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**15th Congress of the
European NeuroEndocrine Association**

**September 12–15, 2012
Vienna, Austria**



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**15th Congress of the European NeuroEndocrine Association - September
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Abstracts



PRIZE LECTURE

FONDATION IPSEN 2012 ENDOCRINE REGULATIONS PRIZE ROLF GAILLARD LECTURE

Leptin and the Homeostatic System Regulating Body Weight

J. Friedman

Molecular Genetics, The Rockefeller University, New York, USA

The discovery of leptin has led to the elucidation of a robust physiologic system that maintains fat stores at a relatively constant level. Leptin is a peptide hormone secreted by adipose tissue in proportion to its mass. This hormone circulates in blood and acts on the hypothalamus to regulate food intake and energy expenditure. When fat mass falls, plasma leptin levels fall stimulating appetite and suppressing energy expenditure until fat mass is restored. When fat mass increases, leptin levels increase, suppressing appetite until weight is lost. By such a mechanism total energy stores are stably maintained within a relatively narrow range.

Identification of a physiologic system that controls energy balance establishes a biologic basis for obesity and further establishes links between leptin and numerous other physiologic responses. Recent studies have explored the relationship between leptin and the reward value of food. In addition, new methods for identifying neurons activated by leptin and other stimuli have been developed.

Recessive mutations in the leptin gene are associated with massive obesity in mice and some humans. Treatment with recombinant leptin markedly reduces food intake and body weight. The low leptin levels in patients with leptin mutations are also associated with multiple abnormalities including infertility, diabetes, and immune abnormalities all of which are corrected by leptin treatment. These findings have established important links between energy stores and many other physiologic systems and led to the use of leptin as a treatment for an increasing number of other human conditions including a subset of obesity, some forms of diabetes including lipodystrophy and hypothalamic amenorrhea, the cessation of menstruation seen in extremely thin women.

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Disclosure: No significant relationships.

PLENARY LECTURES

PL01

NPY: Beyond the Regulation of Energy Metabolism

H. Herzog

Neuroscience, Garvan Institute, Darlinghurst, Sydney, Australia

Many factors and pathways have been implicated in the regulation of appetite and energy homeostasis, however, none of them are as central and essential as the NPY system. NPY is a complex system consisting of 3 ligand genes NPY, peptide YY (PYY), pancreatic polypeptide (PP), and at least 5 different receptors (Y1, Y2, Y4, Y5, and Y6). Whereas central NPY is known to stimulate appetite and feeding behaviour, the mostly peripherally expressed family members PYY and PP have the opposite effect and have been identified as potent satiety factors. Negative energy balance leads to increased hypothalamic NPY expression and the activation of appetite stimulatory pathways and other feeding-related behaviours. However, NPY causes also neuroendocrine and metabolic changes which favour energy storage, including decreased thermogenesis, hyperinsulinaemia, insulin hyper-responsiveness in white adipose tissue, activation of the hypothalamo-pituitary-adrenal axis, and decreased activity of the hypothalamo-pituitary-thyrotropic, -somatotropic, and -gonadotropic axes. However, whole body homeostasis does not only involve the regulation of fat and lean mass but also that of bone mass and coordinating this to bodyweight, eg, larger body mass requires stronger bones. Recently, we have identified the critical role of the NPY system in the regulation of bone formation with reduced central and peripheral Y-receptor signaling leading to elevation in bone formation and bone volume. In addition, elevation in bodyweight and the subsequent development of obesity are associated with low-grade inflammation likely leading to insulin resistance in this tissue. Importantly, the NPY system also plays a critical role in modulating the immune system and thereby influencing particular stress-related immune responses. New outcomes of the analysis of various transgenic models from the NPY family with regard to the regulation of energy and bone homeostasis with a particular focus on stress-induced changes will be presented.

Disclosure: No significant relationships.

PL02

Functional Hypothalamic Amenorrhea: Genetic Basis

No abstract available

PL03

Signaling within the Mammalian Circadian Timing System

S. A. Brown

Institute of Pharmacology and Toxicology, University of Zürich, Switzerland

Human behavior and physiology are governed by a “circadian” biological clock that diurnally modulates the activity of many cell and organ systems. Although a central clock is located in the brain hypothalamus, its mechanism is cell-autonomous and present in most cells of the body. Our laboratory has exploited this redundancy to develop viral tools to explore circadian signaling directly in hu-

man cells from different individuals in real time. So far, we have used this novel technology to explore various aspects of both chronobiology and endocrinology. For example, we have used fibroblast pathway profiling to predict the degree of suppression of the hormone melatonin by light in human volunteers, as well as daily behavioral preferences of the same individuals. Similarly, we have also used these simple methodologies to identify possible therapeutic targets for human sleep disturbance in old age. Altogether, the data that we gain can be a potent predictor of complex behavioral responses in otherwise non-accessible tissues, making them useful biomarkers for personalized medicine.

PL04

Tumor-Targeting Therapies in Pituitary Adenomas: Where Are We Now?

No abstract available

PL05

HRT and Cognitive Function

P. Bouchard

Endocrinology, Hôpital Saint Antoine, Paris, France

A large corpus of preclinical literature suggests a role for steroids in the treatment/prevention of cognitive decline. These convincing data became suspicious following the publication of the WHI studies, mainly the WHIMS. However, recent evidence suggests a role of estrogens and progestins for healing following brain injury and eventually for cognition. The present situation suggests that estrogen therapy is certainly useful in young women who have undergone oophorectomy, a condition which significantly increases the risk of cognitive decline and neurological diseases later in life. Under these circumstances, HT should then be prescribed until the usual age of menopause. Alternatively, in postmenopausal women, the evidence of a positive role of hormones on cognition is still not clear. Preclinical studies suggest a window of efficacy requiring the use of HT at the time of menopause without delay which could result in the loss of response to estrogen treatment probably because of the loss of ERα in critical areas of the brain. While this may be the case in women, HT has not been proven to consistently improve verbal memory and other areas of cognition and the results of clinical and epidemiological studies on neurocognitive impairment are still inconclusive. Progesterone and progestins such as norethisterone have been proven far more effective on neurogenesis, cholinergic projections, and inflammation, with the exception of MPA. Indeed, PR-dependent signaling of endogenous brain progesterone rapidly limits the extent of tissue damage and the impairment of motor functions following traumatic injury and stroke. Alternatively, other steroids are also efficient: allopregnanolone, a ligand of GABA_A receptors, seems also active on brain protection, although its signaling is not mediated through PRs. This data opens the exciting field of treatment of stroke and traumatic brain injury to the already known effects of estradiol mainly in younger women or shortly after menopause.

Disclosure: No significant relationships.

SYMPOSIA

■ **Neuroendocrinology of Addiction**

S01

Ghrelin and Addiction

S. L. Dickson, R. H. Shirazi, M. Alvarez-Crespo, E. Egecioglu, C. Hansson, E. Jerlhag, J. A. Engel, K. P. Skibicka
Institute of Neuroscience and Physiology, The Sahlgrenska Academy at the University of Gothenburg, Sweden

Accumulating evidence suggests that ghrelin's physiological role extends beyond appetite and energy balance to include reward-seeking behavior, involving a direct action at the level of the midbrain dopamine system. Indeed, we found that central ghrelin signaling is important for animals to receive reward from addictive drugs including alcohol. Recently, we explored the role of the central ghrelin signaling system in food preference, food reward, and food motivation. Direct injection of ghrelin into the brain ventricles or the VTA increased the intake of palatable/rewarding food. Preference for palatable food was suppressed by a 7-day peripheral treatment with a GHS-R1A antagonist. The antagonist also suppressed the ability of rewarding food to condition a place preference. Finally, we explored motivated behavior for a food reward in an operant conditioning model (ie, lever pressing for food in a progressive ratio schedule). Ghrelin increased motivated behavior for a sucrose reward when injected icv or intra-VTA but not intra-NAcc. By contrast, ghrelin administration to both the VTA and NAcc increased the free feeding of chow. In a state of overnight food restriction, where endogenous levels of ghrelin are increased, GHS-R1A blockade in the VTA was sufficient to decrease the motivation to work for a sugar reward. Blockade of GHS-R1A in VTA or NAcc was not sufficient, however, to reduce fasting-induced chow hyperphagia. Thus, the VTA but not the NAcc appears to be a direct target site for ghrelin's effects on food motivation. In conclusion, the central ghrelin signaling system is important for reward, not only from chemical drugs such as alcohol, but also for food reward and food-motivated behavior.

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Disclosure: No significant relationships.

S02

Exercise Addiction: Differential Diagnosis, Co-Occurring Disorders and Phases of Addiction

A. Sartorio¹, N. Marazzi², A. E. Rigamonti²

¹Laboratorio Sperimentale Di Ricerche Auxo-endocrinologiche, Istituto Auxologico Italiano, IRCCS; ²Dipartimento di Farmacologia Medica, Università degli Studi di Milano, Milan, Italy

It is well-established that exercise in appropriate quantity and of proper quality contributes significantly to preserve health. In the last decade, there has been increasing awareness that exercise addiction (EA) is becoming a common and worrisome phenomenon. The definition of EA is actually difficult due to the lack of physiological or biochemical markers, the best marker to distinguish between non-abusive habitual exercise and EA being (for the latter) the refusal to discontinue exercise in the face of medical, occupational, social, or familial contraindications. DSM-IV categorizes EA as having ≥ 3 of the following items: tolerance, withdrawal, intention effects, loss of control, time, conflict, and continuance. As occurs for drug addiction, many factors such as availability, genetics, history of "drug" abuse, stress, and life events contribute to the transition from "exercise use" to EA and abstinence. Trying an oversimplification, we can recognize a primary EA when performance of the exercise itself is the end point, with dieting and weight loss used as a means to improve performance (males) and a secondary behaviour, when the aim of excessive exercise is to achieve weight loss or prevent fatness

(females). Several exercise-induced neurochemical changes and disruptions of hormonal axes (dopamine, endorphins, ACTH/cortisol, interleukins, catecholamines, GH) are responsible for the establishment and worsening of EA. Unfortunately, EA frequently co-occurs with other addictions that, if left unrecognized, can complicate the treatment process. When exercise addiction and eating disorders co-occur, the danger is that only the latter problem will be usually treated with the secondary EA remaining hidden. Although there is scant literature on the treatment of EA, like most behavioural addictions some form of cognitive-behavioural therapy is recommended. Addiction specialists must remain attuned to the signs and symptoms of EA, given its co-occurrence with substance use disorders and other common behavioural addictions (ie, gambling, compulsive shopping, sex addiction).

Disclosure: No significant relationships.

S03

Influences of Nutritional State, Gut Hormones, Obesity, and Bariatric Surgery on Food Reward

A. Goldstone

Metabolic and Molecular Imaging Group, MRC Clinical Sciences Centre, Imperial College London, UK

Appetite is controlled by homeostatic processes signaling acute nutritional state and long-term adiposity to the hypothalamus and brainstem including gut and other appetitive hormones and metabolites. These systems interact with higher cognitive processes, food reward, individual psychological traits, genetic factors, and the sensory qualities of food to finally influence eating behaviour. Decision-making, motivation to eat, and the preference for particular foods involve brain reward and cognitive systems including the nucleus accumbens, amygdala, hippocampus, and orbitofrontal, prefrontal, and insula cortices. In addition to hyper- or hypo-responsiveness of brain reward systems to high-calorie foods, other addictive behaviours may also promote overeating in obesity, including impulsivity, compulsivity, impaired inhibitory control, and stress-induced eating. The interaction between these homeostatic and hedonic networks will be illustrated by review of our human functional magnetic resonance imaging studies examining the effects of nutritional state (fasted vs fed), appetitive gut hormones (ghrelin), obesity, and different bariatric surgical treatments (gastric bypass vs gastric banding surgery) on food reward.

Disclosure: No significant relationships.

■ **Ghrelin**

S04

Targeting Neuroendocrine Pathways for the Treatment of Diabetes

No abstract available

S05

The Neuroprotective Role of Ghrelin in Parkinson's Disease

Z. B. Andrews

Physiology, Monash University, Clayton, Australia

Ghrelin is a hormone secreted from the stomach that acts on the brain and peripheral tissues to control appetite, energy metabolism, glucose homeostasis, and hormone secretion. However, recent studies highlight important "non-metabolic" roles for ghrelin and neuroprotection is one such role of ghrelin. This presentation discusses

the role of ghrelin and other gut hormones in Parkinson's disease, as impaired gut function is hypothesized to contribute to Parkinson's disease. We show that ghrelin targets dopamine neurons in the substantia nigra and activates a mitochondrial pathway that prevents cell degeneration in a mouse model of Parkinson's disease. Moreover, negative energy balance (such as calorie restriction) elevates plasma ghrelin and ghrelin mediates the effect of calorie restriction on glycemia and anxiety. We discuss novel unpublished data showing that ghrelin underpins the neuroprotective effect of calorie restriction in a mouse model of Parkinson's disease by stimulating mitochondrial function in the substantia nigra. Human Parkinson's disease patients exhibit significant differences in plasma ghrelin compared to age-matched controls, suggesting ghrelin may be an important target to treat Parkinson's disease. Future studies are required to examine the neuroprotective function of ghrelin in other neurodegenerative disorders such as Alzheimer's disease.

Disclosure: No significant relationships.

S06

Peripheral Effects of Ghrelin in Humans

E. T. Vestergaard

Aarhus University Hospital, Denmark

Traditionally, the pulsatile secretion of GH from the pituitary gland is considered to be under both positive and negative regulation by hypothalamic factors: GH-releasing hormone stimulates GH release, whereas somatostatin exerts an inhibitory influence. More recently, another receptor (GHS-R) has been discovered at the hypothalamic as well as at the pituitary level. Subsequent to this its endogenous ligand, ghrelin, produced primarily by gastric cells, was discovered. The effects of exogenous ghrelin include stimulation of GH, adrenocorticotrophic hormone, and prolactin secretion. Administration of ghrelin stimulates appetite and food intake. The GHS-R is, however, also present in many peripheral organs and tissues for which reason more differentiated direct and pleiotropic effects of ghrelin could be expected and will be the topic of this presentation. Data derived from human models are still sparse and largely based on short-term infusion studies. Ghrelin infusion results in relative insulin suppression and hyperglycemia, and induces insulin resistance. However, it is difficult to dissect direct effects of ghrelin from those caused by its well-known stimulatory effects on GH and ACTH secretion even during concomitant somatostatin infusion. Hypopituitary subjects constitute an attractive model for studying GH- and cortisol-independent effects of ghrelin and infusion studies have demonstrated that ghrelin induces hyperglycemia, lipolysis, and insulin resistance *per se*. In regional arterio-venous perfusion studies of the lower limbs, ghrelin also directly increases circulating concentrations of free fatty acids. In humans, acute ghrelin administration induces a condition similar to reversible diabetes. Longer-term studies are needed to investigate if these metabolic effects persist. Investigations of the metabolic effects of ghrelin are important both to unravel the mechanisms causing type-2 diabetes but also to recognize putative side effects of ghrelin or ghrelin agonists in the future treatment of conditions such as postoperative ileus and gastroparesis.

Disclosure: No significant relationships.

■ New Imaging Techniques in Neuroendocrinology

S07

Radiopharmaceutical Basics

M. Mitterhauser, W. Wadsak

Dept of Nuclear Medicine, Medical University of Vienna, Austria

Radiopharmaceuticals are the chemical tools to enable molecular imaging in PET (Positron Emission Tomography) and SPECT (Single-Photon Emission Computer Tomography) diagnostics. In general, they comprise (1) a molecular vehicle responsible for selective and specific interactions with the respective molecular target (eg, receptors, transporters, enzymes) and (2) a radionuclide exhibiting suitable physico-chemical characteristics for safe application and quantitative assessment. There is a variety of molecular imaging targets in neuroendocrine pathologies like somatostatin receptors (SSTR), amino acid binding sites and uptake processes or specific enzymes. All these targets can be significantly altered and visualized by molecular imaging techniques. The most frequently used radionuclides are F-18, Ga-68, C-11 for PET and Tc-99m and In-111 for SPECT, respectively. Combining these radionuclides with suitable vector molecules generates a radiopharmaceutical, which enables a personalized and targeted diagnosis. These imaging concepts will be explained on the basis of [⁶⁸Ga]DOTA-peptides, [¹⁸F]dopamine analogues/precursors, and [¹¹C/¹⁸F]11b-hydroxylase inhibitors. Especially [⁶⁸Ga]DOTA-peptides have gained wide interest and application in recent years. Nowadays, derivatives radio-labelled with β^- -emitting radionuclides (eg, Y-90, Lu-177) are also widely available, which opens the opportunity of so-called "theranostics" – a complimentary pair of radiotracers, one bearing a diagnostic radionuclide and the other carrying a therapeutically active isotope. Hence, it will be demonstrated that nuclear medicine methods are able to contribute both to personalized diagnosis and therapy of neuroendocrine pathologies.

Disclosure: No significant relationships.

S08

Clinical Use of Radiotracers: Current Practice and Future Options

No abstract available

S09

New Trends in Imaging of the Pituitary

M. Buchfelder

Dept of Neurosurgery, University of Erlangen-Nürnberg, Erlangen, Germany

To date, for its premium soft-tissue contrast, magnetic resonance imaging is generally considered the imaging method of choice to depict normal variants and lesions within and around the pituitary. The goal of all imaging studies is to indicate precisely the localization, extent, and nature of the sella region space occupying intracranial tumours. In large lesions, the challenge is the delineation, the differentiation of enclosed or invasive growth and the assessment of evolution over time. Several methods have been developed to monitor tumour growth and thus not only the natural history but also the influence of various therapies. Volumetry is the most expensive and labour-intensive of these. In small lesions, such as ACTH-secreting microadenomas of the pituitary in Cushing's disease and some TSH-secreting adenomas of minute size, the problem is different, namely the most sensitive depiction. Various attempts have been made to improve visualization of such lesions and several novel techniques will be reviewed, such as spoiled gradient-recalled acquisition in the steady state, dynamic imaging after contrast application and half-dose contrast application in 3T field strength. A new evolving technology is intraoperative MR imaging during surgery of the skull base, hypothalamic and pituitary surgeries. With the frequent use of sophisticated imaging of the head, the number of incidentally discovered abnormalities has also dramatically increased. A few re-

marks concerning the contribution of radiology to assist in the differential diagnosis will be made.

Disclosure: No significant relationships.

S10

Insight from fMRI Studies into Appetite Regulation

No abstract available

■ Pituitary Cell Biology

S11

Mechanisms of Apoptosis Resistance in Pituitary Adenomas

M. C. Zatelli

Dept of Biomedical Sciences and Advanced Therapies, Section of Endocrinology, University of Ferrara, Italy

Pituitary tumours are mostly benign, and locally invasive in rare cases. Deregulation of several genes has been suggested as a possible alteration underlying the development and progression of pituitary tumours. Pituitary tissue homeostasis results from the balance between cell proliferation and programmed cell death or apoptosis; the impairment in cell death mechanisms results in tumorigenesis and progression. Several genes and pathways have recently been demonstrated to deeply influence the apoptotic mechanisms in the normal pituitary, eventually facilitating the development of a pituitary adenoma. Among the possibly altered systems leading to an imbalance between cell proliferation and programmed cell death there is evidence that the KISS1/KISS1R system, which is altered in pituitary adenomas, enhances the apoptotic rate in GH-producing and non-functioning pituitary adenomas (NFA). Similarly, over-expression of the Pitx2 transcription factor or of GADD45 β may play an anti-apoptotic role in NFA and in gonadotroph pituitary adenomas, respectively. The expression status and function of the PI3K/AKT/mTOR pathway have also been shown to be important for the pro-apoptotic effects of the drugs interacting with this pathway. We recently demonstrated that the Magmas gene (mitochondria-associated protein involved in granulocyte-macrophage colony-stimulating factor signal transduction) is highly expressed in ACTH-secreting mouse pituitary adenoma cell lines and in the majority of human pituitary adenomas, where Tim 16, the protein encoded by Magmas, is expressed in the mitochondria. Our data show that Magmas protects pituitary cells from apoptosis, suggesting its possible involvement in neoplastic transformation. We also found that Magmas directly impairs the pro-apoptotic mechanisms by hampering mitochondrial-mediated apoptotic mechanisms, further indicating that Magmas over-expression might represent a possible escape from programmed cell death in pituitary adenomas. This data fits well with the slow-growth potential of pituitary adenomas and their relative insensitivity to chemotherapeutic approaches which are very effective in rapidly growing tumors.

Disclosure: No significant relationships.

S12

Pituitary Stem Cells During Tissue (Re-) Modeling

H. Vankelecom

Dept of Development and Regeneration, Research Unit Embryo and Stem Cells, Laboratory for Tissue Plasticity, University of Leuven (KU Leuven), Belgium

The pituitary gland must appropriately remodel to meet the body's fluctuating hormonal demands. In the past, stem cells were suggested to participate in the generation of new endocrine cells. However, pituitary stem cells remained elusive and have only recently been identified. Our group tracked down multipotent stem cells in the pituitary "side population". These stem cells were found to express Sox2, a well-known gatekeeper of stem-cell pluripotency.

Sox2⁺ cells are located in multiple putative niches, including the cleft-lining marginal zone. Now that pituitary stem cells are identified, their role in pituitary cell (re-) modeling can be explored. We examined 2 paradigms of pituitary (re-) modeling. The rodent pituitary undergoes prominent maturation during the first weeks after birth. We observed an activated phenotype of the neonatal stem cell compartment in terms of abundance, proliferation, topographical arrangement, and stemness expression as well as activity. In adult organs, stem cells play an important role in tissue repair. Whether the mature pituitary is capable of cell regeneration after injury remains unsettled. We developed a transgenic "pituitary injury" model in which cells can be destroyed in a conditional manner, and detected a prompt reaction of the stem cell compartment, including expansion of the Sox2⁺ marginal-zone niche and co-expression of the ablated hormone. Moreover, we observed a substantial degree of regeneration, at least partly driven by the stem cells. Together, our studies provide several arguments that stem cells are involved in pituitary (re-) modeling, both during physiological and pathological conditions. Moreover, they demonstrate that the mature gland holds regenerative competence. Whether stem cells are also implicated in pituitary tumorigenesis and may give rise to so-called "cancer stem cells" is a challenging question. Our studies are expected to provide a better understanding of the mechanisms of pituitary plasticity and tumor pathogenesis, and thus may have important fundamental and clinical implications.

Disclosure: No significant relationships.

S13

MicroRNAs in Pituitary Tumors: Getting a Wee Chance to Grow

H. Butz¹, K. Baghy², I. Liko³, S. Czirjak⁴, P. Igaz⁵, M. Korbonits⁶, A. Zalatnai⁷, T. Krenács², I. Kovács², K. Racz², A. Patócs¹

¹Molecular Medicine Research Group, Hungarian Academy of Sciences; ²1st Dept of Pathology and Experimental Cancer Research, Semmelweis University;

³Gedeon Richter Plc; ⁴National Institute of Neurosurgery; ⁵2nd Dept of Medicine, Semmelweis University, Budapest, Hungary; ⁶Dept of Endocrinology, Bart's and the London School of Medicine, Queen Mary University of London, UK

Background MicroRNAs (miRNAs) are small (~22 nt), non-coding RNA molecules which posttranscriptionally regulate protein expression. Their role has been demonstrated in various processes including tumorigenesis. Deregulation of cell cycle mainly at the G1/S checkpoint has been reported in pituitary adenomas but G2/M transition has not been studied.

Aims Examination of the expression of Wee1 kinase and other genes involved in the regulation of the G2/M transition parallel with miRNA profile in order to identify miRNAs and their targets which may be involved in pituitary tumorigenesis.

Materials and Methods A total of 57 hormonally inactive (NFA) and 15 growth hormone-producing (GH) pituitary adenomas was studied. Expression of Wee1 and G2/M checkpoint genes together with expression of miRNAs were measured by TaqMan low-density arrays and validated by RT-PCR. Multiple, *in silico* target prediction algorithms were applied for identification of miR-mRNA interactions. Protein changes were detected using immunohistochemistry and Western blot. mRNA-miRNA interactions were proven using an *in vitro* luciferase reporter system.

Results Wee1 on protein, but not on mRNA level was decreased in NFA and GH-producing adenomas as compared to normal pituitary tissues. *In vitro* experiments confirmed that of the 5 over-expressed miRNAs, miR-128, miR-155, and miR-516a-3p target the 3'UTR region of the Wee1 transcript and over-expression of these miRs inhibited Wee1 protein expression. Of the 24 other genes involved in G2/M transition, Cdc25A/C and YWHAB were over-expressed while Cdk7, p21, Chk2, and GADD45B were under-expressed in tumor samples compared to normal tissues. miRNAs targeting Cdc25A were under-expressed in adenomas and their expression level negatively correlated with tumor size.

Conclusion In pituitary adenomas, the G2/M transition of the cell cycle is complexly regulated by miRNAs through regulation of ex-

pression of 2 key regulators, Wee1 and Cdc25A. These regulation loops may contribute to tumor growth and represent potential novel therapeutic targets.

Disclosure: No significant relationships.

S14

Vasopressin Receptors in Corticotropinomas: Is There a Diagnostic/Therapeutic Role? A Translational Approach

J. P. Castaño¹, A. Ibáñez-Costa¹, L. López-Sánchez¹, E. Venegas-Moreno², M. Á. Japón³, P. Benito-López⁴, E. Rivero-Cortes¹, L. Jiménez-Reina⁵, A. Soto-Moreno², A. Leal-Cerro², R. M. Luque¹

¹Dept Cell Biology, Physiology and Immunology, Hospital Univ. Reina Sofía, IMIBIC, University of Córdoba; CIBEROBn. Córdoba; ²Division de Endocrinología; ³Dept of Pathology, Hospital Universitario Virgen del Rocío, Sevilla; ⁴Servicio de Endocrinología, Hosp. Reina Sofía; ⁵Dpt Ciencias Morfológicas, University of Córdoba, Spain

Introduction Desmopressin, a synthetic analog of arginine-vasopressin (AVP) commonly used to treat nocturnal enuresis and diabetes insipidus, is also used in the diagnosis and post-surgical test of Cushing's disease (CD) patients as it stimulates ACTH release. However, the universal value of this test and its molecular basis remain controversial. Here, desmopressin effects were tested on ACTH release from pituitary corticotropinoma cells, normal corticotrope cells or cells from other pituitary tumors. We also compared in vitro and in vivo responses to desmopressin and tested its effects on gene expression and proliferation, and explored the underlying molecular mechanisms (eg, type of receptors, signaling) in cultured corticotropinoma cells.

Material and Methods A desmopressin test was performed before surgery in all CD patients. Desmopressin effects on ACTH secretion, Ca²⁺i, AVP receptors (AVPR1a, AVPR1b, and AVPR2) expression, and proliferation were evaluated in vitro on pituitary cells derived from human corticotropinomas, other tumor types, or normal human pituitary.

Results Desmopressin stimulated all functional endpoints tested in vitro, (ACTH release, Ca²⁺i kinetics, proliferation, and receptor expression), exclusively in human ACTH-secreting adenomas, but not in cells from normal pituitary or other tumor types. Corticotropinoma cells displayed higher AVPR1b expression compared to normal pituitary and other adenomas, suggesting that this receptor mediates desmopressin effects. Moreover, expression of all AVPRs directly correlated with basal cortisol levels in CD patients, and AVPR1b expression also correlated with basal ACTH. Incubation with specific AVPR1a or AVPR2 antagonists partially blunted desmopressin-induced ACTH release, and an AVPR1b antagonist fully blocked it.

Conclusion These results indicate that desmopressin directly and selectively stimulates key functional processes (ACTH secretion, gene expression, etc) in corticotropes from pituitary tumors causing CD, most likely via AVPR1b, and thereby provide a plausible molecular basis for this desmopressin action, and invites future investigation of AVPR1b agonists/antagonists as tools to diagnose/treat CD patients.

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■ GH/IGF-I Axis and Aging

S15

Endocrine Regulation of Aging by IGF Signals

A. Bartke

Internal Medicine/Geriatric Research, Southern Illinois University School of Medicine, Springfield, IL, USA

Mice with mutations causing growth hormone (GH) deficiency or resistance exhibit various symptoms of delayed and/or slower aging and live much longer than their normal siblings. Hypoinsulinemia and a dramatic decrease of circulating levels of insulin-like growth factor-1 (IGF-1) in these animals indicate that their remarkable extension of longevity is likely due to suppression of insulin/IGF-like signals (IIS), a mechanism conserved from yeast to mammals and well-characterized in worms and insects. However, in comparison to the effects of GH deficiency or resistance, mutations directly interfering with IGF-1 biosynthesis or action are generally less effective in extending mouse longevity. This focuses attention on GH actions that are not mediated by IGF-1 or differ from IGF-1 effects.

We are particularly interested in the effects of GH on the secretion of pro- and anti-inflammatory cytokines by the adipose tissue, on insulin signaling in various insulin target organs, and on the utilization of metabolic substrates.

Long-lived GH-related mutants have increased levels of adiponectin and reduced expression of interleukin 6 and tumor necrosis factor α . This shift from a pro- to anti-inflammatory profile, together with reduced mammalian target of rapamycin signaling, is believed to mediate the remarkable enhancement of insulin sensitivity at both whole-animal and tissue levels. In the skeletal muscle, enhanced insulin sensitivity appears to be due primarily to reduced inhibitory (serine) phosphorylation of insulin receptor substrate-1. The effects of surgical removal of epididymal and perinephric fat pads indicate that in the absence of GH signals these fat depots represent an important source of adiponectin and promote insulin sensitivity. Alterations of insulin and cytokine signaling in GH-related mutants are associated with reduced serum lipids, increased lipid oxidation, and ambient temperature-dependent reduction of respiratory quotient.

Our findings imply that somatotrophic signaling promotes aging via multiple mechanisms.

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Disclosure: No significant relationships.

S16

Impact of GH Deficiency, GH Excess, and/or Caloric Restriction on Longevity in Mice

J. Kopchick

Edison Biotechnology Institute, Ohio University, Athens, OH, USA

Mice are routinely used for aging studies for several reasons including their genetic similarity to humans, their short lifespan, and the ability to experimentally manipulate their genome. To that end, giant and dwarf transgenic mice that express GH or GH antagonist (GHA) genes have been generated. Also, dwarf GH receptor gene-disrupted (GHR^{-/-}) animals have been generated by targeting exon 4 of the GHR/BP gene. Together, these mice display extremes in GH action. We have found that giant GH transgenic mice are lean and insulin-resistant with relatively short life spans. On the other hand, GHR^{-/-} mice are dwarf, obese, and insulin-sensitive with increased life spans. In fact, they possess the longest life span of any laboratory mouse. Importantly, dwarf GHA transgenic mice are obese and yet possess normal life spans. Consequently, we have uncoupled mouse dwarfism from extended longevity. Caloric restriction (CR) has been shown to extend longevity in a variety of organisms ranging from worms to mice. Importantly, the long-lived GHR^{-/-} mice, when placed on CR diets, do not show a further increase in life span. In terms of obesity, the GHR^{-/-} mice have increased relative fat mass throughout life as compared to littermate controls with a preferential enlargement of the subcutaneous depot. Serum adipokine

changes have been noted in these mice with both total adiponectin and high molecular weight (HMW) adiponectin increased. Interestingly, the ratio of biologically active HMW adiponectin to total adiponectin is increased in the GHR^{-/-} mice and is normal in GHA mice. We believe that this ratio may be important in the insulin-sensitive state found in the GHR^{-/-} mice. Significantly, the GHR^{-/-} mouse mimics many phenotypic aspects of Laron syndrome patients including depressed incidences of cancer and diabetes. A review of results and recent data derived from these mice will be presented.

Disclosure: No significant relationships.

S17

Metabolic Effects of Growth Hormone During Stress and Caloric Deprivation in Humans

N. Møller

Medical Dept Mea, Aarhus University Hospital, Denmark

Phylogenetically, growth hormone (GH) is an ancestral hormone, which has been identified in the pituitary of primitive vertebrates, and STAT 5, a principal intracellular mediator of GH signaling, exhibits a very high degree of homology to invertebrate and prevertebrate STATs reflecting the ancient nature of the GH/STAT signaling system and signifying a prominent role throughout metabolic evolution.

In terms of evolutionary biology, the effects of GH on substrate metabolism in humans are simple: during states of energy surplus, GH, in concert with IGF-I and insulin, promotes nitrogen retention, and when food is sparse, GH alters fuel consumption from the use of carbohydrates and protein to the use of lipids, thereby allowing conservation of vital protein stores. These effects are most conspicuous during metabolic stress, such as fasting, and probably also a number of other metabolic scenarios including most cases of severe illness. A number of recent studies in humans have highlighted the importance of STAT and mTOR activation, stimulation of lipolysis via modulation of adipose triglyceride lipase, perilipin A and G0/G1 switch gene 2 and local promotion of IGF-I in muscle and adipose tissue.

Thus, in many ways the metabolic role of GH in humans is best understood in the long perspective of evolutionary fuel economy and the GH/STAT-governed master fuel switch from carbohydrate and protein utilization to lipolysis and lipid oxidation undoubtedly has played a major role for human survival during conditions of metabolic stress and caloric deprivation.

Disclosure: No significant relationships.

S18

Serum IGF-1 as a Marker for Morbidity and Mortality in Population-Based Studies: Epidemiological Considerations

O. Dekkers

Clinical Epidemiology, Leiden University Medical Centre, The Netherlands

IGF-1 plays a central role in metabolism and growth regulation. Several population-based studies have shown that high IGF-1 levels are associated with increased cancer risk and low IGF-1 levels with an increased risk for cardiovascular events. These morbidity risks translate into an increased mortality risk for people with either low or high IGF-1 levels. Whether the associations between morbidity, mortality, and IGF-1 levels are causal is difficult to determine from observational studies alone. It might be that the associations reflect an underlying condition such as for example bad nutritional state in people with low IGF-1 levels. But there is evidence from basic research supporting a causal association between cancer and cardiovascular morbidity and IGF-1. Based on data from population-based studies, IGF-1 levels around the 50th percentile are associated with the lowest mortality. This suggests that in patients with GH deficiency, GH should be targeted at IGF-1 levels between 0 and 1 SD in order to mimic optimal physiology. However, conclusions about

therapy should be based not only on population-based studies but also on randomized trials.

Disclosure: No significant relationships.

Neuroendocrinology of Circadian Rhythms and Sleep

S19

Role of Melatonin in the Regulation of Human Circadian Rhythms and Sleep

C. Cajochen

Centre for Chronobiology, Psychiatric Hospital, University of Basel, Switzerland

The pineal hormone melatonin is primarily a neuroendocrine transducer of external time (ie, light-dark cycle) promoting an increased propensity for “dark appropriate” behavior. The most unequivocal characteristic of endogenous melatonin is its utility to be used alone or in combination with core body temperature as a phase marker of the endogenous circadian pacemaker located in the suprachiasmatic nuclei located in the anterior hypothalamus. However, there are 3 major reasons which imply that melatonin could also play an important role in the regulation of human circadian rhythms and sleep-wake behavior:

- The endogenous melatonin rhythm exhibits a close temporal association with the endogenous circadian component of the sleep propensity rhythm and thermoregulation.
- There is evidence that exogenous melatonin is able to induce sleep when the homeostatic drive to sleep is insufficient, to inhibit the drive for wakefulness emanating from the circadian pacemaker and to induce phase shifts in the circadian clock such that the circadian phase of increased sleep propensity occurs at a new desired time.
- Light’s acute alerting response depends on its capacity to suppress endogenous melatonin levels during the biological night.

Thus, melatonin’s soporific and chronobiotic properties make it an optimal candidate for treating sleep, in addition to circadian rhythm disorders.

Disclosure: No significant relationships.

S20

Neuroendocrine Consequences of Restricted Sleep: Insights from Controlled Studies in Rats

P. Meerlo

Center for Behavior and Neurosciences, University of Groningen, The Netherlands

Chronically disrupted sleep may have serious repercussions for health and perhaps sensitizes individuals to psychiatric disorders. Indeed, short sleep and insomnia often precede and predict the onset of depression. However, the neurobiological mechanisms through which insufficient sleep may contribute to the development of depression are unknown. We therefore developed an animal model of chronic sleep restriction to study effects of sleep loss on neurobiological and neuroendocrine systems that have been implicated in the pathophysiology of depression. To mimic insufficient sleep as it often occurs in our society, rats in our studies are exposed to a schedule of chronically restricted sleep, allowing them about 4 hours of sleep per day. We then assess the consequences of restricted sleep on neuroendocrine regulation and brain function after one day and several weeks. While one day of sleep restriction has no major effects on most of the systems we examined, a week of restricted sleep altered HPA axis regulation and reduced hippocampal cell proliferation. In addition, after a month of sleep restriction we found a significant reduction in hippocampal volume. These changes may in part be related to alterations in serotonergic signaling since sleep-restricted rats displayed blunted physiological responses to direct serotonin 1A receptor stimulation. This desensitization of the serotonin 1A system persisted for many days even with unlimited recovery sleep. Importantly, control experiments

indicate that the reduction in serotonin 1A sensitivity is not a by-product of stress but a result of sleep loss *per se*. The gradually developing changes in neurotransmitter receptor systems and neuroendocrine reactivity in our model are remarkably similar to what is seen in depressed patients. These experimental studies thus provide support for the hypothesis that chronically disrupted sleep may contribute to the symptomatology of psychiatric diseases.

Disclosure: No significant relationships.

S21

Psychosocial Stress in Children, Sleep, and HPA System Regulation

M. Hatzinger¹, S. Brand², E. Holsboer-Trachsler²

¹Psychiatric Services Solothurn, University of Basel, and Dept of Adult Psychiatry, Solothurn; ²Depression Research, Psychiatric University Clinics, Basel, Switzerland

Objective Dysregulation of the stress system, ie, the hypothalamic-pituitary-adrenocortical (HPA) axis, is associated with psychiatric disorders such as depression. Moreover, aberrant HPA axis functioning is closely related to unfavourable sleep regulation described in depression. Since most of the data available so far are from studies after the disorder's onset, we started a project in children aiming to investigate sleep regulation, HPA axis function, and psychological/behavioural variables in order to identify potential biomarkers early in development.

Methods Parallel to a thorough psychological/behavioural assessment, 67 pre-schoolers (35 boys and 32 girls) aged 5 years underwent sleep EEG monitoring and baseline HPA activity assessment using saliva morning cortisol measurements after awakening. Follow-up investigation took place 3 years later during primary school.

Results In the baseline examination, boys showed significantly more REM sleep when compared to girls. Independent of gender, an unfavourable sleep profile was associated with an increased HPA axis activity. Furthermore, bad sleep regulation was related to more difficult behavioural/psychological dimensions. These findings were stable over time 3 years later: HPA system dysfunction was associated with unfavourable sleep patterns and with psychological/behavioural abnormalities as well.

Conclusions HPA system activity and sleep regulation are associated with psychological/behavioural dimensions. Both neurobiological values are stable over time and are related to psychological/behavioural abnormalities in follow-up. Thus, sleep and HPA axis measures seem to be promising biomarkers for the course and probably also for the onset of clinically relevant psychiatric problems in children.

Disclosure: No significant relationships.

S22

Peptidergic Regulation of Human Sleep

A. Steiger

Dept of Psychiatry, Max Planck Institute of Psychiatry, Munich, Germany

Human sleep is characterized by a neurophysiological and a neuroendocrine component, the Non-Rapid-Eye-Movement- (NonREM-) REM cycle, recorded by sleep electroencephalogram (EEG) and the secretion patterns of various hormones, respectively. In healthy young subjects, during the first half of the night slow-wave sleep (SWS) and growth hormone (GH) preponderate, whereas the second half is dominated by cortisol and REM sleep. During normal ageing and a depressive episode, sleep-endocrine activity is changed similarly, as SWS and GH decrease, REM sleep is disinhibited and cortisol increases particularly during depression. Preclinical studies suggest that peptides are common regulators of sleep EEG and hormones. In a series of studies, we investigated their role in normal and pathological human sleep. Sleep EEG and nocturnal hormone secretion were investigated simultaneously in healthy volunteers and depressed patients after pulsatile administration of various peptides. After GH-releasing hormone (GHRH), SWS and GH increased in young men whereas cortisol was blunted. Corticotrophin-releasing hormone (CRH) exerted opposite effects. These findings point to a reciprocal

interaction of GHRH and CRH in sleep regulation, at least in men. In depressed patients, sleep improved after a CRH antagonist. In women, however, GHRH impaired sleep. Similarly, after ghrelin NonREM sleep was promoted in young and elderly men, but not in women. In both sexes, GH and cortisol were elevated after ghrelin. SWS increased after galanin. The major effect of neuropeptide Y (NPY) is a shortening of sleep latency, pointing to a role in the timing of sleep. Somatostatin impaired sleep, particularly in elder subjects. After vasoactive intestinal polypeptide (VIP), the NonREM-REM-cycle was decelerated. Pituitary adenylate cyclase-activating peptide (PACAP) prompted an increase in the time constant tau of the physiological nocturnal EEG delta-power decline, ie, a less pronounced dynamic of the reduction of delta-power with time. Our data suggest a specific role of various peptides in sleep regulation.

Disclosure: No significant relationships.

Long-Term Consequences in Appropriately Treated Pituitary Patients

S23

Psychopathology and Personality Traits in Cushing's Disease and Acromegaly

C. Sievers¹, C. Dimopoulou¹, M. Ising², H. Pfister², J. Schopoh³, G. K. Stalla¹

¹Internal Medicine, Endocrinology and Clinical Chemistry; ²Clinical Psychology, Max Planck Institute of Psychiatry; ³Medizinische Klinik IV, Campus Innenstadt, University of Munich, Germany

Ever since the "endocrine psychosyndrome" in association with physical illnesses was described by the Swiss psychiatrist Manfred Bleuler in the 1950s, several studies have focused on psychopathological and personality changes associated with pituitary lesions.

Chronic hypercortisolism, as in Cushing's disease (CD), comprises a broad spectrum of psychological and psychiatric manifestations, ranging from anxiety to major depression and psychosis. With regard to personality dimensions, anxiety seems to be more pronounced in patients with CD than adrenal causes of hypercortisolism, outlining the importance of the pituitary mass *per se*. After correction of hypercortisolism, psychopathological variables gradually improve with time. However, increased prevalences of psychopathology and maladaptive personality traits seem to persist even after long-term cure of CD, suggesting irreversible effects of a previous glucocorticoid excess on the central nervous system rather than an effect of pituitary tumors and/or their treatment *per se*.

Also, in acromegaly, patients show a distinct pattern of increased anxiety-related personality traits. The prevalences of psychiatric disorders are elevated and cognitive function is impaired. As in Cushing's disease, biochemical control often fails to restore full quality of life and mental health parameters. The permanent impairment might be due to irreversible neuronal changes in the presence of long-term GH-IGF-1 excess which was suggested by an MRI study in which acromegalic patients exhibited larger grey matter and white matter volumes at the expense of the cerebrospinal fluid volume.

In conclusion, psychiatric dysfunctions are common, also in biochemically cured Cushing's disease and acromegaly, rendering neuropsychological and psychiatric testing essential in the diagnostic work-up and follow-up.

Disclosure: No significant relationships.

S24

Acromegaly and Arthropathy

N. Biermasz¹, K. Claessen¹, S. Ramautar¹, J. A. Romijn¹, H. Kroon², A. M. Pereira¹, M. Kloppenburg³

¹Endocrinology; ²Radiology; ³Rheumatology, Leiden University Medical Center, The Netherlands

Arthropathy is an invalidating complication of acromegaly and has a high impact on quality of life. Currently, little is known about the prognosis and determinants of this joint disease in acromegaly. Pa-

tients with controlled acromegaly have an increased prevalence of osteoarthritis at multiple sites already at a young age. Interestingly, the phenotype of arthropathy differs from primary osteoarthritis with respect to preservation of joint spaces. Joint space width in the hand remained increased despite long-term control of GH excess. The height of IGF-1 at diagnosis was associated with severity of radiological joint disease. Recently, we investigated radiographic progression of arthropathy over 2.5 years in 58 patients (mean duration of control: 17.6 yr). 40 patients were cured by surgery and, if necessary, additional radiotherapy, 18 (31 %) patients were controlled by somatostatin (SMS) analogs. Radiographic progression of joint disease was defined by the Osteoarthritis Research Society International classification on radiographs of the hands, knees, and hips obtained at the first study visit and after 2.5 years. Progression of osteophytes and JSN was observed in 72 % and 74 % of patients, respectively. Higher severity of radiographic arthropathy features at the first study visit was associated with more radiographic progression over 2.5 years. Higher age and presence of d3-growth hormone receptor (d3-GHR) polymorphism predisposed for osteophyte progression. Patients with biochemical control by SMS analogs had more progression of osteophytosis than surgically cured patients (OR = 18.9; $p = 0.025$), independently of age, sex, Body Mass Index, IGF-1 SDS at the first study visit, and d3-GHR. In conclusion, acromegalic patients have progressive arthropathy according to both osteoarthritis features despite long-term biochemical control. Parameters reflecting GH/IGF-1 activity were associated with progressive joint disease. Remarkably, biochemical control by SMS analogs was associated with more progression than surgical cure, which may indicate insufficient GH control according to current criteria and the need of more aggressive therapy.

Disclosure: No significant relationships.

S25

Socioeconomic, Educational, and Marital Status of Hypopituitary Patients

K. Stochholm¹, S. Juul², J. S. Christiansen¹, C. H. Gravholt¹

¹Dept of Internal Medicine and Endocrinology, Aarhus University Hospital; ²Dept of Epidemiology, School of Public Health, Aarhus, Denmark

Introduction Growth hormone deficiency (GHD) has been recognized as a clinical entity for more than 40 years, with a clinical effect of treatment with growth hormone reported decades ago. The primary disease resulting in hypopituitarism has an impact on morbidity and mortality as previously shown in hypopituitary and GHD populations. In questionnaire and interview studies, the consequences of childhood-onset GHD on socio-economic conditions, psychological factors, and on quality of life have been investigated, primarily in selected populations treated with growth hormone. These studies compared GHD patients with controls or same-sex siblings and identified an identical educational level, a reduced or identical employment degree, and a reduced number of individuals living with a partner. However, non-response may bias the results.

Materials and Methods Register study using Danish nationwide registries. 260 males and 156 females with childhood-onset GHD as well as 25,358 male and 15,110 female controls were included. Information was obtained concerning cohabitation, parenthood, education, income, retirement, convictions, and death. Income was analyzed dichotomized by the median among controls and using conditional logistic regression and the other outcomes were analyzed using Cox regression. Subgroups of GHD patients with malignant tumors, craniopharyngioma, idiopathic GHD, and others were investigated separately.

Results Both male and female GHD patients had a significantly worse outcome on all studied socio-economic parameters. Patients with idiopathic GHD had a socio-economic profile similar to controls. Fewer GHD patients lived in partnerships and entered them later, had fewer parenthoods, lower educational level, lower income, higher risk of retirement, and fewer convictions. Crude mortality was increased; however, it was reduced when adjusting for marital and educational status.

Conclusion Socio-economic conditions in adults with childhood-onset GHD are severely affected and impact mortality significantly;

only in the subgroup of patients with idiopathic GHD conditions are similar to the background population.

Disclosure: No significant relationships.

S26

Craniopharyngioma as a Model for Synergistic Effects of Hypopituitarism and Hypothalamic Dysfunction

E. M. Erfurth

Dept of Endocrinology, Clinical Sciences, Medical Faculty, Lund University, Sweden

There is an increased risk for cardiovascular death, especially among females with a craniopharyngioma (CP), when on conventional hormone replacement, but without GH therapy. About 50 % of CP children and adults are obese and hypothalamic damage seems to be a key issue. The exact background is not clear but vagally mediated hyperinsulinemia with autonomic imbalance is a cause as well as reduced sensitivity to endogenous leptin. In a childhood-onset craniopharyngioma population on complete hormone therapy including GH, we recorded increased cardiovascular risk and severe obesity particularly in females and among those with hypothalamic involvement by the tumour (TGTV). In patients with TGTV vs non-TGTV, increased weight, Body Mass Index, fat mass, and muscle mass, s-insulin, insulin/kg fat mass were found. Hypothalamic involvement by the tumour was also associated with a decrease in energy expenditure and disrupted feeding-related signals from leptin, ghrelin, and insulin. In obese CP patients with hypothalamic damage, increased leptin relative to BMI may cause leptin resistance, which in animal models has been correlated to bone loss. In the childhood-onset CP population, both genders had increased serum leptin levels, which correlated significantly negatively with bone mineral density (BMD) at L2–L4, and 45 % of CP women had Z-score levels ≤ -2.0 SDS. Furthermore, 75 % of those with a Z-score ≤ -2.0 SDS had hypothalamic involvement by the tumour. Insufficient estrogen and androgen supplementation during adolescence was a cause, but hypothalamic involvement with consequent leptin resistance was also strongly associated with low BMD in both genders. Further, in the childhood-onset CP population we recorded normal quality of life, which mirrors an adaptation to their present situation. Lower scores of neurocognitive performances were recorded and patients with TGTV had the lowest scores.

Disclosure: No significant relationships.

