciech Zgliczynski
Medical Center of Postgraduate Education, Warsaw, Poland.

Background
Insulin resistance leading to glucose intolerance and even diabetes mellitus is common in acromegaly and is partially caused by pathological high concentrations of growth hormone (GH) and somatomedin C (IGF-1). On the other hand, somatostatin analogues, common treatment option, can cause inhibition of insulin secretion and glucose tolerance disturbances.
Aim
Of the study was to determine impact of prolonged somatostatin analogues administration on insulin resistance in acromegaly.
Material
27 acromegalics 16 women and 11 men, aged 23 to 65, mean 43, previously untreated and with excluded diabetes mellitus was enrolled into this study.
Intervention
Primary octreotide LAR treatment for 6 months prior to neurosurgery.
Methods
Prolonged (0–180') oral glucose tolerance test (OGTT) with glucose, GH and insulin assessment was performed initially, 2 weeks after first octreotide injection and after 6 months of treatment. Insulin resistance was calculated as fasting glucose to fasting insulin ratio (FG/FI), sum of insulin levels during OGTT (sI).

Also, HOMA and Quicki indexes was calculated. Control group consists of healthy volunteers from department database. Disease activity was calculated with clinical symptoms score, GH and IGF-1 levels.
Results
Initially, 21 out of 27 (77%) patients was insulin-resistant (FG/FI <6), HOMA index was significantly higher than in controls (3.2 S.D. 1.4 v. 1.6 S.D. 0.8 $P<0.001$). After 6 months of treatment insulin-resistance presented 16 (59%), insulin levels drop significantly in fasting state and during OGTT (sI 659 S.D. 160 v. 430 S.D. 180 $P<0.05$ initially v. 6 months therapy) whereas glucose levels did not differ significantly ($P<0.01$). HOMA index fall close to controls (2.1 S.D. 0.7), and Quicki was slightly higher than initially (mean 0.329 v. 0.369 respectively), but difference did not reach statistical significance ($P=0.12$).
Conclusions
Somatostatin analogue therapy could improve insulin-sensitivity and did not worsen glucose metabolism in patients with acromegaly.

P602

Endocrine and neuro-ophtalmologic correlates of primary empty sella

Antonio Bianchi[1], Bruno Gregori[2], Eugenia Sacco[1], Antonella Giampietro[3], Domenico Milardi[3], Alessandra Fusco[1], Neri Accornero[2], Alfredo Pontecorvi[1] & Laura De Marinis[1]
[1]Department of Endocrinology, Catholic University School of Medicine, Rome, Italy; [2]Department of Neurological Sciences, University of Rome 'La Sapienza', Rome, Italy; [3]International Institute 'Paolo VI', Catholic University School of Medicine, Rome, Italy.

Background
Primary empty sella (ES) can be asymptomatic or associated with endocrine and neurological alterations, such as visual defects. Studies in a large number of patients is still lacking.
Objective
To study visual deficit in ES and its relationship with hormonal status.
Material and Methods
We recorded visual evoked potentials (VEP) by white/black, red/black, blue/black patterns. Isoluminance between red and blue checks allowed to compare potentials. We measured P1 latencies and computed a chromatic (blue-red/blue+red) contrast index (CC). Chromatic visual field perimetry was performed with a dedicated computerised system which provides quantitative chromatic maps for each eyes.
We included 64 eyes of 32 normal volunteers (age: 44 ± 14.8) and 10 eyes of 10 ES patients with no systemic disease and increased intracranial pressure (age 50 ± 16.1). On basis of clinical and laboratory data, patients were divided in two groups: with (group A) and without (group B) endocrine abnormalities.
Results
VEP and Visual field perimetry studies showed a significant alterations of both P1 latencies and visual field indices in ES patients as compared to controls. In group A visual alterations appeared more pronounced as compared to group B.
Discussion
Chromatic studies can selectively analyse parallel visual pathways which differ in their physiology and susceptibility to visual pathologies. Data suggest a different disorder of visual systems in ES patient with and without endocrine abnormalities. Studies in a large number of ES patients can provide insights in the pathophysiology of syndrome and more accurate indications for treatment.

P603

Growth hormone deficiency in patients with acromegaly after 'successful' transsphenoidal surgery

Milan Petakov[1], Tatjana Isailovic[1], Marina Djurovic[1], Djuro Macut[1], Miloje Joksimovic[2], Bojana Popovic[1], Ivana Bozic[1] & Svetozar Damjanovic[1]
[1]Institute of Endocrinology, Clinical Center of Serbia, Belgrade, Serbia; [2]Clinic for Neurosurgery, Belgrade, Serbia.

The diagnosis of adult growth hormone deficiency (GHD) in patients with pituitary disease relies predominantly on provocative tests of GH secretion. The incidence of GHD in treated acromegalic patients has not been fully documented. Therefore, the aim of the present study was to elucidate GH response to insulin-induced hypoglycaemia (ITT, 0.15 IU/kg i.v.) in a cohort of 10 patients with acromegly considered cured solely by transsphenoidal surgery (6 females and 4 males, mean age 51 ± 2.6 years), and 6 healthy age-matched controls (3 females and 3 males). All patients cured for acromegaly (biochemical criteria for remission-'cure" were the normalization of IGF-I level and GH suppression to less then 1 μg/l during the OGTT) had normal residual pituitary function i.e. had no signs of pituitary ACTH and TSH deficiency. The mean ($\pm$S.E.M) peak GH response to ITT in cured acromegalics was significantly lower in comparison with healthy subjects (8.19 ± 2.05 vs. 17.45 ± 3.1 μg/l; $P<0.05$). In five 'cured' acromegalic patients (50%) we confirmed the presence of severe growth hormone deficiency (peak GH during ITT less then 3 μg/l). In conclusion, it has been increasingly recognized that some patients previously concerned cured after surgery for acromegaly, in fact have the GH deficiency. It is necessary to check GH secretory capacity in every cured patient previously operated for acromegaly even if no other pituitary hormone deficit exist. Possibly, some of so-called cured patients with acromegaly should be treated with GH substitution, concerning the possible premature morbidity and mortality due to GH deficiency.

P604

Excess mortality in women with pituitary disease: results of a meta-analysis

Eigil Husted Nielsen, Jörgen Lindholm & Peter Laurberg
Dept. of Endocrinology, Aalborg Hospital, Aarhus University Hospital, Aalborg, Denmark.

Background
Several studies of rather heterogeneous groups of patients have shown an increased mortality in patients with pituitary diseases. In patients without hypersecretion of growth hormone or ACTH the increased mortality has mostly been attributed to pituitary insufficiency. Some studies have suggested sex-specific differences in standard mortality rates (SMR) whereas others have shown increased cardiovascular and/or cerebrovascular mortality. A recent study of patients who had undergone surgery for non-functioning pituitary adenoma showed a normal SMR in men, whereas SMR was significantly increased in women. We explored this sex related difference by a meta-analysis.
Material and methods
We performed an internet-based meta-analysis using major medical science databases of MedLine, Embase and Web of Science to identify publications on mortality in patients with pituitary disease. Both Thesaurus-term and free-text searches were applied. Articles were required to provide exact information on standard mortality rates in both men and women separately, 95% confidence interval (CI) and a well-defined normal reference population. Studies including patients with Cushing's disease or acromegaly were excluded as were studies with a majority of patients carrying a diagnosis of craniopharyngioma. Sex-specific overall SMR values for men and women in the meta-analysis were calculated as weighted averages of SMR from individual studies, using the inverse variance method. An additional analysis of association between first year of inclusion of new patients and SMR values in each study was also performed.
Results
Six studies fulfilled our criteria for inclusion in the meta-analysis. The weighted overall SMR for men was calculated to 2.06 (CI: 1.94–2.20), whereas weighted SMR for women was 2.80 (2.59–3.02). Mortality rates were thus significantly higher than in the reference population in both men and women, and SMR in women was significantly higher than in men. Analysis of association between first year of inclusion of new patients and SMR showed a statistically significant negative correlation in men reaching a normal value in the most recent study. In women SMR was always higher and did not normalize in recent studies.
Conclusion
Our meta-analysis showed that SMR is increased in both men and women with pituitary disease, with a significantly higher SMR in women than in men. SMR seems to be reaching normal levels in male patients treated in recent decades, whereas SMR is still clearly elevated in women. The reason for this is unknown, but most likely the high mortality in women reflects suboptimal diagnosis and/or suboptimal therapy of pituitary insufficiency.

P605

Familial acromegaly – the role of the AIP gene

M Gueorguiev[1], F Lolli[1], JP Chapple[1], R Quinton[2], A Ribeiro-de-Oliveira[3], MR Gadelha[4], V Popovic[5], JP Monson[1], JAH Wass[6], LA Frohman[7], AB Grossman[1] & M Korbonits[1]
[1]Barts and the London Medical School, London, United Kingdom; [2]Royal Victoria Infirmary and University of Newcastle-upon-Tyne, Newcastle-upon-Tyne, United Kingdom; [3]Federal University of Minas Gerais, Belo Horizonte, MG, Brazil; [4]Clementino Fraga Filho University Hospital, Federal University of Rio de Janeiro, Brazil; [5]University Clinical Centre, Belgrade, Serbia; [6]Churchill Hospital, Oxford, United Kingdom; [7]University of Illinois, Chicago, United States.

Pituitary adenomas are present in ~25% of autopsy samples, and recent studies have also suggested that clinically important pituitary adenomas are some 5 times more common than previously recognised. Acromegaly is almost always due to a sporadic growth-hormone secreting pituitary adenoma, but familial acromegaly has been reported occasionally. Linkage and loss of heterozygosity studies have shown that it is caused by a tumour suppressor gene located at 11q13; very recently 3 families have been reported with a very low penetrance mutation in the gene coding for the aryl hydrocarbon receptor (AhR) interactive protein (AIP), a molecular chaperone, which has been linked to the induction of hepatic detoxifying gene products in response to environmental toxins such as dioxin. However, an additional function appears to be regulation of the cell cycle, suppressing cyclin E and increasing expression of p27, which we have previously shown to be involved in pituitary tumorigenesis.

We studied 19 families with familial pituitary adenoma and identified mutations in the AIP gene in a 4/19, which were either stop codons or mutations disrupting the protein-binding segments of the protein. The penetrance of the disease at the time of the study was 64%, suggesting a much higher level of penetrance than previously reported; in some families there was 100% penetrance. A selected group of young-onset sporadic acromegalic patients, including 3 with gigantism, showed no germline mutations. We found AIP protein expression in normal pituitary and in sporadic pituitary adenomas, while mRNA expression of AIP and its putative partner AhR showed up-regulation, suggesting a compensatory mechanism. Somatic mutations of somatotroph tumours were not seen.

In summary, AIP mutation has been identified in one in four of familial acromegaly kindreds and shows a relatively high penetrance; while mutations of this gene are not involved in the pathogenesis of sporadic somatotroph adenomas, more subtle defects are currently under investigation.

P606

Validation of different insulin sensitivity indices in GH deficiency children using roc curve analysis

R Baldelli[1], S Bellone[2], G Corneli[2], A Giampietro[3], V Cimino[3], A Pontecorvi[3], L De Marinis[3] & G Bona[2]
[1]Regina Elena Cancer Institute, Rome, Italy; [2]University of Piemonte Orientale, Rome, Italy; [3]Catholic University of Sacred Hearth, Rome, Italy.

Insulin sensitivity in GHD patients tends to decrease with age and variations in body composition. Several indices of insulin sensitivity have been considered and among these HOMA, ISI and QUICKI are based on mathematical calculations taking into account glucose and/or insulin levels either in basal conditions or after OGTT. Aim of present study was to validate the different indices in a population of pre-pubertal GHD children ($n=66$) by ROC curve analysis. All patients underwent OGTT with evaluation of glucose and insulin. To validate the different indices the ROC curve analysis has been used with the aim to provide the cut-off limit, sensitivity and specificity for each index. The lowest limit of normality was defined as the value that provided the best pair of highest sensitivity/specificity for HOMA, ISI and QUICKI. Evaluating data derived from ROC curve analysis we have found that ISI index was the most robust index of insulin sensitivity. Using a cut-off of 0.6, HOMA shows a sensitivity of 29% and a specificity of 83.7%; using a cut-off of 0.4, QUICKI shows a sensitivity of 32.3% and a specificity of 88.4%; using a cut-off of 9.2, ISI shows a sensitivity of 43.5% and a specificity of 100%. Applying the cut-off point for ISI, among the patients we found that 42% of GHD children were insulin resistant. This kind of diagnosis was difficult before, because the specific cut-off limits of ISI had not been calculated. Data from the current study demonstrate that ISI was more potent respect to HOMA and QUICKI and represent a convenient test for the diagnosis of insulin resistance.

P607

The transition phase in GHD patients and metabolic alterations: life span variations of insulin sensitivity?

R Baldelli[1], S Bellone[3], G Gorneli[2], J Bellone[3], A Petri[3], S Vannelli[4], C De Sanctis[4], L Benso[4], G Bona[3] & G Aimaretti[2]
[1]Regina Elena Cancer Institute, Rome, Italy; [2]University of Turin, Turin, Italy; [3]Univeristy of Piemonte Orientale, Rome, Italy; [4]Regina Margherita Pediatric Hospital, Rome, Italy.

GH has well documented insulin antagonistic effects. By inference, GHD may be expected to result in increased insulin sensitivity. Young GHD children have a tendency to both fasting and readly provoked hypoglycaemia probably resulting from impaired hormonale counter-regolation. Increased insulin sensitivity could also contribute to their hypoglycaemia; however, this has not been directly demonstrated. Interestingly, susceptibility to hypoglycaemia in GHD children diminishes with increasing age and, paradoxically, GH deficient adults demonstrate insulin resistance even prior to GH replacement therapy. The mechanism underlying this apparent age-related deterioration in insulin sensitivity in GHD subjects is unknown (changes in body composition or metabolic responses to GH, or interaction with pubertal increases in sex steroids e.g.). The transition period is defined as the period between end of linear growth and attainment of full adult somatic development. It can be defined as late teenage years, 'Post-adolescence', 'Young adulthood' with a duration of ~ 3–10 years and the ESPE consensus of december 2003 underline the transition period defined as ending around 25 years. In order to examinate the life span insulin sensitivity index a group of GHD patients have been selected ($n=81$); in particular **group A** ($n=10$) (<25 yrs), **group B** ($n=4$) (26–30 yrs), **group C** ($n=11$) (31–40 yrs), **group D** ($n=14$) (41–50 yrs), **group E** ($n=30$) (51–60 yrs) and **group F** ($n=12$) (>60 yrs). The insulin sensitivity was evaluated using HOMA index (basal insulin levels x blood glucose/22) reflecting, in particular, the 'value' of insulin resistance. Our preliminary results indicated that insulin sensitivity decresed significantly in the group of patients after the transition phase (group B) respect to the other period of life ($P<0.05$, vs A, C, E). We are not aware of any other works evaluating insulin sensitivity in a large group of GHD patients. In our patients reduced insulin sensitivity in the period after transition age could support the hypothesis to treat this patients also in this period of life due to possible high incidence of insulin resistance after the transition period. There is some debate as to whether a reduced insulin sensitivity is only a transiet phenomenon or a persistent one. This data is reflecting somatic immaturity of patients who suffer for two components: developmental existing since childhood (in childhood onset GHD) and metabolic acquired in the transition period so this data support the hypothesis that patients must be treated also in transition phase due to the possible incidence of metabolic alteration in the following period of life.

P608

Growth hormone deficiency in succesfully treated acromegalic patients is not protective from cardiac complications

R Baldelli[1], C Di Somma[3], M Ambrosio[4], A Bianchi[5], R Pivonello[3], V Gasco[2], P Maffei[6], M Appetecchia[1], N Sicolo[6], E degli Uberti[4], L De Marinis[5], S Grottoli[2], A Colao[3] & E Ghigo[2]
[1]Regina Elena Cancer Institute, Rome, Italy; [2]University of Turin, Turin, Italy; [3]Federico II Univeristy of Naples, Naples, Italy; [4]University of Ferrara, Ferrara, Italy; [5]Catholic University of Rome, Rome, Italy; [6]University of Padua, Padua, Italy.