Regulation of Energy Homeostasis

S27

The Fat Mass and Obesity Gene (FTO) and Body Composition

C. Church¹, F. McMurray¹, M. Merkestein², L. Stasiak², S. Lee², J. McTaggart², G. Nicholson¹, L. Teboul¹, D. Andrew¹, F. Ashcroft², B. Cox¹

¹Mammalian Genetics Unit, Medical Research Council, Harwell; ²Dept of Physiology, Anatomy, and Genetics, Henry Wellcome Centre for Gene Function, Oxford, UK

In 2007, single nucleotide polymorphisms within the first intron of the FTO gene were associated in genome-wide association studies (GWAS) with obesity and type-2 diabetes. Subsequently, this has been replicated in many populations. Obesity is thought to be caused by increased energy intake. The FTO gene has Fe- (II-) and 2-oxoglutarate-dependent nucleic acid demethylase enzymatic activity and has recently been linked with regulation of N6-methyladenosine modification of mRNA. We have developed 3 mouse models to investigate the function of the FTO gene. We have a dominant point mutation in the C-terminal of FTO that caused reduced weight, reduced fat mass, and increased energy expenditure. In this model, growth was normal. Secondly, we constructed a conditional exon 3 knockout allele that, when globally germline deleted, showed growth restriction, reduced body weight, and significant perinatal lethality. These mice

showed altered respiratory exchange ratios (RER) and increased energy expenditure. This model is consistent with 2 other published knockout alleles and shows the importance of FTO in growth and body composition. Finally, we have made a conditional over-expression allele that exhibited increased body weight and fat mass as a result of increased food intake. This supports the human studies and a role for FTO in obesity. We are taking a number of approaches to further understand FTO function. These include using our conditional mouse alleles to test the function of the gene in specific tissues: brain, skeletal muscle, liver, and adipose tissue. This is being achieved using Cre recombinase lines driven by tissue-specific promoters. Further, we have addressed the lethality of the global germline knockout by making an adult onset global knockout using a tamoxifen-inducible ubiquitin Cre. These latter mice also showed reduced body weight and changes in body composition.

Disclosure: No significant relationships.

S28

Central Regulation of Peripheral Lipid Metabolism

R. Nogueiras

Physiology, University of Santiago de Compostela, Spain

The central nervous system (CNS) plays an essential role in the regulation of energy homeostasis. In addition to endocrine signaling and nutrient sensing, there is an important neuronal network that connects the CNS with peripheral metabolic processes. Many neuro-anatomical studies have shown that the white adipose tissue is innervated by the autonomic nervous system. For instance, specific neuronal circuits within the CNS respond by adjusting ongoing autonomic nervous system activity to a wide spectrum of organs. During the last years, several reports have demonstrated that signals from the CNS directly control the amount of fat by modulating the storage or oxidation of fatty acids in WAT and also in the liver. Importantly, some CNS pathways regulate these processes independent of food intake, suggesting that those signals possess alternative mechanisms to regulate energy homeostasis. Different neuronal circuits within the hypothalamus, such as leptin-, ghrelin-, insulin-, or GLP1-responsive neurons as well as melanocortins or NPY exert their direct actions on fatty acid metabolism in peripheral tissues. Finally, new systems that control fatty acid metabolism and adiposity continue to be discovered and have potential implications in human obesity. Dissecting the complicated interactions between peripheral signals and neuronal circuits regulating fatty acid metabolism might open new avenues for the development of new therapies for preventing and treating obesity and its associated disorders.

Disclosure: No significant relationships.

S29

Liver Estrogen Receptor as an Integrator of Metabolic and Reproductive Functions

No abstract available

S30

TRH and Other Factors of the PVN in the Regulation of Metabolic Homeostasis

C. Fekete¹, R. M. Lechan²

¹Dept of Endocrine Neurobiology, Institute of Experimental Medicine, Budapest, Hungary; ²Dept of Medicine, Division of Endocrinology, Diabetes and Metabolism, Tufts Medical Center, Boston, USA

The hypothalamic paraventricular nucleus has a critical role in the regulation of energy homeostasis by both establishing circulating levels of thyroid hormone and through effects on autonomic centers in the brainstem. Hypophysiotropic TRH neurons are located in the medial and periventricular parvocellular subdivisions of the PVN and highly responsive to changes in circulating leptin levels, the latter serving as a major signal for central regulation of the hypothalamic-pituitary-thyroid (HPT) axis. Thus, during fasting, the fall in circulating leptin levels activates orexigenic NPY/AGRP and inhibits anorexigenic α -MSH/CART neurons located in the hypothalamic arcuate nucleus.

These neurons directly innervate hypophysiotropic TRH neurons and exert opposing effects on TRH biosynthesis; AGRP and NPY markedly inhibit TRH gene expression whereas α -MSH activates TRH gene expression by regulating CREB phosphorylation. CREB also has an activating effect on hypophysiotropic TRH neurons, but the mechanism remains unknown. A direct effect of leptin on hypophysiotropic TRH neurons has also been proposed, and it may have a role in the activation of the HPT axis in association with obesity through a STAT-3-dependent mechanism. Melanocortin-responsive neurons in the PVN located in the ventral and lateral parvocellular subdivisions are involved in relaying satiety signals to brainstem autonomic centers after fasting animals have been refed. These neurons show rapid c-fos activation during refeeding at the time animals develop satiety and may use glutamate as a neurotransmitter to modulate feeding-related signals transmitted to the nucleus tractus solitarius (NTS) via the vagus nerve. Melanocortin-signaling in the PVN may also lead to the activation of other neuronal groups in the PVN including oxytocin, nesfatin, GRP-producing cells, that may contribute to the anorexic effects of α -MSH.

Disclosure: No significant relationships.

MEET-THE-EXPERT SESSIONS

MTE01

Cushing's Syndrome

No abstract available

MTE02

Treatment of NETs

G. Kaltsas

Pathophysiology, National University of Athens, Greece

Neuroendocrine tumors (NETs) are derived from multipotent cells that belong to the diffuse endocrine system characterized by specific immunohistochemical features such as the expression of markers of NE differentiation, mainly chromogranin A. According to the latest WHO classification, gastroenteropancreatic NETs (GEP-NET) include carcinoid tumors and pancreatic neuroendocrine tumors (pNET) whose incidence is rapidly increasing. According to the site of origin and degree of differentiation the biological behavior and natural history of GEP-NETs differs, necessitating different therapeutic approaches. Some GEP-NETs can secrete biologically active substances causing distinct clinical syndromes; however, the majority of CEP-NETs are non-functioning and as they are well-differentiated (grade 1–2) and slowly growing tumors present late with metastatic, mainly to the liver, disease (stage IV according to the TNM classification system). Surgery remains the best therapeutic approach for local/localized tumors (stage I–III), limited hepatic disease and difficult-to-manage functioning tumors. Treatment with long-acting somatostatin (SS) analogs is used to control the majority of functioning tumors whereas the SS analog octerotide has been shown to exert an antiproliferative effect in ileal carcinoids in a recent prospective study. Streptozotocin-based chemotherapy is active in grade-1–2 pNETs but relatively inefficient in carcinoid tumors; poorly differentiated GEP-NETs (grade 3) are temporarily responsive to cisplatin-based chemotherapy. Tumors with a high uptake of radio-labeled SS analogs can be treated with radiopharmaceuticals such as ¹⁷⁷Lutetium and ⁹⁰Yttrium; however, the efficacy of these therapies needs to be proven in phase-III trials. Recently, molecular targeted therapies with inhibitors such as sunitinib (against tyrosine kinase) and everolimus (against mTOR pathway) have shown considerable efficacy in pNETs in phase-III trials; in addition, everolimus has also shown efficacy in carcinoid tumors. Furthermore, locoregional therapies mainly against hepatic metastases, such as radiofrequency ablation and/or embolization, are extensively utilized to decrease tumor load in patients with grade-1–2 disease.

Disclosure: No significant relationships.

MTE03

Copeptin

M. Christ-Crain

Endocrinology, Diabetes and Metabolism, University Hospital Basel, Switzerland

Copeptin vasopressin is the main hormone involved in the regulation of water homeostasis and osmolality of body fluids. In addition, as a stress hormone, it is a potent synergistic factor of corticotrophin-releasing hormone in stimulating the pituitary-adrenal axis. Unfortunately, the measurement of vasopressin levels is cumbersome because of its instability and short half-life. Vasopressin derives from a larger precursor peptide along with 2 other peptides, neurophysin II and copeptin. Copeptin is released in an equimolar ratio to vasopressin and is more stable in the circulation and easy to measure. Until now it is unknown whether copeptin has a physiological function. As a diagnostic marker, copeptin shows promising

results in differentiating patients with diabetes insipidus from patients with primary polydipsia. High baseline copeptin levels (> 20 pmol/l) identify patients with nephrogenic diabetes insipidus, whereas very low baseline levels suggest complete central diabetes insipidus. Delta copeptin levels measured upon the water deprivation test help to differentiate partial central diabetes insipidus from primary polydipsia. Furthermore, preliminary data suggest that copeptin levels have some utility in the differential diagnosis of hyponatremia. As a stress hormone, it has been shown that copeptin mirrors the individual stress level more subtly than cortisol. Thereby, copeptin levels have prognostic implications in various diseases, eg, in pneumonia, heart insufficiency, myocardial infarction, or stroke. Importantly, in ischemic stroke, copeptin is the first biomarker improving the prognostic assessment of a clinical score alone. Seemingly, copeptin allows tapping an endogenous information system of our body that – through mechanisms still poorly understood – accurately assesses the severity of damage.

Disclosure: No significant relationships.

MTE04

Suprasellar Mass Lesions

V. Popovic-Brkic

Neuroendocrinology, Medical Faculty, University Belgrade & Clinical Center Serbia, Belgrade, Serbia

Mass lesions in the suprasellar region usually belong to one of the following categories: (1) congenital/developmental, (2) inflammatory/infectious, (3) neoplastic, or (4) vascular. Suprasellar masses are discovered on magnetic resonance imaging (MRI) carried out to investigate diabetes insipidus (DI) or optic chiasm compression.

1. Congenital lesions may present on MRI with an ectopic posterior pituitary. Developmental lesions in the suprasellar region are craniopharyngiomas mostly studied in children. They are embryological tumors and the diagnosis is proven by histology. Neuroendocrine dysfunction and metabolic complications before and after treatment together with recurrence of the disease are clinical challenges for the endocrinologist.
2. The most common inflammatory lesion is lymphocytic infundibuloneurohypophysitis (LINH) which presents with DI and pituitary stalk enlargement. Langerhans cell histiocytosis (LHC) is defined as a granulomatous lesion (proliferation of histiocytes) but there is an ongoing debate on whether it should be categorized as a tumor or as an inflammatory process. MRI reveals a hypothalamic mass.
3. Germ cell tumors account for 8 % of pediatric brain tumors and are associated with DI. These tumors occur in younger patients. The most common malignancies that result in suprasellar metastases are breast and lung cancers. They occur in older patients, are locally invasive, and have rapid growth.
4. Suprasellar aneurysms are rare lesions that often mimic pituitary tumors and commonly present with visual disturbances and, if not recognized, may result in catastrophic outcomes.

Clinical cases

1. Isolated childhood-onset GH deficiency and suprasellar mass in an 18-year-old boy.
2. Lymphocytic infundibuloneurohypophysitis and DI in a 12-year-old girl.
3. Somnolence, ophthalmoplegia, and DI in a 10-year-old boy.
4. DI and fever in a 42-year-old female.
5. Visual field impairment in a 62-year-old female with multinodular goiter.
6. Headache in a 61-year-old female who became drowsy, confused, and disoriented.

Disclosure: No significant relationships.

MTE05

Metabolomics in Endocrinology

S. Virtue

University of Cambridge Metabolic Research Laboratories, Institute of Metabolic Science, Cambridge, UK

The aim of this talk is to discuss how to work with systems biologists to get the most out of metabolomic studies. Perhaps most importantly, it will discuss the potential pitfalls that can occur at the experimental design and analysis stages of a metabolomics study.

In the sense metabolomic studies produce large quantities of data, they superficially resemble transcriptomic studies. However, compared to microarray analyses, the level of comprehensiveness (in terms of how much of the metabolome can be measured) and the methods for normalisation of data are far less advanced in metabolomics. This means that to extract biologically meaningful data from a metabolomic study a great deal of care must be paid to the experimental design prior to conducting the study. Secondly, methods for making biological sense of the data are still under development, making it not currently possible to use the kind of comprehensive pathway analysis methods developed for transcriptomics.

This talk will focus principally on lipidomics as an example of metabolomics. It will cover several major methodologies and discuss their advantages and limitations. Critical aspects of obtaining biologically meaningful data from lipidomic studies will be covered including experimental design. Data analysis will be also discussed focusing on how to normalise lipidomic data and appropriate statistical analyses. The talk will also discuss the inherent complexities of metabolomic studies with regard to interpretation of results – with a particular focus on the fact that many metabolites represent intermediaries in biological processes and therefore their levels can be affected by multiple synthesis and degradation steps.

MTE06

Pregnancy and Pituitary Disorders

Z. Karaca

Endocrinology, Erciyes University, Kayseri, Turkey

Pregnancy is a physiological state that has been associated with enlargement of the pituitary gland as a result of lactotroph hyperplasia. Since the placenta becomes an extra source of hormone production, pituitary and target hormone levels as well as binding globulin levels differ from the non-pregnant state. Pituitary adenomas and hypopituitarism are usually associated with infertility, so data regarding pituitary disorders during the gestational period are limited. Pituitary adenomas may pose a potential risk of tumour growth during pregnancy besides adversely affecting pregnancy by hormone secretion. The risk of tumour growth during gestation is not high for pituitary adenomas, either functional or non-functional. Prolactinomas are the most common pituitary adenomas encountered during pregnancy since the disease is common among

women of reproductive age and fertility can be easily restored after medical treatment. Acromegaly is the second most common functional pituitary adenoma seen in relation to gestation after prolactinomas. It is safe to discontinue medical treatment after confirmation of pregnancy in both GH- and PRL-secreting pituitary adenomas. An approach to these patients is summarized in **Figures 1 and 2**. Cushing's disease is rare and most cases with Cushing's syndrome are caused by adrenal adenomas, unlike in non-pregnant women. Prompt diagnosis and treatment are essential for Cushing's disease due to adverse effects of hypercortisolism on both mother and fetus. Spontaneous pregnancy is rare in patients with hypopituitarism due to altered gonadotroph functions. Ovulation induction is essential for fertility in hypogonadotropic patients. But not only gonadotropins, replacement of other deficient pituitary hormones, including GH, play an important role in the preparation of the uterus for implantation. Due to improved assisted reproductive technologies and treatment methods, the number of pregnant women with pituitary disorders seen in clinical practice is increasing which necessitates special attention.

Disclosure: No significant relationships.

MTE07

Acromegaly

P. Chanson

Endocrinology and Reproductive Diseases, Hôpitaux Universitaires Paris-Sud, Le Kremlin-Bicêtre, France

Acromegaly is related to an excessive production of growth hormone (GH) from a pituitary GH-secreting adenoma in most cases. Prevalence is estimated around 100 per million inhabitants. It is characterized by slowly progressive, acquired somatic disfigurement (mainly involving the face and extremities) and systemic manifestations. The rheumatologic, cardiovascular, respiratory, and metabolic consequences determine its prognosis. Diagnosis is confirmed by an increased serum GH concentration, insuppressible by an oral glucose load (GH levels remain upon 0.4 µg/l) and by detection of increased levels of insulin-like growth factor-1 (IGF-1) compared to age-adjusted normal values. Treatment is aimed at correcting (or preventing) tumor compression by excising the disease-causing lesion, and at reducing GH and IGF-1 levels to normal values. When surgery, the usual first-line treatment, fails to correct GH/IGF-1 hypersecretion, medical treatment with somatostatin analogs (and/or eventually with dopamine agonists) is generally used. The GH-receptor antagonist (pegvisomant) is helpful in patients that are resistant to somatostatin analogs. In patients with aggressive adenomas or when medical treatment fails to achieve normal GH/IGF-1 levels and/or when a definitive treatment is intended with the hope of interrupting medical treatment, radiotherapy can be used. Results of gamma-knife or stereotactic radiotherapy, which are now more frequently proposed, are essentially the same as the classic fractionated, conventional radiotherapy but nobody knows if it will decrease side effects of this therapeutic modality. Thanks to this multi-

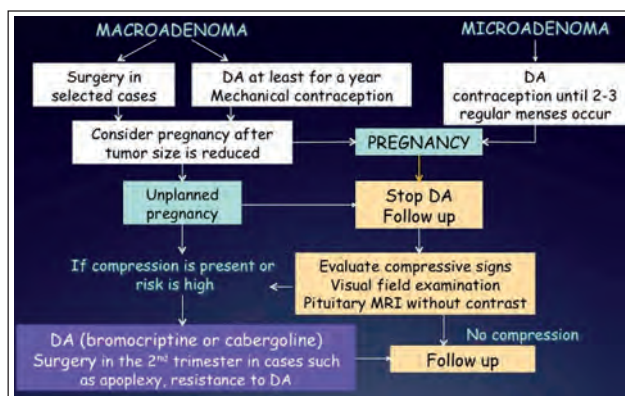


Figure 1. Z. Karaca. Approach to patients with prolactinomas at conception and during pregnancy.

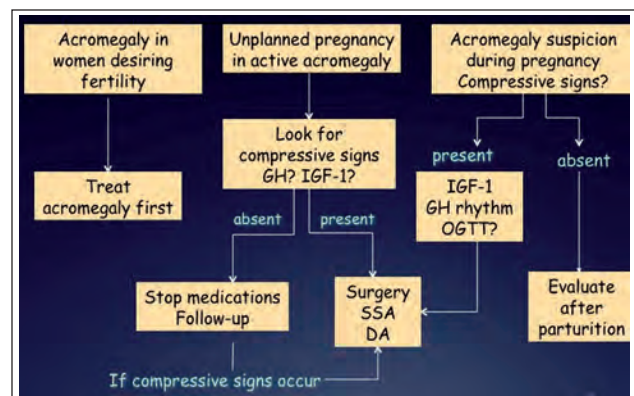


Figure 2. Z. Karaca. Approach to patients with acromegaly at conception and during pregnancy.

step therapeutic strategy, adequate hormonal disease control is achieved in most cases, allowing a normal life expectancy.

Disclosure: No significant relationships.

MTE08

Management of Pheochromocytomas

J. Mysliwiec

Endocrinology, Diabetology and Internal Diseases, Medical University of Białystok, Poland

Pheochromocytomas (PHEO) occur in about 0.1 % of patients with sustained hypertension. However, this percentage is probably underestimated as far as about half of the patients with PHEO have only paroxysmal hypertension or are normotensive. Despite this low incidence, PHEO must always be considered because if identified, they can be cured in the great majority of cases, whereas left untreated, the tumor is likely to be fatal due to catecholamine-induced malignant hypertension, heart failure, myocardial infarction, stroke, ventricular arrhythmias, or malignancy. A pheochromocytoma is usually a benign tumor typically originating from adrenal medullary chromaffin tissue but in about 15 % of cases from extra-adrenal chromaffin cells. Most PHEOs represent sporadic tumors that are usually unicentric and unilateral while familial (inherited) cases are often multicentric and bilateral. Although adrenal tumors are rarely malignant (< 10 %), the mean prevalence of metastases in extra-adrenal PHEOs exceeds 50 %. In the last decade, major advances have been made in understanding the molecular genetic basis of adrenal and extra-adrenal PHEO. As many as 1/3 of all PHEOs are inherited (much more than the 10 % originally estimated), attributable to germline mutations in at least 9 genes (NF1, RET, SDHA, SDHB, SDHC, SDHD, TMEM127, MAX, and VHL), their recognition is critical for optimal patient management. Thus, the identification of a germline mutation can predict risks of malignancy, recurrent disease, associated non-chromaffin tumors, and risks to other family members. The laboratory diagnosis of these tumors is based on the identification of excessive secretion of catecholamines and/or of their derivatives. The only curative treatment for PHEO is complete surgical resection, usually by means of a laparoscopic approach as far as it has become the standard treatment for most PHEOs even larger than 7 cm. Genetic and molecular changes involved in malignant PHEOs are targets of novel treatment approaches.

Disclosure: No significant relationships.

MTE09

Endocrine Side Effects of New Classes of Anti-Cancer Drugs

L. Bastholt

Dept of Oncology, Odense University Hospital, Denmark

Ipilimumab (IPI) is a fully human IgG1 monoclonal antibody against CTLA-4, an immunosuppressive receptor on T cells, which acts to promote an anti-tumour immune response. Phase-III, randomized controlled trials have shown improved overall survival in metastatic cutaneous melanoma [1, 2].

The blockade of CTLA-4, one of the key brake systems in our immune system, may lead to the development of toxicity, with a profile never seen before in oncology. The most common study drug-related adverse events were those classified as immune-related adverse events (irAE), which are seen in 60–70 % of the patients treated with ipilimumab, with severe (grade 3 or higher) irAEs in 10–30 % of patients. The grade of toxicity is highly dependent on the administered dose. The irAEs most often affected are the gastrointestinal tract (diarrhea and colitis) and the skin (pruritus and rash). The most frequent irAE directed against the endocrine system are noted as thyroiditis and hypophysitis.

The cornerstone in handling these patients is early detection of toxicity as being immune-related, modification of dose of IPI, and the decision whether or not to institute treatment with systemic steroids.

Therefore, education of patients and treating physicians is of utmost importance. Guidelines for handling irAEs have been developed based on the experience in the early part of the development program for IPI. Clinical studies indicate that the use of steroids in this setting does not hamper the treatment effect. Based on this it is recommended to start moderate or high-dose steroid therapy as soon as dangerous irAEs (ie, hypophysitis) or moderate/severe irAEs (ie, diarrhea) are detected.

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Disclosure: No significant relationships.

MTE10

Aggressive and Malignant Pituitary Tumors

J. Trouillas¹, G. Raverot²

¹Histologie-Embryologie Inserm U1028; ²Inserm U1028, Faculté de Médecine Lyon-Est, Hospices Civils de Lyon, Cedex, France

Pituitary tumors are generally considered as benign. However, many of them (45 %) invade the sphenoid or the cavernous sinus and some (15 %) are aggressive, with a high proliferation rate and short postoperative time before recurrence. Only tumors with systemic or cerebrospinal metastases (0.2 %) are considered as malignant. Predicting pituitary tumor behavior remains a challenge. In 2004, the WHO classification pointed out “atypical adenomas”, based on invasion and elevated indexes of proliferative markers (Ki-67, mitoses, p53) thought to correlate with aggressive pituitary tumor. Using a combination of radiological (MRI), histological, transcriptomic and comparative genomic hybridization approaches in prolactin tumors, we identified prognostic histological and molecular markers and genomic alterations, especially an allelic loss of chromosome 11, associated with prolactin tumor aggressiveness. We suggested that some “aggressive adenomas” may be non-metastatic carcinomas, thus requiring an “aggressive” therapeutic strategy. Recently, it has been published that some aggressive and malignant pituitary tumors regressed under temozolomide, a new alkylating chemotherapeutic drug used in the treatment of glioblastoma. Three independent groups, including ours, confirmed the efficacy of temozolomide treatment for some, but not all, aggressive pituitary tumors or carcinomas and revealed that the 06-methylguanine-DNA methyltransferase (MGMT) status is a poor predictor of treatment outcome. Since temozolomide treatment is not effective for all aggressive and malignant pituitary tumors, it is therefore necessary to develop new drugs. Recent data indicate that in pituitary adenomas both the Raf/MEK/ERK and PI3K/Akt/mTOR pathways are up-regulated in their initial cascade, implicating a proliferative signal derangement. So, mTOR inhibitors, which have been recently shown to have an anti-neoplastic activity in several human cancers, could be a good alternative for pituitary tumors resistant to temozolomide. The multidisciplinary management and the development of new therapies are essential for the treatment of these aggressive and malignant pituitary tumors with dramatic prognosis.

Disclosure: No significant relationships.

MTE11

Hyperprolactinemia: Pitfalls in the Diagnosis and Treatment

M. Gadelha, A. Moraes, L. Vieira-Neto

Endocrinology Section, Hospital Universitário Clementino Fraga Filho, Universidade Federal do Rio de Janeiro, Brazil

Hyperprolactinemia is the most common endocrine disorder of the hypothalamus-hypophysis axis. The biochemical diagnosis of hyperprolactinemia is straightforward, owing to the simplicity of ordering a prolactin measurement and a single determination is

frequently enough to ascertain the diagnosis. Nevertheless, its etiology comprises several physiological, pharmacological, and pathological conditions, which can often make the diagnosis a challenging task.

In an asymptomatic patient with hyperprolactinemia, the presence of macroprolactin ought to be assessed as it is a common cause of hyperprolactinemia and its routine screening could avoid unnecessary treatment. On the other hand, in a symptomatic subject, prolactin levels may be used to distinguish different etiologies. For example, in patients with prolactinomas, prolactin concentrations normally parallel adenoma volume. Macroprolactinomas present characteristically with prolactin levels superior to 200–250 µg/l. However, this association is not always verified and prolactin concentrations and tumor mass may be dissociated. The “hook effect” and cystic adenomas are the main causes for this discrepancy.

Cabergoline (CAB) is used as first-line treatment for prolactinomas, including invasive giant adenomas. In patients with visual loss, close monitoring of the visual fields is mandatory. Giant tumors are usually a treatment challenge and a multidisciplinary approach may be necessary.

In patients with medical treatment with CAB, unrecognized macroprolactinemia may explain a discrepancy between good clinical response and persistent high prolactin serum levels. Another important treatment topic refers to CAB withdrawal. This may be safely tried in selected patients after 2 years of therapy but the risk of recurrence ranges from 26–69 %. Therefore, adequate surveillance is crucial.

Disclosure: No significant relationships.

MTE12

Hyponatraemia

A. Peri

Dept of Clinical Physiopathology, University of Florence, Italy

Hyponatraemia is the most common electrolyte disorder encountered in clinical practice. Many pathological conditions may be associ-

ated with reduced serum Na⁺. A thorough diagnostic workup is always mandatory in order to define the exact aetiopathogenesis of hyponatraemia, which may have a profound effect on the therapeutic choice. A correct diagnosis relies on several issues, which should be carefully evaluated, such as the clinical history of the patient, his present condition, drug prescription(s), serum tonicity, urine osmolality, and electrolytes as well as volume status. The latter should be carefully evaluated because it is a crucial issue in selecting the best therapeutic strategy. Patients with hypovolemic hyponatraemia should be rehydrated by infusion of isotonic saline solution, whereas patients with symptomatic euvoletic or hypervolemic hyponatraemia should be treated with hypertonic (3 % NaCl) saline solution followed by fluid restriction when serum Na⁺ has reached safer levels (usually ≥ 120 mEq/l) and the patient has become asymptomatic [1]. The risk associated with hyponatraemia itself (eg, development of cerebral edema) should always be weighted against the potential risks of treatment, and in particular the development of osmotic demyelination syndrome, which may result from aggressive correction of serum Na⁺. Vasopressin receptor antagonists, also known as vaptans, represent a very promising alternative approach for the treatment of mild-moderate euvoletic and hypervolemic hyponatraemia. In Europe, tolvaptan has been approved for the treatment of hyponatraemia secondary to the syndrome of inappropriate antidiuresis (SIAD), so far. The safety and effectiveness of tolvaptan in correcting hyponatraemia have been assessed in clinical trials [2, 3] and are currently experienced in clinical practice.

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Disclosure: No significant relationships.

ORAL COMMUNICATIONS

■ Cushing's Syndrome

OC01

Dysregulation of the Hypothalamo-Pituitary Axis in Patients with Adrenal Incidentalomas: Evidence Obtained with the DEXA-CRH Test

M. Tzanela¹, V. Tsalidis², E. Margelou¹, M. Tampourlou², N. Mazarakis³, G. Piaditis³, S. Tsagarakis¹

¹Endocrinology, Evangelismos Hospital; ²Endocrinology, Athens Polyclinic; ³Endocrinology, Genimatas Hospital, Athens, Greece

Introduction Adrenal incidentalomas (AI) are a common finding in patients studied by abdominal imaging and approximately 9–17 % are found bilaterally. So far, the potential role of hypothalamo-pituitary adrenal (HPA) axis dysregulation in the pathogenesis of AI, especially of those found bilaterally, has not been addressed. The DEXA-CRH test has been previously used to detect dysregulation of the HPA axis; here, it was used to assess ACTH and cortisol responses in a large group of patients presenting with unilateral and bilateral adrenal incidentalomas.

Methods and Materials We studied 97 patients (51 with unilateral and 46 with bilateral AI) and 26 normal controls. All subjects underwent a formal low-dose dexamethasone suppression test followed by a CRH test (100 µg iv).

Results Patients with AI demonstrated statistically higher ACTH and cortisol responses to the DEXA-CRH test compared to the controls ($p < 0.05$). Although ACTH responses did not differ between patients with unilateral and bilateral incidentalomas, cortisol responses were significantly higher in patients with bilateral AI ($p < 0.05$). All patients with abnormal DEXA-CRH tests had negative pituitary MRI imaging. Patients were divided into 3 groups according to ACTH responses: group A with $\text{ACTH}_{\text{max}} < 10$ pg/ml ($n = 48$), group B with $\text{ACTH}_{\text{max}} 10\text{--}20$ pg/ml ($n = 34$), and group C $\text{ACTH}_{\text{max}} > 20$ pg/ml ($n = 15$). The proportion of patients with bilateral AI in group C was significantly higher than in group A (66.6 % vs 37.5 %; $p < 0.05$). On the contrary, more patients with unilateral AI were found in group A when compared to group C (62.5 % vs 33.3 %; $p < 0.05$).

Conclusion In conclusion, patients with AI demonstrate higher ACTH and cortisol responses to the DEXA-CRH test compared to normal subjects. This finding appears to be more common in patients with bilateral AI and provides some ground for the potential involvement of HPA dysregulation in the pathogenesis, in at least a subgroup, of AI of patients.

Disclosure: No significant relationships.

OC02

Usefulness of Prolactin IPS/P Ratio and Dominant ACTH/Prolactin Ratio in Bilateral Inferior Petrosal Sinus Sampling with Desmopressin Stimulation in Patients with ACTH-Dependent Cushing's Syndrome (CS)

Z. Belaya¹, I. Sitkin², L. Rozhinskaya¹, L. Dzeranova¹, E. Marova¹, S. Arapova¹, N. Molitvoslova¹, G. Kolesnikova¹, G. Melnichenko¹

¹Neuroendocrinology and Bone Disease; ²Radiology, The National Research Center for Endocrinology, Moscow, Russian Federation

Aim To evaluate prolactin measurement and the newly recommended dominant ACTH/prolactin normalized ratio to improve the diagnostic accuracy of inferior petrosal sinus sampling (IPSS) with desmopressin stimulation.

Methods and Materials We performed IPSS with prolactin measurement in 59 patients with ACTH-dependent Cushing's syndrome (without pituitary adenoma on MRI or adenoma < 6 mm and/or negative high-dose dexamethasone suppression test) and calculated the IPS/P prolactin ratio to the highest IPS/P ACTH ratio after desmopressin administration. 53 patients (49 with Cushing's disease and 4 ACTH-ectopic CS [all bronchial carcinoid]) were surgically treated, after which histological confirmation was given. ACTH (taken 2× before and 3× after IV administration of desmopressin 8 mkg) was measured by electrochemiluminescence assay (Roche, Cobas e601) prolactin (taken once before desmopressin administration) was measured by immunochemiluminescence assay on a Vitros ECI.

Results Patients were 36 (25–51) years old; 24-h UFC: 1903 (1278–3205) nmol/l; 9:00 o'clock ACTH: 106 (73–139); 23:00 o'clock ACTH: 79.6 (54.9–103.5) pg/ml. In 3 patients, an ACTH IPS/P ratio < 2 coexisted with a prolactin IPS/P ratio < 1.8 , helping to identify technical problems during IPSS. However, in 8 patients, prolactin IPS/P ratio was < 1.8 whereas ACTH ratio was > 2 before and/or > 3 after desmopressin administration. The reduced cut-off value for the prolactin IPS/P ratio to 1.5 was beneficial in 4 out of 8 cases. Best diagnostic accuracy for the dominant ACTH IPS/P ratio was achieved at the cut-off point 0.715 (sensitivity 93.9 %, specificity 100 %); area under the curve (AUC): 0.954 (95%-CI: 0.898–1.010) which was not statistically significantly different from ordinary IPSS with desmopressin stimulation: AUC 0.980 (95%-CI: 0.940–1.019).

Conclusion The prolactin IPS/P ratio and dominant ACTH/prolactin-normalized ratio is useful for differential diagnosis of ACTH-dependent CS in the event of an ACTH IPS/P ratio < 2 before and < 3 after desmopressin administration during routine IPSS testing.

Disclosure: No significant relationships.

OC03

Predictors of Late Recurrence of Cushing's Disease after Neurosurgery

M. Barbot¹, N. Albiger¹, F. Ceccato¹, S. Koutroumpis¹, A. Daniele¹, A. C. Frigo², F. Mantero¹, C. Scaroni¹

¹Endocrinology Unit, Dept of Medicine Dimec; ²Dept of Environmental Medicine and Public Health, University of Padova, Italy

Introduction The outcome of transsphenoidal neurosurgery (TSS) for Cushing's disease (CD) is weakened by the possibility of late recurrences. The aim of this study was to find the best predictors of relapse, focusing on desmopressin (DDAVP) and CRH tests during follow-up.

Methods and Materials 57 patients with a diagnosis of CD (mean age 36 yrs) who underwent TSS and subsequently experienced remission ($n = 24$ [R]), late relapse ($n = 15$ [LRE]) or persistent disease ($n = 18$ [PJ]). CRH and DDAVP tests were carried out 6 months after TSS; ROC curves were performed to find the threshold limit to predict recurrence.

Results Mean time to relapse was 40 months (range 8–60). ACTH increase after both DDAVP ($p < 0.0001$) and CRH tests ($p = 0.0008$) was higher in LRE patients. ACTH rise > 9 pg/ml after DDAVP and > 36.7 pg/ml after CRH test showed a respective sensitivity of 93 % and 73 % and specificity of 82 % and 76 % in discriminating patients with LRE. Combining the results of the 2 tests to evaluate if their performance improved over each test alone, the area under the curve for DDAVP+CRH was 0.95, ie, higher than each test alone but without statistic significance. Positive response to both tests resulted in a positive predictive value (PPV) of 100 %, while no response to either test resulted in a negative predictive value (NPV) of 100 %.

Conclusion CD requires a long-term follow-up because the risk of relapse recurs years after TSS. A positive ACTH response to the DDAVP test was highly predictive for later relapse when performed after TSS. Hyper-ACTH responsiveness to the CRH test might be valuable information but with the sensitivity and specificity being lower than for the DDAVP test. Even though we cannot recommend the routine performance of both tests we would underline that, when concordant, they had PPV and NPV of 100 %.

Disclosure: No significant relationships.

OC04

Normal Urinary Cortisol with LCI699 in Patients with Cushing's Disease: Preliminary Results from a Proof-of-Concept Study

X. Bertagna¹, R. Pivonello², M. Fleseriu³, Y. Zhang⁴, P. Robinson⁵, A. Taylor⁶, C. Watson⁷, M. Maldonado⁸, A. H. Hamrahian⁹, M. Boscaro¹⁰, B. M. Biller¹¹

¹Dept of Endocrinology, Centre de Référence des Maladies Rares de la Surrenale, Cochin Hospital, Faculté de Médecine Paris Descartes, Université Paris 5, France;

²Dept of Molecular and Clinical Endocrinology and Oncology, Section of Endocrinology, Università degli Studi di Napoli Federico II, Naples, Italy; ³Dept of Medicine and Neurological Surgery, Northwest Pituitary Center, Oregon Health & Science University, Portland, OR, USA; ⁴Oncology Clinical Pharmacology, Novartis Pharmaceuticals Corp, East Hanover, NJ, USA; ⁵Horsham Research Centre, Novartis Pharmaceuticals UK Limited, Horsham, UK; ⁶Translational Medicine Diabetes and Metabolism; ⁷Exploratory Development, Novartis Institutes for Biomedical Research, Cambridge, MA, USA; ⁸Clinical Development, Oncology Business Unit, Novartis Pharma AG, Basel, Switzerland; ⁹Dept of Endocrinology, Diabetes, and Metabolism, Cleveland Clinic Foundation, Cleveland, OH, USA; ¹⁰Division of Endocrinology, Polytechnic University of Marche, Ancona, Italy; ¹¹Neuroendocrine Clinical Center, Massachusetts General Hospital, Boston, MA, USA

Introduction Clinical features of the Cushing's syndrome result from chronic excess of circulating cortisol. As LCI699 potently inhibits 11 β -hydroxylase, which catalyzes the final step of cortisol synthesis, it is a potential new treatment for all forms of Cushing's syndrome.

Methods and Materials Adult patients with mild-to-severe Cushing's disease (mean of 3 urinary free cortisol [UFC] levels $> 1.5 \times$ ULN [upper limit of normal]) received oral LCI699 for 10 weeks in this proof-of-concept, open-label study. LCI699 was initiated at 2 mg bid, then escalated every 2 weeks to 5, 10, 20, and 50 mg bid until UFC normalized, whereupon this dose was maintained until the end of active treatment (day 70). Patients were monitored until day 84. Dose reduction for intolerance was permitted. UFC was assessed on the second-to-last day of each period. The primary endpoint was UFC $\leq$ ULN or a decrease from baseline at day 70 ≥ 50 %.

Results Twelve patients (25–55 years; 4 men) were enrolled and all completed the study. Baseline UFC ranged from 1.6 – $17.0 \times$ ULN. All 12 patients achieved the primary endpoint; 11 (92 %) had normal UFC levels on day 70. After treatment discontinuation, UFC was $> \text{ULN}$ in all 10 patients with measurements available at day 84. The median LCI699 doses most frequently associated with UFC normalization were 5 and 10 mg bid. Levels of the precursor, 11-deoxycortisol, generally increased during treatment and declined after day 70. Mean systolic and diastolic blood pressures were reduced from baseline by 6.8 and 5.1 mmHg, respectively. LCI699 was generally well-tolerated; the most common adverse events included fatigue (7/12), nausea (5/12), and headache (3/12). No serious drug-related adverse events were reported. ACTH levels increased during treatment; median increase from baseline was 104.0 % (range 11.9–1603.9 %). Four patients experienced study drug-related hypokalemia ($K^+ < 3.5$ mmol/l; min 3.1 mmol/l).

Conclusion LCI699 demonstrated efficacy with a satisfactory safety profile in this proof-of-concept study in Cushing's disease.

Disclosure: No significant relationships.

OC05

Long-Term Use of Pasireotide Results in Sustained Reductions in UFC and Continued Improvements in Signs and Symptoms in Patients with Cushing's Disease

R. Pivonello¹, L. van Gaal², L. R. Salgado³, J. Schopohl⁴, M. Maldonado⁵, A. Trovato⁶, G. Hughes⁵, M. Boscaro⁷

¹Dept of Molecular and Clinical Endocrinology and Oncology, Università degli Studi di Napoli Federico II, Naples, Italy; ²Dept of Endocrinology, Diabetology and Metabolism, Antwerp University Hospital, Belgium; ³General Internal Medicine Service, Hospital das Clínicas, University of São Paulo Medical School, Brazil; ⁴Medizinische Klinik IV, Campus Innenstadt, University of Munich, Germany; ⁵Clinical Development, Oncology Business Unit, Novartis Pharma AG, Basel, Switzerland; ⁶Oncology Clinical Pharmacology, Novartis Pharmaceuticals Corporation, Florham Park, NJ, USA; ⁷Division of Endocrinology, Polytechnic University of Marche, Ancona, Italy

Introduction The large, randomized study of pasireotide in Cushing's disease showed that 12-month pasireotide treatment resulted in rapid and sustained decreases in UFC and significant improvements in signs and symptoms. 24-month efficacy and safety results from an extension to this trial are reported.

Methods and Materials 162 patients with persistent/recurrent or *de novo* (if not surgical candidates) Cushing's disease and UFC $\geq 1.5 \times$ ULN received pasireotide 600 μg or 900 μg sc bid. Patients with UFC $\leq$ ULN or achieving clinical benefit at month 12 were eligible for the extension.

Results 58 patients entered the extension (median exposure 26 months). Using a last-observation-carried-forward analysis, mean percentage change (95%-CI) from baseline in UFC was -54.7 % (-71.8 to -37.6) at month 12 ($n = 58$) and -59.5 % (-68.6 to -50.5) at month 24 ($n = 58$). Among patients who completed the 24-month therapy ($n = 39$), mean percentage change (95%-CI) in UFC from baseline to month 24 was -62.7 % (-74.7 to -50.6). At month 24, 20/58 (34.5 %) patients had UFC $\leq$ ULN. Similarly, plasma ACTH and serum cortisol decreased from baseline to month 12 and were maintained to month 24. Improvements in signs and symptoms were also maintained over 24 months. Mean changes from baseline to month 24 were: SBP -11.3 mmHg (-15.0 to -7.5); DBP -7.2 mmHg (-10.4 to -3.9); weight -9.6 % (-12.1 to -7.2); BMI -9.6 % (-12.1 to -7.2). Over 24 months, common AEs were diarrhea (58.6 %), nausea (52.5 %), hyperglycemia (40.1 %), cholelithiasis (32.1 %), and diabetes mellitus (29.6 %). Mean HbA_{1c} increased from 5.8 % at baseline to 7.2 % and 6.8 % at months 12 and 24. No new patients experienced hyperglycemia AEs between month 12 and month 24.

Conclusion 24-month pasireotide treatment resulted in sustained reductions in UFC, plasma ACTH, and serum cortisol, and improvements in signs and symptoms. The safety profile was similar to that reported at 12 months. These results further support the long-term use of pasireotide for the treatment of Cushing's disease.

Disclosure: No significant relationships.

OC06

Clinical and Patient-Reported Outcomes Associated with Pasireotide in Patients with Cushing's Disease

X. Badia¹, M. Roset¹, A. Forsythe², T. M. Coles³, L. D. McLeod⁴, L. M. Nelson³, S. M. Webb⁴

¹Health Economics and Outcomes Research, IMS Health, Barcelona, Spain; ²Oncology Global Health Economics and Market Access, Novartis Pharmaceuticals Corp, East Hanover, NJ; ³Psychometrics and Health Outcomes Strategy, RTI Health Solutions, Research Triangle Park, NC, USA; ⁴Dept of Endocrinology, Hospital Sant Pau, Ciberer 747, Universitat Autònoma de Barcelona, Spain

Introduction Cushing's disease (CD) significantly impacts signs and symptoms as well as health-related quality of life (HRQoL). Pasireotide demonstrated efficacy in the phase-III study of 162 patients with active CD randomized to pasireotide 600 or 900 μg twice daily [1]. This *post-hoc* analysis assessed the effect of pasireotide on

cortisol, clinical signs and symptoms (diastolic blood pressure, weight, BMI, and depression measured using the Beck Depression Inventory II) and patient-reported outcomes in a subset of 78 patients who completed 12 months of therapy in this trial.

Methods and Materials Treatment groups were combined for analysis. Urinary free cortisol (UFC), CD signs and symptoms, and HRQoL (CushingQoL²) were evaluated. The minimal important difference (MID) was validated to be 10.1 points on the CushingQoL scale. The correlations between changes in CushingQoL and in UFC and signs/symptoms were computed. Correlation coefficients of 0.1, 0.1–0.5 and > 0.5 are considered weak, moderate, and strong associations, respectively.

Results At 6 months, 37 % of patients normalized UFC. 80 % of patients either had a reduction in diastolic blood pressure ≥ 5 mmHg (58 %), decrease in weight ≥ 7 % (42 %), or had improvement $\geq$ MID on CushingQoL (46 %) (mean $\pm$ SD 11.1 $\pm$ 15.2). Improvements in symptoms and HRQoL were maintained at 12 months. Improvement in CushingQoL was correlated ($p < 0.01$) with changes in UFC at 12 months ($r = -0.40$), and at 6 and 12 months with BMI ($r = -0.39$; -0.31), weight ($r = -0.41$; -0.32) and depression ($r = -0.54$; -0.59).

Conclusion Pasireotide leads to meaningful improvements in HRQoL in patients with CD. Improvements in UFC, weight, and depression are associated with improvements in HRQoL. The 11.1 improvement in CushingQoL score observed in this study at 6 months was comparable to the 10-point difference historically reported for patients with uncontrolled versus controlled CD by other modalities [2].

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Disclosure: No significant relationships.

OC07

Drawings Reflect a New Dimension of the Psychological Impact of Long-Term Remission of Cushing's Syndrome

J. Tiemensma¹, N. P. Daskalakis¹, E. M. van der Veen¹, S. Ramondt¹, S. K. Richardson¹, E. Broadbent², J. A. Romijn¹, A. M. Pereira¹, N. R. Biermasz¹, A. A. Kaptein³

¹Endocrinology and Metabolism, Leiden University Medical Center, The Netherlands; ²Psychological Medicine, University of Auckland, New Zealand; ³Medical Psychology, Leiden University Medical Center, The Netherlands

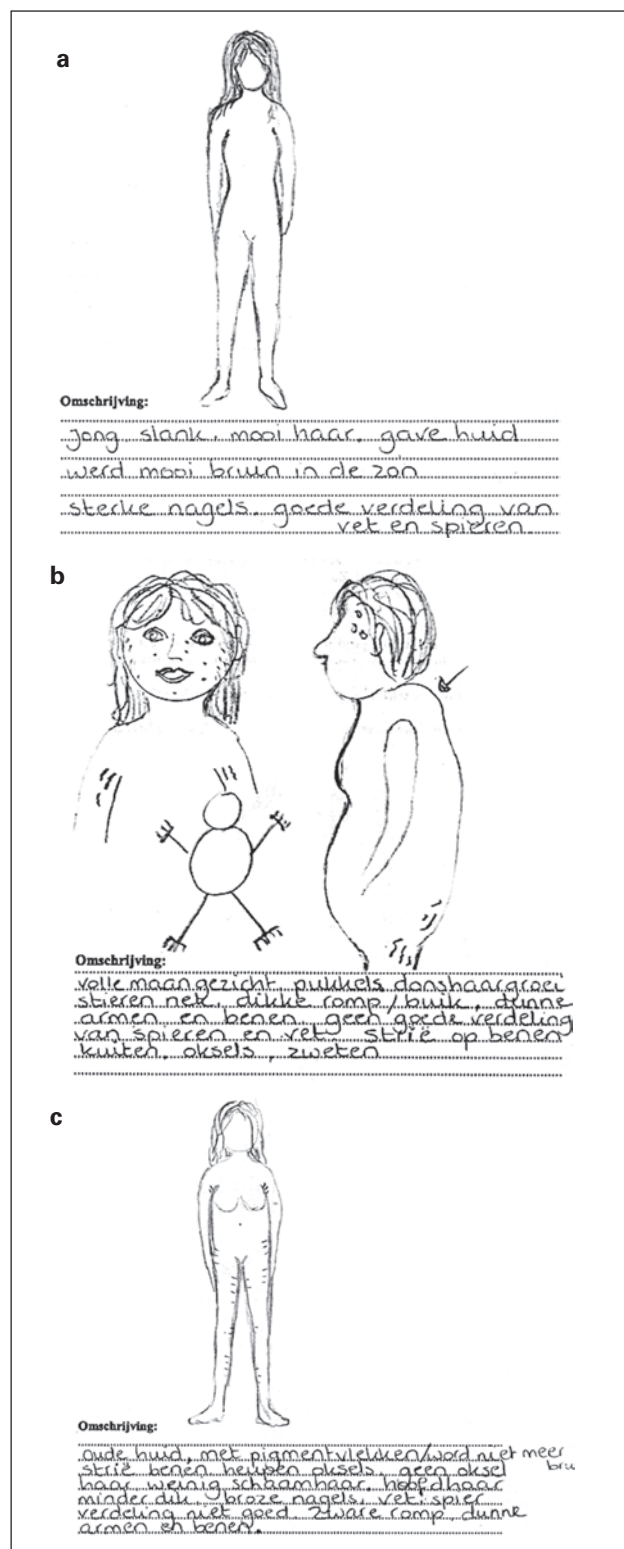
Introduction Drawings can be used to assess perceptions of patients about their disease. We aimed to explore the utility of the Drawing Test and its relation to illness perceptions, quality of life, and clinical disease severity in patients after long-term remission of Cushing's syndrome.

Methods and Materials We included 47 patients with long-term remission of Cushing's syndrome. Patients completed the Drawing Test, the Illness Perception Questionnaire-Revised, the Short-Form 36, the EuroQoL-5D, and the CushingQoL. The Cushing's syndrome severity index (CSI) was scored based on medical records.

Results Characteristics of the drawings were strongly associated with the CSI and severity ratings of health professionals (all $p < 0.02$). In addition, patients perceived a dramatic change in body size during the active state of the disease compared to the healthy state before disease. Patients reported that their body did not completely return to the original size (ie, before disease) after treatment. There were no clear associations between characteristics of the drawings and quality of life (QoL) or illness perceptions. This indicates that drawings and QoL or illness perceptions do not share multiple common properties and measure different aspects/dimensions of the disease process.

Conclusion Drawings (for examples see **Figures 3a–c**) reflect a new dimension of the psychological impact of long-term remission of Cushing's syndrome, because drawings do not share common properties with parameters of QoL or illness perceptions, but do represent the clinical severity of the disease. The assessment of drawings may enable doctors to appreciate the perceptions of patients with long-term remission of Cushing's syndrome.

Disclosure: No significant relationships.



Figures 3a–c: J. Tiemensma et al. Examples of patient drawings.

OC08

Multisystem Morbidity and Mortality in Patients with Cushing's Syndrome before and after Diagnosis and Treatment: A Nationwide Cohort Study

O. Dekkers¹, E. Horvath-Puho², J. O. Jørgensen³, S. C. Cannegieter¹, V. Ehrenstein², J. P. Vandenbroucke¹, A. M. Pereira⁴, H. T. Sørensen²

¹Clinical Epidemiology, Leiden University Medical Centre, The Netherlands; ²Dept of Clinical Epidemiology; ³Endocrinology, Aarhus University Hospital, Denmark; ⁴Endocrinology and Metabolism, Leiden University Medical Center, The Netherlands

Introduction Cushing's syndrome (CS), characterized by long-term overexposure to cortisol, is associated with hypercoagulability, insulin resistance, hypertension, bone loss, and susceptibility to infections. Our population-based cohort study aimed to elucidate long-term risk of mortality and multisystem morbidity in CS patients before and after treatment.

Methods and Materials The source population was the entire population of Denmark from 1980–2010. Data were obtained from the Danish National Registry of Patients covering all Danish hospitals and the Danish Civil Registration System. The study included all patients with a diagnosis of benign CS of pituitary and adrenal origin and an age- and gender-matched population comparison cohort. We used Cox regression for time-to-event analysis, and computed hazard ratios (HR) with 95%-confidence intervals (95%-CI).

Results The study population consisted of 343 CS patients and 34,300 matched comparison cohort members. The mortality rate was increased in CS patients (HR: 2.3; 95%-CI: 1.8–2.9). As well, patients with CS were at an increased risk for venous thromboembolism (HR: 2.6; 95%-CI: 1.5–4.7), acute myocardial infarction (HR: 3.7; 95%-CI: 2.4–5.5), stroke (HR: 2.0; 95%-CI: 1.3–3.2), peptic ulcers (HR: 2.0; 95%-CI: 1.1–3.6), fractures (HR: 1.4; 95%-CI: 1.0–1.9), and infections (HR: 4.9; 95%-CI: 3.7–6.4). About 1 % of patients had a venous thromboembolism in the first 3 months after surgery to cure their CS. This increased multi-morbidity risk was already present in the years before diagnosis; it remained equally strongly present during the first year of follow-up, but was attenuated later. Mortality and risk of myocardial infarction remained elevated during long-term follow-up.

Conclusion Our study showed a clearly increased risk of mortality and multisystem morbidity in patients with CS already before diagnosis, with little signs of early attenuation after diagnosis and treatment.

Disclosure: No significant relationships.

■ Pituitary Basic Studies

OC09

Cyclic Adenosine 3'-5'-Monophosphate (cAMP) Exerts Proliferative and Anti-Proliferative Effects in Pituitary Cells of Different Types by Activating Both CAMP-Dependent Protein Kinase A (PKA) and Exchange Proteins Directly Activated by cAMP (Epac)

E. Vitali¹, E. Peverelli², G. Mantovani¹, A. G. Lania², E. Giardino¹, P. Beck-Peccoz¹, A. Spada¹

¹Dept Medical Sciences, Università degli Studi di Milano, Milan; ²Endocrine Unit, IRCCS Istituto Clinico Humanitas, Rozzano, Italy

Introduction cAMP is implicated in the inhibition or stimulation of proliferation depending on the cell type. Consistent with the frequent expression of gsp mutations, the activation of the cAMP-PKA pathway generates proliferative signals in GH-secreting adenomas whereas this effect is not present, or even reverted in an inhibitory signal, in non-functioning pituitary cells (NFPA). Although cAMP effects were initially attributed to PKA activation, 2 cAMP-activated guanine nucleotide exchange factors (Epac1 and -2) have recently been identified as modulators of cAMP action. The aim of the

present study was to investigate the effects of cAMP in different pituitary cell types on cell proliferation and hormone secretion and to determine the specific role of PKA and Epac in mediating these effects.

Methods and Materials We tested the effects of different cAMP analogs (PKA-selective, Epac-selective or -non-selective) on cell proliferation and hormone secretion on cell lines and primary cultures from pituitary adenomas.

Results We found that non-selective cAMP analogs caused a 40-% stimulation of somatotroph cell proliferation, whereas they exerted an opposite inhibitory effect (~50 %) on lactotrophs and non-functioning pituitary cells. Conversely, cAMP analogs induced a similar increase of GH and PRL release from somatotrophs and lactotrophs, respectively. Stimulatory and inhibitory effects induced by cAMP analogs were mimicked by the PKA- and Epac-selective cAMP analogs. Moreover, by using Epac activator in combination with PKA-selective analogs, we demonstrated that they act synergistically in regulating cell proliferation and hormone secretion.

Conclusion In conclusion, we demonstrated that cAMP stimulated hormone secretion while it exerted opposite effects on the proliferation of different pituitary cell types and these effects are mediated by both PKA and Epac through the activation of different pathways, ie, CREB and Rap1, respectively.

Disclosure: No significant relationships.

OC10

Identification of Coupling Specificity between Somatostatin Receptor 5 (SST5) and G Proteins by Means of a Bioluminescence Resonance Energy Transfer (BRET) Technique: The Role of GoA Protein

E. Peverelli¹, G. Mantovani¹, E. Vitali¹, E. Giardino¹, M. Busnelli², B. Chin², A. G. Lania², P. Beck-Peccoz¹, A. Spada¹

¹Dept Medical Sciences, Università degli Studi di Milano; ²Dept Pharmacology, CNR Neurosciences Institute, Milan; ³Endocrine Unit, IRCCS Istituto Clinico Humanitas, Rozzano, Italy

Introduction In this study, we employed a novel bioluminescence resonance energy transfer (BRET) biosensor to study the coupling specificity of somatostatin receptor 5 (SST5) and its naturally occurring mutant R240W in living cells. Our previous data demonstrated that SST5 carrying the R240W mutation in the third intracellular loop maintained the ability to inhibit intracellular cAMP levels similarly to the wild type but failed to mediate the inhibition of intracellular calcium levels, GH release and cell proliferation, suggesting possible alterations in G protein coupling.

Methods and Materials The BRET biosensor may monitor the activation of G proteins in response to SST5 activation by specific agonist BIM23206. Energy transfer occurs within 2 subunits of the heterotrimeric G protein complex, the energy donor (Galpha-Luciferase) and the acceptor (Ggamma2-GFP10). G protein activation induces a structural rearrangement that increases the distance between the donor and the acceptor with a consequent decrease in the energy transfer. To detect specific G protein activation in living cells, we expressed in HEK293 cells wild-type or mutant SST5 together with different G protein (i1, i2, i3, oA, oB, or q).

Results We demonstrated that wild-type SST5 activated Gi(1–3) and Go(A and B), whereas R240W SST5 maintained the ability to activate all Gi and GoB, but not its splicing variant GoA. As expected, neither wild-type nor mutant receptors activated Gq. To investigate the role of GoA in SST5-mediated signal transduction, we transfected cultured cells from GH-secreting adenomas with SST5 and a pertussis toxin- (PTX-) resistant GoA protein. In PTX-treated cells, GoA restored the ability of BIM23206 to inhibit ERK1/2 phosphorylation and GH secretion.

Conclusion In conclusion, our data first demonstrated the coupling specificity of SST5 and revealed a crucial role for GoA in SST5 signaling in GH-secreting adenomas.

Disclosure: No significant relationships.

OC11

Phosphodiesterases and AIP-Changes in the Expression of Isoform PDE4A8 in Sporadic and AIP-Mutated Pituitary Tumours Suggest an Important Role in Tumorigenesis

G. Trivellini¹, A. Ribeiro-Oliveira Jr², P. Gabrovská¹, C. Lennox¹, M. F. Bizzì³, A. V. Giannetti³, A. B. Grossman⁴, S. V. B. Pinheiro⁵, G. Bolger⁶, M. Korbonits¹
¹Dept of Endocrinology, Bart's and The London School of Medicine, Queen Mary University of London, UK; ²Internal Medicine, Federal University of Minas Gerais; ³Service of Neurosurgery, Hospital das Clínicas, Belo Horizonte, Brazil; ⁴Oxford Centre for Diabetes, Endocrinology and Metabolism and Dept of Clinical Oncology, Churchill Hospital, University of Oxford, UK; ⁵Pediatrics, Federal University of Minas Gerais, Belo Horizonte, Brazil; ⁶Comprehensive Cancer Center, University of Alabama, Birmingham, AL, USA

Introduction Phosphodiesterases (PDEs) are a large group of enzymes responsible for the hydrolysis of cAMP and cGMP. The cAMP pathway is important in somatotroph adenoma pathogenesis, as evidenced by the effects of PRKAR1A and GNAS mutations. Germline mutations in aryl hydrocarbon receptor-interacting protein (AIP) also cause somatotroph adenomas. However, the exact mechanism as to how lack of AIP leads to pituitary tumours is not known. AIP has been shown to interact with PDEs, specifically PDE2A and PDE4A4.

Methods and Materials We studied 46 sporadic pituitary adenomas, 4 AIP-mutated somatotroph adenomas, and 9 normal autopsy pituitaries for the expression of PDE2A, PDE4A4, and PDE4A8. Conventional and Real-Time RT-PCR assays and double-labelling confocal immunofluorescence analyses using mouse monoclonal or rabbit polyclonal antibodies were performed against human PDE2A, PDE4A4, and PDE4A8 as well as pituitary hormones.

Results All 3 PDE isoforms are expressed in normal pituitary. Double-labelling revealed that PDE4A8 is co-localized to GH, PRL, and ACTH cells but at a relatively low level. While PDE2A and PDE4A4 showed similar RNA expression levels in normal pituitary and adenomas, PDE4A8 protein was over-expressed in GH-, PRL- and ACTH-secreting tumour cells in sporadic adenomas when compared to their respective cells from the normal pituitary ($p < 0.01$). However, the analysis of 4 AIP mutant GH-secreting adenomas showed that PDE4A8 was under-expressed when compared to sporadic GH-secreting tumours in 3 out of the 4 analysed mutations (p.Q164X, p.F269_H275dup, and p.R304X; $p < 0.01$).

Conclusion These novel data show that PDE4A8 over-expression, a possible compensatory mechanism in sporadic pituitary tumours, is disrupted by AIP mutations, suggesting a putative role for the PDE4A8 isoform in pituitary tumorigenesis generally, and that PDE4A8 may specifically contribute to the tumorigenic process induced by AIP mutations.

Disclosure: No significant relationships.

OC12

A Possible Role of IGF-1 in the Control of Non-Functioning Pituitary Adenoma Cell Growth

M. Minoia, E. Gentilin, M. Rossi, F. Tagliati, M. R. Ambrosio, E. degli Uberti, M. C. Zatelli
 Dept of Biomedical Sciences and Advanced Therapies, University of Ferrara, Italy

Introduction The main therapeutic approach for non-functioning pituitary adenomas (NFA) is surgery, since radiotherapy has several important side effects and medical therapy is rarely effective. Therefore, understanding the molecular pathways regulating NFA cell proliferation is crucial for future drug development. We here explore the possible role of mTOR inhibitors, everolimus and BEZ235 (which also inhibits the PI3K pathway), on the effects of Insulin-like Growth Factor-1 (IGF-1) in regulating NFA cell growth in primary culture.

Methods and Materials 28 NFA-primary cultures were incubated with or without IGF-1 in the presence or absence of everolimus and

BEZ235, which down-regulates IGF-1 signalling through the PI3K/Akt pathway. We evaluated cell viability, phosphorylation of p70S6K, and AKT phosphorylation.

Results Everolimus and BEZ235 significantly reduced NFA cell viability by 30 % and 40 %, respectively, while IGF-1 enhanced cell viability, an effect completely blocked by mTOR inhibitors. Co-incubation with an IGF-1 receptor-blocking antibody enhanced the antiproliferative effects of everolimus and BEZ235. Phosphorylation of p70S6K, a down-stream effector of mTOR in the PI3K/Akt pathway, was as well enhanced by IGF-1 and reduced by everolimus and BEZ235, indicating that IGF-1 exerts its proliferative effects by inducing this pathway, which, in turn, can be effectively blocked by mTOR inhibitors. We found that AKT phosphorylation is enhanced by IGF-1 treatment and significantly reduced by treatment in particular with BEZ235.

Conclusion In conclusion, our results indicate that IGF-1 directly stimulates NFA cell viability through its own receptor. This effect is blocked by everolimus and BEZ235, which may represent a new medical therapeutic approach for NFA.

Disclosure: No significant relationships.

OC13

PRKCD Is a Target of MIR-26A, a MicroRNA Highly Expressed in ACTH Pituitary Adenoma

E. Gentilin, F. Tagliati, E. degli Uberti, M. C. Zatelli
 Dept of Biomedical Sciences and Advanced Therapies, University of Ferrara, Italy

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

Methods and Materials The aims of this study were to (1) validate a murine ACTH-secreting pituitary adenoma cell line as a possible model to study pituitary miRNA deregulation and to (2) validate and investigate the role of potential targets of differentially expressed miRNAs. We analysed the murine AtT-20/D16v-F2 cell line deriving from a murine ACTH-secreting pituitary adenoma, and normal mouse pituitary for the expression pattern of 11 miRNA, whose expression was found to be different in human pituitary adenomas vs normal pituitary.

Results Our results showed partial agreement (50 %) between an expression trend of these miRNAs in humans and mice. In particular, we found that miR-26a has overlapping expression patterns in humans and mice, being up-regulated in adenomas vs normal pituitary. Our results confirm that the 32 untranslated region of PRKCD, a miR-26a putative target gene, is a functional target of this miRNA and provide evidence, by Real-Time PCR, that this target is transcriptionally suppressed. PRKCD, a member of the PKC subfamily, is dynamically involved in cell apoptosis in a specific stimulus manner. We observed that miR-26a inhibition led to a decrease in cell viability without increasing caspase 3/7 activity.

Conclusion These results indicate that miR-26a is over-expressed in human ACTH pituitary adenoma and can control cell viability in the AtT-20/D16v-F2 cell line by reducing PRKCD expression, playing an important role in pituitary adenoma development. Our study provides new insights into potential contribution of these RNAs to pituitary neoplastic transformation and suggests that miR-26a might be a possible target for therapeutic strategies.

Disclosure: No significant relationships.

OC14

9-CYS Retinoic Acid Induces D2R Expression Increase in an In Vitro Corticotropinoma Model

D. Regazzo¹, N. Albiger¹, L. Denaro², S. Ferasin¹, P. de Lazzari¹, F. Mantero¹, M. Paez-Pareda³, C. Scaroni¹, G. Occhi¹

¹Endocrinology Unit, Dept of Medicine Dimeid; ²Dept of Neurosciences, University of Padova, Italy; ³Max Planck Institute of Psychiatry, Munich, Germany

Introduction Retinoic acid (RA) is recognized to reduce ACTH secretion in pituitary corticotropinomas by activating the nuclear receptors RAR and RXR.

Similarly, dopamine agonists (DA) inhibit ACTH secretion in corticotroph-secreting adenomas depending on dopamine receptor type 2 (DRD2) expression. However, satisfactory results have been obtained only in a minority of patients who usually escape observation after prolonged therapy. Recently, RA-responsive elements have been described in the DRD2 promoter so that it would be useful to investigate wherever RA induces DRD2 expression in corticotropinomas.

Methods and Materials The ACTH-secreting AtT20 cell line was transfected with a vector containing the luciferase gene under control of DRD2 promoter (DRD2). A dose-dependent curve was established after 24-h 9-cis RA treatment. To confirm the direct effect of 9-cis RA on DRD2 expression, cells were co-transfected with either SMRT or N-CoR expressing vectors that are known to inhibit the RA pathway. The effect of DA and RA co-treatment on cell viability was evaluated by MTT.

Results AtT20 cells transfected with DRD2R-Luc and treated with increasing concentrations of 9-cis RA showed a dose-dependent increase of luciferase expression (1.5- and 3-fold increase at 0.1 μ M and 1 μ M, respectively). By co-transfecting with either SMRT or N-CoR we observed the loss of ability of DRD2 promoter to respond to 9-cis RA confirming that such effect is directly mediated by the activation of the RA pathway. MTT test revealed that the DRD2 expressing AtT20 showed that co-treatment reduced cell viability compared to 9-cis RA or DA treatment alone.

Conclusion DRD2 expression is increased through the activation of the RA pathway suggesting that 9-cis RA could be applied to enhance ACTH-secreting pituitary tumor sensitivity and responsiveness to DA. The information obtained by these studies could be important also for other diseases where DA are useful, eg, DA-resistant prolactinomas or Parkinson's disease, diabetes mellitus, and metabolic syndrome.

Disclosure: No significant relationships.

OC15

GnRH Release Failure in Hyperprolactinemia Is Caused by a Kisspeptin Deficiency

N. Binart¹, C. Sonigo¹, J. Bouilly¹, N. Carre¹, V. Tolle², P. Zizzar², A. Caraty³, J. Tello⁴, F. Simon⁴, R. Millar⁵, J. Young⁶

¹Faculté de Médecine Paris-Sud, Le Kremlin-Bicêtre; ²Centre de Psychiatrie et Neurosciences, Paris; ³Université de Tours Nouzilly, Tours, France; ⁴Centre for Integrative Physiology, University of Edinburgh, UK; ⁵UCT/MRC Receptor Biology Unit, University of Cape Town, South Africa; ⁶APHP Endocrinology and Reproductive Diseases, Paris Sud University, Le Kremlin-Bicêtre, France

Introduction Hyperprolactinemia is the most common cause of hypogonadotropic anovulation in women. It is related to an alteration of pulsatile GnRH secretion. This gonadotropic deficiency has been proposed to result from direct suppression of prolactin (PRL) on GnRH release but its mechanism remains unknown. Because GnRH neurons do not express unequivocally the PRL receptor and are stimulated by kisspeptin (Kp) neurons which do express PRL receptors, we hypothesized that GnRH deficiency in this condition could be due to a decrease in Kp secretion.

Methods and Materials We developed and characterized a hyperprolactinemic female mouse model mimicking the human pathology and analyzed the ability of Kp10 ip administration to restore gonadotropin secretion and cyclicity.

Results We demonstrated that a 28-day administration of PRL by micropumps significantly inhibited mouse ovarian cyclicity evaluated by vaginal smears and decreased the number of corpora lutea, reflecting ovulation rate impairment. This anovulation was related to a significant down-regulation of pituitary LHb and FSHb transcripts, indicating a gonadotropin deficiency in this model. Hypothalamic GnRH expression was not altered while there was a significant decrease of Kp mRNA and peptides (hypothalamic staining) suggesting a role of Kp decrease in GnRH release alteration in hyperprolactinemic female mice. We demonstrated that Kp-10 administration restored cyclicity, ovulation, and pituitary gonadotropin expression in hyperprolactinemic mice. Using hypothalamic explants, we also demonstrated that an in vitro decrease of GnRH release induced by PRL was rescued by Kp-10 administration.

Conclusion Together with the recent demonstration that Kp neurons express high levels of the PRL receptor, our data suggest that PRL excess acts directly on Kp neurons to suppress Kp secretion and downstream GnRH secretion. Kp neurons appear, therefore, to be the missing link between hyperprolactinemia and GnRH deficiency in mammals.

Disclosure: No significant relationships.

OC16

Bacterial Lipopolysaccharides (LPS) Stimulate the Production of Angiogenic Factors in Pituitary Tumor Cells under Basal and Hypoxia-Mimicking Conditions

K. Lucia¹, C. Scalfi¹, Y. Wu², M. Buchfelder³, D. Kuhler⁴, G. K. Stalla², U. Renner²

¹Neuroendocrinology Group; ²Clinical Neuroendocrinology, Max Planck Institute of Psychiatry, Munich; ³Neurosurgical Clinic, University of Erlangen-Nürnberg, Erlangen; ⁴Neurosurgical Clinic, Technical University of Munich, Germany

Introduction Whether transient or chronic bacterial infectious or inflammatory processes affect the initiation and progression of tumors is still controversially discussed. We have reported that subsets of pituitary tumor cells express functional toll-like receptors 4 (TLR4), the target of the bacterial cell wall component LPS. As the latter has been shown to influence angiogenic factor production in different tumor types, we have studied whether this is also the case in pituitary tumor cells.

Methods and Materials Pituitary tumor cell lines and human pituitary adenoma cell cultures were treated with LPS under basal and hypoxia-mimicking conditions (CoCl₂ treatment). Hypoxia-inducible factor 1alpha (HIF-1alpha) production was studied by Western immunoblotting. VEGF-A and IL-8 secretion were measured by ELISA.

Results Both LPS and CoCl₂ treatment strongly induced time- and dose-dependent HIF-1alpha protein production and VEGF-A secretion in Tlr4-positive folliculostellate TtT/GF pituitary tumor cells. Additive effects were observed after combined LPS/CoCl₂ treatment. In > 12 human pituitary adenoma cell cultures studied so far, VEGF-A secretion was induced by CoCl₂ whereas only about half of the adenomas responded to LPS. Interestingly, another angiogenic factor, IL-8, was already basically secreted in high amounts in most human pituitary adenoma cell cultures and could not be significantly stimulated any further by LPS or CoCl₂. Only in adenoma cell cultures with low IL-8 secretion rates LPS, but not CoCl₂ could strongly stimulate IL-8 secretion in a time- and dose-dependent manner.

Conclusion LPS could stimulate the production of angiogenic factors in pituitary tumor cells suggesting that elevated LPS levels during transient or chronic bacterial infectious processes might support pituitary adenoma progression by supporting neovascularization.

Disclosure: No significant relationships.

■ Pituitary Tumors

OC17

Safety of Long-Term Treatment with Cabergoline on Cardiac Valve Disease in Prolactinomas

R. S. Auriemma, Y. Perone, L. Ferreri, A. di Sarno, L. F. S. Grasso, R. Pivonello, A. Colao
Dept of Molecular and Clinical Endocrinology and Oncology, Università degli Studi di Napoli Federico II, Naples, Italy

Introduction Cabergoline (CAB) has been found to be associated with an increased risk of cardiac valve regurgitation in Parkinson's disease. This study aimed at evaluating cardiac valve regurgitation in patients (pts) with prolactinomas before and after 24 and 48 months of CAB.

Methods and Materials Forty pts (11 M, 29 F, aged 33.5 ± 12 years) entered the study. The cumulative doses ranged from 12–588 mg (median 48 mg) at 24 months, and 48–1260 mg (median 149 mg) at 48 months. Valve regurgitation was assessed according to the American Society of Echocardiography.

Results At baseline, the prevalence of mitral, aortic, pulmonic, and tricuspid regurgitation was 20 %, 2.5 %, 10 %, and 40 %, respectively. Grading was as following: trace: 17.5 %, 2.5 %, 10 %, and 35 %, respectively, for mitral, aortic, pulmonic, and tricuspid valves; mild: 2.5 % and 5 %, respectively, for mitral and tricuspid valves. CAB-induced PRL decreased by 94 % after 24 months compared to baseline ($p = 0.003$) and a further reduction by 75 % was seen after 48 months ($p = 0.003$), with complete normalization in all pts but one at both evaluations. After 24 months, a slight increase was found in the prevalence of trace mitral (22.5 %; $p = 0.78$) and pulmonic (12.5 %; $p = 0.89$) regurgitations and of mild aortic (2.5 %; $p = 0.89$) and tricuspid (7.5 %; $p = 0.89$) regurgitations compared to baseline. After 48 months, only the prevalence of tricuspid regurgitation was found further slightly increased (45 %) compared to 24 months ($p = 0.82$) and baseline ($p = 0.78$), with trace tricuspid regurgitation being 37.5 % ($p = 0.89$). Neither after 24 months nor after 48 months of CAB did pts develop clinically significant valve regurgitation. No correlation was found between cumulative dose and prevalence or grade of valve regurgitation at both evaluations.

Conclusion CAB does not increase the risk of significant cardiac valve regurgitation in prolactinomas after the first 4 years of treatment.

Disclosure: No significant relationships.

OC18

Complications in Transnasal Transsphenoidal Pituitary Surgery: 200 Retrospective Microscopic Cases vs the First 200 Endoscopic Cases

E. Knosp, A. Micko
Dept of Neurosurgery, Medical University of Vienna, Austria

Introduction Presently, the transsphenoidal microscopic approach is considered the standard for the surgical treatment of pituitary adenomas. In search of minimizing invasiveness and reducing morbidity and with the goal of maximizing resection by improved visualization using angled optics, the endoscopic approach re-emerged in the 1990s and since then has been adopted by many pituitary surgeons. As with any new surgical technique, the endoscopic approach has to prove to be at least equal in terms of endocrine outcome and complication rates. The aim of the present study is therefore to compare the complication rates between the microscopic and endoscopic technique in the hands of experienced pituitary surgeons.

Methods and Materials For this study, the data of 400 patients surgically treated for pituitary adenoma at the Department of Neurosurgery of the Medical University of Vienna were retrospectively reviewed from hospital archives for minor and major complications.

In 200 patients (1992–2002), the microscopic technique was used, the other 200 patients were operated endoscopically (2003–2012). Only cases with primary surgery were included. The complication rates were then compared between the 2 surgical techniques.

Results Neither the rates assessed for major complications in the endoscopic group vs microscopic group (carotid artery injury 0 % vs 0 %, loss of vision 0 % vs 0.5 %, meningitis 1 % vs 1 %, postoperative cerebrospinal fluid leak 2.5 % vs 5.5 %, epistaxis 1 % vs 3 %), nor the rates assessed for minor complications (postoperative anterior pituitary insufficiency 6 % vs 7.5 %, permanent 0.5 % vs 2 % or transient diabetes insipidus 7 % vs 3 %, sinusitis 1.5 % vs 2.5 %, hyponatremia 9 % vs 13.5 %) showed a statistically significant difference.

Conclusion Our data confirms that the endoscopic technique is an equally safe method when compared to the established microscopic transsphenoidal approach for the surgical treatment of pituitary adenomas.

Disclosure: No significant relationships.

OC19

Ocreolin™ Is an Oral Octreotide Formulation to Treat Acromegaly and Other Indications

S. Teichman¹, S. Tuvia², J. Atsmon³, S. Katz⁴, D. Pelled², I. Landau², W. Kramer⁵, C. Strasburger⁶, M. Bidlingmaier⁷, D. Kleinberg⁸, S. Melmed⁹, R. Mamluk²

¹Clinical Research; ²Research and Development, Chiasma, Inc, Jerusalem; ³Tel Aviv Sourasky Medical Center, Clinical Research Center, Tel Aviv; ⁴Regulatory and QA, Chiasma Inc, Jerusalem, Israel; ⁵Pharmacokinetics, Kramer Consulting, LLC, N. Potomac, USA; ⁶Division of Clinical Endocrinology, Charité Universitätsmedizin Berlin; ⁷Medizinische Klinik und Poliklinik IV, Klinikum der Universität München, Munich, Germany; ⁸School of Medicine, New York University, New York, NY, USA; ⁹Dept of Medicine, Cedars Sinai Medical Center, Los Angeles, CA, USA

Introduction Oral octreotide would be a desirable alternative to injectable somatostatin analogs (SRIFa) for chronic administration to patients with acromegaly and other indications. Using Transient Permeability Enhancer (TPE) technology, a novel oral formulation of octreotide (ocreolin) was developed, enabling intestinal octreotide absorption. Pharmacokinetic (PK) studies of ocreolin in healthy volunteers demonstrated that an oral octreotide dose of 20 mg yielded plasma concentrations comparable to 100 µg of subcutaneous octreotide injections. The objective of this study was the assessment of ocreolin effect on basal and stimulated growth hormone (GH).

Methods and Materials Healthy subjects were enrolled in a cross-over design. Basal GH levels were measured in 16 subjects prior to (1 h) and for 2 h following 20 mg oral octreotide dosing. GH-releasing hormone (RH)/arginine was then administered iv and GH levels measured for an additional 2 h.

Results All subjects receiving ocreolin showed blunted GH response to GHRH/arginine with a trend towards increased suppression as drug exposure increased. Compared with the non-dosed period (mean $\pm$ standard error), ocreolin suppressed mean basal GH secretion from 1.3 ± 0.4 to 0.5 ± 0.2 ng/ml (44 %; $p < 0.05$) and mean GHRH-stimulated GH secretion from 56.1 ± 6.0 to 12.2 ± 3.6 ng/ml (80 %; $p < 0.001$). Octreotide plasma levels > 0.5 ng/ml were sustained for a median duration of 8 h. Ocreolin was well-tolerated with no apparent adverse safety events. Side effects of ocreolin were comparable in frequency and severity to those observed after injected octreotide, except for absence of injection site complications.

Conclusion Oral ocreolin enables delivery of biologically active octreotide at therapeutically relevant levels. These results, as well as those obtained in prior trials of injectable octreotide and ocreolin clinical and PK data collected to date form the rationale for a global phase-III trial of ocreolin in acromegaly that is currently underway.

Disclosure: No significant relationships.

OC20

In1-Ghrelin Variant, an Aberrantly Spliced Ghrelin Variant in Human Pituitary Tumors: Presence, Functional Role, and Potential Therapeutic Value

R. M. Luque¹, M. D. Gahete¹, A. Ibáñez-Costa¹, E. Venegas-Moreno², G. F. Taboada³, L. Vieira-Neto⁴, J. Córdoba-Chacón¹, L. Lopez-Sanchez¹, L. Jiménez-Reina⁴, M. A. Japon⁵, M. Á. Gálvez⁶, P. Benito-Lopez⁶, A. Soto-Moreno², M. D. Culler⁷, M. R. Gadelha⁸, A. Leal-Cerro², R. D. Kineman⁹, J. P. Castaño¹

¹Cell Biology, Physiology and Immunology, Instituto Maimónides de Investigación Biomédica de Córdoba (IMIBIC), and Ciberobn, University of Córdoba; ²Division of Endocrinology, Instituto de Biomedicina de Sevilla, Virgen del Rocío University Hospital, Sevilla, Spain; ³Endocrinology Section, Hospital Universitario Clementino Fraga Filho, Universidade Federal do Rio de Janeiro, Brazil; ⁴Morphological Sciences, University of Córdoba; ⁵Pathology, Hospital Universitario Virgen del Rocío, Sevilla; ⁶Endocrinology Service, Reina Sofia Hospital, Córdoba, Spain; ⁷Endocrinology, Ipsen, Milford; ⁸Research and Development Division and Dept of Medicine, Section of Endocrinology, Diabetes and Metabolism, Jesse Brown Veterans Affairs Medical Center and University of Illinois at Chicago, Chicago, IL, USA

Introduction Ghrelin, the natural ligand of the growth hormone secretagogue receptor-1a (GHSR1a), is an acylated peptide mainly derived from the stomach but it is also found in other tissues like the hypothalamus and pituitary, where it fulfills diverse actions. Recently, we identified a novel human ghrelin variant, named In1-ghrelin, which is present in a variety of human tissues and showed that it may play a relevant pathophysiological role in breast cancer. We examined the presence and functional role of In1-ghrelin in normal human pituitaries and pituitary adenomas.

Methods and Materials Ghrelin system expression was analyzed in normal pituitaries and pituitary adenomas. Relationship between In1-ghrelin expression and clinical features of patients with somatotropinomas was analyzed. Pituitary adenoma cell cultures were treated and/or transfected with In1-ghrelin and Ca^{2+} kinetics, hormone secretion, and cell proliferation were analyzed.

Results In1-ghrelin is over-expressed in all types of pituitary adenomas analyzed and is expressed at higher levels than native ghrelin. In1-ghrelin treatment increases free cytosolic Ca^{2+} levels and/or hormone secretion in tumoral cells. Moreover, transfection with In1-ghrelin and treatment with In1-ghrelin peptides increased tumor cell proliferation. Noteworthy, In1-ghrelin expression is higher in non-cured acromegalic patients (treated with SA therapy) and in invasive tumors. Interestingly, expression of the In1-ghrelin variant is positively correlated with that of the GOAT enzyme and truncated GHSR1b (but not with native ghrelin or GHSR1a), suggesting that In1-ghrelin might be the primary substrate for GOAT and be functionally linked to GHSR1b.

Conclusion The In1-ghrelin variant is a potential novel element of the ghrelin family with a plausible pathophysiological role in human pituitary adenomas, wherein it can regulate signaling pathways, hormone secretion, and proliferation. Therefore, In1-ghrelin/GHSR1b/GOAT might provide a useful target to investigate the diagnostic, prognostic and/or therapeutic treatment of pituitary adenomas.

Disclosure: No significant relationships.

OC21

Immunoreactivity Score Using an Anti-SST2A Receptor Monoclonal Antibody in Pituitary Adenomas Strongly Predicts the Biochemical Response to Adjuvant Treatment with Somatostatin Analogs in Acromegaly

F. Gatto¹, R. A. Feelders², J. M. Kros³, R. van der Pas², M. Waaijers², D. Sprij-Mooij², S. J. Neggers², F. Minuto¹, S. W. Lamberts², W. de Herder², D. Ferone¹, L. J. Hofland²

¹Medicina Interna, Endocrinologia, Università di Genova, Genua, Italy; ²Internal Medicine, Division Endocrinology; ³Pathology, Erasmus Medical Center, Rotterdam, The Netherlands

Introduction The currently clinically available somatostatin analogs are the first choice for adjuvant medical treatment of acromegaly. Somatostatin receptor subtype 2 (sst2A) protein expression has been demonstrated to positively correlate with somatostatin analog treatment outcome in GH-secreting adenomas. Recently, a new rabbit monoclonal anti-sst2A antibody (clone UMB-1) has been validated as a reliable method to selectively detect sst2A in formalin-fixed tissues, with a sensitivity equivalent to in vitro receptor autoradiography, in quantifying sst2A protein levels. The objective of this study is to establish whether the evaluation of sst2A protein levels, assessed with a routine reproducible immunohistochemistry protocol using UMB-1 antibody, may predict the successful adjuvant therapy with somatostatin analogs in acromegalic patients.

Methods and Materials A total of 36 acromegalic patients were evaluated. Sst2A expression analysis was performed by immunohistochemistry in 25 and by qRT-PCR in 26 patients. Sst2A immunoreactivity was evaluated using an immunoreactivity score (IRS) which takes into account both the percentage of positive cells and staining intensity. Patients with persistent disease after surgery (25) were treated with somatostatin analogs for a median duration of 6 months. GH and IGF-1 levels before and after postoperative treatment were recorded.

Results IGF-1 normalized in 11 patients. Sst2A IRS showed a significantly positive correlation with both GH ($p = 0.039$) and IGF-1 ($p = 0.001$) suppression by octreotide, was negatively associated with lower IGF-1 levels reached after treatment ($p = 0.001$), and patients who achieved IGF-1 normalization showed significantly higher sst2A IRS compared to the not-normalized group ($p = 0.002$). A sst2A IRS ≥ 5 showed a sensitivity of 86 % and a specificity of 91 % in predicting IGF-1 normalization during adjuvant octreotide treatment.

Conclusion Sst2A IRS with UMB-1 represents a valid tool in clinical practice to identify acromegalic patients likely to respond to adjuvant therapy with the currently available somatostatin analogs.

Disclosure: No significant relationships.

OC22

MGMT Expression in Progressive Regrowing Pituitary Adenomas

S. Wolfsberger, A. Mert, A. Micko, G. Widhalm, E. Knosp
Dept of Neurosurgery, Medical University of Vienna, Austria

Introduction Currently, no effective alternative treatment exists for progressive re-growing pituitary adenomas resistant to conventional multimodality therapy. Temozolomide (TMZ) was proposed as a treatment option for pituitary carcinomas and aggressive pituitary adenomas. Responsiveness of pituitary tumors to TMZ was recently suggested to depend on the immunoexpression of O⁶ methylguanine DNA methyltransferase (MGMT). Therefore, we assessed MGMT expression in a series of patients with progressive re-growing pituitary adenomas to evaluate if TMZ might serve as alternative treatment option.

Methods and Materials Based on postoperative magnetic resonance imaging, 45 patients with progressively re-growing pituitary adenomas were allocated to a progressive re-growing (n = 24) or a

tumor-free group (n = 21) that served as a control. MGMT expression was semiquantitatively assessed by immunohistochemistry (low $\leq 50\%$, high $> 50\%$ immunostained adenoma cells) and compared between the 2 groups.

Results At the time of initial surgery, low MGMT expression was observed in 12 of 24 patients (50 %) of the study group (progressive re-growing pituitary adenomas). In the control group (tumor-free patients), only 5 of 21 patients (24 %) exhibited low MGMT expression. A comparable distribution of MGMT expression was observed in the specimens of repeat surgeries. A shorter interval to second surgery was found in patients with low MGMT expression. Additionally, our preliminary experience with single cases treated with MGMT for progressive re-growing pituitary adenomas is presented.

Conclusion The present data suggest that half of the patients with progressive re-growing pituitary adenomas exhibit low MGMT expression and may be potential candidates for treatment with TMZ. Our findings provide a rationale for the use of TMZ as an alternative treatment approach in this subgroup if conventional therapy including reoperation, radiosurgery, and radiotherapy fails.

Disclosure: No significant relationships.

■ Pituitary Clinical Studies

OC23

“Silent but not Unseen”: Accuracy of T2-Weighted Magnetic Resonance Imaging in Detecting Silent Corticotroph Pituitary Adenomas

L. Cazabat-Sage¹, M. Dupuy², A. Boulton³, M. Bernier⁴, B. Baussart⁵, L. Foubert⁶, M. Raffin Sanson¹, P. Caron², J. Bertherat⁶, S. Gaillard²

¹Endocrinology Dept, Ambroise Pare University Hospital, Université Versailles Saint Quentin, Boulogne; ²Service de Neurochirurgie; ³Service de Neuroradiologie; ⁴Service d'Anatomopathologie, Hôpital Foch, Suresnes; ⁵Service d'Endocrinologie, CHU Toulouse, Hôpital Larrey, Toulouse; ⁶Service d'Endocrinologie, Hôpital Cochin, Paris, France

Introduction Silent corticotroph adenomas (SCA) appear as non-functional macroadenomas. They are defined by an absence of clinical hypercorticism associated with positive ACTH immunostaining. Preoperatively, there are no features that allow to suspect a corticotroph subtype.

Objective To evaluate the diagnostic accuracy of MRI T2 sequences in detecting SCA preoperatively.

Methods and Materials This is a postoperative retrospective monocentric study. The preoperative MRIs of 17 patients with SCA were compared with those of 2 groups: 14 corticotroph-secreting macroadenomas (CSM) and 35 non-functional gonadotroph macroadenomas (NFGM). All pathological specimens and MRIs were reviewed. The neuroradiological interpretation was performed blind to histology.

Results Three aspects are retained in T2: multiple microcysts (MM), doubt on MM aspect, and unremarkable appearance. The MM aspect exists in 76 % of SCA (13/17), in 21 % of CSM (3/14), and in 6 % of NFGM (2/35). Aspects MM and MM doubt are observed for 88 % of SCA, 43 % of CSM, and only 17 % of NFGM. The specificity of MM aspect is 94 % for the diagnosis of SCA with a high likelihood ratio of 13.4.

Conclusion The presence of multiple microcysts in T2 MRI is a good diagnostic tool for silent corticotroph adenoma. Thus, in a non-functional macroadenoma, the presence of this MRI aspect should lead to suspect the corticotroph subtype and to finely study the corticotroph axis in search of a mild biological hypercorticism before surgery.

Disclosure: No significant relationships.

OC24

Copeptin in the Differential Diagnosis of Hyponatremia in Hospitalized Patients: The “Co-Med Study”

N. Nigro¹, B. Winzeler¹, I. Suter-Widmer¹, B. Arici², P. Schütz², C. Nicke³, A. Bock⁴, A. Huber⁵, B. Müller⁶, M. Christ-Crain¹

¹Endocrinology, Diabetology and Metabolism, University Hospital of Basel; ²Internal Medicine and Endocrinology, Kantonsspital Aarau; ³Internal Medicine, University Hospital of Basel; ⁴Nephrology; ⁵Central Laboratory, Kantonsspital Aarau, Switzerland

Introduction Hyponatremia is common and its differential diagnosis challenging. An important mechanism is adequately or inadequately secreted plasma arginine vasopressin (AVP). From a pathophysiological point of view, more or less increased plasma vasopressin levels may help in the differential diagnosis. Unfortunately, AVP measurement is cumbersome and not reliable. Copeptin is secreted in an equimolar ratio to AVP and easy to measure.

Methods and Materials We present data of 44 patients with severe hyposmolar hyponatremia (Na < 125 mmol/l) measured at presentation to the emergency department included in this ongoing, prospective, observational, multicentre study. In all patients, a standardized diagnostic evaluation was performed and patients were treated according to a diagnostic algorithm with fluid restriction or physiologic saline administration, respectively. Copeptin levels were measured and compared between different aetiologies of hyponatremia.

Results Median plasma copeptin levels in patients with primary polydipsia (n = 4) were 7.7 (IQR 3.77–21.16) pmol/l, in the 14 patients with diuretic-induced hyponatremia 13.3 (IQR 6.76–68.70) pmol/l, in patients with SIADH (n = 8) 18.3 (IQR 7.08–27.13) pmol/l, in the 7 patients with hypovolemic hyponatremia 24.5 (IQR 20.44–49.93) pmol/l and in patients with hypovolemic hyponatremia (n = 11) 53.36 (IQR 31.11–104.3) pmol/l. Overall, copeptin tended to discriminate between the various aetiologies of hyponatremia (p = 0.08). A copeptin level > 50 pmol/l allowed a diagnosis of hypovolemic or diuretic-induced hyponatremia requiring saline infusion with a specificity of 94.7 %. A copeptin/U-Na ratio > 2.4 had a specificity of 100 % for the diagnosis of hypovolemic hyponatremia.

Conclusion Plasma copeptin levels add diagnostic information in the differential diagnosis of patients with severe hyponatremia and identify a subset of patients with a clear need for saline infusion. If confirmed in a larger population, copeptin may provide a new tool for a more rapid and targeted treatment of hyponatremia.

Disclosure: No significant relationships.

OC25

Prevalence of Posttraumatic Growth Hormone Deficiency Is Highly Dependent on the Diagnostic Set-Up. Results from the Danish National Study on Posttraumatic Hypopituitarism

M. Klose¹, K. Stochholm², J. Janukonytė³, M. Andersen⁴, L. Frederiksen⁵, P. Laurberg⁶, J. S. Christiansen⁷, U. Feldt-Rasmussen⁸

¹Dept of Endocrinology, Copenhagen University Hospital, Rigshospitalet, Copenhagen; ²Dept of Internal Medicine and Endocrinology, Aarhus University Hospital; ³Dept of Endocrinology, Odense University Hospital; ⁴Dept of Endocrinology, Aalborg Hospital, Aarhus University Hospital, Aalborg, Denmark

Introduction Pituitary screening has been suggested in traumatic brain injury (TBI) based on reports on the high risk of growth hormone (GH) deficiency in particular. We aimed to describe the prevalence of GH deficiency in a cross-sectional unselected national traumatic brain injury (TBI) population.

Methods and Materials We identified 462 patients (18–65 years) hospitalized ≥ 24 h, with more than subtle TBI, indicated by loss of consciousness, amnesia, or cranial/cerebral imaging abnormalities at a Danish hospital in 2008, identified with a head trauma diagnosis from the Danish Board of Health diagnostic code registry. Dynamic assessment of GH reserve was performed in 95 % of the patients

(37 % by 2 different tests) and 89 healthy, age- and BMI-matched controls (HC). GHD was defined as peak GH < 3 mg/l in response to adequate hypoglycaemia, or below BMI-related cut-offs in PD-GHRH or GHRH-arg tests (BMI < 25: < 11 mg/l, BMI 25–30: < 8 mg/l; BMI > 30: < 4 mg/l).

Results Insufficient response to ITT was recorded in 10/200 (5 %) patients, and in 5/86 HC (6 %; $p = 0.78$), whereas insufficient response to a combined test was recorded in 77/407 (19 %) patients, and 10/89 (11 %; $p = 0.05$) HC. Compared to ITT, an insufficient combined test was more frequently recorded in patients ($p < 0.001$) than in HC ($p = 0.28$). Single testing performed in 268 patients showed 57 (21 %) insufficient test responses. Dual testing in 169 patients resulted in 2 (1 %) where both tests were insufficient, whereas 26 (15 %) were discrepant at one of 2 tests, comparable to discrepant results by dual testing in HC (14/86 [16 %]; $p = 0.9$).

Conclusion The prevalence of posttraumatic GHD was highly dependent upon the use of single or confirmed testing. The results stress the importance of a proper reference group and of stringent GH testing in cohorts with low *a priori* likelihood of GHD.

Disclosure: No significant relationships.

OC26

Changes of Myocardial Lipid Metabolism, Cardiac Function, and Insulin Secretion 6 Months after Transsphenoidal Selective Adenectomy in Patients with Acromegaly

Y. Winhofer¹, M. Krssak¹, D. Jankovic¹, P. Wolf¹, A. Gessl¹, S. Wolfsberger², S. Trattnig³, G. Pacini⁴, M. Krebs¹, A. Luger¹

¹Division of Endocrinology and Metabolism, Dept of Internal Medicine III; ²Dept of Neurosurgery; ³Dept of Radiodiagnostics, Centre of Excellence High-Field MR, Medical University of Vienna, Austria; ⁴Metabolic Unit, Institute of Biomedical Engineering, National Research Council, Padova, Italy

Introduction Patients with acromegaly have an increased risk of developing metabolic (ie, diabetes) and cardiovascular complications, which mainly account for morbidity and mortality among this patient population. Hence, the aim of this study was to assess the impact of transsphenoidal selective adenectomy on cardiac and metabolic parameters.

Methods and Materials So far, we have studied 6 patients with acromegaly (ACRO) before and 6 months after transsphenoidal adenectomy and 16 healthy controls (CON), matched for age and Body Mass Index. All participants underwent an oral glucose tolerance test (for assessment of insulin secretion/sensitivity) and cardiac magnetic resonance (for assessment of myocardial lipid content and function). In addition, pituitary hormones were measured.

Results Before surgery, ACRO had significantly increased left-ventricular end-systolic (34.8 ± 8.4 vs 22.7 ± 7.7 ml/m²; $p = 0.004$) and end-diastolic volumes (90.5 ± 11.9 vs 68.8 ± 13.4 ml/m²; $p = 0.002$), increased left-ventricular mass (91.3 ± 10.4 vs 59.9 ± 12.6 g/m²; $p < 0.0001$) and wall thickness (9.7 ± 0.6 vs 5.8 ± 1.2 mm; $p < 0.0001$) compared to CON. Postoperatively, a significant decrease in the disposition index (-0.255 ± 0.007 [−14.4 %]; $p = 0.01$), left-ventricular mass (-11.6 ± 6.4 g/m² [−12.3 %]; $p < 0.007$) and wall thickness (-1.84 ± 0.76 mm [−18.8 %]; $p < 0.006$) as well as the end-diastolic volume (-11.0 ± 10.4 ml/m² [−12.2 %]; $p < 0.05$) was observed in ACRO. IGF-1 decreased by 404.5 ± 220.0 ng/ml (−47.6 %; $p = 0.006$), while pituitary hormones and myocardial lipid content remained unchanged. In comparison to CON, left-ventricular mass and wall thickness remained increased, while cardiac volumes became comparable postoperatively.

Conclusion Our findings indicate that cardiac changes related to growth hormone excess are partially reversible after transsphenoidal adenectomy. Besides, there is actually no evidence that cardiac steatosis might play a pivotal role in the development of cardiomyopathy in patients with acromegaly. In addition, we observed postoperative improvement of insulin secretion, which may decrease the risk of developing metabolic complications.

Disclosure: No significant relationships.

OC27

High Growth Hormone (GH) Activity Is Associated with Increased Serum Estradiol and Reduced Anti-Müllerian Hormone (AMH) in Healthy Male Volunteers Treated with GH and a GH Antagonist

M. Andreassen¹, J. Frystyk², J. Faber¹, L. Ø. Kristensen¹, A. Juul³

¹Endocrine Unit, Dept of Internal Medicine O, Herlev Hospital, University of Copenhagen, Herlev; ²Dept of Endocrinology and Internal Medicine, Aarhus University Hospital; ³Dept of Growth and Reproduction, Rigshospitalet, University of Copenhagen, Copenhagen, Denmark

Introduction The GH/insulin-like growth factor-1 (IGF-1) system may modulate the gonadal axis in males.

Methods and Materials Nine healthy male volunteers (mean 37 years, range 29–49) were treated with GH for 3 weeks (1st week: 0.01, 2nd week: 0.02, 3rd week: 0.03 mg/day/kg) or a GH receptor antagonist (pegvisomant) (1st week 10, last 2 weeks 15 mg/day), separated by an 8-week wash-out. Before and after the 2 treatment periods serum testosterone, estradiol, luteinizing hormone (LH), sex hormone-binding globulin (SHBG), follicle-stimulating hormone (FSH), inhibin b, and AMH were measured.