GH hypersection results in biventricular concentric hypertrophy and a progressive contractile impairment whereas cardiac hypotrophy and impaired diastolic filling and left ventricular function have been reported in GH deficiency (GHD). No information on cardiac performances and structure are available about those acromegalic patients in whom successful treatment made their GH and IGF-I secretion similar to those in GHD patients. In order to study the functional and structural cardiac consequences of optimal treatment for acromegaly, we enrolled 12 active acromegalic patients (group A), 14 post-surgical cured acromegalic patients with selective secondary GHD (group B), 11 cured acromegalic paties under treatment with SS analogs (group C), 21 GHD (group D) and 18 controls (group E). GHD diagnosis was based on GHRH+arginine test. In all the subjects LVMi, EF and E/A was studied by M-B mode echo-Doppler. IGF-I levels were higher in group A respect to groups B, C, D ($P<0.0005$, $P<0.005$, $P<0.0005$, respectively) whereas it was lower in group B than group C ($P<0.005$) but similar to group D. LVMi in group A was higher than in group E ($P<0.0005$) in which it was similar to group D. LVMi in group B were similar than in group A, whereas in group C it was lower than in groups A and in B ($P<0.0005$, $P<0.05$, respectively), still persisting higher than in group D and in group E ($P<0.05$, $P<0.0005$, respectively). EF in group A was similar to group E in whom it was higher

than in group D ($P<0.05$). EF in group B was similar as in group A, while in group C it resulted higher than in group D and E ($P< 0.0005$, $P<0.005$) but still similar to group A. E/A in group A was lower than in group E ($P<0.005$) in which it was higher than in group D ($P< 0.0005$). In group B, E/A was lower than in group A ($P<0.05$) but similar to in group D. In group C, E/A were similar to in group A, but still lower than in group E ($P<0.05$) and similar to group D. In conclusion these data suggest that GH deficiency induced by successful treatment of acromegaly does not per se counteract cardiac abnormalities induced by acromegalic cardiomyopthy. Despite similar GH and IGF-I levels, treatment with SS analogues appears more effective in reducing cardiac mass and to improve diastolic function suggesting a potential GH-independent direct role of SS at the cardiac level.

Reproduction – presented on Tuesday

P609

Implications for molecular mechanisms of glycoprotein hormone receptors using a new sequence-structure-function analysis resource

Gunnar Kleinau[1], Mara Brehm[2], Urs Wiedemann[1], Dirk Labudde[1], Ulf Leser[3] & Gerd Krause[1]
[1]Leibniz-Institut für Molekulare Pharmakologie, Berlin, Germany; [2]FH-Gießen, Gießen, Germany; [3]Institute for Computer Science, Humboldt-Univerität, Berlin, Germany.

Comparison between wild type and mutated glycoprotein hormone receptors (GPHRs) TSHR, FSHR and LHCGR is established to identify determinants involved in molecular activation mechanism. The basic aims of current work are the discrimination of receptor phenotypes according the differences between activity states they represent and hit-assignment of classified phenotypes to 3D-structural positions to reveal functional-structural hotspots and interrelations between determinants that are responsible for corresponding activity states. Since it is hard to survey the vast amount of pathogenic and site-directed mutations at GPHRs and to improve an almost isolated consideration of individual point mutations, we present a system for systematic and diversified sequence-structure-function analysis (SSFA) (http://www.fmp-berlin.de/ssfa). In order to combine all mutagenesis data into one set, we converted the functional data into unified scaled values. This at least enables their comparison in a rough classification manner. In this study we describe the compiled data set and a wide spectrum of functions for user driven searches and classification of receptor functionalities such as cell surface expression, maximum of hormone binding capability, and basal as well as hormone induced Gαs/Gαq mediated cAMP/IP accumulation. Complementary to known databases our data set and bioinformatics tools allow to link functional-, biochemical- specificities with spatial features to reveal concealed structure-function relationships by a semi-quantitative analysis. A comprehensive discrimination of specificities of *pathogenic mutations* and *in vitro* mutant phenotypes and their relation to signalling mechanisms of GPHRs demonstrates the utility of SSFA. Moreover, new interrelations of determinants important for selective G-protein mediated activation of GPHRs are resumed.

P610

A comparison between the efficacy and safety of pegvisomant to that of octreotide LAR in patients with acromegaly

Philip Harris[1], Gwyn D'Souza[1], Anthony Good[1], Gary Layton[1], Annamaria Colao[2], Biller Beverly[3], Anne Klibanski[3] & Ezio Ghigo[4]
[1]Clinical research and Development, Pfizer Ltd, Sandwich, United Kingdom; [2]Department of Endocrinology, Federico II University of Naples, Naples, Italy; [3]Neuroendocrine Unit, Massachusetts General Hospital, Boston, United States; [4]Division of Endocrinology and Metabolism, University of Turin, Turin, Italy.

Two medical therapies are now available for the treatment of acromegaly. Pegvisomant is a growth hormone (GH) receptor antagonist. Somatostatin analogues, in contrast, act by inhibiting the release of GH from the pituitary. The primary objective of this study was to compare the efficacy of pegvisomant (P) to that of octreotide LAR (LAR) in terms of IGF-1 normalisation. The secondary objective was to compare safety and tolerability between the two treatments.

The study was a 52 week, multi-centre, open label, parallel group, randomised trial in acromegalic subjects who were either *de novo*, or post-surgical, with IGF-1 levels $\geq 1.3\times$ upper limit of normal (ULN). Subjects were randomised to either P or LAR, using stratification with respect to baseline severity (mild (IGF-1 ≥ 1.3 ULN; severe (IGF-1 $\geq 2\times$ ULN), The dose of P was started at 10 mg sc and titrated at 8 week intervals to normalise IGF-1 up to a maximum of 40 mg. The dose of octreotide was 50 μg sc three times daily, switching at 4 weeks to 20 mg LAR im monthly. The dose was titrated to normalise IGF-1 at 16 week intervals up to a maximum of 30 or 40 mg monthly, according to local practice. During the study, the Nichols IGF-1 radioimmunoassay (RIA) became unavailable and analysis was switched to the Immulite chemiluminescent assay. The difference in number of subjects who achieved IGF-1 normalisation (responders (R)) between the two treatment groups was analysed by Fisher's Exact test, while changes from baseline in efficacy parameters were analysed by ANCOVA. The R rate was higher in the P group compared to LAR, but the difference was not statistically significant. In P, R rates using the Immulite and RIA assays respectively were 51%, 83%, compared to 34% and 67% in LAR. The number of subjects with treatment-related adverse events was 21 in P and 29 in LAR. Four subjects in both groups had abnormal ($\geq$ 3x ULN $\leq 10\times$) hepatic transaminases. There was a higher incidence of biliary tract abnormalities with LAR.Treatment with P was at least as efficacious as LAR. It is hypothesised that the lower than expected R rates and non-significant difference in IGF-1 normalisation between the 2 treatment groups are due to a change in assay methodology and non-optimal dose titration with P.

P611

Androtest: a structured interview for the screening of hypogonadism in patients with sexual dysfunction

Giovanni Corona[1], Luisa Petrone[1], Edoardo Mannucci[2], Alessandra D Fisher[1], Valerio Chairini[3], Giancarlo Balercia[4], Gianni Forti[1] & Mario Maggi[1]
[1]Andrology Unit, University of Florence, Florence, Italy; [2]Geriatric Unit, Diabetes Section, University of Florence, Florence, Italy; [3]Endocrinology Unit, Maggiore-Bellaria Hospital, Bologna, Italy; [4]Endocrinology Unit, Polytechnic University of Marche, Ancona, Italy.

Objectives
Detecting hypogonadism is crucial in patients with sexual dysfunctions because hypogonadism can have a causal role for them and testosterone (T) substitution represents a milestone for the therapy. At present, three different inventories have been developed for screening of hypogonadism in aging male. All these instruments demonstrated a good sensitivity but low specificity. No inventories are available for the screening of hypogonadism in patients with sexual dysfunction. We wished to set up a brief structured interview providing scores useful for detecting hypogonadism defined as low total T ($<$10.4 nmol/L, 300 ng/dL) in a symptomatic population (sexual dysfunction).
Methods
A minimum set of items was identified within a larger structured interview through iterative ROC curve analysis, with assessment of sensitivity and specificity for hypogonadism in a sample of 215 patients. Sensitivity and specificity were verified in a further sample of 664 patients. Correlation of test scores with PSA, testis volume, and others clinical and psychological parameters, was assessed for concurrent validity.
Results
In the validation sample, the final 12-item version of the interview (ANDROTEST) had a sensitivity and specificity of 68% and 65% with an accuracy of $0.700+0.03$ ($P<0.0001$), in detecting low total testosterone ($<$ 10.4 nmol/l) and of 71% and 65% with an accuracy of 0.716 ± 0.03 ($P<0.0001$), in the screening for low free testosterone ($<$37 pmol/l). Furthermore, patients with pathological test (i.e score $>$ 8) showed higher prevalence of hypogonadism related signs, such as lower testis volume and higher depressive symptoms. Finally, when younger patients only ($<$54 years, which represents the median age of the sample), were considered, Log10 [PSA] levels were significantly lower in those with ANDROTEST score $>$8.
Conclusion
ANDROTEST is a quick, and easy-to-administer interview that provides scores for the screening of male hypogonadism in patients with sexual dysfunction.

P612

Assessment of the relational factor in male patients consulting for sexual dysfunction: the concept of couple sexual dysfunction

Giovanni Corona[1], Luisa Petrone[1], Edoardo Mannucci[2], Angela Magini[1], Francesco Lotti[1], Valdo Ricca[3], Valerio Chiarini[4], Gianni Forti[1] & Mario Maggi[1]
[1]Andrology Unit, University of Florence, Florence, Italy; [2]Geriatric Unit, Diabetes Section, University of Florence, Florence, Italy; [3]Psychiatry Unit, University of Florence, Florence, Italy; [4]Endocrinology Unit, University of Florence, Florence, Italy.

Objectives
To date it is not clear to which extent a clinical, or even a subclinical, sexual dysfunction in the female partner might associate with erectile dysfunction (ED) in the male partner. The present study is aimed at the assessment of clinical features of ED associated with relational disturbances.
Methods
In a consecutive series of 1140 male subjects reporting a stable couple relationship we evaluated the impact of relational factors, as assessed by SIEDY Scale 2 (exploring, as reported by the patient, menopausal symptoms, partner's medical illness interfering with sexual activity and reduced partner desire and climax). SIEDY is an easy to administer instrument for the first screening of ED patient, providing scores for the relational component besides those to quantity the organic and intrapsychic components. Several hormonal, biochemical and instrumental parameters were also studied, along with psychopathology scores (Middlesex Hospital Questionnaire modified MHQ).
Results
We found that SIEDY Scale 2 is significantly and independently from other factors (as the organic ones) associated with ED, delayed ejaculation, hypoactive sexual desire and decreased number of intercourses. In particular, the chance of being affected by severe ED increased by 10 [1–10] % for each increment of SIEDY Scale 2 score ($P<0.05$). SIEDY Scale 2 scores are associated with an advanced age of the partner and a long couple relationship (>10 years), independently from patient's age. In addition, an increased relational factor significantly ($P<0.0001$) correlates with increased extra-marital affairs ($r=0.111$), conflicts in the couple ($r=0.279$), alcohol abuse ($r=0.155$) and presence of depressive symptoms ($r=0.182$), as assessed by MHQ questionnaire.
Conclusion
Our result should encourage the andrologist to consider the context in which the sexual symptom develops, analysing the relationship and partner's behaviour and diseases. Resolving, or at least ameliorating, the relational background and the sexual framework might help in treating male sexual dysfunction.

P613

Effect of hormone replacement therapy apart from growth hormone on the endothelial functions in patients with Sheehan's syndrome

Mithat Bahceci[1], Semir Pasa[1], Hatice O Akay[2], Alpaslan Tuzcu[1], Senay Arikan[1] & Deniz Gokalp[1]
[1]Dicle Univ. School of medicine Department of endocrinology, Diyarbakir, Turkey, [2]Dicle Univ. School of medicine Department of radiology, Diyarbakir, Turkey.

Aim
To examine the endothelial functions of patients with Sheehan syndrome (SS) and to eveluate the effects of hormone replacement treatment except growth hormone on endothelial functions.
Subjects and methods
Twenty-four patients with Sheehan syndrome (PSS) aged 40.83±6.43 yr and 25 healthy control women aged 41.13±6.51 yr (C) were included. Endothelial functions were evaluated with high resolution ultrasonography (flow mediated dilatation:FMD) and serum nitric oxide (NO) levels before and after the treatment [15 months with prednisolon (5–7.5 mg/d), L-thyroxin (100–200 μg/d), and conjugated estradiol (0.625 mg/d)- medroxyprogesteron acetate (5 mg/d) patients <40 years].
Results
1-Before treatment
Baseline (16.87±4.04 μol/L and 11.8±2.14 μol/L) and stimulated NO levels were higher (18.79±4.4 and 14.92±2.44); whereas, baseline arterial diameter (3.74±0.68 mm, 4.62±0.42 mm, $P=0{,}0001$), FMD stimulated NO increment ratio (13.16±5.57% and 26.38±8.89%, $P=0{,}0001$) and arterial dilation ratio (13.42±6.57% and 18.93±5.64, $P=0{,}003$) of PSS were lower than C group.
2- After treatment
Elevation of baseline (17.58±4.3 vs 11.8±2.14) and stimulated NO levels of PSS (21.12±4.85 vs 11.92±2.44, $P=0.0001$) insisted on. On the contrary FMD stimulated arterial dilation ratio of PSS increased to the similar level of C group with treatment. FMD stimulated NO levels (18.79±4.4 vs 21.12±4.85), NO increment ratios (13.16±5.57% and 22.83±8.57%) and FMD stimulated arterial dilation ratio increased with treatment significantly (13.42±6.57% vs 21.73±10.13%) ($P=0{,}0001$).
Conclusions
1- Although patients with Sheehan syndrome had high NO levels, they had small FMD stimulated NO increments and arterial dilation ratio. 2-Increased but little effective NO may responsible for this result. 3- HRT apart from GH may restore endothelial functions in patients with Sheehan's syndrome.

P614

Family history of diabetes mellitus determines insulin sensitivity and beta cell dysfunction in polycystic ovary syndrome

Jana Vrbikova, Katerina Dvorakova, Tereza Grimmichova, Martin Hill, Sona Stanicka & Karel Vondra
Institute of Endocrinology, Pragie 1, Czech Republic.

Aim
To examine the secretion of insulin and glucagon in PCOS in the context of insulin sensitivity.
Patients and methods
13 healthy women (BMI 21.8(2.2) kg/m^2), 21 PCOS without family history of DM2 (FH-); BMI 24.3 (4.4) kg/m^2 and 16 PCOS with the 1st degree relative affected by DM2 (FH+); BMI 26.7 (4.2) kg/m^2. Euglyceamic hyperinsulinaemic clamp (1mIU kg^{-1}.min^{-1}; with the determination of insulin sensitivity index (ISI)) and arginine secretion test to measure insulin (AIR) and glucagon (AGR) secretion after arginine bolus at fasting glycaemia (AIRf and AGRf) and at hyperglycaemia (AGRf and AGRg). Kruskal-Wallis ANOVA followed by Kruskal-Wallis multiple comparisons and Spearman correlations adjusted to a constant BMI were used for data evaluation.
Results
PCOS had higher basal insulin ($P=0.004$) and higher HOMA-R than C ($P=0.002$). Higher basal glucagon ($P=0.005$) and higher glucagon secretion at hyperglycemia (AGRg; $P=0.05$) in PCOS than in C was seen. PCOS FH+ had higher insulin secretion at fasting glycaemia ($P=0.05$) with no difference at hyperglycemia. Insulin sensitivity index (ISI, ISI $_{LBM}$) was lower in PCOS FH+ ($P=0.002$) than in C or PCOS FH-.Concerning beta cell function, disposition indices calculated from ISI and slope I or from AIRg were lower in PCOS FH+ than in PCOS FH- or C ($P=0.05$ for both). Basal glucagon correlated significantly with lean body mass ($r=-0.322$, $P=0.03$), basal insulin ($r=0.308$; $P=0.05$) and AGRg ($r=0.31$; $P=0.04$), with T ($r=0.479$; $P=0.001$), DHEAS ($r=0.335$; $P=0.028$) and with SHBG ($r=-0.356$; $P=0.015$). AGRg correlated with T ($r=0.32$; $P=0.03$), DHEAS ($r=0.40$; $P=0.008$), DHEA ($r=0.36$; $P=0.02$) and with SHBG ($r=-0.28$; $P=0.06$).
Conclusions
Higher basal glucagon levels are present in PCOS irrespective of obesity and family history of DM 2. Insulin resistance and beta cell secretory dysfunction are detectable only in PCOS with the family history of DM 2.
Supported by grant IGA MHCR 8759-3.

P615

Retinol-binding protein-4 in polycystic ovary synrome - relationship with obesity and androgen levels

Jana Vrbikova, Martin Hill, Bela Bendlova, Tereza Grimmichova, Katerina Dvorakova & Karel Vondra
Institute of Endocrinology, Prague, Czech Republic.

Aim and background
Retinol binding protein 4 (RBP 4) is an adipocyte-secreted molecule causing insulin resistance in transgenic animals. RBP was increased in subjects with impaired glucose tolerance and diabetes type 2. The levels of RBP-4 in PCOS were not investigated till now.
Subjects and methods
16 lean PCOS (BMI 21.4 (1.75) kg/m^2, age 24.1 (4.1) years), 25 obese PCOS (BMI 30.3 (4.8) kg/m^2, age 26.3 (5.0) years) and 13 healthy women (BMI 21.5 (1.6) kg/m^2, age 29.4 (7.0) years) were evaluated using euglyceamic hyperinsulinaemic clamp (1 mIU kg^{-1}.min^{-1}) with the determination of insulin sensitivity index (ISI; mmol.kg^{-1}.min^{-1} per mIU l^{-1}). In basal sample, RBP-4 levels (mg/l) were determined using ELISA (ImmundiagnostikAG, Bensheim, Germany). Results are given as mean (SD). ANOVA and multiple backward stepwise regression was used for data analysis. NCSS 2002 statistical software was used for calculations.
Results
Insulin sensitivity index was lower only in O-PCOS (33.2(22)) comparing L-PCOS (70.1 (22)) or C (77.4 (22.7)); ($P=0.0003$). RBP-4 levels were not different between L-PCOS (27.6 (6.8)), C (33.7 (8.2)) or O-PCOS (32.6 (9.9)).
To explain RBP-4 levels in PCOS women, a regression model consisting of ISI, BMI and 17-OHP was suggested. Only ISI ($P=0.04$) and 17 OHP ($P=0.03$) influenced significantly and independently RBP-4 levels; explaining 21.9% of the total variability in the dependent variable. When ISI was taken as dependent variable, and testosterone, RBP-4 and BMI as independent variables, final model contained only BMI ($P=0.0001$) and explained 33.7% of the variability in insulin sensitivity.
In conclusion, RBP-4 levels in PCOS are influenced negatively and independently by both androgen levels and insulin sensitivity. Hence, the RBP-4 levels in PCOS

could not be taken as a marker for the description of insulin sensitivity.
Supported by grants of IGA MH CR 8759-3 and GACR 301/04/1085.

P616

Protein metabolism in a model of premature ovarian failure, Turner syndrome, and the impact of hormone replacement therapy

Claus H Gravholt, Anne-Lene Riis & Jens Sandahl Christiansen
Medical Department M, Århus Sygehus NBG, Århus University Hospital, Århus, Denmark.

Background
Several studies have documented an altered body composition in Turner syndrome (TS), a model of premature ovarian failure. Body fat is increased and muscle mass is decreased. The ovarian failure necessitates substitution with female hormone replacement therapy (HRT) for a number of years, and HRT induces favourable changes in body composition with a decrease in body fat and an increase in fat free mass. It is unknown how HRT affects protein metabolism.
Aim
To study protein metabolism in TS in detail, and evaluate the distinct impact of HRT action.
Design
Randomized crossover study with active treatment (HRT in TS and P-pill in controls) or no treatment for 2 month each.
Material
We studied women with Turner syndrome ($n=8$, age 29.7 ± 5.6 (mean$\pm$S.D.) years), verified by karyotype, and age-matched controls ($n=8$, age 27.3 ± 4.9 years).
Methods
All subjects underwent a 3-h study in the postabsorptive state. After regional catheterization, protein dynamics of the whole body and of the forearm muscles were measured by amino acid tracer dilution technique using [^{15}N]phenylalanine and [$^{2}H_4$]tyrosine. Substrate metabolism was examined by indirect calorimetry.
Results
Estradiol increased and FSH decreased during active treatment in TS. Energy expenditure was comparable among TS and controls, and did not change during active treatment. Whole body phenylalanine and tyrosine fluxes were similar in the untreated situations, and did not change during active treatment. Amino acid degradation (TS vs C: 4.0 ± 0.9 vs 4.8 ± 0.8 μmol*kg^{-1}*h^{-1}, $P=0.1$) and protein synthesis (36.8 ± 5.2 vs 35.2 ± 3.0 μmol*kg^{-1}*h^{-1}, $P=0.5$) was similar in the untreated situations and did not change during treatment. Muscle protein breakdown was similar among groups, and was not affected by treatment. Muscle protein synthesis rate and forearm blood flow did not differ among groups or due to treatment.
Conclusions
Protein metabolism in TS is comparable to controls, and is not affected by a short course of HRT.

P617

Differences in the onset of puberty in selected inbred mouse strains

Otto Pinter, Zoltan Beda, Zsolt Csaba & Ida Gerendai
Neuroendocrine Research Laboratory, Hungarian Academy of Sciences and Semmelweis University, Budapest, Hungary.

Puberty is the final stage of maturation of the hypothalamo-pituitary-gonadal axis and is characterized by changes in circulating gonadotropins and increased levels of sex steroids. There are both genetic and external factors (e.g. nutrition, stress) which can alter the onset of puberty. The aim of this study was to determine the onset of puberty in genetically homogeneous inbred mouse strains. Five strains were used: 129X1/SvJ, DBA/2J, C57BL/6J, CBA/CaJ and A/J. Various pubertal markers were determined: vaginal opening (VO), first vaginal cornification, onset of cyclicity in females, and balanopreputial separation (BPS) in males. There were significant differences between strains in the onset of puberty. The earliest VO was detected in A/J (day 20,8), then CBA/CaJ (day 24,45), DBA/2J (day 25,78), C57BL/6J (day 26,45) and the latest was in 129X1/SvJ (day 29,38). The earliest day for the first vaginal cornification was in CBA/CaJ (day 30,4), followed by C57BL/6J (day 33,18), A/J (day 34,3), 129X1/SvJ (day 36,28) and the latest was in DBA/2J (day 38,33). The earliest onset of cyclicity was detected in CBA/CaJ (day 40,3), then A/J (day40,4), 129X1/SvJ (day 47,19), C57BL/6J (day 48,67) and the latest was in DBA/2J (day 51,11). There was no correlation between the weight and the age at either VO, cornification or the onset of cyclicity among strains. The occurrence of BPS was later in males then the first sign of puberty (i.e. VO) in females. The earliest BPS was in CBA/CaJ (day 27,75), followed by 129X1/SvJ (day 29,37), C57BL/6J (day 29,71), A/J (day 30,8) and the latest was in DBA/2J (day 34). There was no correlation between the weight and the age at BPS among strains. Data indicate significant differences in pubertal parameters of inbred mouse strains.

This work was supported by grant from the EU Commission (PIONEER FOOD-CT-2005-513991).

P618

The investigation of hypothalamo-pituitary-gonadal axis in patients with epilepsy

Fusun Erdogan[1], Sevgi Arik Yuksel[1], Ali Ozdemir Ersoy[1], Fatih Tanriverdi[2], Kursad Ünlühizarci[2] & Fahrettin Kelestimur[1]
[1]Erciyes University Medical School Department of Neurology, Kayseri, Turkey; [2]Erciyes University Medical School Department of Endocrinology, Kayseri, Turkey.

Polycystic ovary syndrome (PCOS) is a common endocrine disorder affecting approximately 4–8% of premenopausal women. The main clinical features are clinical and/or biochemical hyperandrogenemia, oligomenorrhea and polycystic ovarian changes. It has gained a great attention during the last two decades with the realization that this syndrome affects more than the reproductive system. Epilepsy is a common neurologic disorder affecting women during the reproductive years. Seizures and some antiepileptic drugs (AEDs) can compromise reproductive health. During the last years, several reports in the literature suggest a relationship between PCOS and epilepsy. The pathophysiology of the increased prevalence of PCOS and/or hyperandrogenism is not well established in patients with epilepsy and hypothalamus-pituitary-gonadal axis is not investigated in detail.

Forty-eight women with epilepsy were recruited in order to investigate the prevalence of PCOS, glucose intolerance and ovarian functions by using a GnRH analogue buserelin. All the patients were on valproic acid or carbamazepin treatment. Fasting blood chemistry, basal hormone levels (including FSH, LH, estradiol, DHEAS, testosterone, androstenedione SHBG, 17-OHP), OGTT, buserelin test were performed and ultrasonography of the ovaries obtained. Twenty age and BMI matched healthy women served as a control group.

Serum free testosterone and SHBG levels were significantly ($P<0.05$) higher in patients than in the control group. Three patients (7.5%) had glucose intolerance. Glucose and insulin responses to OGTT (either peak or area under the curve: AUC) were significantly ($P<0.05$) higher in the patients than in the control subjects. Patients with epilepsy had sigificantly ($P<0.05$) higher peak and AUC 17-OHP responses to buserelin test. Overall 15 (31.2%) patients had PCOS.

Our results suggest that women with epilepsy treatment have a high prevalance of PCOS, increased insulin resistance and ovarian dysfunction.

P619

Varicocele sclerotherapy improves serum inhibin B levels and seminal parameters

Matteo Baldi[1], Cataldo Di Bisceglie[1], Fabio Lanfranco[1], Angela Bertagna[1], Milena Tagliabue[1], Carlo Gazzera[2], Giovanni Gandini[2] & Chiara Manieri[1]
[1]Div. Endocrinology and Metabolic Diseases, Dep. Internal Medicine, University of Turin, Torino, Italy; [2]Dep. Radiodiagnostics IV, Dep. Medical-Surgical Disciplines, University of Turin, Torino, Italy.

Varicocele is a state of varicosity and tortuosity of the pampiniform plexus around the testis caused by retrograde blood flow. The association between varicocele and male subfertility has been questioned, thus the usefulness of treating varicocele in order to improve fertility is still a matter of debate. Inhibin B levels reflect the functional state of the seminiferous epithelium, and have been found to be a sensitive index of spermatogenesis. Serum inhibin B levels have been reported to increase after surgical varicocelectomy along with the improvement of sperm concentration. The aim of this study was to evaluate variations of seminal parameters and inhibin B concentrations in a group of 38 males affected by varicocele and treated by percutaneous retrograde sclerotherapy. Serum inhibin B, FSH, testosterone levels and seminal parameters were performed before and 6 months after sclerotherapy. Twenty age-matched patients with left varicocele who did not undergo any treatment were studied as controls. A significant increase of inhibin B levels and a significant decrease of FSH levels were observed 6 months after treatment (mean$\pm$S.E.M., 125.8 ± 15.7 vs 106.4 ± 12.7 pg/ml, $P<0.01$; 4.5 ± 0.6 vs 5.6 ± 1.0 mIU/ml $P<0.05$); no significant

change of testosterone levels was observed. After treatment semen analysis showed a significant improvement of sperm concentration (66.3 ± 10.4 vs 39.0 ± 6.6 million/ml, $P < 0.05$) and progressive motility (52.2 ± 3.7 vs 40.2 ± 4.1%, $P < 0.01$); no significant change of sperm normal morphology was observed. In the control group no significant variations of hormonal and seminal parameters were observed 6 months after the basal examination. In conclusion, percutaneous retrograde sclerotherapy in varicocele improves inhibin B levels and seminal parameters, confirming its positive effect on spermatogenesis and Sertoli cell function.

P620

LH receptor gene expression and splicing variants in marmoset (*Callithrix jacchus*) testis and adrenal gland at puberty

Caroline Michel, Jörg Gromoll, Ramesh Chandolia & Manuela Simoni
Institute of Reproductive Medicine, Muenster, Germany.

Background
The LHR is a crucial mediator for normal sexual development and fertility. In the marmoset monkey (*Callithrix jacchus*), LHR type II, lacking exon 10, is the native receptor type. In addition to the testis, the LHR is expressed in the adrenal gland where its function remains unknown.
Aim
To characterise marmoset LHR expression at different stages of puberty in the testis and adrenal gland and examine different splice variants in the testis.
Material and methods
We analysed 25 male marmosets of five age groups ($n = 5$/group): 21.5 ± 0.1, 43.3 ± 0.7, 52.8 ± 0.3, 70.1 ± 0.4 and 116.8 ± 20 weeks (mean ± S.E.M.). Total RNA was isolated from testes and adrenal glands, reverse-transcribed and used for real-time PCR. Splice variants were detected using primers directed to exon 2 and 11. PCR products were analysed by densitometric analysis, cloned into pGEM-T-Easy-vector and sequenced.
Results
The expression levels of the full transcript were lowest at the beginning of puberty and increased progressively both in the testis and in the adrenal gland. The full-length transcript expression values in the testis (2.244 ± 0.9 AU) were 4.2-fold higher compared to the adrenal gland (0.537 ± 0.5 AU). We detected eleven LHR splicing variants in the testes. Seven of these showed exon skipping, lacking one to seven exons, and four were alternatively spliced. As expected, exon 10 was absent in all variants. While each variant is expressed 0.7-fold, the overall amount of all splice variants is much more abundant (6.1 ± 0.5) than the wild type. Two thirds of all isoforms lack four or less exons and densitometric analysis recognized no pubertal-associated variance. Alternative splicing was much less evident in the adrenal glands.
Conclusion
LHR expression increased progressively in both tissues while the splicing patterns itself does not change during puberty, and different splice variants exist in the testis.

P621

Protamine 1 and Protamine 2 sequence variants in teratozoospermia

Sonja Römer, Frank Tüttelmann, Jörg Gromoll, Eberhard Nieschlag & Manuela Simoni
Institute of Reproductive Medicine of the University, Münster, Germany.

Background
During spermatogenesis protamines replace histones in sperm head. Haploinsufficiency of the protamine (*PRM*) 1 or *PRM2* gene causes infertility in mice. A mutation in *PRM1* was associated with increased abnormal sperm morphology in infertile men[1]. We assessed the frequency of mutations and SNPs in the *PRM1* and *PRM2* gene in infertile patiens with normal sperm concentration and reduced morphology, a phenotype similar to that of the *Prm1* deficient knockout mice.
Material and Methods
Using the institutional database (Androbase©) we identified 29 infertile men with normal sperm concentration and severe idiopathic teratozoospermia (<7% normal forms). *PRM1* and *PRM2* were sequenced in the patients and in 29 controls with normal spermatogenesis.
Results
Two single SNPs were identified in the *PRM1* gene. One (A230C) was known (rs737008) as a synomynous polymorphism in exon 2 with a heterozygosity of 0.5, and occured with similar frequencies in teratozoospermic men (heterozygous $n = 11$; homozygous minor $n = 4$) and controls (heterozygous $n = 13$; homozygous minor $n = 3$). We identified a novel synonymous SNP in exon 1 (G54A) in two patients and one control. The G197T mutation in *PRM1* previously reported[1] was not found. A meta-analysis of our and the literature data showed that the mutation G197T is not associated with teratozoospermia. Four SNPs were found in intron 1 of the *PRM2* gene. C298G and C373A are listed in the NCBI database (rs1646022; rs2070923). The remaining two (C366T; C406T) were rare heterozygous SNPs, evenly distributed with a frequency of 3,4% in both groups. The prevalence of all SNPs was similar in infertile men and controls. No SNP was found in the exons.
Conclusion
Mutations of *PRM1* and *PRM2* are rare in teratozoospermic men with normal sperm count. Common polymorphisms of the *PRM* genes are not associated with idiopathic teratozoospermia.
(1) Iguchi, Yang, Lamb, Hecht (2006) *J. Med. Genet*. **43**, 382–384.

P622

Ghrelin effects on spontaneous and stimulated LH secretion in human males

Lorenza Bonelli, Matteo Baldi, Fabio Broglio, Angela Bertagna, Cataldo Di Bisceglie, Milena Tagliabue, Chiara Manieri, Ezio Ghigo & Fabio Lanfranco
Division of Endocrinology and Metabolism, Department of Internal Medicine, University of Turin, Italy, Torino, Italy.