Results During GH treatment, IGF-1 increased (median [IQR] 166 [153–223] vs 585 [557–871] µg/l; $p < 0.001$) together with estradiol (mean $\pm$ 1 SD) 77 ± 22 vs 107 ± 30 pmol/l; $p = 0.012$) and the estradiol/testosterone-ratio ($p = 0.001$). By contrast, LH (4.3 ± 1.9 vs 3.6 ± 1.7 U/l; $p = 0.053$), inhibin b (199 [114–226] vs 170 [109–197]; $p = 0.063$) and AMH (40 ± 15 vs 30 ± 9 pmol/l; $p = 0.013$) decreased. During pegvisomant treatment, there was a decrease in IGF-1 (202 [153–266] vs 105 [97–153] µg/l; $p = 0.001$) and estradiol (86 ± 27 vs 79 ± 23 pmol/l; $p = 0.038$). No significant changes ($p < 0.10$) in testosterone, SHBG, calculated free testosterone (testosterone $\times$ 100/SHBG) or FSH occurred during either of the 2 treatment regimens.

Conclusion Serum estradiol was positively associated with GH/IGF-1 activity, with increased levels during GH treatment and reduced levels during GH receptor blockage. Thus, GH/IGF-1 action may stimulate aromatase activity in vivo. The decrease in LH during GH treatment support increased estrogen activity. High serum GH/IGF-1 during puberty might chance the estradiol-testosterone ratio, thereby contributing to the development of pubertal gynaecomastia. High GH activity reduced AMH levels. AMH measurement has received increasing interest as a marker of Sertoli cell function, but the physiological function and regulating factors are widely unexplored. The present result suggests for the first time a possible influence of GH action on AMH formation in adult males.

Disclosure: No significant relationships.

OC28

Semaphorin 3A (SEMA3A) as a New Gene Responsible for the Kallmann Syndrome

J. Young¹, L. Maione², J. Bouligand³, A. Guiochon-Mante⁴

¹APHP Endocrinology and Reproductive Diseases; ²Endocrinology; ³Molecular Genetics, Pharmacogenomics, Hormonology, Paris Sud University, Le Kremlin-Bicêtre, France

Introduction The Kallmann Syndrome (KS) is defined by congenital hypogonadotropic hypogonadism and anosmia/hyposmia. The discovery of several genetic mutations responsible for KS led to the identification of signaling pathways involved in GnRH neuron migration, but the mutations so far identified account for only 30–40 % of cases. For 3 years, we have tried to identify new KS-responsible genes by using a pangenomic approach.

Methods and Materials From a cohort of 135 KS patients, we selected 61 *propositi* with no mutations in known KS genes. They were analyzed by CGH array, using Agilent 105K oligonucleotide chips with a mean resolution of 50 kilobases.

Results We found a family with autosomally dominant KS in which affected individuals carried a heterozygous deletion of 213 kb at locus

7q21.11, confirmed by real-time qPCR, deleting 11 of the 17 SEMA3A exons. SEMA3A codes for semaphorin 3A, a secreted protein involved in axonal guidance that interacts with neuropilins. Mice lacking semaphorin 3A expression have recently been reported to have a Kallmann-like phenotype. Despite the broad expression of SEMA3A in different neuronal and non-neuronal tissues, KS in this family was not associated with other associated clinical disorders suggesting that the role of semaphorin 3A in neuronal migration is restricted to the olfactory system development and GnRH neuron migration in humans. Since our discovery of SEMA3A as a cause of KS, we found additional KS patients with a point mutation in the SEMA3A gene. These mutations and phenotypes will be discussed in comparison to the mouse phenotype.

Conclusion Our findings definitely validate the role of semaphorin 3A in the development of the olfactory system and GnRH neuronal migration in humans. SEMA3A is a new gene whose loss of function is involved in KS both in humans and in rodents.

Disclosure: No significant relationships.

■ Acromegaly

OC29

High Prevalence of Acromegaly among Patients with Mandibular Prognathism: First Results of a Multimodal Screening Program

H. Schneider¹, R. Hübers², F. Brandenbusch¹, M. Reincke¹, J. Schopohl¹

¹Medizinische Klinik und Poliklinik IV; ²Orthodontics, Ludwig-Maximilians-Universität Munich, Germany

Introduction Acromegaly is often diagnosed late after disease onset. Patients with acromegaly usually develop mandibular prognathism. However, the prevalence of acromegaly in patients with mandibular prognathism is unknown. We hypothesized that the prevalence of acromegaly is increased among patients with mandibular prognathism.

Methods and Materials We established a multimodal screening program for acromegaly: patients presenting with mandibular prognathism at the Department for Orthodontics of the Ludwig Maximilian University Munich were screened for acromegaly by analysis of lateral X-ray scans of the head, a short questionnaire on acromegaly symptoms and digital pictures using face classification software that we established previously. Patients with enlarged sella were referred immediately for endocrine testing. We present the data on the evaluation of lateral X-ray scans.

Results From July 2011 through March 2012, 55 patients presented with mandibular prognathism at the Department of Orthodontics. In 44 patients, lateral X-ray scans were performed. Two of 44 patients had an enlarged sella. Both patients underwent endocrine testing and were classified as acromegalics by our face classification software. Acromegaly with elevated GH and IGF-1 and pituitary macroadenoma was confirmed in both patients. Both patients had typical symptoms of acromegaly including enlargement of hands and feet, headaches, sweating, and sleep disturbance.

Conclusion The prevalence of acromegaly is high among patients with mandibular prognathism. Possibly screening for acromegaly in this group of patients might help earlier diagnosis of acromegaly. However, more data are needed to confirm these preliminary results.

Disclosure: No significant relationships.

OC30

Circulating Patterns of Pegvisomant and Endogenous GH during Prolonged Pegvisomant Therapy in Patients with Acromegaly

M. Madsen¹, S. Fisker², U. Feldt-Rasmussen², C. Hagen³, L. Østergaard Kristensen⁴, H. Ørskov¹, J. O. Jørgensen¹

¹Dept of Endocrinology, Aarhus University Hospital; ²Medical Endocrinology, Rigshospitalet, Copenhagen; ³Dept of Endocrinology, Odense University Hospital;

⁴Dept of Endocrinology, Herlev Hospital, University of Copenhagen, Herlev, Denmark

Introduction The aim of this study was to evaluate the long-term effect of pegvisomant treatment on growth hormone levels, IGF-1 status, and serum pegvisomant.

Methods and Materials The study was a retrospective, multicentre cohort study of acromegalic patients treated with pegvisomant. 17 patients (6 females, 9 males), mean age 47.8 years (range 33.6–76.2), were included. For each patient, 4 hospital visits were identified, the first being an “active disease” visit (no treatment) and the rest obtained during pegvisomant treatment. Eight patients received a somatostatin analog in addition to pegvisomant on the last profile.

Results Mean (range) pegvisomant doses (mg/d) were 10 (10–10), 14.7 (10–15), and 13.8 (10–15) at visits 2, 3, and 4, respectively, and the duration of pegvisomant treatment was 16.0 ± 3.3 months. Serum IGF-1 changed significantly during the treatment period with the highest level at baseline and lowest level at visit 4. Changes in IGF-1 levels were negatively correlated with change in serum pegvisomant levels between visits 1, 2, and 3. GH levels increased in a dose-dependent manner during pegvisomant treatment and decreased at visit 4. Changes in growth hormone levels were positively correlated with a change in serum pegvisomant and negatively correlated with change in IGF-1 levels between visits 1, 2, and 3. A large between-subject variation in serum pegvisomant was observed. Baseline GH was a positive predictor of serum pegvisomant level, whereas gender, age, pegvisomant dose, and weight appeared not to be correlated.

Conclusion (1) Endogenous GH remains stable during long-term pegvisomant treatment in acromegalic patients. (2) Pegvisomant levels during prolonged treatment predict therapeutic outcome. (3) The large inter-individual variation in pegvisomant levels remain to be explained.

Disclosure: No significant relationships.

OC31

Pasireotide LAR and Octreotide LAR Maintain GH and IGF-1 Suppression in Patients with Acromegaly: Extension of a 12-Month Randomized, Double-Blind, Multicenter, Phase-III Study

M. Sheppard¹, M. Gadelha², M. D. Bronstein³, P. Freda⁴, F. Gu⁵, C. Shen⁶, M. Fleseriu⁷, A. J. Farrall⁸, S. J. Neggers⁹, K. Hermosillo Reséndiz¹⁰, M. Ruffin¹¹, K. Asubonteng¹⁰, A. Colao¹²

¹Medical School, University of Birmingham, Edgbaston, Birmingham, UK; ²Division of Endocrinology, Medical School, Federal University of Rio de Janeiro; ³Neuroendocrine Unit, Division of Endocrinology and Metabolism, University of São Paulo Medical School, Brazil; ⁴Dept of Medicine, Columbia University College of Physicians and Surgeons, New York, NY, USA; ⁵Dept of Endocrinology, Key Laboratory of Endocrinology, Ministry of Health, Peking Union Medical College Hospital, Beijing, China; ⁶Faculty of Medicine, School of Medicine, National Yang-Ming University, Taipei, Taiwan; ⁷Northwest Pituitary Center, Oregon Health & Science University, Portland, OR, USA; ⁸SBIRCS, Division of Clinical Neurosciences, Western General Hospital, Edinburgh, UK; ⁹Section of Endocrinology, Dept of Medicine, Erasmus University Medical Center, Rotterdam, The Netherlands; ¹⁰Oncology Clinical Pharmacology, Novartis Pharmaceuticals Corp, Florham Park, NJ, USA; ¹¹Clinical Development, Oncology Business Unit, Novartis Pharma AG, Basel, Switzerland; ¹²Dept of Molecular and Clinical Endocrinology and Oncology, Università degli Studi di Napoli Federico II, Naples, Italy

Introduction In a large, randomized, double-blind study of patients with acromegaly (n = 358), pasireotide LAR was superior to octreotide LAR in providing GH < 2.5 µg/l and normalized IGF-1 at 12

months (31.3 vs 19.2 %; $p = 0.007$). Patients could enter an extension at 12 months; results up to 19 months are reported.

Methods and Materials Medically naïve patients with active acromegaly who were post-surgical or *de novo* diagnosed with a visible adenoma on MRI and completed a 12-month therapy with pasireotide LAR 40–60 mg/28 d or octreotide LAR 20–30 mg/28 d were eligible for the extension. Patients ($n = 120$) with GH $< 2.5 \mu\text{g/L}$ and IGF-1 $\leq$ ULN at 12 months could continue on their randomized therapy.

Results Median GH and IGF-1 levels were normal at 16 and 19 months in both treatment arms in evaluable patients who continued receiving pasireotide LAR ($n = 74$) or octreotide LAR ($n = 46$) after 12 m (Table 1). The most common AEs up to the 19-month data cut-off were (pasireotide LAR and octreotide LAR): diarrhea (39.9 and 45.0 %) and cholelithiasis (29.8 and 39.4 %). Most AEs were mild or moderate. Hyperglycemia-related AEs occurred in 62.9 % (pasireotide LAR) and 25.0 % (octreotide LAR) of patients; 6 and 3 patients discontinued because of hyperglycemia-related AEs. Median glucose and HbA_{1c} levels increased in the first 3 months after pasireotide LAR initiation, remaining stable to 19 months.

Conclusion Pasireotide LAR was superior to octreotide LAR in providing GH $< 2.5 \mu\text{g/L}$ and normal IGF-1 at 12 months; GH and IGF-1 levels remained suppressed to 19 months in both treatment arms. Safety was consistent over 19 vs 12 months. These data suggest pasireotide LAR may be a new treatment option for patients with acromegaly.

Disclosure: No significant relationships.

OC32

Framingham and Agatston Scores Predict Cardiovascular Long-Term (5-Year) Risk in Acromegalic Patients

M. Ragonese¹, O. R. Cotta¹, M. L. Torre¹, A. Alibrandi², A. Albani¹, F. Ferrai¹, S. Puglisi¹, P. D. Romeo¹, F. Trimarchi¹, S. Cannavò¹

¹Section of Endocrinology, Dept of Medicine and Pharmacology; ²Dept of Statistical Science, University of Messina, Italy

Introduction Acromegaly is associated with increased risk of cardiovascular events (CE).

Methods and Materials We evaluated the occurrence of lethal and non-lethal CE over a 5-year follow-up period in 67 patients stratified on the basis of FS and AS. At enrolment (T⁰), acromegaly was active in 38 patients (group A⁰) and controlled in 29 (group B⁰).

Table 1: M. Sheppard et al. Median GH and IGF-1 levels over time in evaluable patients*. After month 12, only patients who remained on the randomized treatment are included.

	Pasireotide LAR n = 176		Octreotide LAR n = 182	
	n	Median	n	Median
GH ($\mu\text{g/L}$)				
Baseline	167	8.8	178	10.1
Month 3	164	2.3	173	2.7
Month 6	151	2.3	165	2.3
Month 9	136	1.8	157	2.3
Month 12	136	1.9	151	2.0
Month 16	64	1.0	38	0.9
Month 19	62	1.1	38	0.9
(IGF-1) $\times$ upper limit of normal				
Baseline	176	2.9	182	2.9
Month 3	164	1.1	174	1.5
Month 6	150	1.0	169	1.3
Month 9	138	1.0	159	1.3
Month 12	136	0.9	151	1.3
Month 16	66	0.7	38	0.9
Month 19	63	0.6	38	0.9

Results Mean FS was similar in the 2 groups ($p = \text{ns}$). Coronary CT was performed in 30 patients of group A⁰ and in 20 of group B⁰. Coronary artery calcifications (CAC) were detected in 10 and 5 patients ($\chi^2 = 0.4$; ns), respectively, and mean AS was not different between the 2 groups ($p = \text{ns}$). At the end of the study (T¹), a lethal CE occurred in 5 acromegalics (2 A⁰, 3 B⁰; ns) and a non-lethal CE in another 4 (2 A⁰, 2 B⁰; ns). A lethal or non-lethal CE occurred in 2 and 1 cases with FS < 10 (5.7 %), in 1 and 3 with ≥ 10 FS < 20 (33 %) and in 2 and 0 with FS ≥ 20 (66.7 %), respectively ($p < 0.05$). Coronary CT was performed in the 5 deceased patients and in 2 out of 4 cases with a non-lethal CE. A lethal or non-lethal CE occurred in 3 cases with AS < 100 (7 %) and in 4 with AS > 400 (57.1 %), respectively ($p < 0.0002$). CAC detection was significantly associated with CE overall ($\chi^2 = 15.0$; $p < 0.0001$) and predicts a lethal CE ($\chi^2 = 10.1$; $p < 0.002$) during the following 5 years. A CE occurred in 18.7 % of patients with permanent active acromegaly and in the 10.5 % of patients with stable disease control.

Conclusion In acromegalic patients, AS > 400 is associated with an increased risk of lethal CE, while very high FS is associated with reduced life expectancy regardless of disease control.

Disclosure: No significant relationships.

OC33

Effect of Different Therapies for Acromegaly on Survival

F. Bogazzi¹, A. Colao², G. Rossi³, M. Lombardi¹, C. Urbani¹, C. Sardella¹, A. Iannelli⁴, I. Scattina¹, L. Manetti¹, S. del Sarto¹, R. Pivonello², L. F. S. Grasso², I. Lupi¹, R. S. Auriemma², G. Lombardi², E. Martino¹

¹Dept of Endocrinology and Metabolism, University of Pisa; ²Dept of Molecular and Clinical Endocrinology and Oncology, Università degli Studi di Napoli Federico II, Naples; ³Unit of Epidemiology and Biostatistics, Institute of Clinical Physiology, National Research Council; ⁴Dept of Neurosciences, University of Pisa, Italy

Introduction Acromegalic patients have increased mortality, which reduces if the disease is controlled. However, a comparison of the effects of different therapies on mortality, including somatostatin analogues (SSA) as primary therapy or as adjuvant to adenomectomy, is unknown.

Methods and Materials Mortality was analyzed in 438 consecutive acromegalic patients from 1966–2009. Patient mortality was compared to that of the general population using the standard mortality ratio (SMR). Predictive factors for mortality and the effect of different therapies on survival were evaluated by Cox regression analysis.

Results 20 patients (4.5 %) died during the study period. The age- and sex-adjusted SMR was 0.70 (95%-CI: 0.43–1.08). Death occurred in 2.4 % (adenomectomy), 2.6 % (adenomectomy plus somatostatin analogues [SSA]) and 11.4 % (SSA alone) patients. Hazard risk (HR) was higher in patients receiving SSA (4.448; 95%-CI: 0.962–20.572; $p = 0.056$) than in all patients submitted to pituitary neurosurgery (adenomectomy and adenomectomy plus SSA); in particular, the increased HR was observed in diabetic patients (22.295; 95%-CI: 0.938–530.106; $p = 0.055$) when compared to non-diabetic subjects (0.482; 95%-CI: 0.011–21.737; $p = 0.707$). On the contrary, HR of patients receiving SSA following adenomectomy (0.322; 95%-CI: 0.044–2.355; $p = 0.264$) did not differ from that of cured patients by pituitary adenomectomy. Multivariate Cox regression analysis revealed that, in the whole population, both general risks factors (age and the physical status at diagnosis) and specific factors for acromegaly (macroadenoma, hypopituitarism, and uncontrolled disease) predicted death. The most compromised patients at diagnosis had higher mortality ($p = 0.001$), which occurred even though acromegaly was controlled.

Conclusion Therapies of acromegaly and its comorbidities have lowered mortality to the level of the general population; SSA following pituitary adenomectomy was comparable to curative neurosurgery on survival; whether SSA as primary medical therapy reduces mortality in diabetic patients is currently questionable and requires further studies.

Disclosure: No significant relationships.

OC34

Treatment Modalities in Acromegaly: Different Effects on Glucose Metabolism and Adipose Tissue Inflammation

N. C. Olarescu¹, T. Ueland², K. Godang³, R. Lindberg-Larsen⁴, J. O. Jørgensen⁵, J. Bollerslev⁶

¹Faculty of Medicine, University of Oslo; ²Research Institute for Internal Medicine;

³Section of Specialized Endocrinology, Oslo University Hospital, Rikshospitalet, Oslo, Norway; ⁴Dept of Dermatology; ⁵Dept of Endocrinology, Aarhus University Hospital, Denmark

Background Active acromegaly is associated with insulin resistance and evidence of inflammation in adipose tissue, but these features may respond differently depending on the treatment modality.

Aim To test the hypothesis that different treatment modalities (transphenoidal surgery [TS], somatostatin analogues [SA], and pegvisomant [PEGV]) induce concordant changes in body composition but discordant effects on glucose metabolism and inflammatory markers.

Methods and Materials We prospectively analyzed a group of 37 patients (18 female, 19 male) with active acromegaly treated with either TS (n = 14), SA (n = 16), or PEGV (n = 7). Body composition, glucose metabolism (glucose, insulin, HOMA-IR), and selected adipokines (leptin, HMW-adiponectin, MCP-1, and VEGF) were measured before and after therapy. Further, in vitro effects of GH/IGF-1 on human mature subcutaneous (SCA) and visceral (VA) adipocytes were investigated.

Results Body fat increased and IGF-1 decreased to the same extent in all 3 groups. However, changes in glucose metabolism differed with treatment. Fasting glucose decreased in the TS group (p = 0.01) and showed an increasing tendency (p = 0.08) in the SA group. Insulin and HOMA-IR decreased in both the TS and SA groups (p < 0.01), while the PEGV group showed no changes in variables of glucose metabolism. VEGF and MCP1 decreased significantly in the TS group only (p = 0.04), while HMW adiponectin increased with PEGV treatment only (p = 0.03). Changes in VEGF levels correlated positively with changes in HMW adiponectin (r = 0.41; p = 0.013), insulin (r = 0.39; p = 0.020), and HOMA-IR (r = 0.40; p = 0.017).

In vitro studies revealed that VEGF and MCP-1 expressions were significantly increased in human mature SCA and VA by GH, but not IGF-1.

Conclusion (1) Glucose metabolism and indices of inflammatory markers improve after treatment of acromegaly despite a concomitant increase in adipose tissue mass; (2) the therapeutic effect does to some extent depend upon the treatment modality; (3) the direct effect of GH on the expression of VEGF and MCP-1 in human adipose tissue in vitro merits further studies.

Disclosure: No significant relationships.

OC35

Canadian Acromegaly Registry: Outcome of Treatment in 649 Patients

S. Vallette¹, S. Ezzat², E. Ur³, C. Chik⁴, A. Imran⁵, S. van Uum⁶, J. Rivera⁷, G. Caspar-Bell⁸, O. Serri¹

¹Endocrinology, CHUM, University of Montreal; ²Endocrinology, University of Toronto;

³Endocrinology, University of British Columbia, Vancouver; ⁴Endocrinology, University of Alberta, Edmonton; ⁵Endocrinology, University of Halifax; ⁶Endocrinology, University of Western Ontario, London; ⁷Endocrinology, McGill University, Montreal; ⁸Endocrinology, University of Saskatchewan, Canada

Introduction To evaluate the quality of care of patients with acromegaly across Canada.

Methods and Materials Retrospective analysis of clinical, biochemical, and treatment outcome data of 649 patients with acromegaly (males: 50.7 %; females: 49.3 %) followed from 1980–2011 in 8 tertiary-care centers from 6 Canadian provinces.

Results In the past 2 decades, referrals for acromegaly have increased from 202 to 303 cases. At the time of diagnosis, diabetes

was found in 28 %, hypertension in 37 %, and sleep apnea in 33 % of cases. Initial IGF-1 levels but not GH levels were correlated with the presence of co-morbidities. Higher IGF-1 levels were associated with diabetes, hypertension, and sleep apnea (p = 0.001). 79 % of patients had macroadenomas of which 36 % were invasive, whereas 21 % had microadenomas. 89 % of patients underwent surgery and 22 % received radiotherapy. Radiotherapy was less used in the past 15 years (16 % of patients) in comparison to 45 % in 1980–1994 (p < 0.0001). Somatostatin analogues (SSA) were used in 61 % of patients, either as adjuvant or primary treatment (15 % of patients); cabergoline and pegvisomant were used as adjuvant treatment alone or in association with SSA in 17 % and 10 %, respectively. Remission after surgery alone was observed in 27 % of patients. Multimodal therapy achieved remission (defined biochemically by normal IGF-1 for age) in 70 % of patients. Patients in remission had lower initial GH (p = 0.04) and IGF-1 levels (p < 0.0001). Hypopituitarism was present in 23 % of patients, cancer in 8.5 %, and mortality was observed in 5.4 %.

Conclusions (1) Referral for acromegaly management to specialized centers in Canada has increased over time and radiotherapy use has declined. (2) At diagnosis, higher IGF-1 level, but not GH, was associated with co-morbidities and was an independent predictor of biochemical outcome. (3) Remission of acromegaly was attained in 70 % of patients with multimodal therapy.

Disclosure: No significant relationships.

OC36

Aging of Newly Diagnosed Acromegalic Patients: Data from the Liège Acromegaly Survey (LAS)

P. Petrossians¹, S. Zacharieva², P. Chanson³, S. J. Neggers⁴, A. Colao⁵, A. Hulting⁶, B. Delemer⁷, T. Brue⁸, V. Hana⁹, G. K. Stalla¹⁰, F. Minuto¹¹, M. Jaffrain-Rea¹², D. Carvalho¹³, C. Fajardo Montañana¹⁴, A. Daly¹, A. Beckers¹

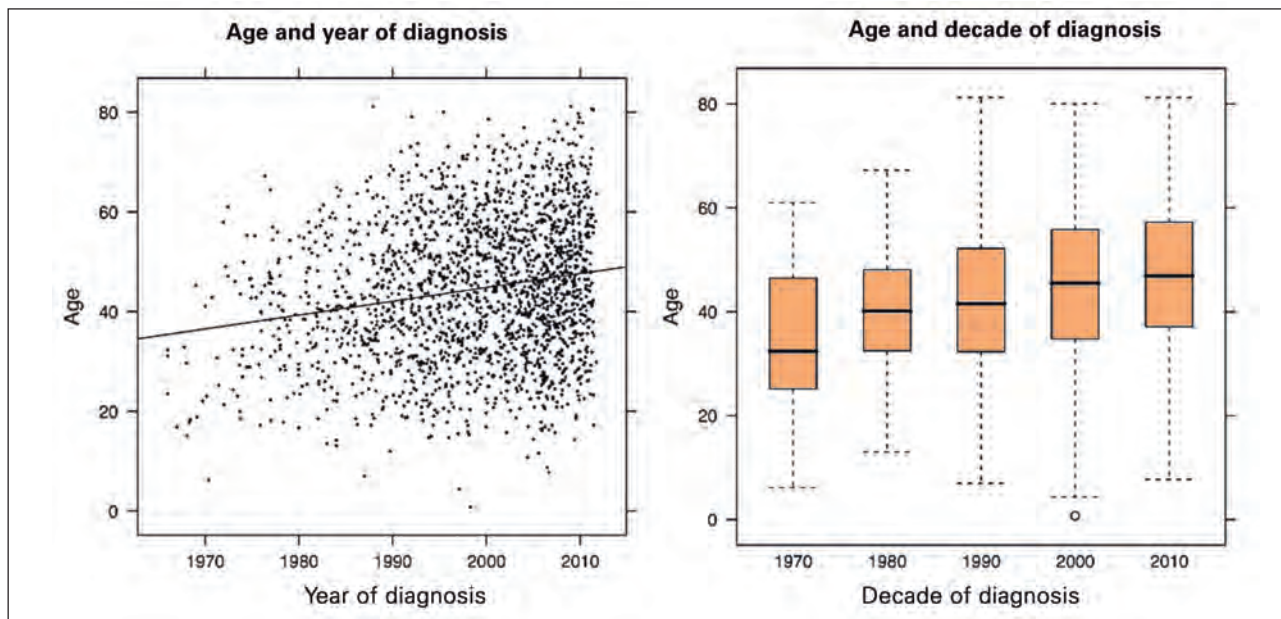
¹Endocrinology, CHU de Liège, Belgium; ²Clinical Centre of Endocrinology and Gerontology, Medical University, Sofia, Bulgaria; ³APHU Endocrinology and Reproductive Diseases, Paris Sud University, Le Kremlin-Bicêtre, France; ⁴Dept of Medicine, Section of Endocrinology, Erasmus University Medical Center, Rotterdam, The Netherlands; ⁵Dept of Molecular and Clinical Endocrinology and Oncology, Università degli Studi di Napoli Federico II, Italy; ⁶Dept of Molecular Medicine and Surgery, Karolinska University Hospital, Stockholm, Sweden; ⁷Endocrinology, CHU de Reims; ⁸Dept of Endocrinology, Centre de Référence des Maladies Rares d'Origine Hypophysaire, Hôpital de la Timone, Marseilles, France; ⁹3rd Dept of Medicine, First Medical Faculty, Charles University, Prague, Czech Republic; ¹⁰Clinical Neuroendocrinology, Max-Planck-Institute of Psychiatry, Munich, Germany; ¹¹Dept of Internal Medicine, University of Genoa, Genova; ¹²Dept of Experimental Medicine, University of L'Aquila, Italy; ¹³Dept of Endocrinology, Diabetes and Metabolism, Centro Hospitalar S. João, Porto, Portugal; ¹⁴Dept of Endocrinology, Hospital Universitario de la Ribera, Alzira, Spain

Introduction The Liège Acromegaly Survey (LAS) is a database of nearly 3000 patients from 14 European centers. In a previous study focusing on patients from Liège (n = 290), we discovered that the age of the population of newly diagnosed patients is rising. With the database extended to 13 other centers, we tried to verify the reality of this trend with a bigger population.

Methods and Materials 2929 patient (1331 males, 1598 females) data from 14 European centers have been recorded in our database. Patients were included with no limitation on the date of diagnosis. In some centers, data were available on patients diagnosed before the eighties.

Results Median age at diagnosis for the whole population was 44.9 years (**Figure 4**). When looking at the patients' age and the year of diagnosis, a trend toward an aging of the population was observed. This trend was more apparent when looking at patients' age by decade (**Figure 5**). In one center, however, (Bulgaria, 815 patients), this trend was not observed although the age of patients diagnosed since 2010 has increased.

Conclusion The increase in the age of newly diagnosed acromegalic patients suggests that with time an increasing number of older patients gets diagnosed and that these patients would have not been diagnosed in previous decades. This evolution could be due to a



Figures 5 and 6: P. Petrossians et al. Trends in age at diagnosis of acromegaly.

better medical follow-up in the general population. One center (Bulgaria) presented this trend only for the last years, which could be related to the change in the local political and economical situations.

Disclosure: No significant relationships.

Metabolism/Translational

OC37

Changes in Central Peptide YY Expression during Embryonic and Postnatal Development and Its Regulation under Different Energy States in Adult Mice

C. Gelegen Van Eijl¹, A. Leiter², H. Al-Qassab³, I. Evans², E. Irvine³, M. Claret³, F. Andreelli⁴, D. Withers³, K. Chandarana³, R. Batterham³

¹Biophysics, Imperial College London, UK; ²Medicine, University of Massachusetts Medical School, Worcester, USA; ³Medicine, University College London, UK; ⁴Diabetology, University Pierre et Marie Curie, Paris, France

Introduction Peptide YY is a gut-derived satiety signal. PYY-expressing neurons have been identified in the hindbrain of river lamprey, rodents, and primates. Despite this high evolutionary conservation, little is known about PYY neurons. In this study, we investigated changes in central PYY expression in mice during the embryonic and postnatal periods and adulthood under different energy states.

Methods and Materials Brainstems and hypothalami were dissected from embryos, postnatal pups, and adult mice in *ad libitum* fed state and following acute starvation, prolonged caloric restriction, and bariatric surgery. Gene expression experiments were undertaken on RNA extracted from brain tissues. Brains were sectioned for immunohistochemistry (IHC) and *in situ* hybridization (ISH). Levels of the PYY1-36 and PYY3-36 isoforms in brainstem extracts from *ad libitum* fed and 24-hour fasted adult mice were evaluated using HPLC and RIA.

Results In adult mice, PYY neurons were identified in the gigantocellular reticular nucleus (Gi) region of the brainstem. In both hypothalamus and brainstem, PYY mRNA was present at E9.5, peaked at P2 and then decreased by 70 % in adult brainstem, while there was no hypothalamic expression by P21. Double IHC on hypothalamic sections from neonatal and adult mice showed co-localization of PYY and vimentin staining. In contrast to the circulation, PYY1-36 was the predominant isoform in the *ad libitum* fed

state. Following a 24-hour fast the amounts of PYY1-36 and PYY3-36 isoforms were similar. Brainstem PYY expression decreased significantly by acute starvation, prolonged caloric restriction, and bariatric surgery.

Conclusion Our findings suggest a role for brainstem PYY in the regulation of energy homeostasis. In addition, hypothalamic expression pattern of PYY and its co-localisation with vimentin suggest a role of PYY in hypothalamic development and neurogenesis.

Disclosure: No significant relationships.

OC38

Deletion of MECP-2 Increases Body Weight by Altering the Transcriptional Control of the Proopiomelanocortin Gene

R. Torres-Andrade, R. Moldenhauer, J. Soto-Covasich, M. Rios-Silva, F. Parada-Flández, B. Kerr

Molecular Physiology, Centro de Estudios Científicos, Valdivia, Chile

Introduction The methyl-CpG binding protein (MECP2) is a transcriptional regulator encoded by the MECP2 gene. MECP2 represses the expression of genes by binding to methylated CpG residues in the DNA, but it is also able to activate gene expression through an association with CREB1. Mecp2-hypomorphic mice exhibit defective body weight regulation and conditional deletion of Mecp2 in Sim1-expressing cells of the paraventricular nucleus induces an increase in body weight. Moreover, Mecp2 mutation, the major cause of the Rett Syndrome, has been associated with altered body weight. However, the molecular mechanism underlying the defective body weight regulation observed in absence of a fully functioning Mecp2 allele has not been elucidated.

Methods and Materials To determine the mechanism through which Mecp2 regulates body weight, we weekly analyzed the phenotype exhibited by Mecp2-null mice and the pattern of hypothalamic gene expression was evaluated at 8 weeks of age. We also determined the expression of transcription factors activated by leptin-signaling and the leptin-induced response on food intake and Pomc expression.

Results The results showed that Mecp2-null mice displayed an increase in body weight since 7 weeks of age, which is accompanied by an increased amount of adipose tissue. To determine the molecular causes of this phenotype, we evaluated the expression of several hypothalamic genes and we found an altered expression of genes and proteins associated to leptin-signaling without any change in the

basal expression of the leptin-signaling target, Pomc. However, when mice were treated with exogenous leptin, Mecp2-null mice failed to induce the increase in Pomc expression and decrease in food intake observed in wild-type mice.

Conclusion The results obtained show that the absence of Mecp2 produces obesity and an inadequate response to peripheral metabolic signals on Pomc expression and food intake. Therefore, Mecp2 is a key factor that controls body weight balance in mice.

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OC39

Hypothalamic Ceramide Levels Regulated by CPT1C Are Involved in Orexigenic Effects of Ghrelin

S. Ramírez¹, L. Martins², J. Jacas¹, P. Carrasco¹, H. Muley¹, M. Pozo¹, D. Serra³, F. G. Hegardt³, R. Nogueiras², C. Diéguez², M. López², N. Casals¹

¹Basic Sciences, School of Medicine and Health Sciences, Universitat Internacional de Catalunya, Sant Cugat del Vallés; ²Cimur, Universidad Santiago de Compostela; ³Biochemistry and Molecular Biology, Universitat de Barcelona, Spain

Introduction Ceramides are part of a big group of lipids that have been related with obesity pathologies. In fact, we have recently demonstrated that ceramides are involved in the satiating response to leptin in the arcuate nucleus of the hypothalamus (ARC). We also have demonstrated that the brain-specific carnitine, palmitoyltransferase 1 (CPT1C), which is located in the endoplasmic reticulum of neurons, enhances the synthesis of ceramides and blocks the anorexic response to leptin. Nevertheless, it is unknown whether CPT1C and ceramides are involved in the hypothalamic response to the orexigenic hormone ghrelin.

Methods and Materials To answer this question we performed intracerebroventricular (icv) administrations of ghrelin to WT and CPT1c KO mice and analyzed food intake and the molecular signaling pathway in the medio-basal hypothalamus (MBH).

Results Results show that the orexigenic effects of ghrelin, as well as associated changes in neuropeptides (NPY and AgRP) and their transcription factors (pCREB and FoxO1) are blocked by the lack of CPT1C. Then, we analyzed whether ceramide levels were regulated by ghrelin administration. Our findings show that icv ghrelin injection produced a rise in ceramide levels within 30 minutes in WT mice, an effect that was not observed in CPT1c KO mice, suggesting that CPT1C is regulating the increase in ceramide levels in response to ghrelin. Finally, we demonstrate that the inhibition of the *de novo* synthesis of ceramides by myriocin blocks the orexigenic effects of ghrelin and that the administration of C6-ceramide to CPT1C KO mice restores it in these mice.

Conclusion Our data indicate that ghrelin increases hypothalamic ceramide levels through CPT1C and that this effect is necessary for its orexigenic action.

Disclosure: No significant relationships.

OC40

The Central Melanocortin System Participates in the Mechanisms Involved in Anorexia Associated with Anxiety

P. Tavares¹, B. Borges¹, R. Soares², F. Guimaraes³, J. Antunes-Rodrigues¹, L. Elias¹

¹Physiology, School of Medicine of Ribeirão Preto; ²Psychology and Education, FFCLRP; ³Pharmacology, School of Medicine of Ribeirão Preto, University of São Paulo, Ribeirão Preto, Brazil

Introduction Anxiety is an emotional state with psychological components and neuroendocrine changes and becomes pathological when it is disproportionate to the trigger, causing eating disorders, such as anorexia. The pathogenesis of anorexia in anxiety is not well established, but studies have pointed out the involvement of the central melanocortin system. Thus, in the present study we evaluated

the involvement of the melanocortin 4 receptor (MC4R) on hypophagia induced by anxiety.

Methods and Materials Male Wistar rats (200–230 g, n = 5–8/group) had a cannula implanted into the lateral ventricle. Six days after, at 8 am, half the animals were subjected to restraint for 2 hours and half were maintained in the cage. At 4 pm, food was removed from all experimental animals. The next day, at 8 am, animals received central injections of vehicle or SHU9119 (MC4R antagonist, 0.1 µg/µl, in 5 µl) and 30 minutes later food was reoffered and food intake was determined at 2, 4, 8, and 24 hours. Another set of animals submitted to the same protocol was subjected to the elevated plus maze for 5 minutes and 90 minutes after the animals were transcardially perfused under anesthesia for collection of hypothalamic tissue and immunohistochemical Fos localization.

Results The rats subjected to restraint showed hypophagia and reduced body weight gain compared to controls. This effect was reversed by injection of MC4R antagonist. The groups that received SHU9119 had the highest number of entries and increased time spent in open arms. The exposure to maize did not increase Fos expression in the paraventricular nucleus in control animals, but Fos-immunopositive neurons were increased in rats treated with SHU9119.

Conclusion The MC4R antagonist reduces anorexia caused by anxiety induced by restraint, suggesting that activation of this receptor may contribute to hypophagia associated with anxiety.

Disclosure: No significant relationships.

OC41

Intracerebroventricular Insulin Infusion Increases Triglyceride Secretion in Vivo

T. Scherer¹, C. Lindtner², J. O'Hare², L. Zielinski², L. Scheja³, C. Buettner²

¹Division of Endocrinology and Metabolism, Dept of Internal Medicine III, Medical University of Vienna, Austria; ²Medicine, Mount Sinai School of Medicine, New York, NY, USA; ³Dept of Biochemistry and Molecular Biology II, University Medical Center Hamburg-Eppendorf, Germany

Introduction Hepatosteatosis and dyslipidemia are both hallmarks of the metabolic syndrome and plasma triglycerides (TG) correlate with insulin resistance (IR). Hepatic lipogenesis is increased in the IR state, thus TG secretion must not be too low in order to prevent steatosis. Insulin action comprises direct effects on peripheral organs, eg, liver and adipose tissue, but also of indirect effects that are mediated via the central nervous system. Systemic insulin decreases very low-density lipoprotein (VLDL) production by the liver, yet it is unknown whether brain insulin can independently regulate TG flux.

Methods and Materials To study the role of brain vs systemic insulin signaling on hepatic TG secretion, we performed tyloxapol studies in male Sprague Dawley rats during systemic or isolated brain hyperinsulinemia. The latter was accomplished by infusing insulin or vehicle for 4 hours into the 3rd ventricle (ICV) or the mediobasal hypothalamus (MBH).

Results ICV insulin infusion increased hepatic VLDL secretion compared to controls (2.59 ± 0.28 vs 1.80 ± 0.2 µmol/kg/min; $p = 0.039$). To the contrary, a hyperinsulinemic euglycemic clamp decreased TG flux (0.85 ± 0.05 µmol/kg/min; $p = 0.020$). Plasma lipid profiling in these animals demonstrated that ICV insulin increased the accumulation of TG-associated FAs such as palmitate and oleate (+30 %; $p < 0.05$). Of note, insulin infusion into the MBH had no effect on VLDL flux vs controls (1.85 ± 0.32 µmol/kg/min vs 1.71 ± 0.32 µmol/kg/min; $p = 0.773$), indicating that a different brain region is integrating the central insulin signal. Conversely, mice that lack the neuronal insulin receptor had reduced hepatic TG flux, which was again assessed by tyloxapol infusion studies (154 ± 6 vs 126 ± 12 µmol/kg/h; $p = 0.038$).

Conclusion While systemic hyperinsulinemia and isolated loss of neuronal insulin signaling both suppress TG flux, ICV insulin infusion acutely increases VLDL secretion. We speculate that the elevated TG production in obesity and diabetes may be due to preserved central insulin effects in a presently unknown brain region.

Disclosure: No significant relationships.

OC42

PTP1B Inhibition Reverses Leptin Resistance and Desensitization of Hypophagia during Long-Term Exposure to Endotoxin

B. Borges, R. Rorato, E. Uchoa, P. Marangon, J. Antunes-Rodrigues, L. Elias
Physiology, School of Medicine of Ribeirao Preto, University of Sao Paulo, Ribeirao Preto, Brazil

Introduction Repeated exposure to endotoxin induces desensitization of hypophagia and an inability of leptin to phosphorylate STAT-3 protein in the hypothalamus. Leptin resistance is induced by the feedback inhibitors of leptin-signaling pathway SOCS3 and tyrosine phosphatase 1B (PTP1B). Reduction in the phosphatase SHP-2 also participates in the development of leptin resistance. Since we observed that SOCS3 is unlikely to underlie the resistance to leptin in the endotoxin tolerance, we investigated the participation of PTP1B and SHP2 in this response.

Methods and Materials We induced endotoxin tolerance, injecting repeated (6LPS) LPS doses (100 µg/kg) in comparison with single (1LPS) treatment. 6LPS, but not single LPS rats, showed higher expression of PTP1B and reduced expression of SHP2 in the medio-basal hypothalamus. We also evaluated the effects of icv injection of PTP1B inhibitor (0.3 nM or 3.0 nM/rat in 5 µl) on food intake and leptin-induced pSTAT3 hypothalamic expression in animals under single or repeated LPS stimulation.

Results Hypophagia and body weight loss induced by 1LPS were potentiated by PTP1B inhibitor. Interestingly, tolerance to hypophagia was reversed by PTP1B inhibitor in the 6LPS group. PTP1B inhibition also reversed the ability of leptin to induce STAT3 phosphorylation in the hypothalamus of animals from 6LPS group, with no additional effect on saline or single LPS group.

Conclusion The present data suggest that PTP1B signaling is essential for the development of leptin resistance after long-term endotoxin exposure. Reduction of SHP2 expression also contributes to this hypothalamic leptin resistance. The present results contribute to the understanding of mechanisms of leptin resistance in obesity, known to be associated with low-grade chronic inflammatory state.

Disclosure: No significant relationships.

OC43

Diminished ACTH Sensitivity and Efficacy in Obese Premenopausal Women

E. Roelfsema¹, H. Pijl¹, J. D. Veldhuis², D. M. Keenan³

¹Endocrinology, Leiden University Medical Center, The Netherlands; ²Endocrine Research Unit, Mayo Clinic, Rochester; ³Statistics, University of Virginia, Charlottesville, VA, USA

Introduction The ACTH-cortisol axis in premenopausal women is activated and associated with decreased ACTH potency, estimated by relating ACTH and cortisol pulse masses.

Methods and Materials Recently, a new and more accurate method for constructing the endogenous dose-response relationship was introduced which is based on the relation between ACTH concentrations and associated cortisol secretion rates within cortisol bursts. We hypothesized that the endogenous dose-response relation between ACTH and cortisol in obesity is changed, leading to diminished responsiveness. We studied 25 obese premenopausal women and 16 normal-weight premenopausal women and estimated ACTH and cortisol secretion rates and analytical dose-response estimates of endogenous ACTH efficacy (maximal cortisol secretion), dy-

namic ACTH potency, and adrenal sensitivity (slope term) from 24-h ACTH-cortisol profiles.

Results The initial potency (negative logarithm) was -7.83 ± 0.75 (mean $\pm$ SEM) in obese women, and -10.14 ± 1.08 in lean women ($p = 0.10$) and the corresponding values for the recovery phase were -26.62 ± 2.21 and -36.67 ± 1.66 ($p = 0.004$). The sensitivity (curve slope) amounted to 0.468 ± 0.05 in obese women and 0.784 ± 0.09 in normal-weight women ($p = 0.004$). The efficacy (maximal value) was 17.6 ± 4.9 nmol/l/min in obese women and 26.3 ± 3.8 nmol/l/min in normal-weight women ($p = 0.009$). No differences were present for basal secretion rate, inflection point and EC_{50} values. Short-term treatment with bromocriptine to improve the metabolic state and to diminish leptin levels did not change the dose-response curve. This was also found during treatment with acipimox, which decreased ACTH levels, but did not change the ACTH-cortisol relationship.

Conclusion The ACTH-cortisol relation in obesity in women is characterized by decreased sensitivity and potency, thus explaining non-elevated serum cortisol concentrations in view of increased plasma ACTH levels. The mechanistic pathways involved are not yet clarified, but do not involve ACTH secretion *per se* or leptin.

Disclosure: No significant relationships.

OC44

Short-Term Treatment with the GLP-1 Agonist Liraglutide Alters Hedonic Perception of High-Calorie Food Items: An fMRI Study in Healthy Volunteers

C. Tugendsam¹, F. Fischmeister², D. Kasses¹, A. Geissler², K. Schindler¹, M. Hilbert², G. Vila¹, M. Wurmig², M. Riedl¹, M. Pinter², K. Tugendsam³, S. Trattnig⁴, A. Luger¹, R. Beisteiner²

¹Division of Endocrinology and Metabolism, Dept of Internal Medicine III; ²Study Group Clinical fMRI, Dept of Neurology, Medical University of Vienna; ³Institute of Science and Technology, Vienna; ⁴Dept of Radiodiagnostics, Centre of Excellence High-Field MR, Medical University of Vienna, Austria

Introduction In the combat against the world-wide obesity epidemic, new therapeutic options are urgently needed. One option currently under study is the use of glucagon-like peptide-1 (GLP-1) agonists, in which weight loss was noted as a side effect when used as an antidiabetic medication. We explored the effects of a short-term, low-dose treatment with the GLP-1 agonist liraglutide on healthy male subjects.

Methods and Materials The study was designed as a double-blind, placebo-controlled crossover trial on 16 male healthy volunteers with a dose of 0.6 mg liraglutide (or saline as placebo) over the course of 3 days. On the following day – after a 12-hour fast – functional Magnetic Resonance Imaging (fMRI) measurements using a visual paradigm with high- and low-calorie food items were performed; afterwards *ad libitum* food intake at a breakfast buffet was recorded. Subjective feelings of appetite, hormonal and stress parameters were also analyzed.

Results We found increased activity in the dorsolateral prefrontal cortex, indicating enhanced cognitive control and a reversal of the response patterns from high to low calorie food items in the caudate/nucleus accumbens and anterior cingulate cortex regions, indicating higher attractiveness of low-calorie food under liraglutide. At a subsequent breakfast buffet, subjects tended to eat less, and subjective ratings of appetite were reduced and ghrelin dynamics enhanced.

Conclusion GLP-1 agonists might achieve their weight-reducing properties by facilitating healthier food choices.

Disclosure: No significant relationships.

POSTER PRESENTATIONS

POSTER SESSION 1 – THURSDAY

■ Acromegaly

P001

Influence of Standard Glucocorticoid Replacement Therapy on Bone Turnover and Bone Mineral Density in Men with Active Acromegaly

M. Stojanovic, D. Miljic, S. Pekic, M. Doknic, V. Popovic
Neuroendocrine Unit, Clinic for Endocrinology, Diabetes and Metabolic Diseases,
Faculty of Medicine, University of Belgrade, Serbia

Introduction Acromegaly is frequently complicated by fragility vertebral fractures, often despite normal bone mineral density (BMD). This observation still needs elucidation. Some acromegalic patients develop hypopituitarism and require glucocorticoid replacement. The impact on bone metabolism is of concern with glucocorticoid treatment. The aim of this pilot study was to examine the effect of hydrocortisone replacement therapy on bone turnover markers and BMD in men with active acromegaly.

Methods and Materials 20 male patients were included, 45.42 ± 3.85 yrs old, BMI 26.99 ± 0.89 kg/m², with active acromegaly (elevated IGF-1 and GH levels), with an average duration of 3.0 ± 9.2 yrs. All patients were eugonadal or on stable testosterone replacement. BMD was measured using dual X-ray absorptiometry (DXA-Hologic) at the lumbar spine (L1–L4) and femoral neck (FN), and bone mineral content (BMC) was assessed by using a whole-body DXA scan. Osteocalcin (OC) and beta-cross-laps (BCL) were assayed as markers of bone formation and resorption.

Results Four patients received hydrocortisone replacement in standard doses, and 16 were without HPA axis deficiency and received no glucocorticoids. No significant difference was observed in either BMD L1–L4 (1.090 ± 0.168 g/cm² vs 1.114 ± 0.050; *p* = 0.953) or BMD FN (0.890 ± 0.079 g/cm² vs 0.945 ± 0.033; *p* = 0.705) or BMC (2.89 ± 0.32 vs 3.08 ± 0.16 kg; *p* = 0.354) between the 2 groups. There was no change in bone turnover markers (OC: 84.36 ± 23.00 vs 50.33 ± 9.17 ng/ml; *p* = 0.163; BCL: 1657.75 ± 529.73 vs 1258.38 ± 243.69; *p* = 0.477) in acromegalic patients receiving hydrocortisone replacement.

Conclusion Standard hydrocortisone replacement in acromegalic patients did not affect bone turnover markers and BMD in a limited number of male patients with active acromegaly. Further investigation on larger series is pending.

Disclosure: No significant relationships.