Acylated ghrelin (AG) participates in the modulation of the hypothalamic-pituitary-gonadal axis functions, with a predominantly inhibitory effect upon the reproductive system in animals. Animal studies have shown that ghrelin suppresses LH secretion *in vivo*, decreases LH responsiveness to GnRH *in vitro* and partially delays the timing of puberty in males. Aim of this study was to evaluate the effects of AG infusion on spontaneous and stimulated gonadotropin secretion in male subjects. In 6 eugonadal males (age mean ± S.E.M.:28.7 ± 1.1 yrs; BMI:22.4 ± 2.1 kg/m^2) we evaluated LH and FSH levels every 15 min during: a) i.v. isotonic saline infusion (SAL) from 0 to +480 min; b) i.v. SAL from 0 to +240 min followed by AG (1.0 μg/kg as a bolus at +240 min, and AG infusion 2 μg/kg/h in 500 ml isotonic saline from +240 to +480 min); c) GnRH test (100 μg i.v. as a bolus at +120 min) during saline or AG infusion from 0 to +240 min. No significant changes in FSH pulsatile secretion were recorded in test sessions a) and b). Under SAL infusion, significant LH pulses were recorded in all subjects. AG infusion significantly decreased LH pulse number and frequency, pulse height (MSPH: 0.04 ± 0.02 mU/ml; −84% vs. SAL) and pulse mass (MSPM: 0.65 ± 0.46 mU/ml; −89% vs. SAL). LH and FSH responses during saline (LH peak 18.2 ± 3.9 mU/ml, FSH peak 12.7 ± 2.6 mU/ml) were similar to those recorded during AG (LH peak 21.6 ± 4.4 mU/ml, FSH peak 11.2 ± 2.9 mU/ml). These findings demonstrate that AG inhibits pulsatile LH secretion but not LH responsiveness to GnRH in males. Therefore ghrelin, at least the acylated form, exerts an inhibitory effect on the gonadal axis in men through a hypothalamic mechanism.

P623

Differential effects of two-week treatment with atorvastatin or elocalcitol, two RhoA/ROK signalling modulators, on erectile function and sildenafil responsiveness in spontaneously hypertensive rats

Annamaria Morelli[1], Xin-Hua Zhang[1], Sandra Filippi[2], Linda Vignozzi[1], Sara Mungai[1] & Luciano Adorini[3]
[1]University of Florence, Dept. of Clinical Physiopathology, Andrology Unit, Florence, Italy, [2]University of Florence, Interdepartmental Laboratory of Functional and Cellular Pharmacology of Reproduction, Departments of Pharmacology and Clinical Physiopathology, Florence, Italy; [3]Bioxell, Milan, Italy.

Increased RhoA/Rho-kinase (ROK) signalling is known to impair erectile function. Spontaneously hypertensive rats (SHR) over-express penile RhoA and show an impaired erectile response. We tested treatments known to inhibit RhoA activation, on erectile function and sildenafil responsiveness in SHR. SHR have been treated for two weeks with atorvastatin (5 and 30 mg/Kg/day), or with elocalcitol (30 μg/Kg/day), a vitamin D receptor (VDR) agonist. The normotensive Wistar Kyoto (WKY) rats have been used as controls. At the selected doses, neither atorvastatin affected cholesterol, nor elocalcitol affected calcaemia in both SHR and WKY. In WKY, sildenafil (25 mg/Kg by oral gavage) greatly increased erectile function, evaluated as intracavernous pressure/mean arterial pressure (ICP/MAP) ratio after electrical stimulation (ES) of the cavernous nerve. In SHR, both basal and sildenafil-stimulated ICP/MAP ratio were depressed. Atorvastatin did not affect basal ICP/MAP at any concentration tested. However, it dose-dependently increased

sildenafil effect on ES-induced erection, significantly potentiated by 30 mg/Kg dosing. At this dose, atorvastatin normalized the over-expression of RhoA mRNA (real time RT-PCR) observed in SHR, without affecting other genes such as ROK1, ROK2, PDE5, nNOS, eNOS. Conversely, elocalcitol, at a dose known to ameliorate bladder overactivity by inhibiting RhoA activation, failed to restore ICP/MAP ratio, sildenafil responsiveness and RhoA expression in SHR. Finally, SHR rats expressed high levels of VDR mRNA in the bladder (almost 5-fold increase over WKY), but not in corpora cavernosa (CC). In conclusion, our data confirm that an increased RhoA signalling impairs erectile function and sildenafil responsiveness in SHR. Atorvastatin, at a dose unable to affect lipids, ameliorates sildenafil effectiveness and down-regulates RhoA expression. Conversely, elocalcitol was ineffective in restoring erectile function in SHR, either alone or with sildenafil. The differential quantitative VDR expression in bladder and CC suggests a plausible mechanism for the tissue-specific effect of elocalcitol on RhoA/ROK contractile pathway.

P624

Testosterone regulates RhoA/Rho-kinase signalling in two distinct animal models of chemical diabetes

Linda Vignozzi[1], Annamaria Morelli[1], Sandra Filippi[2], Stefano Ambrosini[4], Rosa Mancina[1], Michaela Luconi[3], Sara Mungai[1], Gabriella Barbara Vannelli[4], Xin-Hua Zhang[1], Gianni Forti[3] & Mario Maggi[1]
[1]University of Florence, Dept. of Clinical Physiopathology, Andrology Unit, Florence, Italy; [2]University of Florence, Interdep. Lab of Functional and Cellular Pharmacology of Reproduction, Depts. of Pharmacology and Clinical Physiopathology, Florence, Italy; [3]University of Florence, Dept. of Clinical Physiopathology, Endocrinology Unit, Florence, Italy; [4]University of Florence,Department of Anatomy, Histology and Forensic Medicine, Florence, Italy.

The contractile RhoA/Rho-kinase (Rock) signalling pathway is up-regulated in penile tissue in animal models of experimental diabetes and has been proposed to contribute to diabetes-related erectile dysfunction (ED). In previous studies we demonstrated that testosterone (T) restores diabetes-induced ED by influencing the NO/cGMP/PDE5 pathway.
Aim
To investigate the effect of T on the RhoA/Rock signalling in course of diabetes.
Methods
We used two distinct animal models of chemical diabetes (alloxan-induced in the rabbit and streptozotocin-induced in the rat) with or not T supplementation.
Results
In both models, hypogonadism was observed, characterized by reduced T plasma level and androgen-dependent accessory glands atrophy. Diabetic animals showed a significant increase in responsiveness to increasing concentrations of Y-27632, a highly selective Rock inhibitor, as evaluated either by '*in vitro*' contractility study (diabetic-rabbit) and '*in vivo*' as erectile response elicited by intracavernous injections (diabetic-rats). T-substitution (30 mg/kg, weekly) completely reverted hypogonadism and diabetes-induced penile hypersensitivity to Y-27632. To test whether this effect was due to a T-dependent regulation of RhoA/Rock gene expression, we measured RhoA/Rock mRNA. Both isoforms of Rock (Rock1/-Rock2) were analyzed by real time RT-PCR in rat penile samples. We found that Rock1 mRNA was significantly increased ($P < 0.05$) in penile tissues from diabetic animals and restored to the control values by T, as also confirmed by semiquantitative RT-PCR in rabbit. Conversely, RhoA and Rock2 mRNA expression was not influenced neither by diabetic condition and by T administration. Accordingly, Rock1 protein expression, as evaluated by western blot and immunohistochemistry analysis, resulted increased in penile samples from diabetic animals and normalized by T.
Conclusions
Our data further support the hypothesis that the activation of RhoA/Rock signalling contributes to diabetes-related erectile dysfunction. Moreover, treating hypogonadism in course of diabetes, may restore erectile function also by normalizing RhoA/ Rock pathway over-activity.

P625

Effect of sildenafil administration on penile hypoxia induced by cavernous neurotomy in the rat

Linda Vignozzi[1], Annamaria Morelli[1], Sandra Filippi[3], Gabriella Barbara Vannelli[4], Gianni Forti[2] & Mario Maggi[1]
[1]University of Florence, Department of Clinical Physiopathology, Andrology Unit, Florence, Italy; [2]University of Florence, Department of Clinical Physiopathology, Endocrinology Unit, Florence, Italy; [3]University of Florence,Interdepartmental 2Laboratory of Functional and Cellular Pharmacology of Reproduction, Departments of Pharmacology and Clinical Physiopathology, Florence, Italy; [4]University of Florence, Department of Anatomy Histology and Forensic Medicine, Florence, Italy.

Objectives
Radical prostatectomy is an effective therapy for men with clinically localized prostate cancer. A significant number of men develop erectile dysfunction after radical prostatectomy (PPED) due to intraoperatory cavernous nerve injury causing hypoxia and fibrosis of corpus cavernosus. We established an experimental model of bilateral cavernous neurotomy (BCN) in the rat in order to investigate whether sildenafil treatment in PPED patients could prevent penile tissue damage.
Design and methods
One, 5 and 10 days after neurotomy, animals were treated or not with a single dose of sildenafil (25 mg/kg orally) one hour before sacrifice. To analyze penile oxygenation, rats of each experimental group received (one hour before sacrifice) an intraperitoneal injection of the bio-reductive drug pimonidazole hydrochloride (hypoxyprobeTM-1, 60 mg/Kg), which has been recognized as a standard marker for *in vivo* imaging and quantification of hypoxia.
Results
With immunohistochemistry for hypoxiprobeTM, we found that BCN induced massive hypoxia at all times investigated in corpora cavernosa sections from the experimental rats, as revealed by computer-assisted quantitative image analysis. This tissue hypo-oxygenation was significantly reduced in sections from sildenafil treated rats at 1 and 5 days after neurotomy, while at 10 days this reduction was less evident and not significant. In addition, functional studies indicated that hypoxic corpora cavernosa tissues were hypersensitive to the relaxant effect of the endothelin receptor type B (ETB) agonist IRL-1620, due to the previously described hypoxia-induced overexpression of ETB receptors. Accordingly, ETB mRNA expression (real time RT-PCR) was significantly increased in corpora cavernosa from BCN rats, and was restored to control levels by sildenafil administration at all times investigated.
Conclusions
Our results indicate that sildenafil treatment can positively influence penile tissue oxygenation after cavernous nerve injury, with its effect being more evident the earlier it is administered.

P626

Androgenicity, androgen receptor polymorphism and pharmacogenetics

Domenico Canale, Cecilia Moschini, Carolina Caglieresi, Agnese Moscatelli, Silvia Gavioli, Enrico Macchia & Enio Martino
University, Pisa, Italy.

Exon 1 of the androgen receptor (AR) gene contains a variable number of CAG triplets, (CAGr)n 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 the transcriptional activity induced by the AR and therefore modulates target organs responsiveness to androgens.

The (CAGr)n is inversely associated with the transcriptional activity of target genes. The (CAGr)n has been analysed in a variety of cross-sectional studies, investigating its influence on clinical conditions and parameters affected by T action, such as bone density, spermatogenesis, mood variations, cognitive functions and hair development in both men and women. Zitzmann *et al* have correlated the prostate growth induced by T replacement therapy in hypogonadal men with (CAGr)n, demonstrating an impressive modulating effect by the CAGr polymorphism. A role of the (CAGr)n has been also demonstrated in determining the androgenicity of an individual: hypoandrogenized patients compared to a control group have an increased (CAGr)n (24.0 *vs* 21.5) with a significant shift toward higher numbers.

We will two patients affected by the same disease, that is congenital selective hypogonadotropic hypogonadism, treated with similar doses of androgens. Androgenization, though, was completely different, as the pictures will show: one had a 'female' hair pattern, no beard, no hair in the chest and lower abdomen, pubic hair 3, depressed mood, the other one was well androgenized, with 'extraordinary male' hair pattern, good muscular development, married with children. The first one had a (CAGr)n equal to 30 the second one 15.

Our data further support a pharmacogenetic approach which stresses the evaluation of AR polymorphism to be performed before initiating a long term androgen replacement treatment to provide satisfactory androgen effect at target organs.

P627

Characterization and functional role of an androgen-dependent PDE5 activity in bladder

Sandra Filippi[1], Annamaria Morelli[2], Peter Sandner[6], Benedetta Fibbi[2], Rosa Mancina[2], Mirca Marini[4], Mauro Gacci[5], Linda Vignozzi[2], Gabriella Barbara Vannelli[4], Marco Carini[5], Gianni Forti[3] & Mario Maggi[1]
[1]University of Florence, Interdepartmental Laboratory of Functional and Cellular Pharmacology of Reproduction, Departments of Pharmacology and Clinical Physiopathology, Florence, Italy; [2]University of Florence, Department of Clinical Physiopathology, Andrology Unit, Florence, Italy; [3]University of Florence,Department of Clinical Physiopathology, Endocrinology Unit, Florence, Italy; [4]University of Florence, Department of Anatomy, Histology and Forensic Medicine, Florence, Italy; [5]University of Florence,Department of Urology, Florence, Italy; [6]Bayer Healthcare, Wuppertal, Germany.

Benign prostate hyperplasia (BPH) is the most common disease in the aging male, often comorbid with erectile dysfunction (ED). PDE5 inhibitors (PDE5i, sildenafil, tadalafil and vardenafil) decrease lower urinary tract symptoms (LUTS) in patients with ED and BPH. We studied PDE5 expression and activity in the human bladder and PDE5i effects both *in vitro* (human and rat) and *in vivo* (rat). PDE5 is highly expressed in rat and human bladder and immunolocalized in vascular endothelium and muscle fibers. Sildenafil, tadalafil and vardenafil blocked 70% of the total cGMP catabolizing activity, with vardenafil being the most potent ($IC_{50}=0.3$ nM). In human bladder cells and in rat strips, a PDE-resistant cGMP analogue, SP-8-Br-PET-cGMPS, induced, respectively, a consistent anti-proliferative and relaxant effect. In contrast, the nitric oxide (NO) donor sodium nitroprusside (SNP) was almost ineffective. However, blocking PDE5 with vardenafil increased SNP anti-proliferative and relaxant activity up to the level observed with SP-8-Br-PET-cGMPS. We also found that castration decreased, and T supplementation restored, PDE5 gene expression in rat bladder. Accordingly, bladder strips from castrated rats were more sensitive to SNP- induced relaxation than strips from control or T-replaced rats, while in the presence of vardenafil, all groups showed the same SNP sensitivity. To discover whether vardenafil affects bladder activity *in vivo*, the rat bladder outlet obstruction (BOO) model was used. Chronic treatment with 10 mg/kg/d vardenafil significantly reduced non-voiding contractions (47%, $P<0.05$ vs. placebo) up to tamsulosin level (51%). Overall, these results demonstrate that PDE5 regulates bladder smooth muscle tone, strongly limiting the NO/cGMP signalling, and that vardenafil, by blocking PDE5, may be a possible therapeutic option for bladder dysfunction, by ameliorating irritative LUTS.

P628

Testosterone levels correlate positively with HDL cholesterol levels in men with Type 2 diabetes

Roger D Stanworth[1], Dheeraj Kapoor[1], Kevin S Channer[2] & T Hugh Jones[1]
[1]Academic Unit of Endocrinology, University of Sheffield, Sheffield, United Kingdom; [2]Faculty of Health and Wellbeing, Sheffield Hallam University, Sheffield, United Kingdom.

Low testosterone levels are a common finding in men with coronary artery disease and Type 2 diabetes and predict the future development of the metabolic syndrome and Type 2 diabetes in healthy men. Testosterone replacement therapy has been shown to improve insulin sensitivity and glycaemic control in men with diabetes and improves numerous other cardiovascular risk factors. Interest in testosterone as a potential treatment for cardiovascular disease continues to grow. Low HDL cholesterol (HDL-C) levels are now recognised as an independent cardiovascular risk factor and comprise part of the metabolic syndrome. The effect of testosterone treatment on HDL-C in clinical trials has been inconsistent. Testosterone may be acting through differing processes with opposite effects on HDL.

We present data on the link between testosterone levels and blood lipid levels in a sample of 293 men with Type 2 diabetes. Lipids were assessed by standard methods. Total testosterone (TT) and SHBG levels were assessed by ELISA. Bioavailable testosterone (BioT) was measured by ammonium precipitation. Calculated bioavailable (cBioT) and free testosterone (cFT) were also derived using recognised formulae.

Regression analysis revealed that HDL-C levels were positively associated with TT (regression coefficient $r=0.253$, $P<0.001$), BioT ($r=0.172$, $P=0.003$), cBioT ($r=0.219$, $P=<0.001$), cFT ($r=0.139$, $P=0.18$) and SHBG ($r=0.169$, $P=0.004$). Total cholesterol levels were not significantly associated with testosterone levels but there was a trend towards a negative association of testosterone with total cholesterol ($P=0.051$).

Thus, in our group of men with Type 2 diabetes, testosterone is positively associated with HDL-C suggesting that the dominant effect of testosterone in this group may be to increase HDL. Further clinical trials of testosterone replacement therapy in men with type 2 diabetes are warranted.

P629

Mutations of GnRH receptor and GPR54 in a cohort of patients with idiopathic hypogonadotropic hypogonadism

Nicolas Richard[1], Céline Leprince[1], Céline Gonfroy[1], Jacques Young[2], Didier Dewailly[3], Michel Pugeat[4] & Marie-Laure Kottler[1]
[1]CHU Caen, Caen, France; [2]CHU Kremlin Bicêtre, Kremlin bicêtre, France; [3]CHU Lille, Lille, France; [4]Gpmt Hospitalier est, Lyon, France.

Objective
To determine the frequency of mutations of the gonadotropin-releasing hormone receptor (GnRHR) and of the G protein-coupled receptor 54 (GPR54) genes in normosmic idiopathic hypogonadotropic hypogonadism patients (IHH).
Methods
In a retrospective study we analysed the *GnRHR* and the *GPR54* genes of 327 IHH patients including 105 females (36.5%) and 183 males (63.5%). Among the index cases (288 siblings) 267 were sporadic form (92.7%) and the others were included in 21 families (7.3%) with at least two affected siblings. Only 170 patients were tested for *GPR54* mutations. All females were diagnosed with primary amenorrhoea and 30.4% males presented with cryptorchidism. After informed consent, genomic DNA was amplified by PCR to obtain partially overlapping amplicons encompassing the exon-intron boundaries of the GnRHR and GPR54 genes and analyzed by DNA sequencing.
Results
Familial cases: ten of 21 (47.6%) IHH patients tested had mutations in either the *GnRHR* or the *GPR54* gene. Among the eight (38.1%) individuals bearing *GNRHR* mutations, 5 (23.8%) were homozygous or compound heterozygous and 3 (14.3%) were simple heterozygous. Among the 11 remaining patients, mutations of *GPR54* were found in two patients (18.2%): one (9.1%) at the homozygous state and the other one at the heterozygous state. *GnRHR* and *GPR54* mutations account for 7.5% and 2.5% respectively of sporadic cases.

The phenotype is depending on the nature of the genetic defect. GnRH administration fails to stimulate gonadotropin secretion when the biological activity of the mutated GnRHR is abolished (R139H, A129D, R139C) while a response is observed when the defect is partial (Q106R, R262Q).
Conclusion
The prevalence of GnRHR mutations is about three fold higher than that of GPR54. No genetic defect was identified in half of familial cases suggesting that additional genes play an important role in normal puberty.

P630

Assessment of non-enzymatic antioxidant profile of women on contraceptives

Oluyemi Akinloye[1], Oyabiyi S Adewale[2] & Ayo O Arowojolu[3]
[1]Department of Chemical Pathology, College of Health Sciences, Ladoke Akintola University, Osogbo, Oyo State, Nigeria; [2]Department of Chemical Pathology, University College Hospital, Ibadan, Oyo State, Nigeria; [3]Department of Obstetrics and Gynaecology, University College Hospital, Ibadan, Oyo State, Nigeria.

Objective
The use of contraceptives has been reported to interfere with absorption of micronutrients many of which serve as dietary antioxidant. The Changes in antioxidant levels may play significant role in the risk and pathophysiology of diseases associated with the use of contraceptives. This study was designed to assess the comparative antioxidant status of women on oral contraceptive pills (OCP), Injectables (INJ), Intrauterine Contraceptive Devices (IUCD) and Norplant (NP).
Methods
Participants were recruited (with informed consent) from the family planning clinics of the University College and Adeoyo Maternity Hospitals, Ibadan, Nigeria. A total of 118 apparently healthy (no alcohol and cigarette smoking), non-pregnant women consisting of 31 subjects on IUCD, 10 on OCP, 10 on INJ and 9 on NP. Likewise, 58 aged matched women not on contraceptives were recruited from same community as controls. Anthropometric indices and non-enzymatic antioxidant were evaluated using conventional methods. The study was approved by Ethical Committees of the Oyo State Government of Nigeria.
Results
Subjects on OCP had significantly lower vitamin C (50%), vitamin E (25%), albumin (20%), uric acid (31%) and selenium (69%) ($P<0.05$) when compared to

the controls. Significantly higher systolic BP and lower BMI were also observed in these subjects ($P<0.05$). The extend of lipid peroxidation (LPO) as evaluated by the level of malondialdehyde in the serum was significantly higher in subjects on OCP (62%) and IUCD (21%) ($P<0.05$) when compared to the controls.
Conclusions
These results indicate that while INJ and NP have no significant influence on antioxidant profile, IUCD remains the most acceptable in this community. Also, OCP has a tendency to depress the antioxidant status of its users. A routine monitoring of the antioxidant status of women on contraceptives especially OCP and IUCD and possible supplementation with dietary antioxidant may be warranted, particularly in developing countries.

P631

Hypothalamic-pituitary-gonadal axis responses of the male rats short and long time static magnetic fields (50 Hz) exposure

Akram Ahangarpour, Hadi Fathi Moghaddam, Mohammad Javad Tahmasebi Birgani, Hajie Shahbazian & Mohammad Badavi
Department of Physiology, School of Medicine, Jundishapour Medical Sciences, University of Ahwaz, Ahwaz, Iran.

Different studies have been done in the field of effect of magnetic field exposure on the biological organs. The aim of this study was to investigate the effects of static magnetic field (SMF) exposure on the secretion of hypothalamic-pituitary-gonadal axis in the male rats during short and long time exposed to SMFs (50 Hz).
Methods
Experiments have been done in four protocols. Each protocol included four groups (Wistar male rats, same range of age and weight) and each group contains 12 rats. After one-week adaptation they placed in exposure to SMF (0, 6, 12 and 24 mT) for 40 or 120 minutes daily for 17 or 34 days. All of protocols were started from 9:00 a.m. After experiments animals were anaesthetized, their blood has collected in separated tubes. Their serums were removed and kept frozen under -20 °C until use. Hormones were measured using gamma counter equipment with IRMA and RIA methods. The results were analyzed by ANOVA statistical method, followed by Tukey posthoc test ($P<0.05$).
Results
Subchronic exposures (40 min/day for 17 days) to SMFs have no effect on serum testosterone, LH and FSH levels. In contrast, SMFs (2 h/day for 17 days) induces a decrease of serum testosterone sham, vs. 6, 12 and 24 mT respectively (6.97 ± 1, vs. 4.66 ± 1.5; 2.6 ± 0.59 and 2.8 ± 0.64 ng/mL, $P<0.05$) and FSH levels (3.918 ± 1, vs. 2.1 ± 0.8; 0.765 ± 0.037 and 0.715 ± 0.01 mIU/mL, $P<0.05$).
Our results from third and forth protocols of experiments (40 min/day for 34 days) to 6 mT, SMF induces a increase of serum testosterone 6 mT *vs* sham, 12, and 24 mT respectively (7.53 ± 2, vs. 1.84 ± 0.6; 1.78 ± 0.3 and 1.63 ± 0.3 ng/mL, $P<0.05$) and 6 mT, SMF (2 h/day for 34 days) induces a increase of serum testosterone 6 mT *vs* 12 mT respectively (10.99 ± 3, vs. 2.6 ± 1; ng/mL, $P<0.05$).
Conclusions
Our results suggest that SMFs probably causes dysfunction in gonadal axis at the hypothalamic-pituitary level in male rats in different protocols. Subchronic exposure to short duration SMFs failed to alter hormonal levels in rat. In contrast, chronic exposure at low intensities increases testosterone.
Keywords: Magnetic fields; Rat; Testosterone; LH and FSH
All procedures carried out according to current and local National guidelines.

P632

Adrenal rest tissue in gonads in 70 French patients with classical congenital adrenal hyperplasia (21 hydroxylase deficiency)

Pierre Peggy[1], Despert Francois[1], Tranquart Francois[1], Chabrolle Christine[1], Kerlan Veronique[2], Sonnet Emmanuel[2], Metz Chantal[2], Baron Sabine[3], De Kerdanet Marc[6], Coutant Regis[4], Emy Philippe[5], Monceau Francoise[5], Bekka Said[1] & Lecomte Pierre[1]
[1]University, Tours, France; [2]University, Brest, France; [3]University, Nantes, France; [4]University, Angers, France; [5]Hospital, Orleans, France; [6]University, Rennes, France; [7]Hospital, Chartres, France.

Congenital adrenal hyperplasia (CAH) due to 21 hydroxylase deficiency is one of the most frequent endocrine genetic diseases. Adrenal rests have been described and can decrease fertility in men[1]. In a retrospective multi-center study we wanted 1.to evaluate the frequency of adrenal rests in classical forms of CAH (21-OH deficiency) by systematic ultrasonography (US); 2.to try to find the cause of this abnormality looking for a relationship between genotype and phenotype or with their therapeutic equilibrium. All patients with classical form of CAH and who have had an US were studied. In 24 women, none had adrenal rest in their ovaries, in accord with a very few cases published in the literature[2]. On the contrary, adrenal rests were detected in 30,4% of 46 men aged 1 to 38 years. We observed an increased frequency in testicular adrenal rest with age: none in the group less than 10 years, 15% in the group 10–17 years and 66,7% above 18 years. The role of an insufficiency in treatment in the development of adrenal rests has been evaluated. The therapeutic equilibrium is judged upon bone age and growth chart evolution in infants and upon 17 OHP or urinary pregnanetriol in adults. The appropriate equilibrium seems more often observed in patients without testicular adrenal rests: among patients, 55% without testicular adrenal rests were good treated during infancy compared to 14,3% with testicular adrenal rests, 44,4% compared to none during adolescence and 25% compared to 9% during adulthood. No relationship could be figure out between genotype and phenotype but the number of cases was probably too small in this cohort. Among 6 patients with adrenal rests and wishing fatherhood, an azoospermia was observed in 3; 2 had a very low sperm count and only 1 patient was able to procreate without any difficulty (2 children). In conclusion, ultrasonography of the ovaries is not usually necessary. On the opposite, testicular ultrasonography must be done during infancy, puberty and every five years during adulthood. This should reinforce better control of the disease by a more intensive treatment to try to reduce the number and volume of adrenal rests and improve fertility.

P633

Three months exercise training improves cardiopulmonary functional capacity in polycystic ovary syndrome

Francesco Orio[1], Francesco Giallauria[2], Stefano Palomba[3], Teresa Cascella[4], Laura Vuolo[4], Domenico Tafuri[5], Francesco Manguso[6], Gaetano Lombardi[4], Annamaria Colao[4] & Carlo Vigorito[2]
[1]Endocrinology, Faculty of Motor Science, "Parthenope" University of Naples and Department of Molecular & Clinical Endocrinology and Oncology, "Federico II" University of Naples, Naples, Italy; [2]Department of Clinical Medicine, Cardiovascular and Immunological Sciences, Cardiac Rehabilitation Unit, Institute of Internal Medicine and Metabolic Disease, "Federico II" University of Naples, Naples, Italy; [3]Department of Obstetrics and Gynecology, University "Magna Graecia" of Catanzaro, Catanzaro, Italy; [4]Department of Molecular & Clinical Endocrinology and Oncology, "Federico II" University of Naples, Naples, Italy; [5]Teaching and Methods of Sportive Activity, "Parthenope" University of Naples, Naples, Italy; [6]Gastroenterology Unit, "Cardarelli" Hospital, Naples, Naples, Italy.

Introduction and Aim
Polycystic Ovary Syndrome (PCOS) is an endocrine disease closely related to several risk factors for cardiovascular disease. Previous study demonstrated an impaired cardiopulmonary functional capacity in PCOS women. The present study was performed to evaluate the effects of 3-months exercise training (ET) programme on cardiopulmonary functional capacity in young women with PCOS.
Patients and Methods
The study was conducted according to the guidelines of the Declaration of Helsinki, and the institutional ethical committee approved the study protocol. The purpose of the protocol was explained to each subject, and written informed consent was obtained from each patient before beginning the study.

Ninety young PCOS women were randomly subdivided into two groups each composed of 45 subjects: PCOS-T (trained) group (age $=21.7\pm2.3$ years, BMI $=29.3\pm2.9$) underwent 3-months ET whereas PCOS-UnT (untrained) group (age $=21.9\pm1.9$ years, BMI $=29.3\pm3.1$) did not. At baseline and after 3 months, all patients were studied for their hormonal and metabolic profile, and underwent cardiopulmonary exercise test.
Results
After 3-month ET, PCOS-T showed a significant improvement in peak oxygen consumption ($+35.4\%$, $P<0.001$) and in maximal workload ($+37.2\%$, $P<0.001$). In PCOS-T we also observed a significant reduction of BMI (-4.5%, $P<0.001$) and C-reactive protein (-10%, $P<0.001$), and a significant ($P<0.001$) improvement of insulin sensitivity indexes. After 3 months, no changes were observed in PCOS-UnT.
Conclusions
Three-months ET improves cardiopulmonary functional capacity and insulin sensitivity in young PCOS women.

P634

Abnormal heart rate recovery after maximal cardiopulmonary exercise stress testing in polycystic ovary syndrome

Francesco Giallauria[1], Stefano Palomba[2], Teresa Cascella[3], Alessandra Vitelli[1], Luigi Maresca[1], Alessandra Grieco[3], Domenico Tafuri[4], Annamaria Colao[3], Gaetano Lombardi[3], Carlo Vigorito[1] & Francesco Orio[5]

[1]Department of Clinical Medicine, Cardiovascular and Immunological Sciences, Cardiac Rehabilitation Unit, Institute of Internal Medicine and Metabolic Disease, "Federico II" University of Naples, Naples, Italy; [2]Department of Obstetrics and Gynecology, University "Magna Graecia" of Catanzaro, Catanzaro, Italy; [3]Department of Molecular & Clinical Endocrinology and Oncology, "Federico II" University of Naples, Naples, Italy; [4]Teaching and Methods of Sportive Activity, "Parthenope" University of Naples, Naples, Italy; [5]Endocrinology, Faculty of Motor Science, "Parthenope" University of Naples and Department of Molecular & Clinical Endocrinology and Oncology, "Federico II" University of Naples, Naples, Italy.

Introduction and aim
Polycystic ovary syndrome (PCOS) is associated with an adverse metabolic and cardiovascular risk (CVR) profile, including: diabetes, insulin resistance, dyslipidemia and hypertension. Heart rate recovery (HRR) is a measure of autonomic dysfunction and an abnormal HRR is also associated with increased mortality. To date, no evaluation of autonomic function in young PCOS women has been performed, therefore the aim of the present study was to evaluate the HRR in PCOS.
Patients and methods
The study was approved by the local Ethical Committee. Forty-eight PCOS patients matched with 48 healthy women mean age (21.7 ± 2.2 vs. 21.9 ± 1.8, yrs$\pm$SD, respectively) and body mass index (29.5 ± 3.1 vs. 29.7 ± 3.6, kg/m^2 $\pm$ SD, respectively). Hormonal and metabolic pattern, cardiopulmonary functional capacity, as expressed by: maximal oxygen consumption (VO_{2max}) and oxygen consumption at anaerobic threshold (VO_{2AT}), and autonomic function, as expressed by HRR, were evaluated.
Results
In PCOS women we observed a significant ($P<0.001$) abnormal HRR (12.7 ± 2.1 vs. 20.8 ± 3.1 beats/min), and a significant impairment of: VO_{2max} (17.9 ± 2.3 vs. 29.0 ± 3.9, ml/Kg/min) and VO_{2AT} (13.1 ± 2.6 vs. 24.1 ± 3.1, ml/Kg/min) compared to healthy women. In PCOS patients, abnormal HRR was inversely correlated to BMI ($r=-0.700$, $P<0.0001$), HOMA ($r=-0.680$, $P<0.0001$) and AUC_{INS} ($r=-0.640$, $P<0.0001$).
Conclusions
Our data are the first to demonstrate an abnormal HRR after maximal cardiopulmonary exercise stress testing in young overweight PCOS patients, adding HRR as a further potential marker of increased CVR in PCOS.

P635

Age at menarche in relation to adult obesity

Taner Bayraktaroglu, Ayse Sertkaya Cikim, Adil Dogan Azezli & Yusuf Orhan
Istanbul Faculty of Medicine, Endocrinology and Metabolism, Endocrinology and Metabolism, Capa, Fatih/Istanbul, Turkey.