P002

Levels of IGFBP-3 in Patients with Acromegaly Receiving Various Kinds of Therapy

A. Holikova, Z. Halimova
Neuroendocrinology, The Center for The Scientific and Clinical Study of Endocrinology, Tashkent, Uzbekistan

Introduction IGFBP-3 is one of the markers of somatotrophic function. We studied IGFBP-3 levels in untreated patients and those receiving different kinds of therapy.

Methods and Materials 48 patients with GH-secreting pituitary adenoma referred to the CSCSE were studied. Mean age 41 ± 19 years, disease duration 1–23 years. Patients were divided into 4 groups (12 in each): group 1: untreated patients, group 2: undergone surgery (ST; transnasosphenoidal pituitary adenomectomy), group 3: receiving X-ray therapy (XRT; gamma therapy for hypothalamic-pituitary area), and group 4: receiving medicamentous therapy by

dopamine agonists (DA; parlorel, bromergon, bromocriptin) at a dose of 7.5–12.5 mg/day regularly.

Results Results showed high basal GH levels in patients with newly revealed acromegaly (61.78 ± 17.7 mU/l) and in the DA group (61.47 ± 12.1 mU/l), rather low levels in patients after XRT (13.4 ± 2.2 mU/l) and normal GH levels (3.4 ± 1.8 mU/l) in patients after ST. IGFBP-3 levels in patients varied from 13,287–39,694 ng/ml with an average of 2465.5 ± 3211.1 ng/ml. Comparative analysis of IGFBP-3 levels in patients receiving various kinds of therapy showed that the lowest IGFBP-3 levels were revealed in patients after radical tumor removal (13,287 ± 15,151 ng/ml), and the highest in group of patients with XRT (39,694 ± 10,790 ng/ml). Patients having undergone ST had higher IGFBP-3 levels (13,287 ± 860 ng/ml) despite normal levels of GH and IGF-1. At the analysis of correlation between GH and IGFBP-3 in groups, a positive correlation in operated patients (*r* = 0.69), receiving XRT (*r* = 0.63), and in the group of patients with DA (*r* = 0.61), and a weak one in newly revealed patients (*r* = 0.42) was found. A positive correlation was between IGF-1 and IGFBP-3 in operated patients (*r* = 0.74) and in the DA group (*r* = 0.64). A weak correlation was revealed in patients with newly diagnosed acromegaly (*r* = 0.44), and in patients having undergone XRT (*r* = 0.56).

Conclusion The definition of IGFBP-3 has clinical value as it can remain high after subsidence when GH and IGF-1 levels are normal.

Disclosure: No significant relationships.

P003

Immunological Characteristics of Acromegaly Depending on the Disease Activity

M. Dudina, S. Dogadin, A. Savchenko
Internal Disease, Krasnoyarsk State Medical University, Krasnoyarsk, Russian Federation

Introduction Growth hormone (GH) and the insulin-like growth factor-1 (IGF-1) axis play an important role in the functioning of the immune system. It is assumed that an excess of growth factors in acromegaly leads to immunological disturbances that depend on the disease activity.

Objective To study the immune status and functional activity of immune cells in patients with active acromegaly and acromegaly in remission (AR).

Methods and Materials A total of 38 active acromegalics and 9 in AR, mean age 50.4 ± 12.58 years, were examined. GH and IGF-1 were measured by ELISA. The level of CD3⁺, CD4⁺, CD8⁺, CD16⁺, CD19⁺, CD25⁺, and C95⁺-lymphocytes were determined by indirect immunofluorescence. The concentrations of Ig A, M, and G were quantified in gel immunodiffusion. The functional activity of blood neutrophils was detected by chemiluminescence (CL).

Results Patients with active acromegaly and AR have statistically significant (*p* < 0.05) higher levels of CD8⁺, CD16⁺, CD19⁺, and CD25⁺-cells and decreasing IgM (*p* < 0.01). In active acromegaly, the increase of CD4⁺-lymphocytes (*p* < 0.05), decrease of CD3⁺-cells (*p* < 0.05) and CD4⁺/CD8⁺ (*p* < 0.01) were observed. CD95⁺-cell increase was typical for patients with AR. Compared to active acromegaly the number of CD25⁺-cells increased in the AR group (*p* < 0.05). Patients of both groups had reduced time-to-maximum CL index (*p* < 0.01), increased the curve CL area (*p* < 0.05). The increase of the maximum intensity CL index (*p* < 0.05) was noted in AR. The increase of all induced CL indexes (*p* < 0.05) was observed only in active acromegaly.

Conclusion Active acromegaly is characterized by the accumulation of a pool of mature T-lymphocytes and high capacity of neu-

trophilic granulocytes for phagocytosis. AR differs by appearance the increasing number of proapoptotic CD95⁺-cells and regulatory T-lymphocytes, and, also, high activity of phagocytic reactions.

Disclosure: No significant relationships.

P004

Fractionated Stereotactic Radiotherapy in Patients with Acromegaly

A. Balderrama-Soto¹, S. Moreno Jiménez², M. A. Celis-López², N. Velasco-Cortez², A. García Garduño², S. P. Pérez-Reyes³, L. Portocarrero-Ortiz¹

¹Neuroendocrinology; ²Radiosurgery; ³Neurosurgery, Instituto Nacional de Neurología y Neurocirugía M.V.S., Distrito Federal, Mexico

Introduction Acromegaly is associated with increased morbidity and mortality. Transsphenoidal microsurgery is the treatment of choice; however, 10–50 % of patients do not enter remission after surgery and 20 % experience recurrence of acromegaly after surgery. Linear accelerator- (LINAC-) based stereotactic radiation therapy is a treatment alternative.

Methods and Materials 20 patients (12 females, mean age 40 ± 2.4 years) with acromegaly were treated with fractionated stereotactic radiotherapy (FSRT) (dose 59.9 Gy, 25–27 fractions) from January 2003 to December 2008. 15 (75 %) had undergone transsphenoidal adenotomy. Anyone had access to medical therapy. The patients were followed prospectively based on a pre-defined protocol that included Goldman visual field examination, magnetic resonance imaging (MRI) of the sella, and pituitary hormone testing at 3, 6, and 12 months, and then yearly. Acromegaly was defined as in remission when serum IGF-1 levels were within the normal age-adjusted range and serum GH was suppressed at less than 1 µg/l after the oral glucose tolerance test, biochemically controlled if mean GH concentration < 2.5 µg/l and sex- and age-adjusted IGF-1 < 1.3× the upper limit of normal and discordant results if patients had normal GH suppression and abnormal IGF-1 or insufficient GH suppression and normal IGF-1.

Results Of the 20 patients, 14 continue follow-up over 7 years. Three (21 %) patients had biochemical remission, 4 (28 %) patients were biochemically controlled, 5 (36 %) had discordant results, and 2 (14 %) with uncontrolled acromegaly. MRI of the pituitary showed stable or reduced volumes of the remaining tumor tissue in the 14 patients, the volumes of pituitary adenoma decreased by a mean of 40 %. Of the 17 patients, 4 (23 %) had complete hypopituitarism and 8 (47 %) partial hypopituitarism.

Conclusion FSRT seems an effective and safe treatment of acromegaly.

Disclosure: No significant relationships.

P005

Acromegaly Associated with an AIP Mutation but without an Overt Pituitary Adenoma. A Case Report

J. Dal¹, C. Gravholt¹, M. Gaustadnes², J. O. Jørgensen¹

¹Endocrinology; ²Molecular Medicine, Aarhus University Hospital, Denmark

Introduction Germline mutations of the AIP gene are found in about 50 % of patients with familial acromegaly. These patients are predominantly young presenting with large tumors and high GH levels. We present a case of gigantism with an AIP mutation in the presence of only moderate GH elevations and without evidence of a macroadenoma.

Results The patient was referred to us from his GP at the age of 27 with suspected acromegaly. The patient's aunt was diagnosed with acromegaly at the age of 37 and treated with surgical removal of a 1-cm large pituitary adenoma. The patient presented with characteristic symptoms and signs of gigantism (Height: 2.08 m, weight: 118 kg). The diagnosis was confirmed by a GH profile during an OGTT (nadir GH: 0.9 µg/l, mean GH levels: 1.3 ± 0.3 µg/l) and a serum IGF-1 level of 352 µg/l (80–265 µg/l). No pituitary adenoma was evident on MRI and a whole-body PET-CT (GA-68-DOTANOC

tracer) did not indicate an ectopic source. Genetic screening for isolated familial somatotropinoma (IFS) revealed a c.911G>A, P.Arg304Gln mutation in the AIP gene. Family screening revealed 6 persons with the AIP mutation, 4 healthy mutation carriers and the 2 persons with acromegaly.

Conclusion This case is unusual for 2 reasons: (1) only moderately elevated GH and IGF-1 levels and (2) no evidence of a pituitary adenoma. We speculate that this is due to a previous pituitary apoplexy resulting in the shrinkage of the tumor and declining GH secretion at the time of diagnosis. Our case underlines that testing for AIP mutations should also be considered in individual cases despite the absence of a macroadenoma and/or pronounced biochemical disease activity.

Disclosure: No significant relationships.

P006

Improved Biochemical Control in Patients with Acromegaly after Switching from Octreotide LAR to Pasireotide LAR: Crossover Extension of a Randomized, Double-Blind, Multicenter, Phase-III Study

A. Colao¹, M. Fleseriu², M. D. Bronstein³, P. Freda⁴, F. Gu⁵, C. Shen⁶, M. Gadelha⁷, A. J. Farrell⁸, S. J. Neggers⁹, K. Hermosillo Reséndiz¹⁰, M. Ruffin¹¹, Y. Chen¹⁰, M. Sheppard¹²

¹Dept of Molecular and Clinical Endocrinology and Oncology, University of Naples Federico II, Italy; ²Northwest Pituitary Center, Oregon Health & Science University, Portland, OR, USA; ³Neuroendocrine Unit, Division of Endocrinology and Metabolism, University of São Paulo Medical School, Brazil; ⁴Dept of Medicine, Columbia University College of Physicians & Surgeons, New York, NY, USA; ⁵Dept of Endocrinology, Key Laboratory of Endocrinology, Ministry of Health, Peking Union Medical College Hospital, Beijing, China; ⁶Faculty of Medicine, School of Medicine, National Yang-Ming University, Taipei, Taiwan; ⁷Division of Endocrinology, Medical School, Federal University of Rio de Janeiro, Brazil; ⁸SBRCS, Division of Clinical Neurosciences, Western General Hospital, Edinburgh, UK; ⁹Dept of Medicine, Section of Endocrinology, Erasmus University Medical Center, Rotterdam, The Netherlands; ¹⁰Oncology Clinical Pharmacology, Novartis Pharmaceuticals Corp, Florham Park, NJ, USA; ¹¹Clinical Development, Oncology Business Unit, Novartis Pharma AG, Basel, Switzerland; ¹²Medical School, University of Birmingham, Edgbaston, Birmingham, UK

Introduction In a large, randomized, double-blind, phase-III trial, pasireotide LAR was superior to octreotide LAR in providing full biochemical control at 12 months (core phase). Patients without full biochemical control at 12 months could switch treatments (crossover extension).

Methods and Materials Medically naïve patients with active acromegaly (post-surgical or *de novo* with a visible adenoma on MRI) who completed 12-month therapy with pasireotide LAR 40–60 mg/28 d or octreotide LAR 20–30 mg/28 d could enter the 12-month extension phase. Patients with GH ≥ 2.5 µg/L and/or IGF-1 > ULN could switch to pasireotide LAR 40 mg/28 d or octreotide LAR 20 mg/28 d. Dose titration to pasireotide LAR 60 mg/28 d or octreotide LAR 30 mg/28 d was permitted. Efficacy data reported here are from the first 6 months after switching therapy; AEs are from any point after switching therapy.

Results Response rates (95%-CI) 6 months after crossover (pasireotide LAR [n = 81] and octreotide LAR [n = 38], respectively) were: GH < 2.5 µg/l and normal IGF-1: 21.0 % (12.7–31.5) and 2.6 % (0.1–13.8); normal IGF-1: 30.9 % (21.1–42.1) and 7.9 % (1.7–21.4); GH < 2.5 µg/l: 43.2 % (32.2–54.7) and 31.6 % (17.5–48.7); tumor volume further decreased by a mean (95%-CI) of 18.1 % (13.6–22.6) and 12.3 % (3.2–21.4), with 25/59 (42 %) pasireotide LAR and 8/27 (30 %) octreotide LAR patients achieving significant (≥ 20 %) tumor volume reduction. Pasireotide LAR and octreotide LAR had similar safety profiles, except for hyperglycemia-related events (60.5 % and 18.4 %, respectively), which were mostly mild/moderate and managed without treatment discontinuation. Fasting plasma glucose and HbA_{1c} levels decreased to near normal within 3 months in patients switched from pasireotide LAR to octreotide LAR.

Conclusion Switching to pasireotide LAR allowed 21 % of patients inadequately controlled with octreotide LAR to achieve full

biochemical control; 2.6 % switched to octreotide LAR achieved full biochemical control. The safety profile of pasireotide LAR was similar to that of octreotide LAR, with the exception of hyperglycemia-related events. Pasireotide LAR may provide a treatment option for patients inadequately controlled with octreotide LAR.

Disclosure: No significant relationships.

P007

Obstructive Sleep Apnea in Patients with Acromegaly

M. Morozova, V. Pronin, Y. Poteskhin, I. Sokolina
UCH #2, First MSMU I.m. Sechenov, Moscow, Russian Federation

Introduction Obstructive sleep apnea (OSA) is widespread in patients with acromegaly due to swelling and proliferation of connective tissue of the upper respiratory tract. 25 % of all deaths recorded in acromegaly account for respiratory disorders, which is 3× higher than in healthy people.

Methods and Materials A total of 34 patients with acromegaly (10 men, 24 women) aged 22–77 years (mean ± SD: 52.8 ± 13.9). All patients had been held cardiorespiratory monitoring (“Kardio-tehnika”), the levels of GH and IGF-1 were measured, a thorough medical history was taken and all patients completed the questionnaire AcroQol. Based on the results of cardiorespiratory monitoring, patients were divided into 4 groups according to the Apnea/Hypopnea Index (AHI): group 1 (patients without SAS, 11.8 % [AHI < 5]), group 2 (patients with mild SAS [AHI 5–14]), group 3 (patients with SAS of moderate severity, from 29.4 % [AHI 15–29]), and group 4 (patients with severe sleep apnea, 32.3 % [AHI > 30]).

Results GH and IGF-1 in all 4 groups were not statistically different. A positive correlation between disease duration and severity of apnea in groups 2 and 3 ($r = 0.80^*$ and $r = 0.79^*$, respectively) was revealed. The main cause of SAS in group 4 was a high Body Mass Index ($r = 0.56^*$). A high AHI was associated with a lower quality of life (AcroQol) ($r = 0.37^*$). Patients with a higher AHI revealed a correlation with the presence of cardiovascular disease ($r = 0.42^*$) and also with the presence of PVS ($r = 0.57^*$) and SVT ($r = 0.39^*$). All *: $p < 0.05$.

Conclusion (1) The higher the SAS severity the lower the quality of life. (2) The presence of SAS in patients with acromegaly is caused by an obstructive component (an increase of cartilage and swelling of the upper respiratory tract). (3) High weight is a risk factor for SAS. (4) The presence of SAS increases the risk of cardiorespiratory events and reduces quality of life.

Disclosure: No significant relationships.

P008

Thyroid Ultrasound Findings in Acromegalic Patients under Treatment with a Somatostatin Analogue

L. Figueroa¹, B. Mangupl², P. Camperos², Y. Linares³
¹Endocrinology; ²Neurosurgery, Neuroendocrinology Section, Hospital Universitario de Caracas; ³Anatomía Patológica, Instituto de Anatomía Patológica, Caracas, Venezuela

Introduction Acromegaly is characterized by GH and IGF-1 hypersecretion. High prevalence of goiter in acromegalic patients is well-known and several studies revealed an increased frequency of thyroid carcinoma in these patients. The potential role of IGF-1 in the pathogenesis of thyroid neoplasms has been considered.

Objective To demonstrate the relation between acromegaly and increased thyroid volume (TV) and investigate prevalence of thyroid carcinoma.

Methods and Materials We studied 26 acromegalic patients from one referral center with an average age of 48 years (29–68), 58 % females, with a 9-year average follow-up (2–20) actually under sandostatin LAR treatment. Thyroid ultrasonography was performed

by one observer and in those with non-surgical nodules > 5 mm a fine-needle aspiration biopsy guided by ultrasound was obtained and *in situ* cytopathological analysis was performed by the same pathologist.

Results Of 26 patients, 1 had undergone thyroidectomy because of a papillary carcinoma, the remaining 25 all presented echographic abnormalities, 21 with nodular disease (84 %) and 4 with diffuse goiter. 88 % presented goiter. TV ranged from 4.75–41.8 ml with a 17.89-ml average. Half the nodules were < 1 cm. Echoguided biopsy was performed in 10 patients, 9 were benign. Papillary thyroid carcinoma was found in 2 patients (7.7 %), 1 with previous surgery 9 years earlier and another with multinodular goiter that presented suspicious echographic features. The median SD pre-treatment GH was 60.06 µg/l ± 17.2 and post 6.31 ± 9.43 and pre-treatment IGF-1 932.63 ng/ml ± 363.7 and post 522.36 ± 314.78, respectively. No correlation evidence was found between GH and IGF-1 values and TV.

Conclusion Our study confirmed that goiter and nodular thyroid lesions are common in acromegaly but cancer prevalence is still a subject under discussion. We suggest that thyroid ultrasonography be performed in recently diagnosed acromegalic patients and strict follow-up in those with nodular disease.

Disclosure: No significant relationships.

P009

Pasireotide LAR Is Superior to Octreotide LAR in a Large, Randomized, Double-Blind, Phase-III Study in Patients with Acromegaly: Analysis of Response by Baseline GH and Tumor Volume

A. Tabarin¹, A. Barkan², S. Kim³, A. J. Farrall⁴, O. Serr⁵, L. Naves⁶, L. Rozhinskaya⁷, W. Rojas⁸, K. Hermosillo Reséndiz⁹, M. Ruffin¹⁰, K. Asubonteng⁹, L. de Marinis¹¹
¹Dept of Endocrinology, CHU of Bordeaux, France; ²Neurosurgery, Dept of Medicine, University of Michigan, Ann Arbor, MI, USA; ³Dept of Endocrinology, Kyung Hee University, Seoul, Korea; ⁴Brain Research Imaging Centre, Division of Clinical Neurosciences, University of Edinburgh, Western General Hospital, Edinburgh, UK; ⁵Service of Endocrinology, Centre Hospitalier de l'Université de Montréal, Notre-Dame Hospital, University of Montréal, Canada; ⁶Dept of Endocrinology, Internal Medicine, University of Brasília, Brazil; ⁷National Research Center for Endocrinology, Russian Academy of Medical Sciences, Moscow, Russian Federation; ⁸Dept of Endocrinology, Hospital de San Jose Fucs, Bogota, Colombia; ⁹Oncology Clinical Pharmacology, Novartis Pharmaceuticals Corp, Florham Park, NJ, USA; ¹⁰Clinical Development, Oncology Business Unit, Novartis Pharma AG, Basel, Switzerland; ¹¹Section of Endocrinology, Dept of Internal Medicine, Università Cattolica del Sacro Cuore, Rome, Italy

Introduction A large, randomized phase-III trial found that pasireotide LAR is superior to octreotide LAR in providing GH < 2.5 µg/l and normal IGF-1 after 12 months ($p = 0.007$; Colao et al. ECE 2012). We report a *post-hoc* analysis evaluating the relationship between response and baseline tumor volume.

Methods and Materials Patients with active acromegaly who were *de novo* with a visible adenoma on MRI or who had prior pituitary surgery but no previous medical therapy received pasireotide LAR 40 mg/28 d ($n = 176$) or octreotide LAR 20 mg/28 d ($n = 182$) for 12 months. Responders had GH < 2.5 µg/l and IGF-1 ≤ ULN at the 12-month assessment. Baseline tumor volume and GH were divided into quartiles. Macroadenomas were defined as ≥ 523 mm³.

Results In patients receiving pasireotide LAR ($n = 128$) and octreotide LAR ($n = 147$) with baseline GH in the 1st (≤ 5.1 µg/l), 2nd (5.1–9.8 µg/l), 3rd (9.8–21.2 µg/l), and 4th (> 21.2 µg/l) quartiles, 80 % (24/30) vs 37 % (14/38), 57 % (20/35) vs 24 % (9/37), 19 % (5/27) vs 20 % (8/40) and 19 % (7/36) vs 6 % (2/32) were responders. At baseline, 127 pasireotide LAR (83 *de novo*) and 139 octreotide LAR (89 *de novo*) patients had evaluable tumor volumes. In patients receiving pasireotide LAR and octreotide LAR with tumor volumes in the 1st (≤ 372 mm³), 2nd (> 372–1044 mm³), 3rd (> 1044–2656 mm³), and 4th (> 2656 mm³) quartiles, 70 % (23/33) vs 26 % (9/35), 36 % (12/33) vs 22 % (8/37), 42 % (13/31) vs 24 % (8/34) and 23 % (7/30) vs 21 % (7/33) were responders, respectively. Results were similar regardless of prior surgery. Tumors in the 1st quartile were micro-

adenomas, those in 2nd quartile were either micro- or macroadenomas, whereas all tumors in the 3rd and 4th quartiles were macroadenomas.

Conclusion The effects of pasireotide LAR and octreotide LAR on GH and IGF-1 after 12 months of therapy are consistent, irrespective of baseline tumor volume and GH levels.

Disclosure: No significant relationships.

P010

Prevalence of Manifestations, Complications, and Illness in Patients with Acromegaly Compared to Other Pituitary Tumors

F. Guaraldi, M. Maccario, N. Prencipe, S. di Giacomo, V. Gasco, A. Berton, A. Mainolfi, E. Ghigo, S. Grottoli
Endocrinology, San Giovanni Battista di Torino, Turin, Italy

Introduction Pituitary adenomas represent the most frequent cause of acromegaly, a rare disease supported by chronic exposure to excessive levels of growth hormone (GH) and its protein IGF-1. It is associated to somatic and visceral hypertrophy, metabolic alterations, cardiovascular and respiratory complications as well as increased risk of neoplasias.

Methods and Materials Using an at-home standard protocol, 137 acromegalic subjects (52 m, 85 f; age at diagnosis [mean $\pm$ SD] 51 $\pm$ 13.4 yrs), diagnosed between 1980 and 2011 and followed at our Endocrinology Centre, were evaluated for prevalence of clinical manifestations, complications, and associated illnesses. Patients with cured or active disease, treated medically, surgically and/or by radiotherapy were enrolled. Medical history was collected using the questionnaire and electronic medical record system.

Results Data analysis revealed the presence of disease manifestations similar to expected (thyroid nodular hyperplasia in 68 % of the patients, carpal tunnel in 20 %, sleep apnea in 10 %) and remarkable complications (hypertension in 55 % of the patients, cardiomegaly in 24 %, alterations of glucose metabolism in 37 %, alterations of lipid metabolism in 44 %, decreased bone density in 37 %, dolico-colon in 15 %, colic diverticulosis in 20 %, kidney stones in 23 %, gallbladder sludge or stones in 47 %) and neoplasias, benign or malignant (114 total, mostly affecting gut and genitourinary system). GH and IGF-1 levels at diagnosis and follow-up showed a trend ($p = ns$) toward positive correlation with prevalence of disease manifestations and complications; no correlation was found for the prevalence of neoplasias. The same evaluations were performed in the control population affected by different types of pituitary adenomas.

Conclusion Attentive surveillance for manifestations, complications and associated diseases is fundamental at diagnosis and follow-up in acromegaly, independently from biochemical values, with the aim to provide prompt diagnosis and treatment.

Disclosure: No significant relationships.

P011

Optimizing Strategies for Face Classification in the Detection of Acromegaly

B. Frohner¹, R. Kosilek¹, R. P. Würtz², H. Schneider¹, D. Gogas³, A. Lammert⁴
¹Medizinische Klinik Innenstadt, Klinikum der Universität München, Munich; ²Institut für Neuroinformatik, Ruhr-Universität Bochum, Germany; ³Endocrinology and Metabolism, University School of Medicine, Marmara, Turkey; ⁴Endokrinologie und Diabetologie, Universitätsmedizin Mannheim, Germany

Introduction It has been shown that face classification software might help distinguish between subjects with and without acromegaly on regular photographs and, thus, might help improve the early recognition of acromegaly. Our group has previously shown that application of face classification software even outperformed acromegaly experts. In this project, we investigated several aspects that will be necessary and helpful to bring this recognition method closer to clinical application.

Methods and Materials Face classification was based on nodes placed on frontal and side photographs of individuals and on the analysis of the underlying texture and geometric functions. As a first step, we analysed whether omission of nodes considered less relevant changed classification rates in the original database on 57 acromegalics and 60 controls. In a second step, we analysed whether classification was improved in an external data set consisting of 13 acromegalics and 45 controls.

Results Correct classification rates in the original database were 79 % with all nodes and 78 % if irrelevant points regarding the part of the head covered by hair were omitted. Using the same approach in the validation set, correct classification rates were 88 % with all nodes (92 % and 87 % of acromegalics and controls, respectively) and 94 % (100 % and 93 % of acromegalics and controls, respectively) after omission of irrelevant nodes.

Conclusion Reduction of nodes associated with unwanted noise can improve correct classification rates in the detection of acromegaly by face classification software. The improved recognition rates in this data set are very promising for future applications of this technology.

Disclosure: No significant relationships.

P012

Prevalence of Diabetes Mellitus among Acromegaly Patients

A. Dreval, I. Trigolosova, A. Vinogradova, I. Ilvovskaya
Endocrinology Dept, Moscow Regional Scientific Research Clinical Institute, Russian Federation

Introduction Prevalence of secondary diabetes mellitus (DM) in acromegaly rich to 68 % and depends on age, duration, and activity of disease.

Methods and Materials 93 acromegaly patients (mean age 57.5 years [48.0–64.5]), duration of acromegaly 13.0 years [7.5–20.25] were observed. In 27 (29 %) patients, DM was diagnosed before acromegaly. In 66 patients, the oral glucose tolerance test revealed the diagnosis of DM (according to the WHO 1999 criteria).

Results Among 66 patients DM was found in 21 (22.6 %). The total prevalence DM in acromegaly was 51.6 %, that too much more, than prevalence type-2 diabetes mellitus (T2DM) in the world (8.3 %) (IDF 2011). In women, prevalence of DM was more than in men (55.1 and 33.5 %, respectively; $p < 0.05$). Prevalence of DM increases with age: from 14.3 % (36–45 yrs) to 48.3 % (> 56 yrs); $p < 0.05$. The prevalence of DM was increased depending on the duration of acromegaly: < 8 yrs: 40.6 %; 9–15 yrs: 52.2 %; > 15 yrs: 60.5 %. In controlled stage of acromegaly prevalence DM was 57.1 % and in uncontrolled stage 49.2 %; $p > 0.05$. We have not found difference in prevalence DM between somatostatin analogs (SSA) treatment group and group without SSA treatment.

Conclusion The prevalence DM in acromegaly considerably exceeds prevalence D2M and depends on gender, age, and duration of acromegaly. SSA treatment had not affected to prevalence DM.

Disclosure: No significant relationships.

P013

Efficacy of Proton-Beam Therapy in Acromegaly

N. Molitvoslovova, O. V. Manchenko, E. Marova, L. E. Kirpatovskaya
Neuroendocrinology, The National Research Center for Endocrinology, Moscow, Russian Federation

Aim To evaluate the efficacy of proton-beam therapy as a primary method in active acromegaly.

Methods and Materials 112 patients at the age of 37–64 years with active acromegaly, 26 males and 84 females. Pretreatment levels of GH were Me (25–75 %): 34.9 (16.1–72.1) ng/ml; IGF-1: 762.0 (478.0–976.0). The dose for each patient was from 30–79 Gy and was received simultaneously. The period of follow-up is 20 years. Most of patients required additional medical therapy.

Results On the whole, proton-beam therapy leads to decrease of GH and IGF-1 rate in 46 % of patients and normalization of these hormones in 44 %. There were no any positive clinical and hormonal effects in 11 patients. In 5 years after irradiation remission was achieved in 27 % of patients, in 10 years in 32 %, in 15 years in 35 %, and in 20 years in 44 % of patients. The data were shown that proton-beam therapy evoked early and late complications. The early complications are: pituitary oedema (9.5 %), pituitary haemorrhage (3.6 %), visual alterations (6.3 %) with loss of vision in 2.7 %. The late complications are: hypopituitarism (49 %), hyperprolactinemia (23 %).

Conclusion The use of proton-beam therapy in acromegaly as a primary method is not justified due to low level of remission, slowing-down of effect and high frequency of complications.

Disclosure: No significant relationships.

P014

Screening for Acromegaly in Patients with Mandibular Prognathism Using Face Classification Software Based on Standard Digital Photography: Preliminary Data

F. Brandenbusch¹, R. Kosilek², R. Hübers³, R. P. Würtz⁴, G. Mast⁵, H. Schneider²

¹Medizinische Klinik IV; ²Medizinische Klinik Innenstadt, Klinikum der Universität München, Munich; ³Orthodontics, LMU Munich; ⁴Institut für Neuroinformatik, Ruhr-Universität Bochum; ⁵Oral-/Maxillo-Facial Surgery, LMU Munich, Germany

Introduction Prognathism is an accessory symptom of acromegaly. Bite disorder is usually recognized by dentists or physicians and gets a regular orthodontic treatment. However, patients are usually not tested for acromegaly as an underlying disease. The prevalence of acromegaly in patients with mandibular prognathism is not known to date. We hypothesized that face classification software could detect acromegaly in patients with prognathism.

Methods and Materials We used a training set of 57 patients with acromegaly and 60 controls and trained the software as previously described. We prospectively took frontal and profile photographs of 11 patients (5 women, 6 men) who presented for treatment of mandibular prognathism at the Department for Orthodontics of the Ludwig Maximilian University, Munich. Additionally, we analysed pictures that had been taken of 221 patients (120 women, 101 men) treated for mandibular prognathism at the Department for Oral-/Maxillo-Facial Surgery of the Ludwig Maximilian University, Munich. All subjects were older than 18 years. We classified all pictures by means of the software trained with the original training set. Patients classified as acromegals by the software were invited to participate in endocrine testing for acromegaly.

Results The software classified prospectively 72.72 % (5 women; 3 men) and retrospectively 74.20 % (97 women; 67 men) of the analysed subjects as acromegalic. Endocrine testing was planned in all patients classified as acromegals and results were not available until abstract submission.

Conclusion Face classification software might be helpful in screening for acromegaly in patients with mandibular prognathism. However, given the high classification rate of acromegaly, it is likely that acromegaly is overestimated with automatic face classification in this patient group.

Disclosure: No significant relationships.

P015

T2 MRI and Tumour Reduction after Primary Octreotide Treatment of Acromegaly

A. Heck¹, G. Ringstad², J. Bollerslev¹

¹Dept of Endocrinology; ²Radiology, Oslo University Hospital, Rikshospitalet, Norway

Introduction Signal intensity in T2-weighted MRI in somatotrophic pituitary adenomas has been described to correlate with histological granulation pattern. Response to somatostatin analogue (SA)

treatment is associated with both histological subtype and T2-weighted signal intensity.

Objective To test whether T2-weighted images correlate with pre-operative SA response on adenoma volume reduction in newly diagnosed patients with acromegaly.

Methods and Materials Retrospective observational cohort study. 26 patients with newly diagnosed acromegaly were included. T2 signal intensity of the adenomas was compared to grey matter of the adjacent temporal lobe. Adenomas with the same or higher signal intensity were classified as hyperintense. Adenoma volume and invasiveness at the time of diagnosis and after 6 months of primary SA treatment were recorded. Adenoma size was assessed by summation of areas and invasiveness by SIPAP score.

Results Eight of 26 (30 %) adenomas were hyperintense and 18 of 26 (70 %) adenomas were non-hyperintense. At baseline, adenoma volume and invasiveness were not different between the groups of intensity. Following treatment, median tumour volume reduction (% of baseline; interquartile range) was 25 % (12–36) in the total cohort ($p < 0.01$). In the hyperintense group, tumour volume reduction was 16 % (6–26), and in the non-hyperintense group 28 % (17–38) ($p = 0.055$ for difference between groups). Moreover, the SIPAP score demonstrated significantly less invasiveness after treatment ($p < 0.01$) but there was no significant difference in SIPAP score reduction between the groups ($p = 0.25$). However, *post-hoc* power calculation of the volume reduction revealed a high risk for a type-2 error ($p = 0.5$).

Conclusion Primary treatment with octreotide was followed by a significant reduction of tumour size and invasiveness in treatment-naïve patients with acromegaly. T2-hyperintense adenomas tended to be less responsive than non-hyperintense, where the non-significance probably was related to low power.

Disclosure: No significant relationships.

P016

Parameters of Glucose and Insulin Metabolism in Patients with Acromegaly

I. Trigolosova, A. Dreval, A. Vinogradova, I. Ilvayskaya

Endocrinology Dept, Moscow Regional Scientific Research Clinical Institute, Russian Federation

Introduction Somatostatin analogues (SSA) may influence glucose metabolism in different ways, however, there is a lack of detailed data of these effects. To estimate changes of fasting insulin (FI), glycemia, and HOMA-IR in some cases of new-onset acromegaly before and after SSA treatment.

Methods and Materials 47 patients (3 female, 44 male) underwent an oral glucose tolerance test (OGTT) with 75 g of glucose before and 3 months after octreotide prolonged-release 20 mg/4 w treatment. Insulin, glucose, and GH levels were determined before and 30, 60, 90, and 120 min after glucose. HOMA-R and areas under the curve (AUC) for insulin (AUCins) and glucose (AUCglu) were estimated.

Results See Table 2.

All data expressed as “before/after SSA” patients 3 and 4 besides SSA received metformin 1700 mg daily. In our patients the treatment of SSA led to different changes of fasting insulin, however,

Table 2. I. Trigolosova et al. Examples of treatment results.

	F, 69 a	F, 54 a	M, 46 a	F, 64 a
Glucose tolerance	Normal/ Impaired	Impaired/ Impaired	DM/ DM	DM/ DM
GH mU/l	6.1/3.4	27/3.5	29.3/3	6.2/0.3
IGF-1 (% of UNL)	120/75	193.5/56.2	535/103	190/65
HOMA-IR	0.87/1.88	3.97/1.79	8.34/1.72	0.89/1.23
FI pmol/l	24/46	90/43	14.4/40	161/39
AUCins (pmol/l) ²	63,297/ 30,360	53,520/ 14,925	33,660/ 13,560	29,451/ 3130
AUCglu (mmol/l) ²	867/1071	963/1027	1662/1104	1557/1087

AUCins decreased significantly in all cases which reflected inhibiting effect of SSA on insulin production. HOMA-IR increased (1), obviously decreased (2–3) or slightly increased within normal limits (4). AUCglu slightly increased (1), was stable (2) or obviously decreased (3–4, may be due to metformin effect).

Conclusion Thus, these observations demonstrate a multidirectional effect of SSA on glucose metabolism.

Disclosure: No significant relationships.

P017

Lung Function in Acromegaly: Obstruction of Small Airways

S. Störmann¹, B. Gutt², J. Römmeler¹, M. W. Angstwurm¹, J. Schopohl¹

¹Medizinische Klinik und Poliklinik IV, Klinikum der Universität München; ²Dept of Endocrinology, Städtisches Klinikum München GmbH – Klinikum Schwabing, Munich, Germany

Introduction Life expectancy is reduced by 10 years in uncontrolled acromegaly. Respiratory disease could be shown to be a large contributor to early death in acromegaly since the early 1970s. Multiple pathophysiological aspects are thought to influence respiratory disease in acromegaly but they are not fully understood. This is due to a lack of fundamental understanding of changes in lung function.

Methods and Materials We performed lung function tests (spirometry, body plethysmography) in 51 non-smoker acromegaly patients (24 male, 27 female) treated in our outpatient clinic on a regular basis. The median age was 54 years (range: 24–82). 22 of these patients were biochemically normalized using strict criteria of cure: IGF-1 in the age- and sex-adjusted normal range and an hGH profile < 1 µg/dl. 37 patients had undergone transsphenoidal surgery, 12 patients had external pituitary irradiation, and 18 patients were treated with somatostatin analogues.

Results Acromegalic patients showed significantly elevated lung volumes in comparison to healthy controls matched to age, sex, weight, and height: total lung capacity (median 112.5 %; $p < 0.001$), residual volume (116.5 %; $p < 0.001$), RV/TLC ratio (104.5 %; $p = 0.032$), intrathoracic gas volume (110.1 %; $p < 0.001$), and maximal vital capacity (109.8 %; $p = 0.005$). Peak expiratory flow (90.8 %; $p = 0.007$) and expiratory flow rates at 75 % of forced vital capacity (FEF75/MEF25, median 73.3 %; $p = 0.006$) were reduced, characteristic of small airway disease. However, patients under treatment with somatostatin analogues in comparison to no medical treatment of acromegaly had higher FEF75 (median 91.4 % vs 58.6 %; $p = 0.011$) and lower total airway resistance (64.4 % vs 86.2 %; $p = 0.011$), regardless of disease activity. None of the lung function parameters differed significantly between male and female patients.

Conclusion We found signs of small airway disease in acromegaly. In particular, this could be demonstrated in patients not treated with somatostatin analogues.

Disclosure: No significant relationships.

P018

Visual Field Defects in 23 Acromegalic Patients

E. Kan¹, E. Kiliç Kan², B. Çolak², H. Atmaca², A. Atmaca²

¹Eye, Samsun Egitim Arastirma Hastanesi; ²Endocrinology and Metabolism, 19 Mayıs Üniversitesi Tıp Fakültesi Endokrinoloji Bilim Dalı Atakum/Samsun, Turkey

Introduction Pituitary tumors are the third most common primary intracranial neoplasm. Pathologic proliferation of the somatotrophs results as overproduction of growth hormone (GH) presenting as acromegaly. In pituitary adenomas typical visual field (VF) defect is bitemporal hemianopsia but tumor size and optic chiasmal position may cause variable VF defects and VF examinations may remain normal.

Methods and Materials We retrospectively reviewed the medical records of 23 acromegalic patients with pituitary adenomas who received VF tests at the Department of Ophthalmology and Endocrinology, Ondokuz Mayıs University Hospital, between 2000 and

2012. Pituitary tumor volume was calculated after performing measurements of tumor diameter in 3 orthogonal planes using Cavalieri's principle. VF test was performed with a Humphrey field analyzer 750 (Zeiss-Humphrey, Dublin, CA, USA) using a 4-mm² Goldmann size-III stimulus.

Results The mean age of 23 patients (11 males/12 females) was 50.4 ± 11.9 years. 15 patients (65.2 %) had normal VF, 2 patients (9 %) had quadrantanopsia, 3 patients (13 %) had hemianopsia and 3 patients (13 %) had 3 quadrantanopsias. Among the patients with normal VF, 4 patients had a suprasellar mass that was elevating the chiasm and spreading to the optic tracts. Beside this in a few cases, we observed visual field defects typical of a chiasmal compression even though no suprasellar extension was detected. Tumor volume in patients with VF defects was significantly larger than in patients with normal VF ($p = 0.02$).

Conclusion Tumor volume and suprasellar height of the tumor are important parameters in the VF defects. Advanced neuroimaging assessments should always be complemented in patients even with normal VF.

Disclosure: No significant relationships.

P019

Acromegalic Cardiomyopathy in an Extensively Admixed Population: Is There a Role for the GH/IGF-1 Axis?

G. Nascimento, M. Oliveira, V. Carvalho, M. H. Lopes, A. Ferreira, P. Ferreira, M. Faria

Internal Medicine, Hospital Universitário Presidente Dutra, Federal University of Maranhão, Sao Luis, Brazil

Introduction A specific acromegaly-related cardiomyopathy has been described in the literature, largely in Caucasians, which is independent of other risk factors, mainly hypertension. This study assessed the cardiac changes in acromegalics of significant ethnic diversity and also the relevance of the aetiopathogenic factors involved, such as disease activity and hypertension. It is a cross-sectional study with a comparative control group.

Methods and Materials In this study, 37 acromegalic patients (20 brown, 14 blacks, and 3 whites) and 74 controls matched by age, gender, and hypertension were evaluated. Cardiac morphology and function were addressed using echocardiography parameters.

Results The mean age of patients was 46.9 ± 12.8 years, with 67.6 % being female and 43.2 % hypertensive. The prevalence of left ventricular hypertrophy (LVH) between acromegalics was 56.8 % vs 10.8 % in the control group ($p < 0.001$). About 86 % of patients with LVH had active disease ($p = 0.023$). Logistic regression revealed that disease activity presented a stronger association (OR: 5.925; CI: 1.085–32.351; $p = 0.040$) with LVH than hypertension (OR: 3.237; CI: 0.702–14.924; $p = 0.132$). When black acromegalics were compared to brown ones, no statistically significant differences were observed.

Conclusion The chronically hyperactive somatotrophic axis remains an independent and determining factor in the development of LVH, as it is more associated with this condition than hypertension in a largely admixed population with a high degree of African ancestry.

Disclosure: No significant relationships.

P020

Cathepsin B, Matrix Metalloproteinase 2 and 9 Levels in Active and Controlled Acromegaly Patients and Relationship with IGF-1

A. C. Karcı¹, Z. Cantürk¹, E. Karcı², I. Tarkun¹, B. Çetinarslan¹

¹Endocrinology and Metabolism; ²Internal Medicine, Kocaeli University Faculty of Medicine, Turkey

Introduction In the follow-up of acromegaly patients, status GH and IGF-1 should be evaluated together to determine disease activity.

ity. There is discordance between GH and IGF-1 measurements in 30 % of patients and a need for other tests to evaluate patients who have shown discordant results. The aim of this study was to show a relationship between acromegaly disease activity and serum levels of matrix metalloproteinase 2 (MMP-2), MMP-9, cathepsin B that was thought to play role in pituitary adenoma pathogenesis.

Methods and Materials In the present study, 64 acromegaly patients (34 men, 30 women) were divided into 2 groups with active (24 patients) and controlled disease (40 patients) according to the latest consensus criteria. Serum MMP-2, MMP-9, and cathepsin B levels were measured by ELISA.

Results Serum MMP-2 levels were significantly higher in patients with active disease than in patients with controlled disease (150.1 ± 54.5 vs 100.2 ± 44.6 ng/ml; $p < 0.0001$). There was no significant difference in MMP-9 and cathepsin B levels between the 2 groups ($p = 0.205$; $p = 0.598$). In order to evaluate disease status GH and IGF-1 measurements were superior to MMP-2, MMP-9, and cathepsin B. To determine active disease MMP-2 at levels 118.3 ng/ml has been found to have 75 % sensitivity and 77.5 % specificity. The risk of active disease was nearly 3-fold in patients with MMP-2 > 118.3 ng/ml than in patients with MMP-2 < 118.3 ng/ml.

Conclusion In this study, it was shown that MMP-2 levels are increased in active acromegaly patients and a cut-off level was defined for MMP-2 to determine active disease. These findings suggest the use of MMP-2 in patients with discordant GH and IGF-1 results to confirm disease status. Further studies are required to support these findings.

Disclosure: No significant relationships.

P021

Correlation of GH and IGF-1 Values with the AcroQoL Questionnaire in Acromegalic Patients before and during Treatment with a Somatostatin Analogue

R. Mangupli, P. Camperos

Neurosurgery Neuroendocrinology Section, Hospital Universitario de Caracas, Venezuela

Introduction The aim of acromegaly treatment is to control the disease by suppressing the clinical and biochemical consequences of GH hypersecretion. Evidence demonstrates that lowering GH and IGF-1 values can improve quality of life in the acromegalic patient. The AcroQoL questionnaire designed by Susan Webb is a tool to evaluate psychological and physical functions in these patients. It comprises 22 questions, each one scored out of 5 and with the maximum score of 110 reflecting best QoL. The objective of this study was to evaluate the AcroQoL questionnaire score and its correlation with 2 biochemical markers GH and IGF-1 before and during treatment with a somatostatin analogue.

Methods and Materials This is a retrospective observational study of 28 acromegalic patients who completed in a 4-year period (6–48 months) the AcroQoL questionnaire before and during treatment with sandostatin LAR 20 or 30 mg IM monthly.

Results The AcroQoL score was low in the 28 acromegalic patients before pharmacological treatment and this score was related with disease activity. The AcroQoL score improved significantly ($t = 6.948$; $p = 0.000$) with an average increase of 17.5 points (42.3 %) in 93 % of individuals who completed at least 2 questionnaires before and during treatment with sandostatin LAR. This improvement correlated with GH and IGF-1 values ($t = 4.008$; $p = 0.000$) with an average decrease of $9.91 \mu\text{g/l}$ (50.5 %) for GH and $t = 6.207$ ($p = 0.000$) average decrease of 360.1 ng/ml (44.3 %) for IGF-1.

Conclusion We consider that quality of life should be assessed in acromegalic patients using the AcroQoL questionnaire before and during pharmacological treatment. The AcroQoL score improved with treatment and correlates well with biochemical parameters, making it a valuable clinical tool to monitor therapeutic effectiveness.

Disclosure: No significant relationships.

P022

Acromegaly and Cabergoline: Synergetic Risk for Cardiac Valve Disease?

L. Maione¹, C. Garcia¹, A. Bouchachi², P. Maison¹, N. Kallel¹, S. Salenave¹, J. Young¹, P. Assayag², P. Chanson¹

¹APHP Endocrinology and Reproductive Diseases; ²APHP Cardiology, Paris Sud University, Le Kremlin-Bicêtre, France

Introduction The effects of cabergoline on cardiac valves have been extensively studied in Parkinson's disease and hyperprolactinemia but not in acromegaly, a condition at risk of cardiac valve abnormalities. Our objective was to examine the prevalence of heart valve disease and regurgitation in a series of patients with acromegaly treated with cabergoline, by comparison with matched patients who had never received this drug. This evaluation has been conducted in a cross-sectional and longitudinal manner in a single referral center.

Methods and Materials 42 patients who had received cabergoline at a median cumulative dose of 203 mg for a median of 34.5 months were compared to 46 patients with acromegaly who had never received cabergoline and who were similar for age, sex, and disease duration. A subgroup of patients receiving cabergoline ($n = 26$) was evaluated longitudinally before and during cabergoline treatment and compared to a group not receiving cabergoline and followed during the same time ($n = 26$). Two-dimensional and Doppler echocardiographic findings were reviewed by 2 cardiologists blinded to the treatment.

Results Demographic and clinical features were not significantly different between the groups. Compared to acromegalic controls, cabergoline treatment was associated neither with a higher prevalence nor with a higher incidence of valve abnormalities. A slightly higher prevalence of aortic valve regurgitation and remodeling was found in the controls relative to the cabergoline-treated patients ($p < 0.02$ and $p < 0.03$, respectively) but this was related to the presence of an aortic dilatation.

Conclusion Cabergoline therapy in acromegalic patients is not associated with an increased risk of cardiac valve regurgitation or remodeling at the doses used in this study.

Disclosure: No significant relationships.

P023

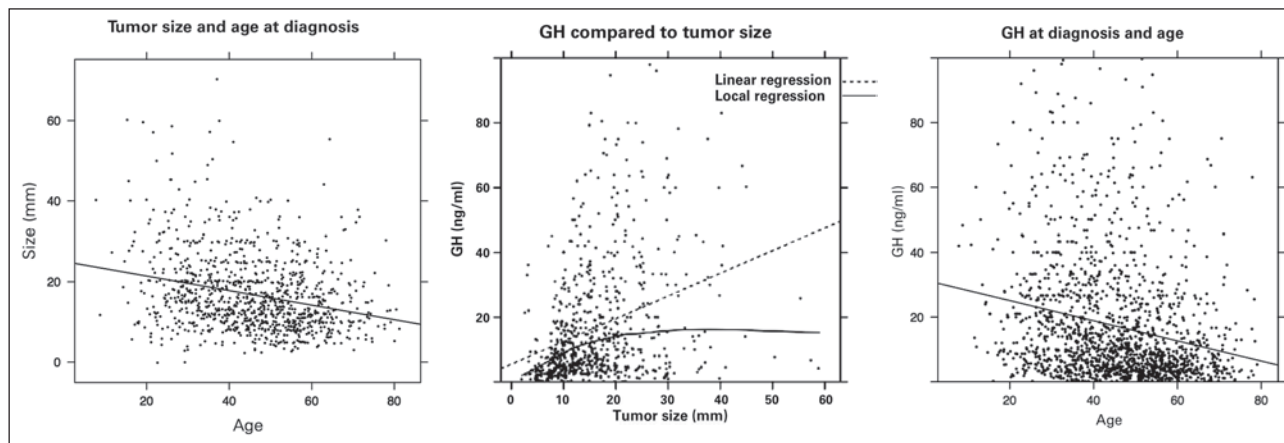
Age, GH, and Tumor Size: The Triangular Relation in Acromegaly. Data from the Liège Acromegaly Survey

P. Petrossians¹, S. Zacharieva², P. Chanson³, S. J. Neggers⁴, A. Colao⁵, A. Hulting⁶, B. Delemer⁷, T. Brue⁸, V. Hana⁹, G. K. Stalla¹⁰, F. Minuto¹¹, M. Jaffrain-Réa¹², D. Carvalho¹³, C. Fajardo Montañana¹⁴, A. Daly¹, A. Beckers¹

¹Endocrinology, CHU de Liège, Belgium; ²Clinical Centre of Endocrinology and Gerontology, Medical University, Sofia, Bulgaria; ³APHP Endocrinology and Reproductive Diseases, Paris Sud University, Le Kremlin-Bicêtre, France; ⁴Section of Endocrinology, Dept of Medicine, Erasmus University Medical Center, Rotterdam, The Netherlands; ⁵Dept of Molecular and Clinical Endocrinology and Oncology, Federico II University, Naples, Italy; ⁶Dept of Molecular Medicine and Surgery, Karolinska University Hospital, Stockholm, Sweden; ⁷Endocrinology, CHU de Reims, France; ⁸Dept of Endocrinology, Centre de Référence des Maladies Rares d'Origine Hypophysaire, Hôpital de la Timone, Marseille, France; ⁹3rd Dept of Medicine, 1st Medical Faculty, Charles University, Prague, Czech Republic; ¹⁰Internal Medicine, Endocrinology and Clinical Chemistry, Max Planck Institute of Psychiatry, Munich, Germany; ¹¹Dept of Internal Medicine, University of Genoa, Genova, Italy; ¹²Dept of Experimental Medicine, University of l'Aquila, Italy; ¹³Dept of Endocrinology, Diabetes and Metabolism, Centro Hospitalar S. João, Porto, Portugal; ¹⁴Dept of Endocrinology, Hospital Universitario de la Ribera, Alzira, Spain

Introduction Literature data suggest that younger acromegalic patients have a more aggressive disease. We looked at diagnosis data from the LAS (Liège Acromegaly Survey) database to assess if the age of patients correlated with MRI and biological data.