Introduction
Age at menarche reflects various health aspects including the timing of sexual maturation, growth and nutritional status, and enviromental conditions. This study carried out assesment obesity risk and the relationship between obesity, metabolic parameters and age at menarche in a series of Turkish women.
Materials and methods
In a survey of obesity outpatient clinic, 4212 women who have mean age 38.5 ± 12.1 years and ages at menarche between 9 – 18 years enrolled tertiles to the study: Group I, age at menarche 9 – 11 years ($n=476$, 11.3%); group II, 12 – 14 years ($n=2319$, 55.1%); group III, > 14 years ($n=1417$, 33.6%). According to ages at menarche, body size variables (height, weight, BMI, waist circumferences) and metabolic parameters were determined and compared between groups.
Results
There were 270 (6.4%) subjects with BMI < 25 kg/m^2, 800 (19.0%) with overweight (BMI 25 – 30 kg/m^2) and 3142 (74.6%) with obesity (BMI > 30 kg/m^2). Mean adult height was shorter (157.7 ± 6.3 yr, 158.4 ± 6.2 yr and 158.9 ± 6.4 yr, respectively) and BMI values (35.6 ± 7.4 kg/m^2, 34.8 ± 7.2 kg/m^2 and 34.6 ± 7.3 kg/m^2, respectively) were greater in group I with the lowest age at menarche than others ($P<0.05$). However, blood pressures, fasting glucose, insulin, total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides levels, waist to hip ratio and HOMA values were not different between groups.
Conclusion
Few studies have examined inverse association of age at menarche with adult BMI and the tendency of BMI to track between childhood and adult life. Age at menarche may simply be a marker for the pace of sexual maturation, leads to differences in adiposity that track into adult life. Our data suggest that children with earlier ages at menarche should be nearest follow-up to prevent the adulthood obesity.

P636

The multi-PDZ domain protein MUPP1: a scaffolding protein controlling the acrosome reaction in mammalian spermatozoa

Frauke Ackermann[1], Dorke Meyer[1], Diana Heydecke[1], Beate Wilhelm[2], Thomas Gudermann[1] & Ingrid Boekhoff[1]
[1]Department of Pharmacology, Philipps University, Marburg, Germany; [2]Department of Anatomy and Cell Biology, Philipps University, Marburg, Germany.

Upon adhesion to the zona pellucida, mammalian sperm undergo regulated exocytosis of the acrosome. Despite the difference in size, some parallels can be drawn concerning the signal transduction processes controlling the sperm acrosome reaction and synaptic vesicle exocytosis. Since components of signal transduction pathways are often organized in multiprotein signalling complexes, attempts were made to identify scaffolding proteins expressed in the acrosomal region of mammalian spermatozoa. Using RT-PCR approaches and immunohistochemical experiments, the Multi-PDZ domain Protein MUPP1, which comprises 13 potential protein interaction modules, was identified in mouse testis. Immunocytochemical experiments combined with immunogold electron microscopy revealed that MUPP1 is exclusively detectable within the acrosomal region of different mammalian spermatozoa and that the MUPP1 protein is most prominent at the outer acrosomal membrane. To assess the possible function of MUPP1, the acrosome reaction was monitored using the photosensitive calcium chelator NP-EGTA-AM and an inhibitory anti-MUPP1 antibody. This functional assay revealed that antibody treatment significantly reduces acrosome reaction compared to control conditions. These results together with the observation that MUPP1 co-migrates in detergent-insoluble lipid rafts along with proteins involved in acrosomal exocytosis, like syntaxin-2, indicates that MUPP1 in different mammalian species may assemble similar, if not identical signaling molecules controlling acrosomal exocytosis.

P637

Expression of the G-protein α-subunit gustducin in mammalian spermatozoa

Dorke Meyer[1], Johanna Fehr[1], Heike Borth[1], Patricia Widmayer[2], Beate Wilhelm[3], Thomas Gudermann[1] & Ingrid Boekhoff[1]
[1]Department of Pharmacology, Philipps University, Marburg, Germany; [2]Department of Physiology, University of Hohenheim, Stuttgart-Hohenheim, Germany; [3]Department of Anatomy and Cell Biology, Philipps University, Marburg, Germany.

The G protein subunit α-gustducin is generally accepted as a marker for chemosensitive cells. Since chemosensation is especially important for the navigation of sperm towards the egg, attempts were made to explore whether α-gustducin might also be expressed in spermatozoa. RT-PCR experiments revealed that a gustducin PCR specific RNA fragment with the predicted size could be amplified from total mouse and rat testis. To identify the testicular cell type in which α-gustducin is expressed, immunohistochemical experiments were performed with an anti-gustducin-specific antibody. The most intense immunoreactivity was visible in differentiating spermatids localized in the lumen of the seminiferous tubules whereas no staining was detectable in spermatogonia. To verify whether α-gustducin is still expressed in mature spermatozoa, mouse and rat sperm were subjected to immunocytochemistry as well as electron microscopy. A strong staining of the innerdense fibres was obtained within the flagellum. Similarly, analyzing human sperm for α-gustducin staining also revealed a strong labeling of the midpiece of the flagellum whereas the principle piece remained unstained. The observation that α-gustducin is expressed in the tail of mammalian spermatozoa may now motivate to identify the corresponding signaling cascade, probably defining the functional role of α-gustducin in spermatozoa.

P638

Delayed puberty with extreme uterine hypotrophy: do not conclude too early to the absence of the uterus

Edwige Yollin, Sophie Catteau-Jonard, Anne-Céline Reyss, Christine Cortet & Didier Dewailly
Lille University Hospital, Lille, France.

Objective
To emphasize the difficulties to distinguish between uterine agenesis and extreme uterine hypotrophy in the context of primary amenorrhea with delayed puberty.
Patients and methods
Among adolescents who consulted our center because of primary amenorrhea, from 1997 to 2005, three patients were referred for a suspicion of Mayer-Rokitansky-Kuster-Hauser Syndrome, after ultrasonography had failed visualizing the uterus. The 3 patients underwent endocrine and genetic evaluations. Pelvic examination was performed by transabdominal ultrasonography and MRI. Patients were placed under estrogen treatment.
Results
Endocrine evaluation indicated Primary Ovarian Failure for patient 1, and Hypogonadotrophic Hypogonadism for patients 2 and 3. Karyotype was 46,XX in all patients. Initial pelvic ultrasonography revealed the absence of uterus. MRI allowed vizualizing prepubertal uterus for patient 1, a hypotrophic uterus for patient 3 and concluded to uterine agenesis for patient 2. In all cases estradiol substitutive therapy induced uterine growth and confirmed retrospectively the diagnosis of extreme uterine hypotrophy.
Conclusion
Pelvic ultrasonography can be misleading in the evaluation of primary amenorrhea. No visualization of uterus on ultrasonography can occur in the context of delayed puberty and should not induce a premature diagnosis of Mayer-Rokitansky-Kuster-Hauser Syndrome. Indeed, such a diagnosis has therapeutic, reproductive and psychological consequences.

P639

Screening and treatment of gestational diabetes and impaired glucose tolerance in Georgia

Natalia Asatiani[1], Ramaz Kurashvili[2], Mzia Dundua[3], Elena Shelestova[1], Liana Tsutskiridze[1], Moche Hod[1], Sergey Smirnov[1] & Victor Vlasov[1]
[1]Georgian Diabetes Center, Tbilisi, Georgia; [2]Dep.of Obstetrics and Gynecology, Rabin Medical Center, Tel-Aviv, Israel; [3]Novo Nordisk A/S, Copenhagen, Denmark.

Background and Aim
One of the greatest problem in pregnancy, complicated by diabetes is screening and appropriate care for women, whose diabetes is manifested during pregnancy. The study is aimed at gathering epidemiological data on gestational diabetes mellitus (GDM) and evaluating the of appropriate therapy in this condition.
Materials and Methods
Screening for GDM was carried out in – 692 pregnant women. GDM was observed in 3.3% (22 women) and impaired glucose tolerance (IGT) - in 5.05% (35 women). 57 women with GDM and IGT comprised the Group 1 (Gr.). Fifteen pregnant women, who were not screened timely and attended the Center at 10-34 week of gestation with fasting hyperglicemia and ketoacidoses comprised the Gr.2.
Results
In Gr.2 HbA1c ($9.5 \pm 1.7\%$) levels at entry were statistically higher, than in Gr.1 ($6.3 \pm 0.93\%$)($P=0.000$). By the end of the 3rd trimester those indices dropped (($6.3 \pm 0.72\%$; $5.45 \pm 0.74\%$ respectively). In Gr.1 following pathologies were observed: pre-eclampsia had 2(3.5%), preterm delivery – 2(3.5%), macrosomia – 9(15.7%), perinatal deaths – 0. In Gr.2: pre-eclampsia had 4(26.6%), preterm delivery – 3(20%), macrosomia – 3(20%), perinatal deaths – 4(26.6%). Mean infants' birth weight (g) was 586 ± 323 (Gr.1) and $3\,260 \pm 445$ (Gr2).
Conclusion
Following epidemiological data were obtained for Georgia: IGT (5.05%) and GDM (3.3%). Good glycemia control during pregnancy not always prevents macrosomia, though significantly reduces the risk of pre-eclampsia, preterm delivery and perinatal deaths.

P640

The effects of cycloheximide, actinomycine D and indomethacin on progesterone release stimulated by PACAP 38 from cultured rat ovarian granulosa cells

Elzbieta Wasilewska-Dziubinska, Magdalena Chmielowska, Ewa Wolinska-Witort, Lidia Martynska, Wojciech Bik & Boguslawa Baranowska
Medical Centre of Postgraduate Education; Neuroendocrinology Department, Warsaw, Poland.

Neuropeptide PACAP 38 expressed in steroidogenic ovarian cells of rat could be an auto- or paracrine regulator of progesterone synthesis. Moreover, it has been shown that PACAP38 can affect ovarian secretion of prostaglandins. The first and rate-limiting step in the biosynthesis of progesterone is the transfer of cholesterol into mitochondria which is facilitated by the cycloheximide-sensitive steroid acute regulator (StAR) protein. StAR protein has been established as an essential factor required for the acute response of steroidogenic cells to trophic stimulation. It seems that PACAP 38 stimulates ovarian progesterone synthesis directly or indirectly influencing on StAR protein activity. In the present study we examined the effects of cycloheximide (an inhibitor of StAR protein synthesis), actinomycine D (an inhibitor of RNA synthesis) and indomethacin (an inhibitor of prostaglandins synthesis) on progesterone release stimulated by PACAP38 from primary culture of ovarian granulosa cells obtained from adult cyclic rat (diestrus). As exogenous substrate for progesterone synthesis 20-hydroxycholesterol, which can readily diffuse across the mitochondrial membranes to the P450 scc was used. Progesterone concentrations in supernatants were assayed by RIA method. After 2 h incubation progesterone release stimulated by PACAP38 was totally inhibited by cycloheximide and partially inhibited by actinomycine D. After 24 h incubation progesterone release stimulated with PACA38 was totally inhibited by actinomycine D and also by indomethacin. These data suggest that ongoing StAR protein synthesis is partially inhibited by actinomycine D during 2 h incubation, but that during 24 h incubation continuing synthesis requires transcriptional activity.
Conclusion
in primary culture of rat ovarian granulosa cells stimulatory effect of PACAP38 on progesterone release is connected with stimulation of StAR protein synthesis and may be mediated by local synthesis of prostaglandins.
This study was supported by CMKP grant 501-1-1-28-32/05

P641

XX-male syndrome: clinical, hormonal and molecular genetic findings in comparison to Klinefelter patients and normal men

Elena Vorona, Michael Zitzmann, Joerg Gromoll, Manuela Simoni & Eberhard Nieschlag
Institute of Reproductive Medicine of the University, Muenster, Germany.

Background
The rare 46, XX-male syndrome has to be distinguished from more frequent forms of hypogonadism, especially the Klinefelter syndrome (47, XXY). We report 11 cases of SRY-positive XX-males in comparison to 101 age-matched Klinefelter patients and 78 age-matched normal men in a case-control study.
Methods
The comparison included results from the physical examination, endocrinological data, semen analysis, cytogenetic and molecular genetic findings. X-chromosome inactivation analysis with inactivation of the androgen receptor (AR) alleles was performed in 10 heterozygous XX-male patients and the findings were compared to the X-chromosome inactivation pattern in Klinefelter patients and in women.
Results
The XX-males were significantly smaller than Klinefelter patients or normal men. The incidence of maldescended testes and gynecomastia was significantly higher than in both control groups. Most XX-male patients were hypogonadal and require testosterone replacement therapy. All investigated XX-males were infertile. The absolute X-chromosome inactivation in XX males was significantly different from random. Seven out of ten XX-male patients showed skewed X-chromosome inactivation ratios ($<20\%$ or $>80\%$) with an equal proportion (distribution) of the X-inactivation on the short and on the long AR alleles. Two had highly skewed ratios of 2:98 and 99:1. There was no preference towards the longer or the shorter AR allele. The patients with skewed inactivation ratios showed no tendencies towards any special diseases.
Conclusions
Our study demonstrates that XX-males are distinct from other patients with hypogonadism due to chromosome disorders with two X chromosomes, such as Klinefelter patients. This is reflected by decreased body height and increased rate of maldescended testes. Two thirds of XX males in our group had non-random X-chromosome inactivation ratios. A reason for the skewed X-chromosome inactivation in these patients may be an X-chromosome abnormality, namely the translocated SRY gene.

P642

Expansion of $CD4^+CD25^+$ regulatory T cells during murine pregnancy is not driven by pregnancy-associated hormones

Anne Schumacher, Catharina Thüre, Juliane Rau, Hans-Dieter Volk & Ana Claudia Zenclussen
Institut für Medizinische Immunologie, Berlin, Germany.

The physiological state of pregnancy is characterised by the tolerance of the maternal immune system towards the paternal alloantigens expressed by the foetus. Recently, $CD4^+CD25^+$ regulatory T cells (Treg) were described to play an essential role for the generation and maintenance of the tolerance state. Several research groups showed that normal pregnancy in humans and mice is associated with an augmentation in the number of Treg in different organs whereas females suffering from abortion displayed diminished numbers of Treg. We showed that the adoptive transfer of Treg from normal pregnant CBA/J ($H2^k$) females previously mated with BALB/c ($H2^d$) males into abortion-prone mice (DBA/2J-mated CBA/J females) is able to protect the semiallogeneic ($H2^d/H2^k$) foetus from maternal immune rejection. In addition, we could confirm that Treg from virgin mice could not rescue from abortion. In the light of these results, we postulated that the expansion of Treg is either driven by the presence of paternal/fetal antigens or by pregnancy-associated hormones. We therefore mated CBA/J females either with BALB/c- or DBA/2J males and determined the levels of progesterone and estradiol by chemiluminescence at different time points of pregnancy (day 0, 2, 5, 8, 10 and 12). In addition, we defined the levels of progesterone in Treg-treated mice on day 14 of pregnancy. We observed comparable levels of progesterone, estrone and estradiol in both, normal and abortion-prone animals. Treg treatment, which was effective in diminishing the abortion rate, did not modify the hormonal levels. Our data suggest that pregnancy-associated hormones are not crucial for the expansion of the Treg population and that this is rather driven by specific paternal alloantigens.

P643

The clinical outcomes of stimulation of ovulation in patients with idiopathic hypogonadotropic hypogonadism (IHH) caused by mutations of GnRH receptor

Slawomir Wolczynski, Monika Zbucka & Katarzyna Jarzabek
Department of Reproduction and Gynecological Endocrinology, Medical University, Bialystok, Poland.

We report pregnancies obtained after stimulation by gonadotropins in 3 patients with IHH caused by homozygous or compound heterozygous mutations of GnRH receptor: (R139H/R262Q, R139C/ R139C, R139H/ Phe 308 del.);

Gonadotropin response to GnRH was observed in the first patient. All the patients were stimulated with gonadotrophins according to the protocole step up with the initial dose of 150IU FSH and 75 IU LH a day. The luteal phase was suported by hCG and progesterone. After 14 days of stimulation in the patient with R139H/R262Q mutated receptor, the estradiol concentration was 540 pg/ml and two mature follicles were observed. That patient was pregnant and gave birth. Patient with R139C/ R139C mutated receptor required higher doses and much longer stimulation, 225 IU FSH and 150 IU LH for 21 days. Compare to the estradiol concentration (620 pg/m) she developed three mature follicles and lot of small follicules. She conceived with triple pregnancies. The first trimester was complicated with OHHS. She miscarried at 22 weeks. In the second stimulation with the same doses for 21 days the estradiol concentration was 580 pg/ml, she was pregnant, the first trimester was also complicated with OHHS and she had twins.

The patient with R139H/ Phe 308 del required 225IU FSH and 150IU LH for 22 days and the estradiol concentration was 560 pg/ml and in the ovary three mature follicles and lots of small follicules was observed. She was pregnant,the first trimester was complicated with OHHS. Right now she is in 27 weeks of amenorrhea.

Conclusions

Patients with the mutations of GnRH receptor type loss off require much longer stimulation with higher doses comparing to IHH patients without GnRH receptor mutations. Despite low estradiol concentration the risk of OHSS and multiple pregnancy is high

P644

Atherogenic indexes in women with premature ovarian failure-POF

Ljiljana Marina, Svetlana Vujovic, Miomira Ivovic, Milos Stojanovic, Milina Tancic-Gajic, Marija Barac & Milka Drezgic
Institute of endocrinology, Belgrade, Serbia.

POF is a disease of unclear origin affecting women under age 40. They are at high risk of cardiovascular diseases (CVD).

The objective of this study was to compare the lipid status and atherogenic indexes - total cholesterol/high density lipoprotein (TC/HDL) and low density lipoprotein/HDL (LDL/HDL) - of women with POF with healthy women of the same age and the same BMI.

We evaluated 54 women in two groups. 1st group: 31 women with POF, mean age 29.55 ± 4.3 years, mean BMI 22.4 ± 2.78 kg/m^2, with laboratory proven menopausal levels of FSH and LH and on hormone replacement therapy (HRT). 2nd group: 23 healthy women, mean age 26.73 ± 6.08 years, mean BMI 20.95 ± 2.66 kg/m^2. Statistical analysis was performed with *t*-Test.

There was no difference in age and BMI between the groups, $P > 0.05$. Mean TC in the 1st group was 5.33 ± 0.71 mmol/l and in the 2nd 4.34 ± 0.58 mmol/l. Mean HDL in the 1st group was 1.34 ± 0.35 mmol/l and 1.42 ± 0.29 mmol/l in the 2nd. Mean LDL in the 1st group was 3.53 ± 0.47 mmol/l and 2.6 ± 0.55 mmol/l in the 2nd. In the 1st group mean triglycerides were 1.21 ± 0.53 mmol/l and in the 2nd 0.83 ± 0.27 mmol/l. Mean TC/HDL in the 1st group was 4.21 ± 1.09 and in the 2nd 3.16 ± 0.57 and mean LDL/HDL in the 1st group was 2.83 ± 0.87 and 1.89 ± 0.51 in the 2nd. The difference between the 1st and the 2nd group was highly significant for TC, LDL and triglycerides as well as for atherogenic indexes TC/HDL and LDL/HDL, $P < 0.01$. There was no difference between the groups for HDL.

Our results show that women with POF are at higher risk of CVD than healthy women of the same age and the same BMI. HRT is of essential importance for these women and according to our study it is necessary to check their lipid status on regular basis.

P645

Effect of age and testosterone on sleep related erections

Antonio R M Granata, Vincenzo Rochira, Bruno Madeo, Valeria Pugni, Lucia Zirilli & Cesare Carani
Integrated Department of Medicine, Endocrinology, Metabolism and Geriatrics, University of Modena and Reggio Emilia, Modena, Italy.

Introduction

In order to study the effect of age and testosterone on sleep related erections, we enrolled 209 men (122: age 30–49 years; 87: age over 50 years), including mild and severe hypogonadal subjects (129) and eugonadal subjects (80).

Subjects and methods

The subjects were assigned to four groups, according to their testosterone serum levels. All the subjects underwent nocturnal penile tumescence and rigidity monitoring (NPTRM). The following sleep-related erection parameters were analyzed: total number of valid erections, total duration of rigidity $> 60\%$, total duration of increase in penile tumescence > 30 mm, maximum rigidity and maximum increase in penile tumescence.

Results

Total number of valid erections, total duration of rigidity $> 60\%$ and total duration of increase in penile tumescence > 30 mm showed constant lower values in the 4 groups of men over 50 years, when compared with the 4 groups of men with age range 30–49 years and with the same testosterone level. Moreover, when comparing groups of men with same age but different testosterone levels, a threshold was identified still for the previous 3 parameters: the more the T is lower than 8 nmol/L, the more sleep-related erections are impaired, but this pattern is lost when T is higher than 8 nmol/L. On the other hand, maximum rigidity and maximum increase in penile tumescence showed the same trend of the other parameters when groups with different age range are compared, but these 2 parameters were uninfluenced by testosterone levels.

Conclusions

Aging has an impairing role on sleep-related erections both in hypogonadal and eugonadal men, while testosterone has an higher effect only on some of the parameters we investigated.

P646

Hormonal and seminal parameters in patients with testicular neoplasia or lymphoproliferative disorders: two year follow up

Cataldo Di Bisceglie, Angela Bertagna, Fabio Lanfranco, Matteo Baldi, Anna Maria Barberis, Lucrezia Zurzolo & Chiara Manieri
Division of Endocrinology and Metabolic Diseases, Department of Internal Medicine, University of Turin, Turin, Italy.

Semen quality and reproductive hormones may be affected in men with testicular neoplasia (TN) and lymphoproliferative disorders (LD). We evaluated these

parameters before, 6, 12 and 24 months after the end of the oncological treatments in 60 patients with TN, and in 35 patients with LD. The patients were divided on the bases of the basal sperm concentration (A< and B≥ 10 million/ml). FSH, LH, testosterone (T) and inhibin B levels and sperm parameters were evaluated in all patients. The patients with TN showed a significant reduction of inhibin B levels and a significant increase of FSH levels 6 and 12 months after the end of the oncological treatments; LH levels showed a significant increase after 6 and 12 months only in patients of group A; T levels showed no variations after the treatment. Sperm concentration showed a significant reduction after 6 and 12 months only in patients of group B. The patients with LD showed a significant reduction of inhibin B levels after 6 and 12 months and a significant increase of FSH levels after 6, 12 and 24 months; LH and T levels showed no variations after the treatment. Sperm concentration showed a significant reduction after 6 and 12 months only in patients of group B. After 24 months reproductive hormones, except for FSH levels in LD, and sperm concentration showed no significant differences compared to basal levels. The other sperm parameters were not significantly affected by the treatment in all patients (TN and LD). In conclusion, the effect of the oncological treatments on sperm concentration is less evident in patients of group A, probably due to a predominant influence of the neoplastic condition. After 24 months we observed an improvement of the hormonal and seminal parameters in TN, except for a persistent iatrogenic effect in LD.

P647

Immunohistochemical evaluation of ghrelin expression in polycystic ovaries in patients with PCOS.

Hanna Komarowska[1], Ryszard Wasko[1], Katarzyna Iwanik[2], Przemyslaw Majewski[2], Alina Warenik Szymankiewicz[3], Ludmila Rafinska[4], Magdalena Jaskula[1] & Jerzy Sowinski[1]
[1]Departament of Endocrinology, Metabolism and Internal Medicine, Poznan University of, Poznan, Poland; [2]Depatment of Clinic Pathomorphology, Poznan University of Medical Sciences, Poznan, Poland; [3]Department of Gynecological Endocrinology, Poznan University of Medical Sciences, Poznan, Poland; [4]Department of Histology and Embryology, Poznan; University of Medical Sciences, Poznan, Poland.

Ghrelin is an endogenous ligand of the GH secretagogue receptor. The influence of ghrelin on different organs has been studied recently e.g. in the regulation of pituitary hormone release, regulation of energy homeostasis, glucose metabolism and insulin secretion, cell proliferation and reproductive function.

The etiology of PCOS has not been firmly explained, althought several pathways have been implicated – the regulatory pathways of steroid hormone synthesis, regulatory pathways of gonadotropin and GH-IGF-1 axis action, the insulin signaling pathway and pathways regulating body weight. Ghrelin seems to link these pathways.

The aim of our study was to estimate the presence of ghrelin in polycystic ovaries cells and evaluation of the relationship betwen ghrelin occurence and cells proliferation.

Methods

Ten polycystic ovaries and ovaries without pathology as the control group were compared. The ghrelin was detected using two different immunohistochemical metods with the polyclonal rabbit anti-ghrelin antibodies (Phoenix Pharmaceuticals Inc.). The cells proliferation was estimated by Ki 67 proliferation index.

Results

Ghrelin immunostaining was demonstrated in cytoplasm of ovarian secondary interstitial cells and in regressing corpora lutea. The cell nuclei were ghrelin positive in granulosa and theca layers of follicular cyst in both groups and in luteal cells of young corpora lutea in healthy ovaries. Ki 67 immunostaining was observed in granulosa and theca layers of follicular cyst in polycystic and healthy ovaries.

Conclusions

It is possible that local ghrelin expression plays an important role in the direct control of ovarian development and function and ghrelin may participate in patomechanism of PCOS.

The local Ethical Committee approved the study.

P648

Ovarian hyperstimulation syndrome during IVF induction revealing a gonadotroph adenoma

Pierre Lecomte[1], Christine Chabrolle[1], Peggy Pierre[1], Michel Jan[1] & Jacqueline Trouillas[2]
[1]Chru Bretonneau, Tours, France; [2]Université Laennec, Lyon, France.

Gonadotroph adenomas are usually detected by their local mass effects. Spontaneous ovarian hyperstimulation syndrome (OHS) has rarely been described as the main manifestation of gonadotroph adenomas in young women. We present a case with a prolonged OHS occurring during IVF ovarian induction leading to the discovery of a FSH pituitary tumour.

Case report

A 36 year-old, normal weight woman with 2 years primary infertility linked to oligomenorrhea and anovulation was included in an IVF program. PRL, androgens and gonadotrophins evaluation before ovulation induction was normal. She had presented a few weeks before a mild OHS after a five days single tablet of clomifene citrate. Before IVF induction, FSH and LH levels were 5 and 3 UI/l. Daily Decapeptyl treatment was started on January 7th for 12 days. Then long-acting Decapeptyl 3 mg was injected on January 18th after hormonal control. E2 level was very high (7300 ng/l) and enlarged ovaries were discovered with transvaginal u.s.: right 87×60 mm and left 69×50 mm with follicles and cysts (15–35 mm). Two days later, pelvic pain and more enlarged ovaries were treated with puncture but cysts quickly reappeared. One month after long-acting GnRH analog injection, E2 and inhibin B were elevated (2300 ng/l and 343 ng/l) and FSH and LH still detectable: 3 and 1.1 UI/l. Since OHS persisted, a gonadotroph adenoma was suspected. A 10 mm adenoma was found in the right part of the pituitary with MRI. Before surgery, FSH and αSU were elevated with no response after GnRH test, in contrast to LH which increased. At the end of March, the surgeon removed a right microadenoma and the pathologist confirmed a gonadotroph adenoma: all cells stained for βFSH and 5% reacted with anti- αSU and anti-βLH antisera. Shortly after surgery, hormone levels normalized and an ovulatory cycle was observed but ovarian size was persistently increased (30 mm cysts) 4 months after initial stimulation.

Conclusion

This case is unusual: OHS observed during an IVF program persisted and elevated FSH after GnRH long-acting analog allowed discovering a FSH pituitary adenoma cured by surgery. Enlarged ovaries were still detected 4 months after initial ovulation induction.

P649

Insulin levels and lipid profile in lean women with polycystic ovary syndrome

Mirjana Stojkovic, Milos Zarkovic, Jasmina Ciric, Biljana Beleslin, Slavica Savic, Djordje Marina, Bozo Trbojevic & Milka Drezgic
Institute of endocrinology, Belgrade, Serbia.

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. Insulin resistance is one of the features of PCOS and potentially affect lipid metabolism.

Objectives

The aim of this study was to compare basal insulin levels and lipid profile in lean women with PCOS with weight matched healthy controls.

Methods

The study group consisted of 64 women divided in two subgroups (1. PCOS group, $n=48$; age 25.7 ± 6.2; BMI 21.3 ± 1.9 kg/m^2. 2. group of healthy controls, $n=16$, age 26.8 ± 6.4; BMI 20.3 ± 1.6 kg/m^2). Data were analyzed by the t test.

Results

Mean basal glucose levels were 4.38 ± 0.46 mmol/L vs. 4.54 ± 0.23 mmol/L, without statistically significant difference between groups. Mean basal insulin levels were significantly higher in PCOS group than in healthy controls (24.82 ± 16.34 mIU/L vs. 6.47 ± 3.19 mIU/L; $P=0{,}001$;). Cholesterol, HDL and LDL cholesterol levels did not reach statistically significant difference between groups, while triglyceride levels were significantly higher in PCOS group than in healthy controls (1.05 ± 0.44 mmol/L vs. 0.73 ± 0.22; $P=0{,}009$;).

Conclusions

These data suggest that PCOS per se, without obesity, affects insulin secretion and lipid metabolism, mainly in triglyceride levels which enhances atherogenic potential in this subjects.

P650

Demonstration of estrogen receptor–β in human gonadotropin-releasing hormone neurons

Erik Hrabovszky[1], Imre Kallo[1], Nora Szlavik[2], Eva Keller[2], Istvan Merchenthaler[3] & Zsolt Liposits[1]

[1]Laboratory of Endocrine Neurobiology, Institute of Experimental Medicine, Hungarian Academy of Sciences, Budapest, 1083 Hungary, Budapest, Hungary; [2]Department of Forensic Medicine, Semmelweis University, Budapest, Hungary; [3]Department of Epidemiology and Preventive Medicine, University of Maryland, Baltimore, MD, United States.

The gonadotropin-releasing hormone (GnRH) neurosecretory system represents the final common hypothalamic pathway in the neuroendocrine control of reproduction. Changing levels of the ovarian sex steroid hormone 17β-estradiol (E_2) tightly regulate the activity of GnRH cells via feedback actions. Recently, our group has localized the second isoform of estrogen receptors (ER-β) within GnRH neurons of the rat brain, indicating that GnRH cells are capable of directly sensing circulating estrogens. To address the issue of whether GnRH neurons of the human hypothalamus also contain ER-β, we have carried out dual-label immunocytochemical studies on autopsy samples. Research protocols to obtain and handle tissues were reviewed and approved by the Regional Committee of Science and Research Ethics (TUKEB 49/1999). Combined technical efforts that minimized *post-mortem* interval (<24 h), optimized fixation conditions (use of a mixture of 2% paraformaldehyde and 4% acrolein) and sensitized the immunocytochemical detection (application of silver-intensified nickel-diaminobenzidine chromogen) allowed the visualization of nuclear ER-β immunoreactivity in 10.8–28.0% of GnRH neurons in the preoptic/hypothalamic area of male human individuals. The demonstration of ER-β in human GnRH cells, which lack the classical ER-α receptor isoform, indicate that estrogens may exert direct actions upon GnRH cells selectively through ER-β. In the light of the differing ligand binding characteristics of ER-β from those of ER-α, this discovery offers a potential novel approach to influence estrogen feed-back mechanisms to GnRH neurons through the recently available ER-β-selective ligands.

P651

Asymmetric dimethylarginine levels and carotid intima media thickness in patients with polycystic ovary syndrome

Baris Pamuk, Mustafa Kulaksizoglu, Ayse Nur Torun, Derun Taner Ertugrul, Ozgur Ciftci, Emel Ozcimen, Ayla Uckuyu, Sevsen Kulaksizoglu, Nilufer Bayraktar & Nilgun Guvener Demirag
Baskent University, Ankara, Turkey.

Increased plasma concentration of asymmetric dimethylarginine (ADMA), an endogenous inhibitor of nitric oxide synthase, has been associated with cardiovasular risk factors. The aim of this study was evaluate plasma ADMA levels and subclinical atherosclerosis in patients with polycystic ovary syndrome (PCOS) and healty controls.

Thirty-five patients with PCOS and age, body mass index (BMI) matced thirty-one healty subjects were included in the study.PCOS was defined according to Rotterdam criterion. Serum levels of follicle stimulating hormone (FSH), luteinizing hormone (LH), dehydroepiandrosterone sulfat (DHEAS), free testosterone and total testosterone were measured. Serum insulin and plasma glucose levels measured at baseline before the oral glucose tolerance test. Insulin resistance was evaluated by homeostasis model assesment (HOMA IR). Also serum concentrations of total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL C), triglycerides (TG), homocysteine, fibrinogen, C reactive protein(CRP) were determined. Plasma ADMA levels were measured. Intima media thickness (IMT) assessment of the common carotid artery (CCA) were performed ultrasonographically.