Methods and Materials Of the 2929 patients included in the database, valid data on tumor diameter and GH levels were obtained for 722 patients.



Figures 6–8. P. Petrossians et al (P023). Results from the linear regression analysis.

Results Linear regression model fitted with a negative slope between the age of patients and the size of the pituitary adenoma. GH showed a positive slope with the size of the tumor, there was also a negative slope between the age of patients and GH (Figures 6–8).

Conclusion At diagnosis, a relation between the age of patient, tumor size, and GH concentration was observed. Older patients had smaller tumors with lower GH. This could suggest that younger patients have a more aggressive disease. Another explanation can also be proposed.

Disclosure: No significant relationships.

P024

GH or IGF-1: Which One Raises Blood Glucose? Hints from the Liège Acromegaly Survey

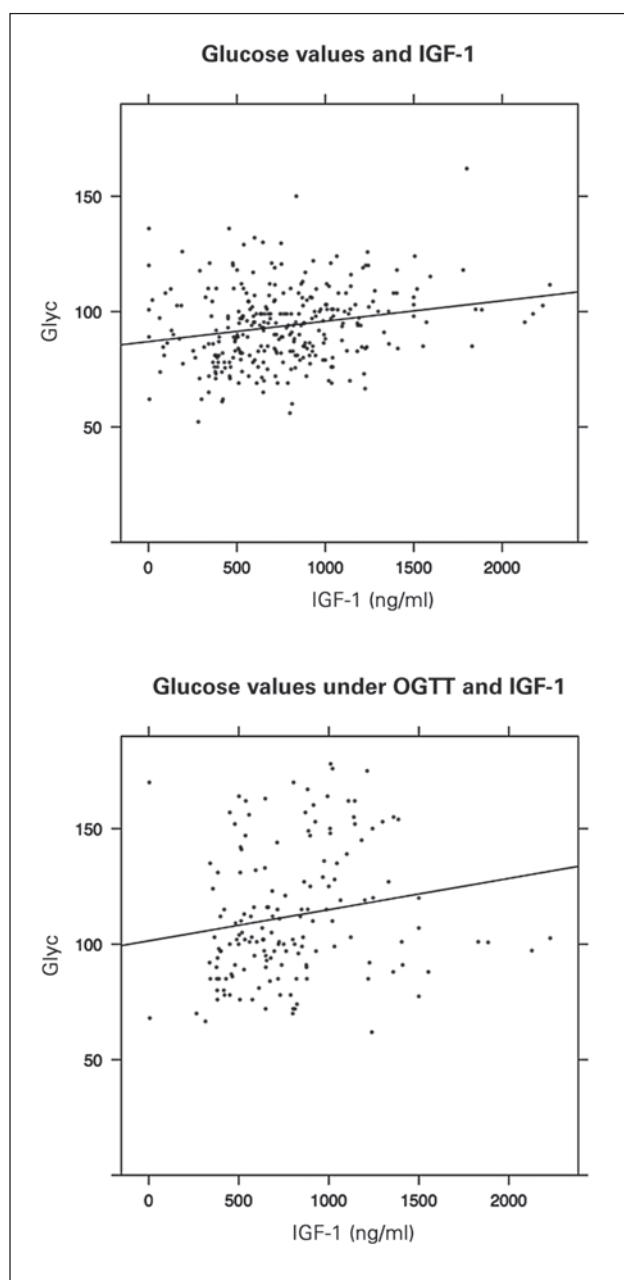
P. Petrossians¹, S. Zacharieva², P. Chanson³, S. J. Neggers⁴, A. Colao⁵, A. Hulting⁶, B. Delemer⁷, T. Brue⁸, V. Hana⁹, G. K. Stalla¹⁰, F. Minuto¹¹, M. Jaffrain-Réa¹², D. Carvalho¹³, C. Fajardo Montañana¹⁴, A. Daly¹, A. Beckers¹

¹Endocrinology, CHU de Liège, Belgium; ²Clinical Centre of Endocrinology and Gerontology, Medical University, Sofia, Bulgaria; ³APHP Endocrinology and Reproductive Diseases, Paris Sud University, Le Kremlin-Bicêtre, France; ⁴Section of Endocrinology, Dept of Medicine, Erasmus University Medical Center, Rotterdam, The Netherlands; ⁵Dept of Molecular and Clinical Endocrinology and Oncology, Federico II University, Naples, Italy; ⁶Dept of Molecular Medicine and Surgery, Karolinska University Hospital, Stockholm, Sweden; ⁷Endocrinology, CHU de Reims, France; ⁸Dept of Endocrinology, Centre de Référence des Maladies Rares d'Origine Hypophysaire, Hôpital de la Timone, Marseille, France; ⁹3rd Dept of Medicine, 1st Medical Faculty, Charles University, Prague, Czech Republic; ¹⁰Internal Medicine, Endocrinology and Clinical Chemistry, Max Planck Institute of Psychiatry, Munich, Germany; ¹¹Dept of Internal Medicine, University of Genoa, Genoa, Italy; ¹²Dept of Experimental Medicine, University of L'Aquila, Italy; ¹³Dept of Endocrinology, Diabetes and Metabolism, Centro Hospitalar S. João, Porto, Portugal; ¹⁴Dept of Endocrinology, Hospital Universitario de la Ribera, Alzira, Spain

Introduction Acromegaly perturbs glucose metabolism. However, it is not clear whether the main factor is GH or IGF-1. Using data from non-diabetic patients from the LAS, we tried to discriminate which hormone is the diabetogenic factor.

Methods and Materials Initial data from the 2929 patients included in the LAS were screened. Patients known as diabetic or those whose initial OGTT glucose values conformed with WHO criteria for diabetes were removed from the study group. A total of 733 patients with valid GH, IGF-1, and glucose values were retained. A linear regression model for fasting glucose vs GH and IGF-1 was calculated.

Results We found no correlation between basal fasting glucose or 120 mn glucose on OGTT and GH levels (Figures 9 and 10). Glucose values correlated significantly with a positive slope with IGF-1 values. This correlation was still observed when the model was recalculated after stratification for different GH levels.



Figures 9 and 10. P. Petrossians et al (P024). Relationships between IGF-1 and glucose values at baseline and during OGTT.

Conclusion In non-diabetic patients, we found a significantly positive linear relation between IGF-1 and glucose concentrations whether fasting or measured at 120 mn on OGTT. This correlation was also observed when patients were grouped based on different GH levels. No correlation was observed between GH and glucose. IGF-1 appears thus as the main diabetogenic factor in acromegaly. Disclosure: No significant relationships.

P025

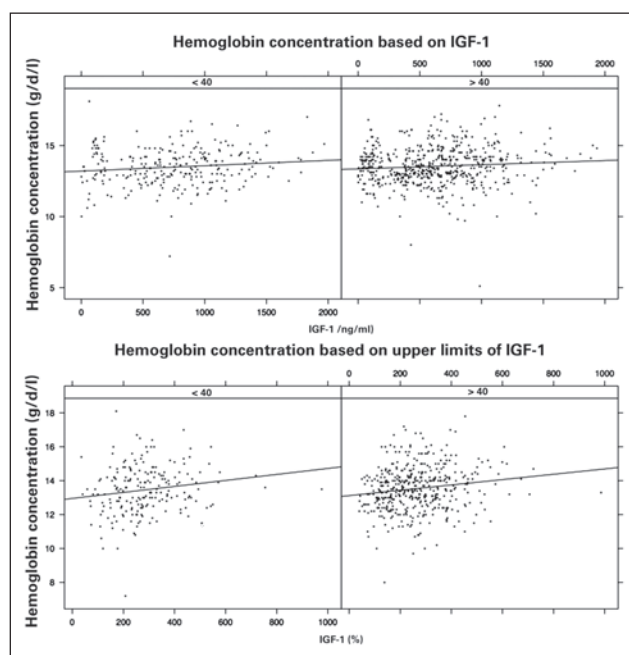
Erythropoiesis in Acromegaly: Effect of GH or IGF-1? Data from the Liège Acromegaly Survey

P. Petrossians¹, S. Zacharieva², P. Chanson³, S. J. Neggers⁴, A. Colao⁵, A. Hulting⁶, B. Delemer⁷, T. Brue⁸, V. Hana⁹, G. K. Stalla¹⁰, F. Minuto¹¹, M. Jaffrain-Rea¹², D. Carvalho¹³, C. Fajardo Montañana¹⁴, A. Daly¹, A. Beckers¹

¹Endocrinology, CHU de Liège, Belgium; ²Clinical Centre of Endocrinology and Gerontology, Medical University, Sofia, Bulgaria; ³APHP Endocrinology and Reproductive Diseases, Paris Sud University, Le Kremlin-Bicêtre, France; ⁴Section of Endocrinology, Dept of Medicine, Erasmus University Medical Center, Rotterdam, The Netherlands; ⁵Dept of Molecular and Clinical Endocrinology and Oncology, Federico II University, Naples, Italy; ⁶Dept of Molecular Medicine and Surgery, Karolinska University Hospital, Stockholm, Sweden; ⁷Endocrinology, CHU de Reims, France; ⁸Dept of Endocrinology, Centre de Référence des Maladies Rares d'Origine Hypophysaire, Hôpital de la Timone, Marseille, France; ⁹3rd Dept of Medicine, 1st Medical Faculty, Charles University, Prague, Czech Republic; ¹⁰Internal Medicine, Endocrinology and Clinical Chemistry, Max Planck Institute of Psychiatry, Munich, Germany; ¹¹Dept of Internal Medicine, University of Genoa, Genova, Italy; ¹²Dept of Experimental Medicine, University of L'Aquila, Italy; ¹³Dept of Endocrinology, Diabetes and Metabolism, Centro Hospitalar S. João, Porto, Portugal; ¹⁴Dept of Endocrinology, Hospital Universitario de la Ribera, Alzira, Spain

Introduction In order to assess the prevalent role of GH and IGF-1 in the production of red blood cells (RBC), we compared RBC count and hemoglobin concentrations of acromegalic patients with GH and IGF-1 levels.

Methods and Materials From the initial 2929 patients included in the LAS, 835 patients presented with valid data and were used in a linear regression model between hemoglobin and RBC count vs GH and IGF-1 levels. The model was then checked in 2 groups of patients, < and > 40 years.



Figures 11 and 12. P. Petrossians et al (P025). Relationship between hemoglobin concentrations and IGF-1.

Results Neither hemoglobin nor RBC count correlated with GH concentrations. Both variables showed a positive linear regression with IGF-1 (**Figures 11 and 12**). The regression model was still significant for patients divided in 2 groups of < and > 40 years or by sex.

Conclusion IGF-1 appears as the main erythropoietic factor in acromegalic patients, irrespective of age. Hemoglobin concentration and RBC count correlated significantly in a linear regression model with IGF-1 levels. No correlation was found between GH concentration and hemoglobin levels or RBC count.

Disclosure: No significant relationships.

P026

Sleep Breathing Disorders in Patients with Acromegaly

M. Agaltsov¹, A. Dreval², S. Fedorova³, I. Illovayskaya²

¹Prophylaxis of Noninfectious Diseases, National Research Center for Preventive Medicine; ²Therapeutic Endocrinology; ³Clinical Physiology, Moscow Regional Research & Clinical Institute, Moscow, Russian Federation

Introduction Acromegaly is associated with many co-morbidities including sleep breathing disorders (SBD). However, detailed data about SBD in patients with acromegaly are limited. Evaluation of frequency and types of SBD at patients with active acromegaly was the aim of this study.

Methods and Materials We examined 20 patients with active acromegaly, aged 28–76 years, median 55 (45–64), GH levels 27 (5–53) ng/ml, IGF-1 294 (125–345) % from upper normal limit, duration of acromegaly 9 (8–15) years. SBD study was conducted using cardiorespiratory monitoring for all patients. Numbers of sleep obstructive and central apnea, index of apnea-hypopnea (IAH) were estimated. Data are expressed in median (25 %; 75 %).

Results Severe or moderate SBD were found in 16/20 (80 %) patients. Number of obstructive and central sleep apnea was 91 (41–318) and 20 (3–37), respectively; IAH 36 (21–48) ep/hour. Medium saturation levels 93 % (91–94) and peak of desaturation 78 % (62–86). Central sleep apnea totaled 4 % (3–11) from all SBD. Correlations were found between IGF-1 levels and sleep breathing parameters: IAH ($r = 0.6^*$), number of obstructive ($r = 0.62^*$) and central ($r = 0.6^*$) apnea episodes, saturation levels ($r = -0.89^*$) and desaturation peak ($r = -0.73^*$). Saturation levels also negatively correlated with duration of acromegaly ($r = -0.62^*$), gravity of hypertension ($r = -0.55^*$) and BMI ($r = -0.47^*$) so these parameters can be considered as worsening factors for saturation. BMI of patients correlated with the number of central apnea ($r = 0.51^*$). Severity of IAH correlated with patients' age ($r = 0.6^*$) and gravity of hypertension ($r = 0.48^*$). Number of central and obstructive apnea correlated with each other ($r = 0.89^*$). All *: $p < 0.05$.

Conclusion Sleep breathing disorders were found in the majority (80 %) of observed patients with acromegaly. Severity of SBD was associated with patients' age, IGF-1 levels, acromegaly duration and gravity of hypertension.

Disclosure: No significant relationships.

P027

Long-Term Results of Fractionated Stereotactic Radiotherapy in the Treatment of Acromegaly

A. Diallo¹, P. Colin², C. F. Litre³, M. Diallo¹, I. Nakibi¹, A. C. Hecart¹, H. Grulet¹, B. Higele¹, P. Rousseaux³, B. Delemer¹

¹Endocrinology, Diabetes, Nutrition, Robert Debré Hospital; ²Radiotherapy, Courclancy Hospital; ³Neurosurgery; ⁴Radiology, Robert Debré Hospital, Reims, France

Introduction The long-term results of fractionated stereotactic radiotherapy (FSR) in acromegaly are unknown. In our center, the RSF has been operational since 1991; we evaluated all patients treated until 2011.

Methods and Materials 30 patients (sex ratio 1, mean age 43 [5–65]) with an adenoma of 20 mm (12–76; 14 invasive) treated imme-

diately by RSF (n = 4) or after surgery (n = 26) by an accelerator CLINAC 2 delivering 50 Gy in 27 sessions of 1.85 Gy. Indications were absence of hormonal control despite treatment with somatostatin analogs (n = 23), contraindication or refusing surgery (n = 4), refused medical treatment (n = 1), and progressive tumour (n = 2), the hormonal control being defined by mean GH < 2.5 ng/ml and IGF-1 normal for age.

Results After a mean follow-up of 132 months (12–240): hormonal control without treatment (remission) 8 patients (27 %) at 49 months, new control under medical treatment (with reduced dosage in half) 15 patients (50 %) at 44 months, tumour control in all patients. Differences for patients in remission: longer follow-up (months) 157 vs 101; $p = 0.02$; lowest mean GH and IGF-1 (ng/ml) before radiotherapy 5.6 vs 43.4 and 524 vs 667; $p = 0.001$; smaller residual adenoma (mm) 10 vs 17.21; $p = 0.0001$; side effects reported to RSF: 12 patients with new anterior pituitary deficiency at 72 months (12–168).

Conclusion RSF in acromegaly is a third-line treatment, adapted to the supra-centimetric target, efficient and well-tolerated for both tumour and hormonal control.

Disclosure: No significant relationships.

P028

Late Diagnosis of Gigantism and Resulting Problems

P. Crocchi¹, D. Ludecke²

¹Paediatric Endocrinology and Diabetes, John Hunter Children's Hospital, Newcastle, Australia; ²Dept of Neurosurgery, University Hospital, Hamburg, Germany

Introduction Gigantism is rare and seems to be a difficult diagnosis in children, with a relatively long delay, given their young age, from onset of symptoms to diagnosis and therapy.

Methods and Materials We reviewed the data of 19 patients with gigantism, presenting to a specialised pituitary neurosurgeon from 1978–2005, to elucidate the pitfalls of diagnosis, problems of surgery, and long-term outcome. All patients had been operated by direct intranasal approach using irrigation-suction-mirror technique.

Results Only 4 patients had microadenomas at diagnosis and 15 had macroadenomas (3 had clearly not completely resectable tumours). There was no mortality and no severe complications.

Characteristic Cases **Case 1:** 2.8-year-old girl presenting with increased growth and precocious puberty. MRI showed a very large pituitary tumour. Prolactin and GH hypersecretion were found. Dopamine agonist therapy was partially effective. After difficult, complete adenoma resection at 3.5 years there was long-term resolution of symptoms and acromegalic features.

Case 2: Nine-year-old boy presenting with precocious puberty and 4 years of height acceleration. Acromegalic features noted and GH and IGF1 elevated. MRI showed intra- and parasellar macroadenoma. Tumour resected with only a small rest at the right cavernous sinus. Somatostatin analogue given post-op. Second surgery (by DKL) at age 11 normalised IGF1 for 8 years. Recent intermittent elevations of IGF1.

Case 3: 13-year-old boy having had MRI for headache and a haemorrhagic, partially invasive pituitary adenoma detected. Previously 7 years of accelerated growth undiagnosed. GH hypersecretion was diagnosed. Cabergoline was not tolerated and somatostatin analogue partially effective. Post-operative normalization of hypersecretion and no hypopituitarism at age 15. At age 19, intermittent IGF1 elevation. Now being treated with pegvisomant.

Conclusion Earlier accurate diagnosis of abnormal growth would almost certainly improve long-term outcome. Experience in adults with micro-adenomas has shown a nearly 100-% cure rate with microsurgery (series of DKL).

Disclosure: No significant relationships.

P029

Predictive Value of the IGF-1 Level in Acromegaly Patients Treated with Surgery and Somatostatin Analogue

A. Baldys-Waligorska, A. Krzentowska, F. Golkowski, G. Sokolowski, A. Hubalewska-Dydejczyk

Endocrinology, Jagiellonian University Medical College, Krakow, Poland

Introduction We evaluated the predictive value of IGF-1 against hGH in the treatment outcome.

Methods and Materials 47 patients (mean age 41.1 ± 12.9 years) requiring octreotide LAR treatment (SSLAR) following incomplete surgery were studied prospectively. hGH and IGF-1 concentrations were measured 3 months after surgery and every 3, 6, 9, 12, 18, and 24 months after introducing SSLAR.

Results Following surgery, respective median values of hGH and IGF-1 concentrations were 5.55 ng/ml (IQR = 7.1) and 512.7 ng/ml (IQR = 379.5). After 6, 12, and 24 months of SSLAR treatment, median values of hGH decreased significantly to 2.95 ng/ml (IQR = 5.5; $p < 0.05$), 2.95 ng/ml (IQR = 4.4; $p < 0.05$) and 2.00 ng/ml (IQR = 3.6; $p < 0.001$), respectively. After 6, 12, and 24 months of SSLAR treatment, the respective median IGF-1 concentrations significantly decreased to 384.5 ng/ml (IQR = 312.2; $p < 0.01$), 323.0 ng/ml (IQR = 230.3; $p < 0.001$), and 334.0 ng/ml (IQR = 328.9; $p < 0.01$). The differences between median hGH and IGF-1 concentrations at 12 and 24 months were not significant. A statistically significant correlation was found between IGF-1 concentration prior to and after surgery ($R = 0.61$; $p < 0.05$) and prior to SSLAR treatment and IGF-1 concentration 24 months later ($R = 0.49$; $p < 0.05$). No such correlation was observed for hGH.

Conclusion The level of IGF-1 prior to surgery and prior to SSLAR treatment is a better predictor of the treatment outcome than hGH. Octreotide LAR was most effective over the first 12 months of treatment. No further significant decrease of hGH or IGF-1 levels was observed past this period.

Disclosure: No significant relationships.

P030

Hyperphosphatemia Is a Common Marker of Active and Severe Acromegaly

L. F. S. Grasso, M. Galdiero, L. Ferreri, T. Mannarino, A. Colao, R. Pivonello

Dept of Clinical and Molecular Endocrinology and Oncology, University of Naples Federico II, Italy

Introduction GH and IGF-1 are physiologically involved in the regulation of electrolyte balance. Changes in calcium (Ca) and phosphorus (Ph) metabolism have been reported in patients with acromegaly, however, no systematic data are presently available. The aim of the current study was to investigate Ca and Ph metabolism in patients with acromegaly, during active disease, and after disease remission.

Methods and Materials 30 patients with acromegaly (12 f, 18 m, 27–72 yrs), and 30 gender- and age-matched controls were retrospectively evaluated at diagnosis and 2–5 years after hormone normalization. Serum and urinary Ca and Ph, serum PTH, and 25-hydroxy-vitamin D levels were evaluated in patients and controls.

Results At diagnosis, patients with acromegaly had significantly higher serum Ph ($p < 0.001$) and Ca levels ($p = 0.03$) as well as urinary Ca ($p < 0.001$) and Ph ($p < 0.05$) levels compared with controls. Serum PTH ($p < 0.05$) and 25-hydroxy-vitamin D ($p = 0.000$) levels were significantly reduced compared with controls. In particular, 8 (26.6 %) patients had borderline serum Ph levels (4.0–4.5 mg/dl) and 14 (46.6 %) had clearcut hyperphosphatemia (> 4.5 mg/dl), whereas only 2 patients (6.6 %) had clearcut hypercalcemia (> 10.5 mg/dl). The prevalence of hyperphosphatemia was significantly correlated with GH ($r = 0.4$; $p = 0.02$) but not with IGF-1 levels. Moreover 4 (13.3 %) patients had a history of micronephrolithiasis; 3 (75 %) of them had an associated hyperphosphatemia; a signifi-

cant association was found between hyperphosphatemia and micronephrolithiasis ($p < 0.05$; $\chi^2 = 3.81$). After disease remission, serum Ph ($p < 0.001$), Ca ($p < 0.05$) and urinary Ca ($p < 0.001$) levels significantly decreased, PTH and hydroxyvitamin D levels increased slightly but not significantly; hyperphosphatemia completely recovered in all patients.

Conclusion The results of this study demonstrate that acromegaly is commonly associated with hyperphosphatemia, which seems to be a specific marker of active disease. This hyperphosphatemia is mainly dependent on GH excess, and directly correlated with GH levels, suggesting that it could be considered a marker of disease severity.

Disclosure: No significant relationships.

P031

Triple Neoplasm in a Single Acromegalic Patient

S. Akin, S. Dagdelen, T. Erbas

Dept of Endocrinology and Metabolism, Hacettepe University School of Medicine, Ankara, Turkey

The incidence of malignant tumours in patients with acromegaly is higher than expected for the general population. We report a rare case of acromegaly complicated with multiple tumors, including renal cell carcinoma, well-differentiated follicular thyroid neoplasm, and colonic tubular adenomas with low-grade dysplasia. In 1987, a 24-year-old man presented with acromegaly with facial changes and headache. Laboratory data showed elevated GH levels and GH did not fall after OGTT. He underwent transsphenoidal surgery for removal of the pituitary macroadenoma. The patient was referred to our institution 10 years after initial surgery and MRI of the pituitary revealed the presence of a residual pituitary adenoma (12×10 mm). Second transsphenoidal surgery was performed. Immunohistochemical examination was consistent with GH-positive adenoma. After surgery, in 2001, he underwent stereotactic radiosurgery. Long-term treatment with octreotide normalized his plasma GH-IGF 1 concentration. In 2006, MRI of the abdomen showed a right renal mass (28×24 mm) suggestive for renal cell carcinoma. Right nephrectomy was performed. Histopathological examination of the resected tumor confirmed the diagnosis of renal cell carcinoma. In 2007, the patient had a thyroid ultrasound which showed an enlarged thyroid lobe containing multiple vascularised, hyper- and hypoechogenic nodules (24×17 mm). Fine-needle aspiration cytology of a dominant thyroid nodule suggested indeterminate results. After thyroidectomy, histopathological examination revealed a well-differentiated follicular neoplasm of uncertain malignant potential. In 2011, colonoscopy revealed multiple small sessile polyps in the transverse and descending colons. Microscopic study demonstrated tubular adenomas with low-grade dysplasia. Multiple neoplasms may have developed in the patient due to the permissive effects of IGF-1 and GH on tumorigenesis. Acromegaly is associated with an increasing risk of malignant tumours. Therefore, periodic assessment with colonoscopy, thyroid ultrasonography, abdominal BT, and mammography should be performed at regular intervals for screening and early detection of malignant tumours in acromegalic patients.

Disclosure: No significant relationships.

■ GH-IGF1 Axis

P032

GH Response Induced by Respiratory Muscle Endurance Training in Healthy Subjects

A. Sartorio¹, F. Agosti¹, A. Patrizi¹, E. Comprì¹, E. E. Müller², S. G. Cella², A. E. Rigamonti²

¹Laboratorio Sperimentale di Ricerche Auxo-endocrinologiche, Istituto Auxologico Italiano, IRCCAS; ²Dipartimento di Farmacologia Medica, Università degli Studi di Milano, Milan, Italy

Introduction To date, the vast majority of studies evaluating growth hormone (GH) response to acute physical exercise has been

performed involving gross muscle groups. To the best of our knowledge, none has evaluated the effects of a respiratory muscle endurance training (RMET) on hormonal secretions, particularly on GH release, though some respiratory devices have been widely used in athletes to train respiratory muscles and to improve cardiopulmonary function and physical performance.

Methods and Materials Eight healthy men underwent an incremental progressive RMET protocol of 11 daily sessions, obtained through the use of a specifically designed respiratory device (Spiro-Tiger®, Idig, Fehraltorf, Switzerland). The 12th session of RMET (15 min duration: 1 min at a respiration rate of 28 acts/min, 5 min at 32 acts/min, 5 min at 34 acts/min, 4 min at 36 acts/min) was associated with blood samplings for determination of GH, cortisol, ghrelin, glucose, and lactate (LA) levels.

Results GH and cortisol responses significantly increased after a 15-minute RMET session, which, in contrast, inhibited ghrelin secretion. There was a minimal, though significant, increase in LA levels with a significant elevation in glycemia.

Conclusion In conclusion, a 15-minute RMET session, administered after an 11-day incremental progressive RMET protocol, was capable of stimulating GH and cortisol release and suppressing ghrelin secretion. Optimization of incremental progressive RMET protocols would be important to maximize the positive chronic effects of this intervention on somatotrophic function and muscle performance.

Disclosure: No significant relationships.

P033

Relation between Insulin-Like Growth Factor-1 and Insulin-Like Growth Factor-Binding Protein-3 and Blood Pressure in a German and Danish Study

N. Friedrich¹, T. Torben Jørgensen², H. Wallaschofski¹, A. Linneberg²

¹Institute for Clinical Chemistry and Laboratory Medicine, University Medicine Greifswald, Germany; ²The Capital Region of Denmark, Research Centre for Prevention and Health, Glostrup, Denmark

Introduction Hypertension represents one of the major health problems and an independent risk factor of cardiovascular disease and mortality. Studies showed that never-treated hypertensive patients and patients with essential hypertension have an excess of insulin-like growth factor 1 (IGF-1). We aim to analyze the association between IGF-1 or IGF-binding protein 3 (IGFBP-3) and blood pressure (BP) in 2 independent population-based studies.

Methods and Materials Data of 3357 Danish subjects (Health 2006) and of 4048 German subjects (Study of Health in Pomerania [SHIP]) were investigated. Serum IGF-1/IGFBP-3 levels were determined by immunoassays and systolic/diastolic BPs were measured. Hypertension was defined as a systolic BP ≥ 140 mmHg, diastolic BP ≥ 90 mmHg or the use of antihypertensive medication. Analyses of variance (ANOVA) and logistic regression analyses adjusted for confounders were performed.

Results ANOVA revealed a positive association between IGFBP-3 levels and diastolic BP in SHIP, whereas in Health2006 the association barely missed statistical significance. With respect to hypertension, again high IGFBP-3 levels (4th vs 1st quartile; SHIP: Odds Ratio [OR]: 1.37 [95%-confidence interval {CI}: 1.11–1.70]; $p < 0.01$; Health2006: OR 1.61 [95%-CI: 1.10–2.35]; $p = 0.01$) were related to a higher risk in both study populations. After exclusion of subjects taking antihypertensive medication, the relation between IGFBP-3 and increased BP was still apparent. For IGF-1, we also observed a trend toward a positive association with hypertension (SHIP: OR: 1.21 [95%-CI: 0.98–1.50]; $p = 0.07$; Health2006: OR 1.39 [95%-CI: 0.95–2.04]; $p = 0.09$).

Conclusion Positive associations between IGFBP-3 levels and blood pressure or hypertension were found. The underlying physiological mechanisms need to be further elucidated.

Disclosure: No significant relationships.

P034

Reduced Growth Hormone (GH) Responsiveness to the Ghrelin Stimulation Test and Combined Administration of GH-Releasing Hormone (GHRH) and Growth Hormone Peptide-6 (GHRP-6) in Patients with Prader-Willi Syndrome (PWS)

M. Doknic, D. Micic, D. Miljic, S. Pekic, M. Stojanovic, V. Popovic
Neuroendocrine Unit, Clinic for Endocrinology, Diabetes and Metabolic Diseases,
Faculty of Medicine, University of Belgrade, Serbia

Introduction Short stature in PWS is thought to be a result of a GH/IGF-1 axis deficiency. While impaired GH secretion to different GH stimuli has been documented, GH response to ghrelin in PWS has not been reported yet. It is not clear whether blunted GH response in PWS is due to true GH deficiency or a consequence of obesity. Furthermore, ghrelin is thought to act at the hypothalamic level while the combined GHRH+GHRP6 test primarily acts at the pituitary level. GHRP-6 is a ghrelin analogue.

Aim To investigate GH response to 2 provocative tests: to ghrelin alone or to combined GHRH+GHRP-6 in patients with PWS.

Methods and Materials We investigated 12 patients with PWS (6 males, aged 12–23 years, height 149.3 ± 12.2 cm; BMI 44.2 ± 8.1 kg/m²). GH response to the ghrelin test (cut-off 7 µg/l) was studied in 6 patients while in the other 6 patients GH secretion was investigated after the combined GHRH+GHRP-6 test (cut-off 10 µg/l). Both tests were performed in 2 patients. Blood samples for GH were taken at –15, 0, 30, 45, 60, and 90 minutes after ghrelin (1 µg/kg iv) and after combined GHRH+GHRP-6 (GHRH 100 µg + GHRP-6 90 µg iv). IGF-1 concentrations were measured in all PWS patients.

Results Four of 6 pts with PWS (67 %) had severe GH deficiency (0.7 ± 0.1 µg/l) after the ghrelin provocative test. Similarly, in group of pts with PWS after combined test, GH response was abnormal (2.5 ± 0.3 µg/l) in 4/6 pts (67 %). In 2 patients who had both tests, discordant results were obtained, blunted GH response to ghrelin with preserved GH response to combined test. Circulating IGF-1 levels were decreased (98.3 ± 12.4 ng/ml) in all patients.

Conclusion Most patients with PWS have reduced GH responsiveness to either the ghrelin test or to the combined GHRH+GHRP-6 test. Since 2 patients showed normal GH response after GHRH+ GHRP-6 administration and the lack of GH secretion after ghrelin provocation, it confirms that ghrelin acts primarily at the hypothalamic level.

Disclosure: No significant relationships.

P035

Body Composition (Lean, Fat, and Bone Tissue) and Bone Mineral Density in Patients with Acromegaly

Y. Poteskhin, V. Pronin, I. Sokolina, E. Gitel, M. Morozova
UCH # 2, First MSMU I.m. Sechenov, Moscow, Russian Federation

Introduction GH and IGF-1 are important regulators of bone metabolism and body composition. High levels of GH/IGF-1 stimulate bone turnover and change body composition.

Methods and Materials We examined 71 acromegaly patients (16 males, 55 females). Estimated body weight, BMI, GH, IGF-1, osteocalcin, beta-crosslaps, body composition, and densitometry. Control group was 18 healthy persons (6 men, 12 women) with no significant violations of the phosphorus-calcium metabolism are similar in age and BMI.

Results The entire cohort of patients was divided into 2 groups: group 1 (active acromegaly) and group 2 (drug biochemical compensation) with an additional third group of healthy individuals serving as controls. It was found an increase in lean tissue mass in group 1 vs group 2* and the control group*. Fat mass is higher in the 1st and 3rd groups compared with the 2nd* and not statistically different in the 1st and 3rd groups. When comparing the BMC in the 3 groups revealed increase of this index in group 1 compared to 2nd* and control groups*, BMC, in turn, positively correlated with levels of IGF-1* ($r = 0.32$). Positive correlation was found between IGF-1

and osteocalcin levels* ($r = 0.49$), as well as a weak positive correlation between GH and osteocalcin* ($r = 0.30$). In group 1, GH/IGF-1 correlation with markers of bone resorption have been identified, the level of osteocalcin was significantly higher than in group 2*. In group 2, the negative correlation of GH and beta-crosslaps* ($r = -0.35$). Posterior longitudinal ligament ossification and increasing the diameter of the vertebrae were identified. All *: $p < 0.05$.

Conclusion

- Active GH/IGF-1 leads to an increase in osteocalcin, indicating metabolic effect on bone.
- Osteocalcin can be used as a marker of bone biochemical activity in acromegaly.
- The early achievement of biochemical compensation promotes the prevention of disorders affecting the quality of life of patients.
- Ossification of ligaments and widening of vertebrae may mask lowering BMD.

Disclosure: No significant relationships.

P036

Growth Hormone Protects Breast Cancer Cells from Chemotherapy by Hampering Cytotoxic-Induced Apoptosis in Estrogen Receptor-Negative Breast Cancer Cells

M. Minoja, E. Gentilin, M. Rossi, F. Tagliati, M. R. Ambrosio, E. degli Uberti, M. C. Zatelli
Dept of Biomedical Sciences and Advanced Therapies, University of Ferrara, Italy

Introduction Growth hormone (GH) and insulin-like growth factor (IGF-1) play important roles in breast cancer (BC) development. IGF-1 has been shown to importantly influence estrogen effects on BC, suggesting that estrogen receptors (ER) may also mediate GH effects. We previously demonstrated that GH and IGF-1 protect the ER-positive BC-derived MCF7 cell line against the cytotoxic effects of doxorubicin (D), independently of IGFI. We evaluated whether this holds true also in the ER-negative BC-derived MDA-MB-231 cell line and in the normal MCF-12A breast cell line. In addition, we investigated the possible mechanisms implicated in the protective action of GH towards the cytotoxic effects of D.

Methods and Materials We evaluate the effects of GH on cell viability, cell cycle, apoptosis, and JNK phosphorylation in breast cancer cell lines.

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

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

Disclosure: No significant relationships.

P037

Growth Hormone and IGF-1 Alterations in Children and Adolescents with Epilepsy

Z. Viksna¹, R. Ligere²
¹Private, Darza Street 18, Jelgava; ²Dept of Endocrinology, Riga Stradin's University Hospital, Latvia

Introduction Growth hormone and IGF-1 (insulin-like growth factor-1) are very important in somatic growth and can relevantly influence both growth and maturation of a child. Since epileptic seizures

may affect hormonal output and regulation, it is essential to diagnose such peculiarities as early as possible.

Aim To evaluate changes in mean levels of growth hormone and IGF-1 in blood serum in children and adolescents with epilepsy.

Materials The retrospective, case-control research involved 51 patients (26 girls [51 %]) aged 6 months to 18 years with diagnosed “idiopathic epilepsy” or “symptomatic epilepsy”. Age groups: 0–10 years (16 girls, 18 boys), 11–18 years (10 girls, 7 boys).

Methods We determined mean levels of GH and IGF-1 in blood serum 30 minutes up to 24 hours after epileptic attacks using the enzyme-linked immunosorbent assay.

Results In 13 of the 51 observed patients (25 %) we found hormonal changes. Mean levels of IGF-1 were reduced in 4 boys (16 %) and 3 girls (11.5 %). GH was increased in 2 boys (8 %) and 1 girl (3.8 %), reduced in 2 boys (8 %) and 1 girl (3.8 %).

Conclusion Clinical observations reveal that children with epilepsy demonstrate growth hormone and IGF-1 alterations, particularly in the age group 0–10 years, claim for accurate analysis, including clinical data.

Disclosure: No significant relationships.

P038

Influence of IGF(CA)19 Gene Polymorphism on Metabolic Parameters in Adults with GH Deficiency (GHD) before and after rhGH Therapy

E. Ferrante¹, E. Sala¹, C. Giavoli¹, E. Verrua¹, G. Mantovani¹, E. Profka¹, L. Olgiati¹, E. Malchiodi¹, M. Filopanti¹, S. Bergamaschi¹, M. Arosio², B. Ambrosi³, A. Spada¹, P. Beck-Peccoz¹

¹Unit of Endocrinology and Diabetology, Dept of Medical Sciences, University of Milan, Fondazione IRCCS Ca' Granda, Ospedale Maggiore Policlinico; ²Unit of Endocrinology, University of Milan, Ospedale S. Giuseppe Multimedica; ³Endocrinology and Diabetology Unit, Dept of Medical and Surgical Sciences, University of Milan IRCCS, Policlinico S. Donato, Milan, Italy

Introduction Recent data showed a correlation between a highly polymorphic microsatellite in the IGF-1 gene promoter, composed of variable cytosine-adenine (CA) repeats (n = 10–24) and IGF-1 serum levels in normal, acromegalic, and GHD subjects. As the role of this polymorphic variant has not been well elucidated, the aim of this study was to investigate the influence of such a polymorphism on clinical and biochemical characteristics of adult patients with GHD (n = 97) at baseline and after 12 months of rhGH replacement therapy.

Methods and Materials IGF-1 levels, body composition (BF%), lipid profile, and glucose homeostasis were evaluated. Different genotypes were studied using the microsatellite method. According to the most frequent 192 bp allele (equivalent to 19 CA repeats) patients were divided in 3 genotype groups: homozygous for 192 bp allele (192/192, n = 7, 7.2 %, group A), heterozygous for the 192 bp allele (192/X, n = 68, 70.1 %, group B), and non-carriers of the 192 bp allele (X/X, n = 22, 22.7 %, group C).

Results The IGF-1 genotype did not influence the clinical and biochemical phenotype of GHD adults at baseline. However, when analyzing 12-month rhGH effects separately in the 3 groups, the increase in IGF-1 levels and decrease in BF% was similar, while a worsening of insulin sensitivity, documented by a significant increase in glucose levels and HOMA-IR and by a significant decrease of QUICKI, was observed only in groups carrying at least a wild type allele (192/192 and 192/X).

Conclusion 12-month follow-up of each group of patients showed that the absence of wild type allele in IGF-1 gene promoter seems to protect adult patients with GHD from the well-known short-term worsening of insulin sensitivity induced by rhGH replacement therapy.

Disclosure: No significant relationships.

P039

Falsely Elevated IGF-1 Levels: Still a Dilemma

L. Bancos¹, D. Erickson¹, T. Nippoldt¹, A. Algeciras-Schimmich²
¹Endocrinology; ²Laboratory, Mayo Clinic, Rochester, USA

Introduction As insulin-like growth factor-1 (IGF-1) measurements are increasingly performed in evaluation of endocrine and non-endocrine disorders, levels above the reference range are frequently encountered and incompletely explained despite extensive validation of age and sex appropriate cut-offs for normal IGF-1 values.

Methods and Materials A retrospective review of Mayo Clinic patients with IGF-1 levels above reference range between July 2007 and May 2010 was performed. IGF-1 was measured at Mayo Clinic Laboratories using the Siemens Immulite IGF-1 assay (Siemens Healthcare Diagnostics, Deerfield, IL). Patients with diagnosis of acromegaly were excluded from analysis.

Results Out of 2747 unique patients (4085 IGF-1 tests), 117 patients (4.2 %) (54 women/63 men), mean age of 51.5 years had “falsely” elevated IGF-1 levels. Mean BMI was 27.98. Median IGF-1 % increase above upper level of normal was 12.27 % (0.3–121 %). There was no relationship of percentage of increase in IGF-1 and age, sex, BMI, comorbidities, medications, gonadal status, GH, or IGBP-3 levels. Abnormal IGF-1 levels led to an oral glucose tolerance test for GH suppression in 21 patients and repeat IGF-1 measurements in 52 patients. From these 34 (65 %) had subsequent normal IGF-1 levels (median of 36 months follow-up). Intra-individual IGF-1 variability was high (median 22 %, ranges 2.06–69 %). The effect of pre-analytical sample handling was evaluated to determine if IGF-1 levels will increase after sample collection. Analysis of serum samples (n = 20) obtained from healthy donors demonstrated that in a subset of patients (7/20; 35 %) IGF-1 levels increase > 20 % from the baseline value when serum was left at ambient temperature between 2 and 72 hours. In contrast, if the samples were refrigerated upon collection no significant changes on IGF-1 levels from baseline occurred.

Conclusion Although all the interfering factors causing “falsely” elevated IGF-1 remain to be elucidated, some results can be explained by an intra-individual IGF-1 variability and pre-analytical sample handling.

Disclosure: No significant relationships.

P040

Positive Associations between Serum Levels of IGF-1 and Subcutaneous Fat Depots in Young Men. The Odense Androgen Study

M. Andersen¹, K. Brixen¹, C. Hagen¹, J. Frydlyk², T. Nielsen¹

¹Dept of Endocrinology, Odense University Hospital; ²Dept of Endocrinology and Internal Medicine, Aarhus University Hospital, Denmark

Introduction Serum levels of IGF-1 are of growing interest due to the associations with morbidity and mortality. Despite markedly suppressed GH secretion, total IGF-1 levels are often within normal range in obese adults.

Aim To study associations between IGF-1 and estimated muscle mass in the Odense Androgen Study population and to investigate associations between serum IGF-1 and regional fat depots.

Methods and Materials The Odense Androgen Study is a population-based, cross-sectional study of 776 randomly selected men aged 20–29 years. Regional lean and fat masses were measured by dual-energy X-ray absorptiometry, whereas regional muscle and fat areas were assessed by magnetic resonance imaging.

Results Age-adjusted IGF-1 levels correlated significantly with different estimates of muscle mass (r-values between 0.15 and 0.19; p < 0.001). Using multiple linear regression, serum IGF-1 correlated positively with subcutaneous adipose tissue on the abdomen (SAT) after controlling for visceral adipose tissue (VAT) in the whole group and in the subgroup of men with normal waist circumference (r-values between 0.13 and 0.15; p < 0.03). In addition, IGF-1 correlated positively with subcutaneous thigh fat area (TFA) after con-

trolling for intramyocellular lipid (imcl) ($r = 0.18$; $p < 0.004$) and IGF-1 correlated negatively with TFAimcl in the whole group and in the subgroup of men with normal waist circumference.

Conclusion SAT and subcutaneous TFAs were positively associated with IGF-1 in regression analyses. Conversely, imcl of the thigh was inversely associated with IGF-1 levels. These findings emphasize the differential associations between IGF-1 and regional fat deposits. Future studies may provide further insight regarding the interplay between circulating IGF-1 levels and regional muscle and fat mass.

Disclosure: No significant relationships.

P041

Targeting the IGF-1R System in Pituitary Tumors in Vitro: Antiproliferative Action and Pitfalls

M. Theodoropoulou¹, M. Riebold¹, M. Tichomirowa², J. L. Monteserin-Garcia¹, M. Buchfelder³, H. Rubinfeld⁴, G. K. Stalla¹

¹Endocrinology, Max Planck Institute of Psychiatry, Munich; ²Dept of Endocrinology and Metabolism, 1st Medical Clinic, University of Mainz; ³Neurosurgical Clinic, University of Erlangen-Nürnberg, Erlangen, Germany; ⁴Institute of Endocrinology and Erlangenstein Medical Research Center, Rabin Medical Center, Petach Tikva, Israel

Introduction IGF-1 receptors (IGF-1R) and their aberrant signaling cascade contribute to the pathogenesis of several solid cancers. Pituitary adenomas express IGF-1R and present with overactivation of the IGF-1R pathway. The aim of the study was to identify the potential antiproliferative action of small molecule inhibitors targeting IGF-1R signaling cascade in pituitary adenomas.

Methods and Materials The IGF-1R tyrosine kinase inhibitor NVP-TAE226, the PI3K/mTOR inhibitor NVP-BEZ235 and the allosteric mTOR inhibitor everolimus were used on acromegalic ($n = 11$) and non-functioning pituitary adenomas (NFPA; $n = 15$) in primary cell culture. Changes in cell viability were determined by thymidine incorporation and a non-radioactive colorimetric assay.

Results TAE226 at the 100 nM concentration suppressed cell viability by $> 20\%$ in 9 out of 15 NFPA and in 10 acromegalic tumors, while at 10 nM it was efficacious in 8 NFPA and 4 acromegalic tumors. Inhibiting the PI3K pathway in the same tumors, using BEZ235 suppressed cell viability at 10 nM concentration in all NFPA and in 2 acromegalic tumors. In contrast, mTOR inhibition with everolimus was efficacious in all but one acromegalic cases, but in only 4 out of 15 NFPA. IGF-1R inhibition in acromegalic tumors in vitro was accompanied by increased GH secretion, which was evidenced when GH got normalized to cell viability assay counts. Similar GH increase was observed after mTOR, indicating a previously unsuspected role for the mTOR pathway on GH synthesis.

Conclusion Altogether, these data indicate a tumor-specific dependency on the different IGF-1R signaling branches, with NFPA being sensitive to PI3K inhibition and acromegalic tumors to mTOR inhibition. Targeting IGF-1R pathways have a potential as antiproliferative agents for the treatment of NFPA. In contrast, their potent antiproliferative action in acromegalic tumors is compromised by the concomitant increase in GH secretion.

Disclosure: No significant relationships.

■ Prolactinomas

P042

Heart Valve Disease among Patients with Hyperprolactinemia: A Nationwide Population-Based Cohort Study

C. Steffensen¹, M. Maegaek²

¹Dept of Endocrinology, ²Clinical Epidemiology, Aarhus University Hospital, Denmark

Introduction Increased risk of heart valve disease during treatment with certain dopamine agonists, such as cabergoline, has been

observed in patients with Parkinson's disease. The same compound is used to treat hyperprolactinemia, but it is unknown whether this also associates with heart valve disease.

Objective To assess the incidence of diagnosed heart valve disease and cardiac valve surgery among patients with hyperprolactinemia compared to a general population cohort in Denmark.

Methods and Materials We identified 2381 hyperprolactinemia patients with a first-time diagnosis recorded from 1994–2010 in the registry, with no previous hospital diagnosis of heart valve disease. Each patient was compared to 10 age- and gender-matched comparison cohort members from the general population. The association between hyperprolactinemia and heart valve disease was analyzed with Cox's proportional hazards regression, controlling for potential confounding factors. To assess the risk of cardiac valve surgery and avoid ascertainment bias, sub-analysis was made in a cohort of 2387 hyperprolactinemia patients with no previous cardiac valve surgery and 23,870 comparison cohort members.

Results 19 (0.80 %) hyperprolactinemic patients were diagnosed with heart valve disease during a total of 17,759.8 years of follow-up, compared to 75 (0.31 %) persons in the comparison cohort during 179,940.6 years of follow-up (adjusted hazard ratio [HR]: 2.27 [95%-CI: 1.35–3.82]). Seven of the 10 patients treated with cabergoline and diagnosed with heart valve disease were asymptomatic and diagnosed on the basis of an echocardiography performed as a safety measure. However, only 2 patients (0.08 %) with hyperprolactinemia underwent surgery, compared to 28 (0.12 %) persons in the general population cohort (adjusted HR: 0.55 [95%-CI: 0.13–2.42]).