The PCOS group had higher levels of androgens, TG, homocysteine, insulin, and HOMA IR. ($P<0.05$). There were no significant differences in ADMA levels and IMT between two groups. Also FPG, TC, HDL C, LDL C, fibrinogen and CRP levels were not different among the groups ($P>0.05$). IMT was significantly correlated with DHEAS but no association was determined with ADMA.

To our knowledge, this is the first study that assessed ADMA levels in patients with PCOS. The results of the study showed that ADMA levels and IMT were not different in PCOS patients from healty controls.

P652

Ghrelin levels in obese patients with polycistic ovary syndrome

Danica Stamenkovic-Pejkovic, Dragan Micic, Mirjana Sumarac-Dumanovic, Aleksandra Kendereski, Goran Cvijovic, Svetlana Zoric & Maja Georgiev
Institute of Endocrinology, Diabetes and Diseases of Metabolism, Belgrade, Serbia.

It was shown that obesity and insulin resistance may influence ghrelin levels. Contraversial results were observed considering ghrelin levels in women with polycystic ovary syndrome (PCOS). The aim of our study was to investigate the basal ghrelin levels in obese patients with PCOS and to evaluate possible correlations between ghrelin levels, insulin resistance and, androgen levels. In 10 obese PCOS patients (BMI 32.50 ± 1.57 kg/m^2, age: 21.4 ± 0.85 years) and 8 obese controls (BMI 32.54 ± 1.95 kg/m^2, age: 28.12 ± 1.51 years) basal ghrelin (RIA, Linco, USA, pg/ml) and testosteron (RIA, INEP, nmol/l) levels were determinated. Insulin sensitivity was determinated using euglycemic hyperinsulinemic clamp (M index).

Results

There was significant difference in ghrelin levels between PCOS patients and controls (42.65 ± 26.91 vs 96.33 ± 37.34, $P<0.05$), while M index was lower in PCOS patients but there was no significant difference (2.39 ± 0.59 vs 3.46 ± 0.92, $P>0.05$). There was negative correlation between ghrelin and testosterone levels ($r=-0.78$, $P<0.05$) and there was no correlation between ghrelin levels and M index ($r=-0.12$, $P>0.05$). In conclusion, obese PCOS patients have lower ghrelin levels than obese heathy women. In addition, a negative correlation between ghrelin and testosteron levels might suggest an interaction between ghrelin and steroid synthesis or action.

P653

Ghrelin levels in lean patients with polycistic ovary syndrome

Maja Georgiev[1], Dragan Micic[1], Mirjana Sumarac-Dumanovic[1], Aleksandra Kendereski[1], Danica Stamenkovic-Pejkovic[1], Goran Cvijovic[1], Svetlana Zoric[1] & Nebojsa Zecevic[2]
[1]Institute of endocrinology, diabetes and diseases of metabolism, Belgrade, Serbia; [2]Clinic for Gynecology and Obstetric NARODNI FRONT, Belgrade, Serbia.

It was speculated that androgen levels and insulin resistance may have influence on ghrelin levels. Elevated, normal and low ghrelin levels were reported in women with polycystic ovary syndrome (PCOS). The aim of our study was to investigate the basal ghrelin levels in lean patients with PCOS and to evaluate possible correlations between ghrelin levels, insulin resistance and, androgen levels. In 10 lean PCOS patients (BMI 20.45 ± 0.51 kg/m^2, age: 21.4 ± 0.85 years) and 8 lean controls (BMI 20.92 ± 0.69 kg/m^2, age: 25.37 ± 2.41 years) basal ghrelin (RIA, Linco, USA, pg/ml) and testosteron (RIA, INEP, nmol/l) levels were determinated. Insulin sensitivity was determinated using euglycemic hyperinsulinemic clamp (M index).

Results

There was significant difference in ghrelin levels (51.82 ± 26.83 vs 120.11 ± 58.42, $P<0.05$) and M index values (3.68 ± 0.66 vs 7.84 ± 1.28, $P<0.05$) between PCOS patients and controls. There were no significant correlations between ghrelin and testosterone ($r=0.40$, $P<0.05$), as well between ghrelin and M index values ($r=-0.12$, $P>0.05$). In conclusion, we observed lower lower ghrelin levels in lean PCOS patients than in camparative controls. Insulin resistance might have influence on low ghrelin levels in this group of patients.

P654

Adrenal morphology on CT-scan in patients with congenital adrenal hyperplasia

Anne Bachelot[1], Fabio Roffi[2], Agnès Rouxel[1], David Eiss[2] & Philippe Touraine[1]
[1]Pitié-Salpétrière Hospital, Paris, France; [2]Necker Hospital, Paris, France.

Congenital adrenal hyperplasia (CAH) due to 21-hydroxylase deficiency is one of the most common autosomal recessive diseases. Decreased production of cortisol leads to increased secretion of CRH and ACTH, resulting in overproduction of androgens and hyperplastic adrenals. 21-OH deficiency has thus been speculated to predispose for the formation of morphological adrenal abnormalities. However, studies are rare, the most relevant showing a high incidence of adrenal masses in 82% CAH patients. We then decided to evaluate adrenal morphology on CT-scan in CAH patients. We performed adrenal helicoidal CT scan with contiguous 3-mm-thick slices in 42 patients (33 females and 9 males; mean age, 27.6 yr (14–47 yr)). Twenty one had a salt-wasting form (SW), 11 a simple virilizing one (SV) and 10 a non-classical form (NCF). We found adrenal hyperplasia in 17 patients (40%), 12 with SW and 5 with SV form. Bilateral adrenocortical adenomas were observed in 2 of them. Subjects with adrenal hyperplasia were older (31.4 ± 1.7 years versus 26.5 ± 1.5 years, $P=0.04$), and had higher levels of 17OHprogesterone (105.2 ± 24.5 ng/ml versus 11.1 ± 4.9 ng/ml, $P<0.0001$) androstenedione

(10.7±2.7 ng/ml versus 2.3±0.6 ng/ml, $P=0.0006$) and renin levels (116± 45 ng/ml versus 18±2 ng/ml, $P=0.008$) than subjects with normal adrenal CT-scan. Total glucocorticoids dose is in current evaluation in both groups. Our results suggest that morphological adrenal abnormalities are not so common in CAH patients; however, they seem to be associated with increasing severity of the enzymatic defect and undertreatment may play an important role in their development. CT-scan should then be proposed in adult CAH patient, but to avoid radiation exposure, adrenal MRI should then be proposed in the follow-up of patients with adrenal abnormalities. Moreover, we propose that CAH should always be ruled out in the case of incidentally detected adrenal masses.

P655

Anti-oxidant activity of seminal plasma in fertile and infertile men

Salah Fayed & Ashraf Ahmed
Mansoura Faculty Of Medicine, El-Mansoura, Egypt, Urology & Nephrology Center, El-Mansoura, Egypt.

To evaluate the seminal plasma anti-oxidant activity in normal and infertile men and its relation to semen quality. 58 men with idiopathic infertility problems were selected, were divided according to their sperm count into two subgroups, infertile asthenospermic group ($n=31$) and infertile oligo-asthenospermic group ($n=27$). 14 proved fertile men were selected as a control group. Semen samples were collected by masturbation, examined by conventional method. Then free seminal plasma samples were separated by centrifugation and stored at −20 C till analyzed for total anti-oxidant activity (Rice-Evans & Miller1994), total thiol concentration (Hu 1994) and the thiobarbituric acid reactive substances (TBRs) by the metod of Walker & Shah (1988).

In the present study, the seminal plasma anti-oxidant activity in infertile groups was significantly higher than in control group (P 0.014), asthenospermic versus controls (P 0.016), oligoasthenospermic versus controls (P 0.036). No significant changes were observed in total thiol concentration and thiobarbituric acid reactive substances in the seminal plasma among the different groups. TBRs showed a positive significant correlation with semen volume and a negative significant correlation with percentage of abnormal forms. It could be concluded from the present study that there is a well developed system of anti-oxidants in the seminal plasma which is activated by increased levels of reactive oxygen species and products of semen lipid peroxidation. So the high levels of seminal plasma anti-oxidant activity observed in infertile groups of our study has been considered as a compensatory protective mechanism to minimize the spermatozoa membrane damage caused by the hazardus effects of free radicals on the membrane high content of polyunsaturated fatty acids.

P656

Reproductive health of women born to bromocriptine-treated mothers

Evgenia Rashidova, Valentina Kokolina, Tatiana Romantsova & Alexej Bobrov
Moscow Medical Academy, Moscow, Russia.

A retrospective cohort study was undertaken using a reproductive health survey of 25 girls aged 14–27 years (Me 20,5 (16;23)) born to bromocriptine-treated mothers. The control group consisted of 25 women born after spontaneous pregnancy of the same age, mother age, region of residence. They were all seen in the clinic for health and psychological interviews (Multiscale Personality Assessment Test- MMPI). All of them were blood analysed for LG, FSG, prolactin, TSH, anti-TPO, testosterone and DHEAS levels; ultrasonography of the mammary gland, internal genitalia with calculation of ovarian volume was presented. Pearson Chi-Square and Fisher's Exact test were used for comparing results in two groups. No difference between two groups ($P>0,05$) was found in hormonal levels, the incidence of menstrual cycle disorders and gynaecological desease, all women had normally developed internal and external genitalia. One of them has prolactin-secreting microadenoma, receives parlodel. Women born to bromocriptine-treated mothers had earlier menarche (Me 12 (12;13)) comparing to control group (Me 13 (12;14))($P=0.046$). We found a high frequency of primary hypothyroidism in women born to bromocriptine-treated mothers -20% (5 women- 3 with subclinical and 2 with overt). The early age of manifestation (9 to 18 years) and absence of anti- thyroid antibodies are their remarkable features. Different pscopatological syndroms and psychosomatic disorders were found in 9 from 16 women (62.5%) who underwent psicological testing using MMPI compairing to 3 from 18 (16.6%) in control group ($P=0.015$) 8 women born to bromocriptine-treated mothers had spontaneous pregnancies and 7 of them have healthy children. 6 were born in term, one child was born pretermed because of intrauterine infection. The study provides additional evidence that in utero exposure to bromocriptine doesn't have severe adverse effects on later health outcomes including reproductive function. The prevalence of psycopatological syndromes may be due to specific family education.

P657

The difference in the level of lipids, CRP, androgens and prolactins between PCOS women with metabolic syndrome and PCOS women without metabolic syndrome

Zelija Velija-Asimi
University Clinical Centre of Sarajevo, Bosnia and Herzegovina.

The polycystic ovary syndrome (PCOS) is characterized by insulin resistance with compensatory hyperinsulinemia. Insulin resistance also plays a role in the metabolic syndrome. PCOS women with metabolic syndrome have more hyperandrogenism and menstrual cycle irregularity than women with PCOS only.

The aim of the study was to determine the difference in the level of lipids, CRP, androgens and prolactins between PCOS women with metabolic syndrome and PCOS women without metabolic syndrome.

Methods

The study included 47 women with PCOS evaluated in our clinic. The women were divided into two groups: 1) women with PCOS and the metabolic syndrome ($n=26$, age 30.9±8 yr, BMI = 30.7±2.1 kg/m^2; WHR = 0.9) and 2) women with PCOS without metabolic syndrome ($n=20$, age 29.5±7.5 yr, BMI = 23.7± 1.7 kg/m^2; WHR = 0.8). Laboratory evaluation included lipids, CRP, TSH, PRL, FSH, LH, E2, progesterone, testosterone, androstendion, DHEAS, insulin levels during OGTT.

Results

PCOS women with metabolic syndrome had significantly higher levels of serum testosterone (3.23±0.81 *vs*. 2.2±0.67 nmol/l, $P<0.05$) than women with PCOS without the metabolic syndrome. Levels of total cholesterol (6.56±0.91 vs. 5.6± 0,9 mmol/l), LDL cholesterol (4.63±1.2 vs. 3.3±0.7 mmol/l), CRP (5.6±1.2 vs. 2.7±1.1 mg/l) and prolactin (623±179 vs. 373±121 uIU/ml) were also higher in PCOS women with metabolic syndrome. Menstrual cycle irregularity was frequently in group PCOS women with metabolic syndrome.

Conclusion

The high level of lipids, CRP, androgens and prolactin suggest that the metabolic syndrome in women with PCOS increased risk for cardiovascular disease.

P658

Presented as S23.2

P659

Presented as S18.2

P660

Presented as S18.4

P661

Expression of p63 and Notch system in the rat testis and vaso-epididymal system during postnatal development

T Hayashi, R Ohno, N Ishii, T Matsuda, T Terao, M Morozumi & T Yamada
Saitama Medical Center, Saitama Medical University, Kawagoe, Saitama, Japan.

The testis and epididymis collaborate in the male gamete development. The testis has the specific function to generate spermatozoa and spermatozoa undergo numerous changes passing through the epididymis. p63 in the basal layer of epithelium plays a key role in maintaining cellular populations, whereas Notch 1 and its ligand Jagged 2 have an important role in the cell differentiation and

Jagged 2 is up-regulated by TAp63, which transactivates p53 target genes and induces apoptosis. However, the role of p63 and its relationship with Notch system in the testis have not been examined. Therefore, we investigated the postnatal expression of p63, Jagged 2 and Notch 1 in the testis in comparison with the vaso-epididymal epithelium by Northern blot analysis and immunohistochemistry. In the testis, TAp63 mRNA expression increased at day 14 after birth and the expressions of Jagged 2 and Notch 1 mRNAs increased at day 16, whereas p63 protein was detectable in spermatocytes and Jagged 2 and Notch 1 proteins were in spermatids, suggesting TAp63-mediated Jagged 2 induction activates the Notch system. On the other hand, deltaNp63 mRNA expression was already recognized in the vas deferens at day 0 and advanced chronologically along the duct to the caput epididymis, whereas Jagged 2 and Notch 1 mRNAs were maintained at a low level. The current study has identified that testis and vaso-epididymal system express different p63 isoforms. Moreover, our data raises the probabilities that TAp63 has an important role for maintenance of germ cell numbers, triggering or balancing the development, differentiation and apoptosis of germ cells by activating both Notch system and p53 target genes, and that the chronological differences of deltaNp63 expression result in the morphological and functional differences in the mesonephric tubule.

P662

Transcripts expressed in the mouse testis during sex-determining period

T Hayashi[1], KL Loveland[2], T Yamada[1] & DM de Kretser[2]
[1]Saitama Medical Center, Saitama Medical School, Kawagoe, Saitama, Japan; [2]Monash Institute of Medical Research, Monash University, Melbourne, Victoria, Australia.

In order to understand the mechanisms that underpin gonadal development, we have conducted a subtractive screen to identify transcripts expressed differentially during the sex-determining period. Suppression subtractive hybridization PCR was performed on cDNA derived from 12.5 dpc male and female gonadal ridges. Clones were tested for differential expression by RNA whole mount *in situ* hybridization. Those localizing to testis cords were further tested on germ cell-depleted testes, and we examined the pattern of expression of four clones with male germ cell dependent expression by *in situ* hybridization in postnatal mouse testes. Four clones showed germ cell dependent expression during sex determining period, and we examined their pattern of expression in postnatal mouse testes by *in situ* hybridization. One of these, K1, encodes a protein closely related to the kinesin-like protein, KIF2. At the onset of spermatogenesis, the transcript signal was intense in the gonocyte cytoplasm and weak in Sertoli cells. This continued until the first onset of meiosis when the signal gradually shifted from spermatogonia to spermatocytes and then to spermatids; the Sertoli cell signal disappeared entirely during the first wave of spermatogenesis. The other three clones, H21 (encoding ADP-ribose polymerase), K22 (cleavage & polyadenylation specificity factor 1) and A12 (KIAA0890) were recognized in gonocytes and Sertoli cells with strong intensity at the onset of spermatogenesis. Although the signals persisted in germ cells throughout the first wave of spermatogenesis and into adulthood, the Sertoli cell signals were lost. In adult testis, all three mRNAs were detected in spermatogonia and spermatocytes. This is the first report that demonstrates the highly regulated expression of these male germ cell dependent gene products in both somatic and germ cells throughout testis development and in adulthood.

Index of Authors