Conclusion Data from the present register-based study do not support that hyperprolactinemia or its treatment is associated with an increased risk of clinically significant heart valve disease.

Disclosure: No significant relationships.

P043

Giant Invasive Prolactinomas: Long-Term Follow-Up

S. P. Pérez-Reyes¹, A. Balderrama-Soto², L. Portocarrero-Ortiz², I. Perez-Ner³

¹Neurosurgery; ²Neuroendocrinology; ³Neurochemistry, Instituto Nacional de Neurología y Neurocirugía M.V.S., Distrito Federal, Mexico

Introduction Invasive giant prolactinoma, an extreme subset of prolactinoma, is characterized by large size, high aggressiveness, massive extrasellar involvement, and very high prolactin levels; it is prevalent in young men.

Methods and Materials From May 1998 to December 2011, we had 140 macroprolactinomas; 36/140 (25.71 %) met the criteria for giant prolactinoma (diameter > 4 cm, plasma prolactin levels > 1000 ng/ml, and clinical signs of hyperprolactinemia and mass effect). The patients were followed for a mean period of 4.56 years.

Results 28 men (77.77 %) and 8 women (22.22 %) were included; mean age 35.41 years (15–62). The most frequent clinical debut were headache in 35 patients (97.22 %) and visual disturbances in 34 patients (94.44 %); the atypical debuts were: tonic-clonic seizures ($n = 1$), third cranial nerve palsy ($n = 1$), hydrocephalus ($n = 1$), pyramidal syndrome due to compression of cerebral peduncles ($n = 1$), and cerebrospinal fluid leakage ($n = 1$). 34/36 patients (94.44 %) received as first-line treatment DA, 2/36 (5.56 %) required surgery for cerebrospinal leakage and V-P shunt for hydrocephalus; 27 patients were treated with cabergoline and 9 patients with bromocriptine. Before treatment mean prolactin was 12,216 ng/ml (1176–18,000), 1 month after initiation of treatment prolactin levels decreased to 87.62 %, mean prolactin was 1511.6 ng/ml (8–13,327.6). At 6 months of treatment with DA, 2 patients required surgery, 1 patient presented with pituitary apoplexy and 1 patient with LCR leakage. Mean tumoral diameter was 5 cm (range 4–8.2 cm). MRI showed tumoral reduction in all patients; 16/36 patients (44.44 %) without tumoral evidence in our time of follow-up.

Conclusion Giant prolactinomas are rare lesions, accounting for 0.5 % of all pituitary tumors and having complex management issues; their response to treatment and long-term follow-up has only

been addressed in isolated case reports. We report our experience in the management of giant prolactinomas over 13 years, with excellent response to DA and low complication rates.

Disclosure: No significant relationships.

P044

Predictors of Neuropsychiatric Side Effects of Dopamine-Agonist Therapy in Patients with Prolactinomas

A. P. Athanasoulia¹, C. Sievers¹, M. Ising², A. Brockhaus³, A. Yassouridis³, G. K. Stalla¹, M. Uhr⁴

¹Internal Medicine, Endocrinology and Clinical Chemistry; ²Molecular Psychology;

³Biostatistics; ⁴Pharmacokinetics, Max Planck Institute of Psychiatry, Munich, Germany

Introduction Treatment with dopamine agonists in patients with prolactinomas and Parkinson's disease is associated with central side effects. Central side effects may depend on a substance's ability to pass the blood-brain barrier which can be actively controlled by transporter molecules such as the P-glycoprotein encoded by the ABCB1 gene. In the present study, we aimed to determine whether cabergoline is transported by the P-glycoprotein and whether polymorphisms of its encoding ABCB1 gene predict central side effects of cabergoline therapy in patients with prolactinomas.

Methods and Materials (1) In an experimental mouse model lacking the homologs of the human ABCB1 gene (*abcb1ab* double-knockout mice), we examined whether cabergoline is a substrate of P-glycoprotein. (2) In a human case-control study, we investigated the association of 4 selected ABCB1 gene SNPs (rs1045642, rs2032582, rs2032583, rs2235015) with the occurrence of central side effects under cabergoline therapy in 92 prolactinoma patients treated at the Max Planck Institute of Psychiatry in Munich.

Results (1) In the experimental mouse model, we observed that brain concentrations of cabergoline were 10-fold higher in mutant mice compared to their wild-type littermates implying that cabergoline is indeed a substrate of the transporter P-glycoprotein at the blood-brain-barrier level. (2) In humans, we found significantly negative associations for the C-carriers and heterozygous CT-individuals of SNP rs1045642 with 2 central side effects (frequency of fatigue and sleep disorders) and for the G-carriers of SNP rs2032582 with the enhancement of dizziness under cabergoline. For the SNPs rs2235015 and rs2032583, no associations with central side effects under cabergoline were found.

Conclusion This is the first study demonstrating that individual ABCB1 gene polymorphisms reflecting a different expression and function of P-glycoprotein could predict the occurrence of central side effects under cabergoline. Our findings can be viewed as a step into personalized therapy in prolactinoma patients.

Disclosure: No significant relationships.

P045

Cataplexy in Prolactinoma Patients

M. Nikolic-Djurovic¹, D. Pavlovic², M. Petakov¹, D. Jankovic¹, S. Damjanovic¹

¹Neuroendocrinology, Institute of Endocrinology, University Clinical Center;

²Neurology, Institute of Neurology, Clinical Center of Serbia, Belgrade, Serbia

Introduction Cataplexy and sleep paralysis can be regarded as attacks of motor inhibition with loss of muscle tone and areflexia. Cataplexy diagnosis relies on clinical grounds and history and is rarely confirmed by video-polygraphy. This case describes a female patient with a prolactin-secreting pituitary tumor (macroprolactinoma) who developed attacks of cataplexy.

Methods and Materials This case describes a female patient with a prolactin-secreting pituitary tumor (macroprolactinoma) who developed attacks of cataplexy.

Results A 53-year-old female, smoker, with a prolactin-secreting pituitary tumor (macroprolactinoma) and a history of acute myocar-

dial infarction presented at our institute because of sudden episodes of weakness with tone loss leading to falls and injuries. Her glycaemia levels as well as arterial blood pressure were normal. 24-hour electrocardiogram and blood pressure monitoring were also normal. When seen by a psychiatrist she denied psychotic phenomena and suicidal ideas. MRI of sellar region was described as empty sella with an expansive intrasellar mass with para- and infrasellar propagations. The patient was treated with dopamine agonist therapy. During neurological examination, mild to moderate obstructive hypopnea/apnea during sleep was shown. The architecture and continuity of sleep were dislocated. The described phenomenology was responsible for the patient's temporary somnolence. Also, EEG monitoring during sleep and at daytime did not show epi potentials. It was concluded that abrupt actuality of tone loss with subsequent falling to the ground does not have epi characteristics, but characteristics of collapse-cataplexy. Recommended HLA standardization was done and positive (HLA-DR3,16; DR 51; DQ 1,2), so the diagnosis of cataplexy was confirmed.

Conclusion Cataplexy and sleep paralysis can be regarded as attacks of motor inhibition with a loss of muscle tone and areflexia. This is the first case of a prolactin-secreting pituitary macroadenoma associated with cataplexy. The mechanism by which the pathology is related to the symptoms is still not clear.

Disclosure: No significant relationships.

P046

Pituitary Apoplexy in a Macroprolactinoma Associated with Thrombocytopenia and Cabergoline Treatment. A Case Report

B. García-Guzmán¹, A. Balderrama-Soto¹, L. Portocarrero-Ortiz¹, J. Navarro-Bonnet², A. Dorantes-Argandar²

¹Neuroendocrinology; ²Neurosurgery, Instituto Nacional de Neurología y Neurocirugía MVS, Distrito Federal, Mexico

Introduction Pituitary apoplexy is a rare and life-threatening entity due to possible infarction and/or hemorrhage. Its incidence is 1.2 per million inhabitants. Multiple risk factors have been described.

Methods and Materials A 34-year-old male with an 18-month history of headaches and progressive visual loss until amaurosis right-sided. Physical examination proved neurological integrity and right optic atrophy. MRI showed a pituitary lesion with suprasellar extension, optic chiasm compression, and third-ventricle effacement. Prolactin 3713 ng/ml (2.6–13.1 ng/ml), FSH 0.2 mUI/ml (1.5–12.4 mUI/ml), LH 0.2 mUI/ml (1.7–8.6 mUI/ml), testosterone 0.08 ng/ml (2–8 ng/ml), serum cortisol 3.1 mcg/dl (8.7–22.4 µg/dl), GH 0.1 ng/ml (0.003–0.97 ng/ml), platelets $71 \times 10^3/\text{U}$ ($150\text{--}450 \times 10^3$). He was started on levothyroxine, prednisone, and cabergoline 0.5 mg/week, afterwards raised to 1 mg/week. 15 days later he presented with an intense and abrupt headache, left visual loss, and a platelet count of $79 \times 10^3/\text{U}$. The MRI showed pituitary tumor growth with intratumoral hyperintense areas. He was started on stress-dose steroids. He developed tonic-clonic generalized seizures and decreased mental status. The new MRI showed hyperintense areas on the perfusion sequence in the vascular territory of the right anterior cerebral artery and ipsilateral diffuse brain edema.

Results Urgent decompressive surgery was undertaken through a transphenoidal approach, with torpid evolution to brain death.

Conclusion We present a case of a pituitary apoplexy in a macroprolactinoma with cabergoline treatment, this association is infrequent. In this case, we consider that the presence of thrombocytopenia associated with cabergoline treatment was the main factor related to the development of pituitary apoplexy.

Disclosure: No significant relationships.

P047

Gender-Related Differences in Patients with Prolactinomas*E. Doudinskaya¹, L. Dzeranova², I. Barmina², E. Pigarova²*¹National Research Center for Preventive Medicine; ²Neuroendocrinology and Bone Disease, The National Research Center for Endocrinology, Moscow, Russian Federation

Introduction Hyperprolactinemia is the most frequent reason of male and female endocrine infertility. In the general population the prevalence of hyperprolactinemia is about 0.5 % in women and 0.07 % in men, which gives the predominance of all forms of hyperprolactinemia in women by 7–10 times.

Objective of this study was to identify gender differences in patients with prolactinomas.

Methods and Materials 286 patients (148 female, 138 male) with hyperprolactinemia (Prl > 600 mU/l) were studied. Presenting clinical symptoms, total Prl, monomeric Prl levels and tumor size at MRI were measured.

Results Men were older than women (median 35 vs 30 years; $p = 0.001$). Tumor-related hyperprolactinemia was revealed in 182 patients (63.3 %): in 71 women (48 %) and in 107 (76 %) men. 40 women and 29 men had microadenomas, 31 women and 78 men had macroprolactinomas. 36 patients (9 women and 27 men) had symptoms of invasive adenomas. The prevalence of headache was higher in men with macroprolactinomas (7/31 vs 48/78; $p = 0.0001$) and with microprolactinomas (8/40 vs 17/29; $p = 0.006$). Galactorrhea was noted more often in women with micro- and macroprolactinomas ($p = 0.001$ and $p = 0.003$, respectively). Prolactin levels were higher in men than in women, whether exhibiting macro- (4901 mU/l [1708–14,025] vs 1888 mU/l [1044–3904]; $p = 0.04$) but without clinical significance for microadenomas (2026 mU/l [405–3460] vs 954 mU/l [615–1352]; $p = 0.15$) and the size of the adenoma was larger in men than in women irrespective of macro- (20.4 vs 16.2 mm; $p < 0.0001$) or microadenoma (8.0 vs 76.9 mm; $p = 0.03$).

Conclusion Prevalence of macroprolactinomas is higher in men; microprolactinomas and non-tumoral hyperprolactinemia appeared to be more frequent in women. Macroprolactinomas in men were larger than in women. Clinical symptoms differed according to gender, with galactorrhea more frequent in women, but headache is more frequent in men. Prl levels are higher in men with tumoral hyperprolactinemia.

Disclosure: No significant relationships.

P048

Tamoxifen in Patients with Prolactin-Secreting Pituitary Tumors Resistant to Dopamine Agonists*N. Bykanova, L. Dzeranova, E. Pigarova*

Neuroendocrinology and Osteopathies, Endocrinology Research Centre, Moscow, Russian Federation

Introduction Monotherapy with dopamine agonists (DA) for prolactinomas is effective in most cases, but approximately 10 % of patients cannot achieve normalization of serum prolactin levels, indicating pharmacoresistance. The expression of estrogen receptors in prolactinomas introduced a new approach to medical treatment using selective estrogen receptor modulators (SERMs) but information on the efficiency and tolerability is very limited. Thus, we performed a pilot study assessing efficiency and safety of SERMs as an additional agent for the treatment of dopamine-resistant prolactinomas.

Methods and Materials The study included 6 patients with prolactinomas resistant to treatment with dopamine agonists aged 23–38 years. Resistance was defined as absence of normalization of total and monomeric prolactin and maintenance of pituitary adenoma on MRI during cabergoline treatment in doses > 3.5 mg/week for > 6 months. All patients received 20 mg of tamoxifen per day, the dose of cabergoline remained stable during the 3-month study pe-

riod. Prolactin and safety lab tests were assessed at baseline and monthly during the study period. There was an elevation of tamoxifen dose to 40 mg in patients without a 50-% decrease of prolactin levels at 1 month of treatment.

Results There was a significant reduction of prolactin levels in all patients after initiation of 20 mg tamoxifen (33.4 ± 13.9 %) at one month, but without complete normalization. Elevation of tamoxifen to 40 mg/day resulted in a further but very small decrease in prolactin levels (18 ± 8 %). We noted a normalization of menstrual cycle in 5 of 6 patients, normalization of libido in 3 of 6 patients. One patient achieved pregnancy. Endometrial hyperplasia was the most common side effect and required initiation of didrogesterone in 2 patients. Hot flashes were seen only in one patient. We did not register any other adverse effects of tamoxifen.

Conclusion Tamoxifen is mildly effective in reducing prolactin levels in patients with dopamine-resistant prolactinomas, but it seems to improve reproductive success.

Disclosure: No significant relationships.

P049

Frequency of Autoimmune Thyroid Disease in Functional Pituitary Adenomas*O. Soyuk, B. Ekiz Bilir, S. Yaman*

Division of Endocrinology, Dept of Internal Medicine, Faculty of Medicine, Istanbul University, Turkey

Introduction There are many studies showing that prolactin, secreted from the lactotroph cells of the anterior hypophysis, plays an important role in autoimmune diseases. Therefore, we aimed to evaluate the frequency of autoimmune thyroid disease (ATD) and its relationship with prolactin levels in functional pituitary adenomas (prolactinoma and acromegaly) causing hyperprolactinemia.

Methods and Materials Prolactinoma ($n = 66$; 53 F/13 M, mean age: 33 years) and acromegaly ($n = 78$; 40 F/38 M, mean age: 42 years) patients of our pituitary outpatient clinic with thyroid function tests (free T4, TSH, Anti-TPO and/or Anti-Tg) and thyroid ultrasonography were evaluated retrospectively.

Results The frequency of ATD (Hashimoto's thyroiditis) was found to be 16 % ($n = 13$) in acromegaly patients and 31 % ($n = 21$) in prolactinoma patients. Frequency of autoimmune thyroid disease in prolactinoma patients was significantly higher than in acromegaly patients ($p = 0.03$).

Conclusion As Hashimoto's thyroiditis is more frequently seen in patients with hyperprolactinemia due to prolactin-secreting tumors. We suggest to measure not only thyroid hormones but also thyroid autoantibodies in prolactinoma patients.

Disclosure: No significant relationships.

P050

Pregnancy Outcomes Following Cabergoline Treatment in Hyperprolactinemic Patients: An Observational Survey Study*R. S. Auriemma, L. F. S. Grasso, A. di Sarno, Y. Perone, R. Pivonello, A. Colao*
Dept of Molecular and Clinical Endocrinology and Oncology, University of Naples Federico II, Italy

Introduction Cabergoline (CAB) and other dopamine agonists are usually discontinued shortly after hyperprolactinemic patients become pregnant. Data on maternal and foetal exposure to CAB and on recurrence of hyperprolactinemia after pregnancy are still limited. This study aimed at elucidating the safety of exposure to CAB during early pregnancy and the recurrence rate of hyperprolactinemia after pregnancy.

Methods and Materials 68 pregnancies in a cohort of 59 patients with hyperprolactinemia (age 37.2 ± 5.5 years), including 51 patients with prolactin-secreting microadenomas, 6 with macroadenomas, and 2 with non-tumoral hyperprolactinemia, were evaluated. In

all patients, CAB therapy was recommended to be discontinued when pregnancy was confirmed. Pregnancies were monitored until delivery or termination according to routine clinical practice.

Results Outcomes examined include the incidence of abortions, premature delivery, and foetal malformations or abnormalities, as well as the recurrence rate of hyperprolactinemia after pregnancy. Pregnancies resulted in 10 (15 %) spontaneous abortions and 58 (85 %) live births. No neonatal malformations and/or abnormalities were recorded in our cohort. Only in 5 out of 59 patients treatment with CAB had to be restarted after pregnancy because of recurrence of hyperprolactinemia, whereas in 91.5 % of cases no further therapy was required and patients were classified as in complete clinical and biochemical remission at last follow-up (48 months).

Conclusion Foetal exposure to CAB at the time of conception and/or during pregnancy does not induce any increase in the risk of miscarriage or malformation. CAB withdrawal does not increase the risk of recurrence of hyperprolactinemia after pregnancy.

Disclosure: No significant relationships.

P051

Macroprolactinoma Associated with Antipsychotics. A Case Report

I. Illovayskaya¹, A. Dreval¹, G. Stashuk²

¹Therapeutic Endocrinology; ²Diagnostic Radiology, Moscow Regional Research & Clinical Institute, Russian Federation

Introduction There is a lot of data about prolactinoma shrinkage after treatment with the selective dopamine agonist, cabergoline. However, there is lack of data about the treatment of prolactinomas in patients with psychiatric disorders.

Methods and Materials We present a woman who suffered from schizophrenia from the age of 25 and accepted cyclodol and clopixon at different doses for a long period.

Results At the age of 27, her menses stopped and she noticed transitory galactorrhea. However, these symptoms were not any further investigated. At 45, she started to complain of vision deterioration. After routine vision correction she did not notice any improvement, so visual fields were examined and bitemporal hemianopsia diagnosed. Brain MRI: macroadenoma 32 × 36 × 33 mm (19,008 mm³) with supra-latero-infrasellar extension, suprasellar cistern, and pituitary stalk were not visualized, deformation of the chiasma and bottom of the 3rd ventricle; initial prolactin levels were 3700 mU/l (120–540), however, after dilution 156,300 mU/l (“hook” phenomenon). Cabergoline 3 mg/weekly was started. After 3 months of treatment she reported visual improvement, prolactin levels 63,000 mU/l. After 12 months of treatment: normal visual fields, prolactin levels 3210 mU/l, brain MRI: tumor size 24 × 27 × 26 mm (8424 mm³) with supra-latero-sellar extension without infrasellar component; suprasellar cistern and pituitary stalk were partly visualized, no deformation of the chiasma and the bottom of the 3rd ventricle. On the cabergoline treatment she had no worsening of schizophrenia.

Conclusion It is difficult to suppose when macroprolactinoma started to grow. However, the absence of clinical symptoms before antipsychotics could be considered as a sign of hormoprolactinemia. On antipsychotic treatment prolactin levels should be monitored regularly. In case of menstrual disorders and significant elevation of prolactin levels a brain MRI should be performed. In our case, cabergoline treatment in high doses was safe and rather effective with a considerable tumor-suppressing effect.

Disclosure: No significant relationships.

P052

Specific Clinical Features of Hyperprolactinemia According to Gender

F. Nasybullina, G. Vagapova, B. Pashaev, V. Danilov

Endocrinology, Kazan State Medical University, Russian Federation

Introduction The importance of the problem of hyperprolactinemia is associated with its high prevalence and major role in the genesis of male and female infertility.

Methods and Materials A total of 150 patients with prolactinomas (87 % female, 13 % male) were examined using generally accepted laboratory and instrumental methods of diagnosis.

Results The main clinical symptoms in women were irregular menstrual cycles (73 %) and lactorrhea (59 %). Chiasmatic syndrome was diagnosed in 14 % of women. Among men clinical signs of hypogonadism were observed in 69 % of patients and expressed as decreased libido (100 %), erectile dysfunction (80 %), infertility (6 %), and depression (98 %). Chiasmatic syndrome was diagnosed in 47 % of men. Microprolactinomas in women were detected in 79 %, small adenomas in 14 %, medium adenomas in 4 %, large adenomas in 2 %, giant prolactinoma was observed in 1 %. Microprolactinomas in men were observed in 37 %, small adenomas in 16 %, medium adenomas in 21 %, large adenomas in 16 %, and giant adenomas were observed in 10 %. According to the generally accepted indications, surgery was administered to 23 % of men and 6 % of women. Primary drug therapy allowed in most cases (90 % female and 95 % male) to restore the copulative function and the reproductive system, as well as to reduce tumor size. Different types of resistance to bromocriptine were observed in 24 % and to cabergoline in 11 %. The ratio of men and women was 1:9. In 80–85 %, prolactinomas resistant to bromocriptine achieved prolactin normalization on cabergoline.

Conclusion The absence of specific symptoms of hyperprolactinemia in men resulted in late diagnosis on the stage of neurological disorders and required surgical therapy in 23 % of men vs 6 % in women. In order to avoid surgery, exclusion of hyperprolactinemia should be carried out in all cases of hypogonadism in men.

Disclosure: No significant relationships.

P053

Management of a Macroprolactinoma in 2 Successive Pregnancies

A. K. Uzum, B. Canbaz, S. Ciftci, N. Colak Ozbey, F. Aral

Division of Endocrinology and Metabolism, Dept of Internal Medicine, Faculty of Medicine, Istanbul University, Turkey

Introduction Progressive increase in serum prolactin levels with a parallel increase in adenoma size occurs during pregnancy. Therefore, prolactin measurement is not recommended during the management of prolactinoma in pregnancy.

Methods and Materials We report on a patient with macroprolactinoma who had 2 successive pregnancies in short term and discuss the problems during gestational periods. A 34-year-old female receiving L-thyroxine for autoimmune thyroiditis complained of menstrual irregularity and galactorrhea. Increased serum prolactin levels and a 17 × 11 mm macroadenoma on sella magnetic resonance imaging (MRI) led us to diagnose a macroprolactinoma. Cabergoline (CBG) 0.5 mg/week was started and increased to 1.5 mg/week at follow-up. Because pregnancy had developed, CBG was shifted to bromocriptine (BRC) 10 mg/day. The antenatal period was uneventful, she was followed up with visual field examinations and serum prolactin levels increased slightly until the 21st week of gestation in which headache, loss of vision at left eye developed. Sella MRI indicating macroadenoma filling suprasellar cistern, compressing to optic chiasm was thought to have progressed in adenoma size. Serum prolactin showed a peak level of 948 ng/dl. We increased BRC to 15 mg/day. No further progression in visual field deficit and tumor size was detected during pregnancy. Prolactin levels remained high until vaginal delivery at week 38. She de-

livered a healthy baby. The postpartum period was uneventful, CBG was restarted but 3 months later she became pregnant again. Sella MRI without gadolinium at week 13 showed no progression and visual field examination was normal. She is currently 20 weeks pregnant under BRC 10 mg/day.

Results Patients with macroprolactinoma should be followed up closely for tumor expansion during pregnancy.

Conclusion Visual field examination is the better diagnostic tool to detect tumor progression in macroprolactinoma, sharp increases in prolactin levels may be remarkable for increases in tumor size.

Disclosure: No significant relationships.

■ Pituitary/Hypothalamic Tumors

P054

Follow-Up Protocol for Non-Functioning Pituitary Adenomas of the Hospital Universitario Virgen del Rocío, Sevilla

A. Soto-Moreno¹, M. C. Tous Romero¹, E. Venegas-Moreno¹, R. Oliva Rodriguez¹, E. Dios Fuentes¹, N. García Hernandez¹, A. Madrazo Atuxta¹, M. A. Japon¹, M. Polaina Bailon², A. Leal-Cerro¹, I. Martín Schrader²

¹Unidad de Gestión, Clínica de Endocrinología y Nutrición/Instituto de Investigación Biomédica; ²Unidad de Neurocirugía, Hospital Universitario Virgen del Rocío, Sevilla, Spain

Aim To describe the protocol implemented at our centre for handling non-functioning pituitary adenomas (NFPA) and to evaluate the compliance with the protocol in our representative patient sample and the results of its implementation.

Methods and Materials Descriptive retrospective study of surgical patients of NFPT between 1996 and 2011. A random representative sample of the patients was selected. Age of the patients, pathologic anatomy, and the actions taken (follow-up, second surgery, and/or radiotherapy) complying with protocol. The size, location, and accessibility of the remainder are the factors determining the second surgical intervention; the use of radiotherapy depends on the age of the patient, histology, atypicality, size, and location of the residual tumor.

Results The behavior of 65 NFPT was analyzed. After the first surgery in 13 patients there are no residual tumors. Of the 52 with remainders, a follow-up was chosen in 19 patients. In 15 patients, second surgery was performed, all with a considerable size (> 2 cm) and accessible for surgery; 4 of them also with campimetric affection potentially reversible. 19 of the patients were selected for radiotherapy after first surgery and another 14 subjects after second surgery; radiotherapy was indicated for patients with a non-favorable pathologic anatomy and/or non-surgical remainders. In 3 patients, the protocol was not implemented. In 2 patients treated with radiotherapy, second surgery was performed. The 19 patients under follow-up are stable.

Conclusion Multidisciplinary work and the creation of working protocols are useful and necessary in this kind of pathologies where first-level scientific evidence is not available. Preliminary results show that the degree of compliance with our protocol is high. In relation with the results of the application of the protocol, the prospect is promising although a larger follow-up is necessary in order to validate it and compare it with similar protocols.

Disclosure: No significant relationships.

P055

Non-Functioning Pituitary Adenoma Hyper-Uptake at ⁶⁸Ga-DOTANOC PET/CT

S. Piacentini¹, G. Perotti², F. Lugli¹, D. Iacovazzo¹, A. Fusco¹, E. Giovannini², G. Treglia², V. Rufini², L. de Marinis¹

¹Endocrinology; ²Nuclear Medicine, Catholic University, Rome, Italy

Introduction Pituitary adenomas are among the most common brain tumors, with a prevalence ranging from 78–94 cases per

100,000 inhabitants, 68 % of which are macroadenomas. Case series have documented that up to 20 % of macroadenomas grow significantly during follow-up. Patients with non-functioning pituitary adenoma (NFPA), if symptomatic, are generally referred for a transsphenoidal surgical approach with proven low morbidity and mortality and an overall recurrence rate of about 5 %. High expression of somatostatin receptors SST2 and SST5 in NFPA (70–90 %) represents the rationale for using radiolabelled somatostatin analogues (SSAs) in the diagnosis and in case of tumor relapse; moreover, there is some evidence for the possible use of somatostatin analogues in the medical treatment of NFPA. Indium-111-pentetreotide scintigraphy has been frequently used to diagnose NFPA relapse with a sensitivity between 80 and 90 %.

Methods and Materials A 52-year-old male patient was referred to our institution in April 2011 with onset of severe headache and visual-field defects. An MRI showed a voluminous expansive process at the sellar and suprasellar regions, with bilateral cavernous sinus involvement, and extension into the 3rd ventricle, compression of the brain stem, and dislocation of the optic chiasm. The patient underwent partial removal of the lesion through transsphenoidal approach. Histological examination showed a null-cell pituitary adenoma with a Ki-67 of 3 %. After 10 months, a follow-up MRI showed the presence of the residual pituitary adenoma localized at the sellar-suprasellar region. The patient underwent brain ⁶⁸Ga-DOTANOC PET/CT.

Results Brain ⁶⁸Ga-DOTANOC PET/CT showed intense focal uptake corresponding to the lesion visualized at the MRI. Therefore, SSA treatment was started.

Conclusion ⁶⁸Ga-DOTANOC PET/CT can be a useful tool not only in confirming the diagnosis of residual NFPA but could also plays a role in selecting patients for medical treatment with SSAs.

Disclosure: No significant relationships.

P056

Pituitary Carcinoma Recurrent to the Posterior Fossa and Spinal Space. A Case Report

S. Yarman, O. Soyuluk, R. Tanakol, H. Azizlerli

Division of Endocrinology, Dept of Internal Medicine, Faculty of Medicine, Istanbul University, Turkey

Introduction Pituitary carcinomas are still very rare and the prognosis is poor as the average survival time is < 4 years. In this case, spinal and posterior fossa metastases from an adrenocorticotrophic hormone- (ACTH-) secreting pituitary carcinoma were detected 16 years after first diagnosis.

Methods and Materials 16 years prior to presentation at our clinic, a 21-year-old woman was diagnosed with a pituitary adenoma which was treated with bilateral adrenalectomy followed by radiation of the pituitary at that time. Due to persistently very high ACTH levels and regrowing invasive pituitary adenoma, she underwent 3 pituitary surgeries followed by one conventional radiotherapy and 2 gamma-knife therapies during the 16 years. Afterwards, she had neurologic symptoms and MRI showed a mass lesion in the posterior fossa and multiple lesions at the spinal channel. The histopathological evaluation of the lesion in the posterior fossa revealed ACTH (+) pituitary cells with dural infiltration. So the diagnosis of a metastatic pituitary carcinoma was made. During the next 6 years she underwent radiotherapy to the metastatic lesions.

Results After regrowing of the pituitary rest adenoma, temozolomide therapy was started and her ACTH level fell significantly, her hyperpigmentation improved, and neurological symptoms disappeared. Her primary and metastatic mass lesions were stable under chemotherapy for 3 months.

Conclusion In this unusual case, metastases occurred 16 years after first diagnosis and the survival was better than predicted. Pituitary carcinoma should be suspected in patients with persistent or recurrent disease.

Disclosure: No significant relationships.

P057

Two Cases of TSH-Secreting Pituitary Adenomas

*T. Kroker Kogoj, K. Mlekuš Kozamernik, A. Sabati Rajic, M. Pfeifer
Dept of Endocrinology, Diabetes and Metabolic Diseases, University Medical
Centre Ljubljana, Slovenia*

Introduction We present 2 patients with TSH-secreting pituitary adenoma, which is rather rare, accounting for 1 % of pituitary adenomas.

Methods and Materials It is a rare cause of hyperthyroidism that is challenging to treat.

Case Presentations The first patient, a 24-year-old female, presented with symptoms of hyperthyroidism and a 6-year history of elevated free thyroxine (fT₄), triiodothyronine (fT₃) and inappropriately normal TSH. She also had elevated sex hormone-binding globulin (SHBG) and bone turnover markers which were assessed to rule out resistance to thyroid hormones. TSH response to the TRH stimulation test was blunted. A pituitary microadenoma (6 mm) was seen on MRI. Good response to octreotide was observed; therefore, preoperative therapy with long-acting octreotide was initiated. This restored euthyroid state but did not affect tumour size. A transsphenoidal pituitary operation was performed 6 months later. A TSH adenoma was confirmed by positive immunostaining. After surgery, TSH was suppressed but the euthyroid state has since been established.

The second patient, a 53-year-old female, had clinical signs of hyperthyroidism which were first attributed to Hashimoto's thyroiditis. Therefore, she received thyrostatic therapy. Because elevated TSH persisted, she was evaluated for suspected secondary hyperthyroidism or resistance to thyroid hormones. No family history of thyroid disorder was reported. A pituitary macroadenoma (27 mm) was discovered by MRI. She had no symptoms or signs of mass effect, and a good response to short-acting octreotide. We began treatment with long-acting octreotide during which euthyroid state was achieved after a month and the tumour regressed to half its original size in 6 months. Because of progression of Hashimoto's thyroiditis to hypothyroidism she required substitution with levothyroxine. She has been receiving octreotide LAR for a year and the decision when to perform transsphenoidal pituitary operation is still pending.

Disclosure: No significant relationships.

P058

Prognostic Role of Ki-67 in Pituitary Tumors

*S. Chiloio¹, A. Bianchi¹, A. Giampietro¹, A. Fusco¹, L. Tartaglione¹, V. Cimino¹,
M. Celico¹, F. Doglietto², L. Lauriola³, G. Rindi³, C. Anile², G. Maira², A. Pontecorvi¹,
L. de Marinis¹*

¹Endocrinology; ²Neurosurgery; ³Anatomic Pathology, Catholic University, Rome, Italy

Introduction Pituitary adenomas are usually benign. However, these tumors can infiltrate surrounding tissues. Some authors report a positive correlation between pituitary adenoma Ki-67 expression and pituitary adenoma invasiveness and recurrence.

Methods and Materials We collected 295 consecutive patients retrospectively who had been referred to the Hypothalamic-Pituitary Disease Department at the Catholic University of Rome over the past 7 years. All patients underwent surgery for a pituitary tumor at the Department of Neurosurgery of the same institution. In the preoperative period, all patients had undergone basal and dynamic endocrine evaluation of the hypothalamic-pituitary function as well as ophthalmologic and neuroradiological examinations including pituitary MRI. All tissue specimens were examined for anterior pituitary hormones via immunohistochemistry and for proliferative activity by anti-Ki-67 monoclonal antibody. During postoperative follow-up, all patients periodically performed endocrine re-evaluation and pituitary MRI.

Results 158 patients are cured at the moment (54 %); 99 patients are controlled without regrowth of residual disease (33 %); 38 patients are uncontrolled for persistence of hormonal hypersecretion

and/or radiological signs of recurrent disease or regrowth of residual disease (13 %). Higher values of Ki-67 were associated with TSH-secreting adenomas and with PRL-secreting adenomas ($p = 0.02$). Ki-67 is higher in tumors with parasellar extension as compared with intrasellar tumors ($p < 0.0001$). Invasion of cavernous sinus is related to higher values of Ki-67 by comparison with cavernous sinus non-invasive adenomas. Bilateral invasion of cavernous sinus is associated with higher values of Ki-67 as compared to the condition of unilateral invasion ($p < 0.0001$). High values of Ki-67 were observed in subtotal surgical resection of the tumor, differently from total surgical resection ($p < 0.0001$). Ki-67 was significantly higher in recurrent adenomas in comparison to non-recurrent adenomas ($p < 0.0001$) and Ki-67 was inversely correlated to disease-free survival ($p = 0.006$; $r = -1.30$).

Conclusion The Ki-67 index can predict tumor aggressiveness and clinical outcome. It might be considered a useful guide for post-surgical management.

Disclosure: No significant relationships.

P059

Evaluation of Metabolic Parameters in Patients with Craniopharyngiomas Using the Visceral Adiposity Index

*M. C. Savanelli, V. Brunelli, E. Scarano, L. Vuolo, P. Contaldi, A. Colao, C. di Somma
Dept of Molecular and Clinical Endocrinology and Oncology, Federico II University, Naples, Italy*

Introduction Craniopharyngiomas are benign tumors of the parasellar and sellar regions. Patients with craniopharyngioma, treated with surgery, have comorbidities such as obesity and multiple pituitary deficiencies.

Aim To evaluate the metabolic parameters of patients with craniopharyngioma treated with surgery.

Methods and Materials We recruited 20 patients with craniopharyngioma ($M = 11, 46.6 \pm 12.6$ a) and 20 patients ($M = 10, 53.5 \pm 8.8$ a) with non-functioning adenomas (NFA) followed up at the Department of Endocrinology of the University Federico II of Naples as controls. The majority of patients have multiple pituitary deficiencies. In all patients, the following parameters were assessed: blood glucose, HbA_{1c}, insulin, total, HDL, and LDL cholesterol, triglycerides, and BMI. We also calculated the Visceral Adiposity Index (VAI), a sex-specific mathematical index indirectly expressing visceral adipose function and insulin sensitivity.

Results Patients with craniopharyngioma showed lower levels of HDL (1.14 ± 0.24 mmol/l vs 1.40 ± 0.28 mmol/l; $p < 0.04$) and higher blood glucose (93.1 ± 9.72 vs 85.7 ± 9.72 mg/dl; $p < 0.021$) compared with patients with NFA. There was no significant difference between the 2 groups in BMI (32.65 ± 5.92 vs 29.47 ± 5.03), total cholesterol (191.9 ± 43.09 vs 209.1 ± 33.05 mg/dl), LDL cholesterol (111 ± 42.88 vs 122.5 ± 28.19 mg/dl), triglycerides (1.8 ± 1.49 vs 1.66 ± 0.48 mmol/l), insulin (7.43 ± 5.97 vs 9.9 ± 5.79 ng/ml), and HbA_{1c} (5.55 ± 0.5 vs 5.55 ± 0.8 %). No significant difference was found in the VAI index between 2 groups (2.55 ± 2.32 vs 2.12 ± 0.93 ; $p = 0.441$). However, the VAI index was higher in craniopharyngioma (2.55 ± 2.32 vs 1.92) and in NFA patients (2.12 ± 0.93 vs 1.93) when compared to an age-matched standard population. Finally, there are no significant differences in the presence of the metabolic syndrome in the 2 groups of patients (35 % vs 15 %; $p = 0.27$).

Conclusion Our study shows no significant differences in metabolic parameters between patients with craniopharyngioma and patients with NFA. However, a difference was in VAI between 2 groups of patients and the general population. Thus, VAI can be considered an accurate index of visceral adiposity in these patients.

Disclosure: No significant relationships.

P060

Linear Accelerator Radiosurgery for Non-Functioning Pituitary Adenomas

L. Portocarrero-Ortiz¹, A. Balderrama-Soto¹, J. C. Fernández Jiménez², M. A. Celis-López³, A. García-Garduño³, S. Moreno-Jiménez³, S. P. Pérez-Reyes²

¹Neuroendocrinology; ²Neurosurgery; ³Radiosurgery, Instituto Nacional de Neurología y Neurocirugía M.V.S., Distrito Federal, Mexico

Introduction Surgery is the treatment of choice for patients with pituitary adenomas, but the degree of resection is often limited by tumor extension, mainly by invasion into the cavernous sinus. Long-term tumor control rates after surgery vary from 50–80 % of patients. Radiation therapy is effective in controlling tumor mass in the majority of patients, new radiation techniques such as linear accelerator- (LINAC-) based radiosurgery may prove to be safer.

Aim To assess the efficacy and side effects of LINAC-based radiosurgery for the treatment of non-functioning pituitary macroadenomas (NFPA).

Methods and Materials From January 2003 to December 2009, 77 patients with NFPA were treated with radiosurgery. The patients were followed prospectively based on a pre-defined protocol that included Goldman Visual Field Examination, magnetic resonance imaging (MRI) of the sella, and pituitary hormone testing at 3, 6, 12 months, and then yearly.

Results 77 patients, 40 females, mean age 49.2 years (17–75 years), were treated with a mean dose of 14.32 Gy (7.6–26.2). 70 (91 %) patients had undergone transsphenoidal surgery and in 7 (9 %) patients radiosurgery was the first-line treatment because of a contraindication of surgery or because the patient did not accept surgery. The mean time between surgery and radiosurgery was 1.9 years (2 months to 8 years). The initial tumor volume was $2251 \pm 350 \text{ mm}^3$, after 3 years the tumor volume was $1532 \pm 432 \text{ mm}^3$, with a reduction of 40 %. In 10 (12.98 %) patients, we observed an increase of tumor volume in the first year *post* radiosurgery; later the tumors stabilized and decreased. Four years after treatment, 1 patient presented a third-nerve palsy. 37 (48 %) patients developed hypopituitarism.

Conclusion LINAC-based radiosurgery is effective for tumor control in NFPA, the most common adverse event was hypopituitarism.

Disclosure: No significant relationships.

P061

Aryl Hydrocarbon Receptor-Interacting Protein- (AIP)- Homologue in the Fruitfly

E. D. Aflora¹, C. Chen², B. Klapholz³, A. B. Grossman⁴, N. Tapon⁵, N. H. Brown³, R. Stanewsky², M. Korbonits¹

¹Dept of Endocrinology, Bart's and The London School of Medicine; ²School of Biological and Chemical Sciences, Queen Mary University of London; ³The Gurdon Institute and Dept of Physiology, Development and Neuroscience, University of Cambridge; ⁴Oxford Centre for Diabetes, Endocrinology and Metabolism and Dept of Clinical Oncology, Churchill Hospital, University of Oxford; ⁵Apoptosis and Proliferation Control Laboratory, Cancer Research UK, London Research Institute, UK

Introduction AIP mutations in humans lead to an incompletely penetrant autosomal dominant disease, familial isolated pituitary adenomas (FIPA). The exact mechanisms by which AIP inactivation promotes pituitary tumorigenesis remain unknown. The *Drosophila melanogaster* homologue of AIP is CG1847 (located on the X chromosome of the fly), coding for a structurally similar protein. Currently, there are no published studies on CG1847.

Methods and Materials We generated *Drosophila* CG1847-mutants using 2 different approaches: RNA-interference (using animals with a universal actin-GAL4 driver and 2 CG1847 RNAi lines with the same RNAi construct present on chromosome-II [line R1] and chromosome-III [line R2] of the fruitfly), and a P-element (imprecise excision of a P-element, a transposable element which can be used to create gene loss in the vicinity of this element).

Results When actin-GAL4 flies were crossed to the CG1847-R2 RNAi line, no viable adult offspring were observed, suggesting that complete AIP-knockdown is lethal. When using the second RNAi line (CG1847-R1), we obtained viable offspring, most likely because AIP expression was reduced to a lesser extent in the R1 line. To confirm these results with a different approach, we generated imprecise excisions of a P-element that is inserted within the 5'UTR of CG1847. One of the excisions deletes 1497bp between exons 1 and 3, thereby creating a loss of function mutation of *Drosophila* AIP. This mutant is lethal in males while females are viable as heterozygotes for the CG1847 mutation, confirming that CG1847 is an essential gene.

Conclusion We have demonstrated that the loss-of-function of the *Drosophila* AIP is lethal, similar to results in AIP-KO mice. These experiments will allow us to study organ-specific knockouts of the gene to identify phenotypes which could lead to pathways important for AIP function, and which can be tested further in mammalian species.

Disclosure: No significant relationships.

P062

Childhood Onset Craniopharyngiomas Have Normal Self-Rating Quality of Life but Impaired Neurocognitive Function in Adult Life

S. Fjalldal¹, H. Holmer², B. Ekman³, K. Österberg⁴, E. M. Erfurth¹

¹Dept of Endocrinology, Skånes University Hospital, Lund; ²Dept of Internal Medicine, Central Hospital, Kristianstad; ³Dept of Medical and Health Sciences, Linköping University; ⁴Dept of Behavioural Medicine, Skånes University Hospital, Lund, Sweden

Introduction Hypothalamic damage in craniopharyngiomas (CP) is associated with poor functional outcome. Data on quality of life (QoL) in CP on complete hormone replacement, including growth hormone, is missing. The aim of this study was to assess QoL and neurocognitive function in childhood onset (CO) CP on complete hormone substitution.

Methods and Materials 42 patients (20 women) aged ≥ 17 years were recruited from the South Medical Region of Sweden (population 2.5 million). Patients were surgically treated for a CO CP between 1958 and 2000. 86 % were receiving GH therapy, panhypopituitarism was present in 32 patients and 48 % had received cranial radiotherapy. Three self-rating questionnaires were applied: the Symptom Checklist-90 (SCL-90), the Interview Schedule for Social Interaction, and the social network concept. All subjects were examined with a battery of neurocognitive tests with high sensitivity for subtle brain dysfunction.

Results No statistically significant group differences were observed across any of the 9 SCL-90 subscales. CP patients had lower performance in neurocognitive tests, reaching statistical significance in 11 of the 20 test variables. The patient group had a lower summary measure of performance ($p = 0.004$) with this difference becoming insignificant when extracting patient with tumour growth towards the 3rd ventricle (TGTV) ($p = 0.18$), while patients with TGTV, compared to controls, had a significantly lower mean total score ($p = 0.006$). A significantly negative correlation was recorded between mean Z score of neurocognitive performance and years since operation ($r = -0.331$; $p = 0.049$).

Conclusion Normal QoL was shown in this first study of GH-substituted adult survivors of CO CP. Lower scores of neurocognitive performance were recorded and patients with TGTV had the lowest score. Therapeutic and rehabilitative efforts are highly warranted in the follow-up of CP patients.

Disclosure: No significant relationships.

P063

Evaluation of an FGFR4 Polymorphism as a Possible Predictor of Disease Penetrance in AIP Mutation Carrier FIPA Patients

L. C. Hernandez Ramirez, M. Korbonits

Centre for Endocrinology, William Harvey Research Institute, Bart's and The London School of Medicine and Dentistry, Queen Mary University of London, UK

Introduction Familial isolated pituitary adenoma (FIPA) is characterised by pituitary adenomas in ≥ 2 members of a family without other associated clinical or genetic abnormalities: 20 % of FIPA families bear mutations in AIP, showing an autosomal dominant pattern of inheritance with incomplete penetrance (15–30 %). Currently, there are no predictive factors to identify subjects who will develop the disease, and therefore long-term surveillance is suggested in AIPmut carrier subjects. A common polymorphism of FGFR4 (G388R, rs351855), with a minor allele frequency of 0.3, is a predictor of progression and poor prognosis in a variety of human neoplasms. A role for rs351855 as a promoter of somatotroph cell tumorigenesis has recently been proposed. As somatotrophinomas are the predominant pituitary adenoma type among AIPmut FIPA patients, we hypothesised that rs351855 might increase the penetrance of somatotroph adenomas within these individuals.

Methods and Materials rs351855 was assessed using restriction fragment length polymorphisms in a subset of 38 AIPmut carriers (21 affected and 17 currently unaffected [4–76 years, median 51]) from our FIPA cohort.

Results Genotyping was successful in 35 individuals: 20 (57 %) had GG and 15 (42 %) had GR haplotypes. Of the 20 affected individuals screened, 12 (60 %) had GG and 8 (40 %) had GR haplotypes. This distribution was similar within unaffected/not clinically tested carriers (GG: 53 % and GR: 46 %).

Conclusion rs351855 is not related to the penetrance of pituitary adenomas in AIPmut carriers. Further efforts will hopefully identify predictive genetic factors determining the penetrance of FIPA in AIPmut individuals.

Disclosure: No significant relationships.

P064

Clinical Features and Risk Factors of Clinical and Subclinical Apoplexy

N. Cinar¹, Y. Tekin², S. Dagdelen¹, T. Erbas¹¹Dept of Endocrinology and Metabolism; ²Dept of Internal Medicine, Hacettepe University School of Medicine, Ankara, Turkey

Introduction The clinical features of pituitary hemorrhage vary from asymptomatic to catastrophic presentations.

Methods and Materials By reviewing charts of 720 patients with pituitary adenoma, we analysed incidence, symptoms, and outcome of clinical and subclinical pituitary apoplexy (PA) patients and we aimed to find out the the risk factors.

Results A total of 103 patients (14.3 %) suffered from PA (53 female, 50 male; mean age 43.5 ± 14.3 years). Five (4.8 %) had microadenoma and the rest had macroadenoma. All underwent surgery. Of these, 30 patients (29.1 %) were diagnosed as clinical PA, while the remaining 73 patients (70.9 %) were classified as subclinical PA. Twelve of the pituitary adenomas were non-functional (11.7 %), 4 of them were necrotic (3.8 %), and the rest were clinically active adenomas (84.5 %). Among the clinically active adenomas, gonadotropin-secreting adenomas (n = 16) and prolactinomas (n = 16) had the highest frequency. Mean tumor size was 2.4 ± 1.5 cm (0.6–7.8 cm). Clinical manifestations include headache (n = 31; 30.1 %), vision loss, and visual-field defects (n = 42; 40.8 %), pituitary dysfunction (n = 26; 25.2 %), and nausea and vomiting (n = 13; 12.6 %). Among the potential risk factors, 16 patients had diabetes mellitus and 29 patients had hypertension. Dopamine agonist therapy was found in 12 patients. One patient had radiotherapy history, one had hormone stimulation test, and 2 patients were pregnant. 62 patients

developed apoplexy in the absence of any precipitating factor. 26 patients (25.2 %) developed panhypopituitarism. Clinical PA patients were significantly older than subclinical PA patients (47.9 ± 14.1 vs 41.7 ± 14.0 ; $p < 0.05$) and most of the adenomas were non-functional (n = 7), gonadotropin-secreting adenomas (n = 7), and mixed adenomas (n = 7). No significant difference was observed in sex and tumor size between the 2 groups ($p > 0.05$).

Conclusion The incidence of subclinical PA is higher than that of clinical PA. Macroadenoma, clinically active adenomas, diabetes mellitus, hypertension, and dopamine agonist therapy might be precipitating factors for apoplexy.

Disclosure: No significant relationships.

P065

Quality of Life in Patients with Non-Functioning Pituitary Adenoma: The Oxford Experience

C. Capatina, C. Christodoulides, A. Fernandez, A. Grossman, J. A. H. Wass, N. Karavitaki

Endocrinology-OCDEM, Oxford University, UK

Introduction Data on the quality of life (QoL) of patients with non-functioning pituitary adenoma (NFA) are sparse and conflicting. We have evaluated the QoL in patients with NFAs followed up in a tertiary UK referral centre.

Methods and Materials Three validated questionnaires (Short Form 36 [SF36], Nottingham Health Profile [NHP], and European Quality of Life Scale [EuroQoL]) were offered to consecutive NFA subjects attending outpatient clinics over 6 months: 180/193 responded (93.3 %; 110 males; median age 64 years [range 33–90]; 98.9 % had surgery, 42.9 % radiotherapy for primary/recurrent disease). Results were compared with age-related UK reference values.

Results Overall, QoL scores of the total group for SF36 and EuroQoL were not significantly different from those of the general population, except for lower self-perceived health status (assessed by visual analogue scale [VAS]). With NHP, men did not show compromised scores and women had significantly impaired scores only on energy levels, health-related problems with interests/hobbies, and with holidays. Linear regression analysis including age, gender, radiotherapy, recurrence, and untreated hypogonadism as independent variables revealed that age was an independent predictor for reduced physical ability and role limitations due to physical problems (RP), sex an independent predictor for RP and compromised mental health, recurrence for lower VAS score and for anxiety/depression and non-replaced hypogonadism for social isolation and worse scores in interests/hobbies.

Conclusion Overall, the health-related QoL and perception of subjective health in patients with NFA was not compromised to any major extent suggesting that we can now offer the prospect of treatment and replacement which will provide a normal or near normal QoL. Specific groups are affected in various dimensions, necessitating measures to compensate for predisposing factors.

Disclosure: No significant relationships.

P066

Pediatric Pituitary Adenomas in a Specialist Hospital in the North of México. A Retrospective Study

M. Márquez¹, L. Torres García¹¹Oncology-Pathology, Karolinska Institutet, Stockholm, Sweden; ²Endocrinology, Hospital Regional de Altas Especialidades del Noreste de México # 25 IMSS, Monterrey, NL, Mexico

Introduction Pituitary adenomas (PA) are rare in the pediatric population. The incidence is 1–10 % of all childhood brain tumors and 2–6 % of all surgically treated adenomas. The prevalence is ~1:1,000,000 children.

Methods and Materials A review of the prevalence, pathology, and therapeutic outcome of pediatric PA at an endocrinology clinic in Monterrey, México, from 1995–2009.

Results Out of 557 treated patients, 19 were children (17 female, 2 male). The mean age at diagnosis was 16.6 years. Mean follow-up was 5.2 years. Patients presented with headaches (n = 7), visual disturbances (n = 7), amenorrhea (n = 10), galactorrhea (n = 9), acromegaly (n = 3), weight gain (n = 9), hypothyroidism (n = 8), and hypopituitarism (n = 4). Pathology analysis showed 11 macroadenomas and 8 microadenomas. Of 13 adenomas stained for prolactin, 3 were non-functioning adenomas, 1 growth hormone- (GH-) positive and 2 mixed adenomas stained for growth hormone and prolactin (PRL/GH). Mean serum prolactin concentration at the time of diagnosis was 162.6 ng/ml. Twelve prolactinoma patients were treated with dopamine agonists and 6 had surgery. PRL/GH patients were treated with sandostatin LAR and dopamine agonists. One of the PRL/GH patients, 2 of the non-functional adenoma patients and one with growth hormone-secreting adenoma had surgery. Five patients had radiotherapy. There seemed to be a correlation between a high Body Mass Index (BMI) and macroadenomas (> BMI patients more frequently had macroadenomas). Seven out of 8 microadenomas and 5 out of 11 macroadenomas were considered cured.

Conclusion The prevalence of PA was 3.4 %. The majority was secretory (16/19), with prolactinomas (13) being the most common type. Three cases of very rare non-secretory adenoma were diagnosed. 63 % of the patients were considered cured.

Disclosure: No significant relationships.

P067

Skull Base Reconstruction during Endonasal Pituitary Surgery

B. Pashaei¹, G. Vagapova², D. Bochkarev¹, V. Danilov³, A. Alekseev¹

¹Neurosurgery, Interregional Clinical Diagnostical Center; ²Endocrinology, Kazan State Medical Academy; ³Neurosurgery, Kazan State Medical University, Russian Federation

Introduction Prevention of CSF-leak, water-tight closer of the bone defects after endonasal endoscopic approaches to the sellar region, especially extended approaches to the large pituitary tumors and craniopharyngiomas with extrasellar extension is a real challenge for neurosurgeons. To summarize and analyze the efficiency of different reconstruction techniques is the main topic of this abstract.

Materials and Methods Several methods of skull-base reconstruction and CSF-leak prevention during endonasal approaches to skull base were used:

- Tamponation of the sella turcica with spongostan, dura reconstruction with a vikryl patch and fibrin glue after tumor resection.
- Tamponation of the sella turcica with a fat graft, dura reconstruction with a piece of fascia lata and fibrin glue.
- Skull base reconstruction with a vascular pedicle mucosal flap of the nasal septum (Hadad-Bassagasteguy [HB] flap).
- Skull base ventral surface reconstruction with a vascular pedicle pericranial flap.

Results Via a transnasal transsphenoidal approach, 226 patients were operated. 182 (80.5 %) with pituitary tumors, 12 (5.72 %) with craniopharyngiomas, 2 (0.9 %) with Rathke cleft cysts, and 30 with other lesions. Reconstruction with artificial materials was performed in 152 (67.25 %) patients; reconstructions with fat graft and fascia lata were made in 18 (8 %) patients; skull base reconstruction with vascular pedicle mucosal flaps of the nasal septum was applied in 56 (24.7 %) patients, and in 3 cases it was used iteratively; in 1 (0.44 %) patient endonasal endoscopic reconstruction of the skull base with a vascular pedicle pericranial flap was performed.

Conclusion Usage of different methods for skull base reconstruction in endonasal surgery reduces risks of CSF-leak postoperatively. Vascular pedicle flaps could be used for water-tight closing in cases of extended approaches to the sellar region and skull base. They heal more quickly and could be used iteratively.

Disclosure: No significant relationships.

P068

HGF/C-MET System Targeting PI3K-AKT and STAT3 Pathways in Pituitary Adenomas: Immunohistochemical Characterization

M. Trovato¹, M. L. Torre², M. Ragonese², A. Simone¹, R. Scarfm¹, V. Barresi¹, G. Giuffrè¹, S. Benvenega², F. Trimarchi², G. Tuccari¹, R. M. Ruggeri², S. Cannavò²

¹Dept of Human Pathology; ²Section of Endocrinology, Dept of Medicine and Pharmacology, University of Messina, Italy

Introduction Downstream signals of HGF/c-met activation are responsible for cell growth and invasiveness in several tumour types. In addition, therapeutic agents targeting HGF and c-met have been shown to inhibit tumour growth and angiogenesis. Recently, the expression of c-met and HGF has been documented in pituitary adenomas (PA).

Methods and Materials We analyzed by immunohistochemistry the expression of HGF/c-met and of the downstream molecules PI3K, phospho-Akt, STAT3, phospho-STAT3 in an archival series of 30 PA (12 non-functioning and 18 functioning; 25 macroadenomas and 5 microadenomas).

Results All PA were HGF/c-met/PI3K/pAkt/STAT3/pSTAT3⁺. Only tumor epithelial cells expressed the full pathway, whereas peri-tumoral normal tissue did not express these molecules. The percentages of epithelial cells expressing HGF, c-met, and pAkt were significantly higher than those expressing PI3K, STAT3, and pSTAT3 (HGF 34 % ± 17 %, c-met 49 % ± 19 %, pAkt 39 % ± 16 %, PI3K 1.3 ± 0.5, STAT3 14 % ± 8 %, and pSTAT3 7 ± 6 %; p < 0.01). There was a significant correlation between HGF and c-met expression (r = 0.86; p < 0.001), as well as between c-met and pAkt (r = 0.51; p < 0.01) or HGF and pAkt (r = 0.42; p < 0.05). Furthermore, an additional intense pAkt immunoreaction restricted to endothelial cells (16 % ± 7 %) was detected on vascular surface area in 17 PA (57 %), mostly macroadenoma (14/17). It was correlated with tumor size (r = 0.7; p < 0.01).

Conclusion C-met and its ligand HGF are widely over-expressed in PA. Their expression, which could involve a paracrine and/or autocrine activation loop, has been mainly associated with enhanced expression and phosphorylation of the downstream molecule Akt. The HGF/C-met/Akt signalling pathway may be involved in tumor growth and angiogenesis, as supported also by the finding of the endothelial pAkt expression on the vascular surface area. This finding could have practical implications for the targeted medical therapy of PA.

Disclosure: No significant relationships.

P069

Characterization of 4 AIP Gene Mutants in Somatotroph Cells

A. R. García-Rendueles¹, E. Díaz-Rodríguez¹, G. Trivellin², M. García-Lavandeira¹, T. Vila Vila¹, C. Diéguez¹, M. Korbonits², C. V. Alvarez¹

¹Physiology, CIMUS-IDIS, Santiago de Compostela, Spain; ²Endocrinology, Queen Mary University of London, UK

Introduction Aryl hydrocarbon receptor-interacting protein (AIP) gene mutations (mAIP) have been identified in about 30 % of all familial isolated pituitary adenoma (FIPA) families and 50 % of acromegaly families (Cazabat, 2007). The functional role of AIP in somatotroph cells is unknown. In pituitary adenomas, mAIPs are usually found as loss of heterozygosity suggesting that AIP may act as a tumor suppressor gene (Vierimaa, 2006), a role that is lost in different mAIPs. 49 AIP variants have been identified to date (Chahal, 2010).

Aim Our aim is to study the effects of R16H, R304Q-AIP, and R304X-AIP in comparison to V49M-AIP in proliferation and apoptosis in somatotroph cells.

Methods and Materials We used the somatotroph rat pituitary cell line GH4C1 that expresses basal levels of AIP. The cells were transiently transfected by nucleofection. As a standard of transfection

tion efficiency a vector expressing GFP under a CMV promoter was co-transfected. Cells were counted with a cytometer. Apoptosis was detected by Hoechst staining.

Results The nucleofection provided transfection efficiency > 90 %. In the presence of complete medium with growth factors we observed no difference in the proliferation between the empty vector, wtAIP, or any mAIP. In deprived medium (0.1-% serum) the cells transfected with V49M-AIP presented a 40-% apoptosis. The rest of the mAIPs has no significant differences in apoptosis. There was no relevant apoptosis in cells transfected with an empty vector or with wtAIP.

Conclusion In somatotroph cells, mAIPs may not affect proliferation but V49M-AIP induces apoptosis. In cells expressing endogenous AIP this mutant seems to act as a toxic mutation. This would explain why this mutant is in heterozygosis. The results also suggest that pro-tumoral effects of mAIP require other factors still unknown.

Disclosure: No significant relationships.

P070

Acute Presentation of Craniopharyngioma in Children and Adults

E. H. Nielsen¹, J. O. Jørgensen², M. Andersen³, P. Bjerre⁴, U. Feldt-Rasmussen⁵, L. Poulsen⁶, L. Ø. Kristensen⁷, J. Astrup⁸, J. Jørgensen⁹, P. Laurberg¹

¹Dept of Endocrinology, Aalborg Hospital, Aarhus University Hospital, Aalborg;

²Endocrinology, Aarhus University Hospital; ³Dept of Endocrinology, Odense University Hospital; ⁴Dept of Neurosurgery, Aarhus University Hospital; ⁵Medical Endocrinology; ⁶Dept of Neurosurgery, Rigshospitalet, Copenhagen; ⁷Endocrine Unit, Dept of Internal Medicine O, Herlev Hospital, University of Copenhagen, Herlev; ⁸Dept of Neurosurgery, Glostrup Hospital, University of Copenhagen, Glostrup; ⁹Dept of Neurosurgery, Aalborg Hospital, Aarhus University Hospital, Aalborg, Denmark

Introduction We wished to study the occurrence of acute-onset symptoms in patients presenting with childhood-onset or adult-onset craniopharyngioma, and to investigate whether some patient groups were more likely to present with acute symptoms.

Methods and Materials Medical records of 189 consecutive patients (39 children, 150 adults) presenting with craniopharyngioma from 1985–2004 were manually reviewed and data regarding initial symptoms, neuroimaging results, visual performance, and pituitary function were systematically collected. Note was made of acute symptoms preceding hospital admission. Data were analysed focusing on subgroup analyses based on age, gender, calendar year period, and various tumour characteristics.

Results Acute symptoms were reported in 24 (13 %) patients. Acute visual symptoms, headache, nausea, or vomiting were most frequently reported, followed by reduced consciousness, cranial nerve palsy, or hyperpyrexia. Acute symptoms were more frequent among children (28 %) than among adults (9 %; $p < 0.01$). There were no differences in sex or calendar year period. Hydrocephalus was present in approximately half of childhood cases as compared to 1/5 of adult patients ($p < 0.001$). Radiologic evidence of intra-tumour haemorrhage was seen in only 2 cases. Acute symptoms were more frequent among patients with tumours occupying the third ventricle ($p < 0.01$) or in case of radiologic signs of calcification ($p < 0.05$) or hydrocephalus ($p < 0.01$).

Conclusion Craniopharyngioma presented with acute symptoms in 13 % of patients and was more frequent among children than adults. Severe third-ventricle involvement, calcification, and obstructive hydrocephalus were associated with acute symptoms at presentation. Intra-tumour haemorrhage was rare.

Disclosure: No significant relationships.

P071

Pituitary Adenomas with MR Invasion of the Cavernous Sinus Space: A Classification Based on Direct Endoscopic Visualization

E. Knosp, A. Micko

Dept of Neurosurgery, Medical University of Vienna, Austria

Introduction Invasiveness into the space of the cavernous sinus (CS) is the most important prognostic factor for surgical outcome and recurrence of pituitary adenomas. To predict invasiveness by radiological criteria, we previously described a 4-tiered classification based on MRI: the extent of parasellar tumor growth related to the intercarotid lines on coronal MRI was correlated with the surgical findings as seen through an operating microscope. Recently, endoscopic techniques have allowed for a direct close-up inspection of the medial wall of the CS with the aid of angulated lenses. The aim of this study was to re-evaluate our classification based on endoscopic technique.

Methods and Materials We evaluated 151 consecutive, purely endoscopically operated pituitary adenomas with parasellar extension on at least one side according to the previously published classification.

Results Compared to our previous data we found no difference in the rate of invasiveness in adenomas with parasellar extension grades 0, 1, and 4. In grade 2, however, the CS was invaded in 9.6 % with endoscopic vs 88 % with microscopic evaluation ($p < 0.001$). In grade 3, the difference was 37.9 % vs 86 % (endoscopic vs microscopic; $p = 0.002$). A further classification of grade-3 adenomas showed a statistically significant (26.5 % vs 70.6 %; $p = 0.001$) difference between invasiveness into the superior (grade 3A) vs into the inferior compartment of the CS (grade 3B).

Conclusion The proposed classification proved to be a reliable predictor of increasing likelihood of invasiveness with increasing grades of parasellar invasion. Direct endoscopic visualization provides better detection of invasiveness into the CS. Further evaluation of grade-3 adenomas showed that there is a higher rate of invasiveness into the inferior than into the superior compartment of the CS.

Disclosure: No significant relationships.

P072

Pituitary Microadenoma and Rathke Cleft Cyst: A Not-So-Rare Association

J. F. Bonneville

Neuroradiology, University Hospital, Besancon, France

Introduction Association of pituitary microadenoma and intrasellar Rathke cleft cyst is not rare and frequently makes the MR diagnosis difficult.

Methods and Materials From 2000–2012, 24 intrasellar pituitary microadenomas, mostly prolactinomas, were found with coexisting intrasellar Rathke cleft cysts. MRI sequences included sagittal T1, coronal T2 and T1, but axial T1 was the most informative.

Results Most Rathke cleft cysts are hyperintense on T1 and usually present with a small hypointense hyperproteinic nodule on T2. Differential diagnosis includes hemorrhagic pituitary adenoma. Presence of a pituitary microadenoma gives rise to a mass effect on the sellar floor, sellar diaphragm, and pituitary stalk but not Rathke cleft cysts.

Conclusion Coexisting pituitary microadenoma and Rathke cleft cysts are not rare. Precise evaluation of MRI features, particularly on axial T1-weighted MR sequences, is mandatory for the diagnosis.

Disclosure: No significant relationships.

P073

Overexpression of Transcription Factor GLI1 in Human Pituitary Adenomas

K. Lampichler¹, A. Ilhan-Mutlu², E. Knosp³, G. Vila¹, A. Luger¹, L. Wagner⁴, S. Baumgartner-Parzer¹

¹Division of Endocrinology and Metabolism, Dept of Medicine III; ²Internal Medicine I; ³Dept of Neurosurgery; ⁴Division of Nephrology, Dept of Internal Medicine III, Medical University of Vienna, Austria

Introduction The Hedgehog signalling pathway is an essential regulator of multiple embryonic and developmental processes including morphogenesis, differentiation, and stem cell self-renewal. It is frequently reactivated in various types of human cancer and modulates tumor growth, cancer stem cell fate, and survival rates. GLI1 is the terminal mediator of the Hedgehog pathway and activates several target genes through direct DNA binding. Although the cancer stem cell model seemed to be only relevant in malignant tumors, recent data suggest that cancer stem cells could also be of relevance for the pathogenesis of benign tumors.

Methods and Materials The present study was designed to evaluate a potential role of GLI1 in the pathogenesis of pituitary adenomas. We studied the expression level of GLI1 in 25 human pituitary adenomas (13 non-functioning and 12 hormone-secreting, respectively) by qRT-PCR. The percentage of GLI1 copy numbers relative to the copy number of the house-keeping gene GAPDH was calculated. Expression levels were verified on the protein level using immunofluorescence confocal microscopy.

Results 13 out of 25 tumors (52 %) showed a GLI1 expression in terms of copy numbers of > 1 % of the GAPDH expression (1–10 %: 8 adenomas, mean $3.68 \pm \text{SD } 1.83$; > 10 %: 4 adenomas, mean $21.68 \pm \text{SD } 8.85$; > 100 %: 1 adenoma, 108.11 %). 61.5 % of the GLI1 expressing tumors were non-functioning. We found GLI1 to be mainly located in the cell nucleus which is in line with previous reports that GLI1 translocates into the nucleus upon activation.

Conclusion Our data thus suggest an important role of tumor-initiating stem cells in pituitary adenoma development. It is important to note that the first Hedgehog-signalling inhibitor vismodegib was only recently approved. On the basis of our data, vismodegib could be a promising treatment option for pituitary adenomas expressing GLI1.

Disclosure: No significant relationships.

Neuroendocrine Tumors

P074

Risk Factors of Puberty Retardation in Adolescents with Different Tumors of the Hypothalamic-Pituitary Area

Y. Urmanova¹, M. Shoraimova¹, U. Mavlonov²

¹Endocrinology, Tashkent Pediatric Medical Institute; ²Endocrinology, Bukhara Medical Institute, Bukhara, Uzbekistan

Aim To analyze risk factors of puberty retardation in adolescents with different tumors of the hypothalamic-pituitary area.

Methods and Materials We observed 12 adolescents (mean age 13.8; 7 boys, 5 girls). All patients had undergone the spectrum of analyses, including endocrine status assessment, clinical, biochemical, hormonal (GH, LH, FSH, prolactin, TSH, testosterone, and others), radiological (X-ray of hand and Turkish saddle, CT/MRI of Turkish saddle in 18 patients), anthropometry (desired height, centile, growth rate, height and weight SDS, and others) according to the international height-weight card of Tanner-Whitehouse, assessment of sexual development according to Tanner, cariotyping, and other analyses.

Results Patients were divided according to their tumor type: craniopharyngioma (n = 5), corticotropinoma (n = 4), pinealoma (n = 2), and prolactinoma (n = 1). Puberty retardation was revealed in all patients. It was revealed that in patients with Turkish saddle tumors among other risk factors of PR the most widespread were brain traumas (n = 7;

38.8 %), birth traumas (n = 5; 27.7 %), stresses (n = 3; 16.6 %), and smoking (5 %).

Conclusion (1) A variety of risk factors of PR for Turkish saddle tumors was revealed. (2) The most widespread among the risk factors were brain and birth traumas.

Disclosure: No significant relationships.

P075

Correlation of Aggressiveness of Course and Histological Structure of Non-Functional Pituitary Adenomas (NFPA)

Y. Urmanova, G. Saydaliyeva

Endocrinology, Tashkent Pediatric Medical Institute, Uzbekistan

Aim Our aim was to study the correlation between aggressiveness of the course and histological structure of NFPA.

Methods and Materials We observed 11 patients with IPA (5 male, 6 female; mean age 37.8). All patients had undergone surgical treatment by transsphenoidal access in RSSPMCE MoH RU at the neuroendocrinology department. All patients had undergone the spectrum of analyses, including endocrine status assessment, clinical, biochemical, hormonal (GH, LH, FSH, prolactin, TSH, testosterone, and others), radiological (CT/MRI of the Turkish saddle), and histological studies. Depending on the type of cells found on the histological study, patients with IPA (chromophobic adenomas) were divided into 3 groups: (1) small-cell (undifferentiated) chromophobic adenoma (n = 5), (2) large-cell chromophobic adenoma (n = 6), and (3) oncocytoma (n = 0).

Results Preliminary analysis of research showed that among the observed patients those most disposed to invasive total growth were patients from group 1 with a small-cell histological NFPA structure. Besides, these patients incurred more frequent tumor relapses in the post-operative period (n = 3; 27.3 %), had acute disease manifestations with general cerebral symptoms and neuroendocrine disturbances (secondary amenorrhea in females, potency and libido decrease in males, metabolic syndrome, visual disturbances, and others). Two female patients aged 27.5 from group 1 had to undergo repeated selective pituitary adenomaectomy 3×.

Conclusion (1) Small-cell NFPA show the most aggressive growth and tumor relapse. (2) Following research is necessary to study the markers of aggressiveness in all 3 groups.

Disclosure: No significant relationships.

P076

An Unusual Presentation of an Uncommon Neuroendocrine Disease

A. Zacharof

^{1st} Dept of Internal Medicine, Hellenic Red Cross Hospital, Halandri, Athens, Greece

Introduction A case of a middle-aged woman with pheochromocytoma, dilated cardiomyopathy, and ventricular arrhythmia.

Methods and Materials We report the case of a 36-year-old woman who was diagnosed to have dilated cardiomyopathy and who had had episodes of syncope in the past. Ventricular arrhythmia was detected on Holter monitoring. A history of breathlessness on exertion, fatigue, loss of weight, and fearfulness for 3 years was reported. She also had several episodes of syncope in this period for which a computerized tomography (CT) of the brain was found to be normal.

Results Her blood pressure was 200/120 mmHg and heart rate 120 bpm. Hematology, electrolytes, calcium, phosphorus, magnesium, fasting blood glucose, urea, creatinine, liver, and thyroid function tests were all normal. Her baseline electrocardiogram (ECG) showed a prolonged QTc interval. The echocardiogram revealed global hypokinesia with an ejection fraction of 34 %. A diagnosis of dilated cardiomyopathy was made and she has started on betablockers, angiotensin-converting enzyme inhibitors, and diuretics. Her Holter

monitoring revealed ventricular arrhythmia (torsades de pointe). She was found to have a paroxysmal rise in blood pressure associated with sweating and panic attacks. The possibility of a pheochromocytoma was considered. An abdomen ultrasound revealed a 4 × 3 cm echogenic mass in the left adrenal region. Her 24-hour urine vanillylmandelic acid (VMA) excretion was 17.7 mg in 2340 ml urine. A CT scan and a whole-body ¹²³I m-iodobenzyl-guanidine (¹²³I MIBG) scan confirmed the diagnosis. She was operated and biopsy confirmed the diagnosis. Three weeks after surgery the ECG was normal and her echocardiogram showed an improvement in ejection fraction from 34 % to 45 %.

Conclusion QT prolongation interval in ECG can occur in patients with pheochromocytoma and may give rise to dangerous arrhythmias. Our case illustrates that pheochromocytoma can be a rare, reversible cause of such a cardiac arrhythmia.

Disclosure: No significant relationships.

P077

Long-Term Survival after Combined Therapy of Ectopic Cushing's Syndrome Caused by a Well-Differentiated ACTH-Secreting Neuroendocrine Carcinoma of the Pancreas

M. Vetrì¹, M. Lodi², G. Giannone²

¹Dept of Endocrinology; ²Surgery Oncology, Hospital Garibaldi Nesima, Catania, Italy

Introduction Ectopic adrenocorticotrophic hormone (ACTH) production by a pancreatic neuroendocrine tumor (p-NET) is relatively rare, those tumors are responsible for about 15 % of ectopic Cushing's syndrome, occur most frequently in adults and patients with this disease face a poor prognosis.

Methods and Materials We present the case of a 48-year-old man with Cushing's syndrome due to an ectopic ACTH-secreting p-NET. He had markedly elevated plasma ACTH (355.0 pg/ml) and urinary free cortisol (UFC 800.00 µg/24 h) levels associated with hypokalemia (2.7 mEq/l), diabetes mellitus, hypogonadism, and hypothyroidism. Computed tomography revealed a solid mass in the head-body of the pancreas and multiple masses in the liver. Tumor and liver metastases were somatostatin receptor-positive as revealed by octreoscan.

Results Distal pancreatectomy and, later, bilateral adrenalectomy were performed to control the effects of ACTH-secreting liver metastases. After kenoconazole and somatostatin analogue injections, peptide receptor radionuclide therapy with ¹⁷⁷Lu-(DOTA-TATE) was also used. After 5 years of liver metastasis stabilization, both at biochemical (ACTH, UFC) and anatomical (number and dimensions) levels, the disease became active again with ACTH > 1500.0 pg/ml. A new evaluation with CT- and octreoscan revealed increases in dimension and number of some liver metastases and their partially lost capability of the SSRS radioligand uptake. The patient then underwent combined treatment with somatostatin analog plus everolimus (10 mg/day), an inhibitor that targets the protein kinase mammalian target of rapamycin (mTOR).

Conclusion For functioning p-NETs, long-term survival is challenging in the presence of metastases. With advances in medical management and radiolabelled somatostatin therapy, symptoms may be controlled to optimize quality of life. This case illustrates long-term survival and the beneficial effect of new and different types of treatment in the management of an ectopic ACTH syndrome when a single conventional therapy fails to control the disease.

Disclosure: No significant relationships.

P078

Role of ⁶⁸Ga-DOTATATE PET-CT in Patients with Medullary Thyroid Carcinoma with Persistent or Relapsing Disease after Surgery

F. Marciello¹, A. C. Carratù¹, C. de Luca¹, R. Esposito¹, L. Alo², A. Colao¹, S. Lastoria², A. Faggiano¹

¹Dept of Molecular and Clinical Endocrinology and Oncology, University of Naples Federico II; ²Nuclear Medicine, National Cancer Institute Fondazione G. Pascale, Naples, Italy

Introduction Different radiotracers (pentavalent DMSA, ¹²³I-MIBG, and ¹¹¹In-octreotide) have been tested in medullary thyroid carcinoma (MTC) to improve diagnostic performance of US, CT, and MRI in patients not cured by surgery. However, these techniques show a variable and relatively low sensitivity due to poor spatial resolution and image noises caused by physiological uptake.

Aim Our aim was to evaluate the role of ⁶⁸Ga-DOTATATE PET-CT in MTC patients with persistent/relapsing disease after surgery.

Methods and Materials Twelve subjects having undergone surgery for MTC were enrolled. ⁶⁸Ga-DOTATATE PET-CT was performed in all subjects 6–12 months after surgery. This procedure was performed by acquiring whole-body studies 40–60 min after radioligand iv injection (74–111 MBq). Tumour persistence or relapse after surgery was based on assessment of detectable and progressively increasing serum calcitonin levels, associated or not with tumour lesions identified at the radiological work-up (including US, CT, MRI).

Results After surgery, 9/12 patients had tumour persistence/relapse while 3/12 were considered disease-free. The combined use of US, CT, and MRI detected tumour lesions in 7 of 9 cases, while ⁶⁸Ga-DOTATATE in 5 of 9 cases. No lesions were detected by either modality in the remaining 2 patients. ⁶⁸Ga-DOTATATE PET-CT showed a sensitivity and specificity of 55 % and 100 %, respectively. No correlation was found between SUVmax and calcitonin levels.

Conclusion ⁶⁸Ga-DOTATATE PET-CT is highly specific in detecting tumour relapse or persistence in patients operated for MTC. However, its sensitivity is not superior to conventional imaging techniques in case of occult disease. A possible role for ⁶⁸Ga-DOTATATE PET-CT may be the identification of somatostatin receptor-positive MTC lesions to treat with cold or radiolabelled somatostatin analogues.

Disclosure: No significant relationships.

P079

Different Response to mTOR Inhibitors in Human Bronchial Carcinoid Cell Lines

T. Gagliano, R. Rossi, D. Molè, E. Gentilin, F. Tagliati, M. Minoia, E. degli Uberti, M. C. Zatelli

Dept of Biomedical Sciences and Advanced Therapies, University of Ferrara, Italy

Introduction Bronchial carcinoids (BC) are tumors originating from endocrine cells dispersed in the respiratory epithelium that can be divided into typical (TBC) and atypical (ABC) BCs. TBCs are generally benign, while ABCs are aggressive. mTOR has a central role in regulating cell growth and metabolism. Everolimus, an mTOR inhibitor, reduces cell viability of selected human primary cultures of BCs with higher levels of mTOR, suggesting that the level of mTOR pathway components might predict response to mTOR-targeted therapies.

Methods and Materials Evaluation of the effects of mTOR inhibitors on human BC-cell lines, verification of the hypothesis that differential mTOR expression leads to a different sensitivity to mTOR inhibitors. NCI-H727 (TBC) and NCI-H720 (ABC) cells were treated with everolimus or NVP-BEZ235 (PI3K/mTOR-inhibitor). Cell viability, apoptosis, cell-cycle, mTOR pathway protein were evaluated.

Results We found that 100 nM everolimus inhibits cell viability by ~40 % in NCI H720 cells and by ~10 % in NCI H727 cells

($p < 0.01$); 100 nM NVP-BEZ 235 has similar effects on NCI H727, but has greater effects on NCI H720 (-50% ; $p < 0.01$). Everolimus does not induce apoptosis but promotes an accumulation in G1/G0 ($+5\%$ in NCI H727 and $+8\%$ in NCI H720 cells vs ct). NVP-BEZ 235 does not promote apoptosis in NCI H727, but induces this process in NCI H720 ($+150\%$), and causes G1/G0 accumulation in both NCI H727 ($+8\%$) and NCI H720 ($+11\%$), with an activation of GSK3 β and a parallel decrease in cyclin D1 levels, and ERK1/2 phosphorylation. In addition, mTOR expression is greater, at both protein and mRNA levels, in NCI H720 cells as compared to NCI H727 (1.5-fold).

Conclusion These data demonstrate that mTOR and the PI3K pathway are differentially activated in human BCs cell lines, depending on the type (TBC vs ABC), and this different activation results in an increased sensitivity to treatment with mTOR inhibitors of the ABC cell line. These data support the hypothesis that the analysis of mTOR expression might be a prognostic tool in planning BCs medical therapy, with, in this framework, everolimus and NVP-BEZ235.

Disclosure: No significant relationships.

P080

An Important Role of PKC δ in Human Medullary Thyroid Carcinoma Cell Viability

D. Mole, F. Tagliati, E. degli Uberti, M. C. Zatelli

Dept of Biomedical Sciences and Advanced Therapies, University of Ferrara, Italy

Introduction Protein kinase C (PKC) is a family of serine-threonine kinases that regulate many cellular processes including proliferation and survival. Previous evidence has shown that PKC is involved in the control of human medullary thyroid carcinoma (MTC) proliferation and survival by modulating apoptosis, with a mechanism that implicates PKC β II inhibition and translocation in different subcellular compartments.

Methods and Materials Transfection was performed through electroporation. Cell viability was investigated by ATPlite kit, apoptosis by caspase 3/7 assay, total and phosphorylated Akt, p70 S6K and STA5 protein levels were measured by AlphaScreen SureFire assay kits and PKC-delta expression by Western Blot.

Results In this study, we investigated the role of PKC-delta (PKC δ) signaling in the proliferation of human MTC cell lines (TT cells) by PKC δ inhibition. We found that pharmacological inhibition of the PKC δ pathway reduces caspase 3/7 activity in time course. Using an shRNA vector system, which offers more than 70 % gene expression inhibition, we show that PKC δ -defective-TT cells grow more than those induced with mock-transfected cells, this difference being significant after 3 days. In addition, the expression of the pAKT (Ser473), p70 S6K(T389) and STAT5(Y694-699), a downstream target of PKC pathway involved in cell growth, cell cycle, and proliferation were reduced compared to mock-transfected cells.

Conclusion These observations indicate for the first time that the PKC δ pathway plays an important role in the growth control of human MTC with a mechanism likely involving Akt, p70 S6K and STAT5 signaling.

Disclosure: No significant relationships.

P081

Antiproliferative Effects of NPV-BEZ 235 in Medullary Thyroid Carcinoma Primary Cultures and Cell Lines

M. Rossi, E. Gentilin, M. Minoia, F. Tagliati, E. degli Uberti, M. C. Zatelli

Dept of Biomedical Sciences and Advanced Therapies, University of Ferrara, Italy

Introduction The phosphatidylinositol 3-kinase (PI3K)/AKT/mammalian target of rapamycin (mTOR) pathways is abnormally activated in human Medullary Thyroid Carcinoma (MTC) cell lines. NPV-BEZ 235 is a potent dual inhibitor of the PI3K/mTOR cascade with antiangiogenic activity.

Methods and Materials We evaluated the effects of this drug on cell viability in MTC cell line (TT cells) and in MTC primary cultures in the presence or absence of a proliferative stimulus such as Vascular Endothelial Growth Factor (VEGF).

Results Both TT cells and MTC primary cultures showed a dose-dependent reduction in cell viability when treated with increasing concentrations of NVP-BEZ 235 (10 nM–5 μ M). A significant antiproliferative effect was observed after 48 h of treatment, already at a concentration of 25 nM. The maximum effect ($> 60\%$; $p < 0.01$) was observed at concentrations ≥ 100 nM and 1 μ M in TT cells and in MTC primary cultures, respectively. On the other hand, treatment with VEGF 10 ng/ml significantly enhanced cell viability ($\sim 40\%$), an effect completely blocked by BEZ 235. Moreover, the latter significantly ($p < 0.01$) reduced VEGF secretion in MTC cells at concentrations ≥ 10 nM ($\geq 20\%$).

Conclusion In conclusion, our data demonstrate that NVP-BEZ235 reduces MTC cell viability with a mechanism which involves VEGF signalling, suggesting that it might represent a possible medical treatment for persistent/recurrent MTCs.

Disclosure: No significant relationships.

P082

Vitamin D Pathway in Neuroendocrine Tumors

V. Ramundo, L. Vuolo, A. Negri, M. Samataro, R. Pivonello, C. di Somma, F. Marciello, M. del Prete, V. Marotta, R. Esposito, A. C. Carratù, C. de Luca di Roseto, A. Faggiano, A. Colao

Dept of Clinical and Molecular Endocrinology and Oncology, University of Naples Federico II, Italy

Introduction Vitamin D exerts anti-proliferative effects by binding to the vitamin D receptor (VDR), an intracellular nuclear receptor. There is evidence of the association between cancer and specific polymorphisms of VDR. With regard to neuroendocrine tumors (NETs), in murine and human insulinoma cell lines the 1-25(OH) $_2$ D has been shown to inhibit cell proliferation, induce apoptosis, inhibit insulin secretion, down-regulate the expression of insulin gene and up-regulate the expression of VDR. The aim of this study was to investigate expression and prognostic role of the vitamin D pathway in patients with NETs.

Methods and Materials 33 patients with NETs (15 sporadic and 18 hereditary MEN1-associated; 14 with recurrent/progressive disease and 19 with remission/stable disease) were evaluated and compared to 33 age- and sex-matched controls. Serum 25(OH)D levels were measured by chemiluminescence (CLIA) with dosing limits in the range 4–150 ng/ml. VDR polymorphisms evaluated included Fok-1 and Taq-1.

Results Mean serum 25(OH)D levels were significantly lower in patients with NETs (19.9 ± 2.0 ng/ml) than in controls (30.4 ± 1.9 ng/ml; $p < 0.0001$). Allelic variants of the VDR polymorphisms, Fok-1 and Taq-1, were similarly distributed in patients and controls ($p = ns$). Serum 25(OH)D levels were significantly lower in patients with recurrent/progressive disease than in those with remission/stable disease (16.2 ± 2.0 vs 23.3 ± 2.8 ng/ml; $p < 0.05$). No significant differences were observed in the distribution of allelic variants of the VDR polymorphisms between sporadic and hereditary MEN1-associated NETs and between patients with recurrent/progressive disease and those with remission/stable disease.

Conclusion VDR polymorphisms, Fok-1 and Taq-1, do not differ between sporadic and hereditary NETs and do not influence the prognosis of these tumors. Serum 25(OH)D levels are significantly lower in patients with poor-prognosis NETs, suggesting a potential therapeutic use of vitamin D in these patients.

Disclosure: No significant relationships.

P083

Metabolic Disorders in Patients with Adrenal Incidentalomas

G. Alimoukhamedova¹, Z. Khalimova², S. Safarova¹¹Neuroendocrinology, Republican Specialized Scientific-Practical Medical Centre of Endocrinology; ²Neuroendocrinology, Center of Endocrinology, Tashkent, Uzbekistan

Introduction Metabolic disorders are common in adrenal incidentalomas.

Methods and Materials 98 patients with adrenal incidentalomas were observed; clinical, biochemical, and hormonal tests were performed.

Results 30 % had no metabolic disorders, median age 30.3 ± 1.83, male 53 %, female 47 %. 63.3 % had arterial hypertension with an average duration of 2.68 ± 0.79 years. Average BP was 166.6 ± 6.33/106.6 ± 3.53 mmHg. BMI was 22.7 kg/m². Average hormone levels did not differ from controls though 13.3 % had raised levels of cortisol, 6.67 % aldosterone, 73.33 % epinephrine, 56.67 % norepinephrine, and 20 % dopamine. Lipid levels were within normal values and did not differ from the control group. Deviations in carbohydrate metabolism indicators were not revealed. 70 % of patients were included into the group with metabolic syndrome, median age 39.1 ± 1.58 years. Frequency of metabolic disorders was identical, both for men and women. 82.4 % had arterial hypertension. Duration of arterial hypertension was significantly higher ($p < 0.05$) in comparison with the group of patients without metabolic syndrome (5.68 ± 0.91). Average SBP level was 171.9 ± 4.84, DBP 105 ± 2.78 mmHg. Though average hormone levels did not deviate from normal values, there was isolated increase of some hormones in some cases. 25 % of patients had hypercortisolism, hyperaldosteronism 13.34 %, increase of epinephrine 83.8 %, norepinephrine 61.7 %, dopamine 30.8 %. Moreover, it was associated with an increase of total cholesterol level in 38.2 %, triglycerides in 35.3 %, LDL in 30.9 %, and decrease of HDL in 58.8 %. BMI was increased in 85.3 % (average 28.6 ± 0.43 kg/m²). Carbohydrate metabolism disorders were revealed in 42.6 %, including impaired fasting glycemia in 10.2 %, impaired glucose tolerance in 17.6 %, and diabetes in 14.7 %.

Conclusion Metabolic disorders are important components of adrenal incidentaloma clinics; dyslipidemia, increase of BMI, age, and deviation of hormones and glycemia levels doubtlessly play a role in its development. Significantly accurate association of frequency of metabolic disorders with presence of subclinical hormonal activity, especially with increase of cortisol and aldosterone level was found.

Disclosure: No significant relationships.

P084

Results of a Prospective Observation of Patients with Adrenal Incidentalomas in the Republic of Uzbekistan

G. Alimoukhamedova, Z. Khalimova, S. Safarova

Neuroendocrinology, Republican Specialized Scientific-Practical Medical Centre of Endocrinology, Tashkent, Uzbekistan

Introduction Observation is important in the management of patients with adrenal incidentalomas.

Methods and Materials 98 patients with adrenal incidentalomas were observed for 5 years with annual clinical-hormonal and CT tests.

Results After primary diagnosis, 25.5 % of patients were operated whereas 74.5 % were not operated and instead underwent dynamic observation. Clinical examination, including estimation of hemodynamic indicators (BP, BMI, fasting and postprandial blood glucoses, blood electrolytes and lipids, hormonal tests [blood aldosterone, cortisol, plasma renin activity], CT of adrenal glands) was performed at every visit. For estimation of dynamics of changes of tumors and their structure, size, and density at primary and control scans

were compared annually within 5 years. So, of 73 patients undergoing supervision, 87.7 % had no changes observed, 4.1 % had a variation of the tumor size, 5.5 % had hyperfunction of adrenal glands without change of the tumor size, and 2.7 % had an increase in size together with hyperfunction. During observation none of the patients had malignisation. The analysis of the results of surgical treatment of 34 patients revealed enough high efficiency of operative intervention. The overwhelming majority of the operated patients with adrenal incidentalomas with subclinical hormonal secretion had good clinical effect with regress of the basic symptoms of the disease and decrease in basic hormonal indicators. Normalization of BP was observed in 64.3 % of cases, improvement of metabolic indicators in 78.6 %, and increase of efficiency of medicamentous therapy in 21.4 %. No one of 34 operated patients had tumor relapse revealed.

Conclusion Prospective observation of patients with adrenal incidentalomas timely revealed dynamic changes of tumors sizes, hormonal activity (12.3 %) that has served their timely removal. Surgical intervention is the method of choice in the treatment of adrenal incidentalomas, allowing normalization of BP in 64.3 % of cases, improvement of metabolic control in 78.6 %, and increase of efficiency of medicamentous therapy in 21.4 %.

Disclosure: No significant relationships.

P085

Investigation of the Diagnostic Value of Chromogranin A in Neuroendocrine Tumors in the Hungarian Population

J. Gardi¹, Z. Valkusz¹, I. Földesi¹, T. Nyár², T. Wittmann¹¹First Dept of Internal Medicine; ²Dept of Medical Informatics, University of Szeged, Hungary

Introduction Chromogranin A (CgA) is expressed in the secretory granules of most normal and neoplastic neuroendocrine cells. Elevated circulating CgA levels have been found in neuroendocrine tumors (NETs), but the usefulness of this marker is still controversial. The aim of the present study was to evaluate the role of CgA in the diagnosis of NETs and in treatment responses.

Methods and Materials 25 patients with NETs and 28 assumed normal controls were included in this study. Serum CgA levels were measured by IRMA or ELISA. Receiver-operating characteristic (ROC) curve method was applied to find cut-off values to distinguish patients from healthy individuals.

Results We found good correspondence between values determined by IRMA and ELISA. Area under the ROC curve was 0.976 at 116.8 ng/ml with a sensitivity and specificity of 92.0 % and 96.4 %, respectively. Patients with somatostatin analogue treatment had a remarkable (70–90 %) decrease in CgA levels compared to the baseline values as a response to the therapy. Increasing serum CgA concentrations during treatment correlated to the progress of the disease.

Conclusion Serum CgA levels showed a high sensitivity and specificity in the diagnosis of NETs. Since changes in CgA levels are related to treatment responses it could be a helpful biochemical marker during follow-up.

Disclosure: No significant relationships.

P086

RSUME Is Implicated in HIF-1-Induced VEGF-A Production in Neuroendocrine Pancreatic Bon-1 Cells

Y. Wu¹, K. Lucia¹, C. Auernhammer², G. K. Stalla¹, E. Arz³, U. Renner¹¹Neuroendocrinology Group, Max Planck Institute of Psychiatry; ²Dept of Internal Medicine II, University of Munich, Germany; ³Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Argentina

Introduction RSUME, a small RWD-containing SUMOylation Enhancer protein, was reported to increase overall SUMO-1, -2, and -3 conjugation by interaction with the SUMO conjugase Ubc9. RSUME is expressed in various tissues, with high levels in the cer-

ebellum, pituitary, heart, kidney, liver, stomach as well as in the pancreas. Previous research indicates that RSUME can activate the protein levels of hypoxia-inducible factor-1 α (HIF-1 α). The latter is the oxygen-regulated subunit of HIF-1, the most important transcription factor of the cellular adaptive processes to hypoxic conditions. It is also a major regulator of vascular endothelial growth factor-A (VEGF-A), which is critically involved in the complex process of tumor neovascularisation.

Methods and Materials In this study, the expression and role of RSUME in a neuroendocrine pancreatic tumor cell line were studied using real-time PCR, western blot, immunofluorescence, and ELISA.

Results We found that after hypoxia-mimicking CoCl₂ treatment RSUME was up-regulated in pancreatic tumor cells (Bon-1) both in mRNA and protein levels. HIF-1 α did not differ in mRNA levels but HIF-1 α protein levels increased significantly upon CoCl₂ treatment indicating that RSUME may target the posttranslational modification of HIF-1 α in hypoxia condition. As a consequence, VEGF-A production was also elevated upon CoCl₂ treatment in Bon-1 cells. When RSUME expression was specifically down-regulated by siRNA, the CoCl₂-induced increase VEGF-A secretion was strongly impaired which was shown to be a consequence of the RSUME knockdown-associated reduction of HIF-1 α synthesis.

Conclusion In summary, RSUME plays an important role in initiating pancreatic tumor neovascularisation through regulating HIF-1 α levels and subsequent VEGF-A production and may therefore be critically involved in pancreatic adenoma progression.

Disclosure: No significant relationships.

P087

Long-Acting Somatostatin Analogues Induce Objective Tumor Responses in Patients with Early-Stage MEN-1-Related Neuroendocrine Tumors

A. Faggiano¹, V. Ramundo¹, M. del Prete¹, V. Marotta¹, F. Marciello¹, L. Camera², V. Napolitano³, L. de Luca⁴, A. C. Carratù¹, R. Esposito¹, C. de Luca di Roseto¹, A. Colao¹

¹Dept of Clinical and Molecular Endocrinology and Oncology; ²Biomorphologic and Functional Sciences, University of Naples Federico II; ³Internal Medicine, 2nd University of Naples; ⁴Gastroenterology and Digestive Endoscopy, Pellegrini Hospital, Naples, Italy

Introduction Somatostatin analogues (SSA) represent one of the main therapeutic options in patients affected with functioning well-differentiated neuroendocrine tumors (NET). There are no studies specifically focusing on NET associated to Multiple Endocrine Neoplasia type 1 (MEN-1).

Methods and Materials All first-degree relatives of MEN-1 subjects, genetically diagnosed for MEN-1 before the clinical diagnosis of NET and with evidence of one or more duodeno-pancreatic NET < 15 mm in size were enrolled. 22 patients with MEN-1-related duodeno-pancreatic NET (age range 21–42 years) were treated with octreotide LAR (30 mg/28 days). Treatment duration ranged 1–7 years. At radiological evaluation (performed by multidetector-row computed tomography and endoscopic ultrasound), multiple duodeno-pancreatic NET (range 1–8), sized 3–14 mm, were detected.

Results An objective tumor response was observed in 18 %, stable disease in 78 %, and progression of disease in 4 % of cases. In 5 patients with abnormally increased chromogranin-A and/or gastrin serum concentrations, a significant hormonal response occurred in 100 % cases and was stable along the time.

Conclusion Therapy with SSA is highly effective in patients with early stage MEN-1 duodeno-pancreatic NET, resulting in long-time suppression of tumor and hormonal activity and 18 % objective response.

Disclosure: No significant relationships.

P088

Functional Imaging in Glomus Tumors and Paraganglioma with ⁶⁸Ga-DOTA-TOC and ¹⁸F-DOPA PET/CT

A. Kroiss¹, D. Putzer¹, A. Frech², C. Uprimny¹, L. Posch², R. Gasser³, C. Goetsch³, G. Fraedrich², B. Shulkin⁴, I. Virgolini¹

¹Dept of Nuclear Medicine; ²Dept of Vascular Surgery; ³Endocrinology, Medical University of Innsbruck, Austria; ⁴Nuclear Medicine, St. Jude Children's Research Hospital, Memphis, USA

Introduction We compared functional imaging modalities in the diagnosis and staging of glomus tumors and paragangliomas, using ⁶⁸Ga-DOTA-TOC and ¹⁸F-DOPA PET/CT. Diagnostic CT imaging referred as reference standard.

Methods and Materials Two male and 12 female patients (age range 28–73 years) with anatomical and/or histologically proven disease were included in this study. Three female patients were suffering from paraganglioma, and 11 patients from glomus tumors. Comparative evaluation included morphological imaging with CT, functional imaging with ⁶⁸Ga-DOTA-TOC PET and ¹⁸F-DOPA imaging. Imaging results were analyzed on a per-patient and on a per-lesion basis.

Results On a per-patient basis, both ⁶⁸Ga-DOTA-TOC PET and ¹⁸F-DOPA showed a sensitivity of 100 % when compared with anatomical imaging. In paraganglioma patients, on a per-lesion basis, the sensitivity of ⁶⁸Ga-DOTA-TOC was 100 % and that of ¹⁸F-DOPA PET was 50 %. In glomus tumor patients, on a per-lesion basis, both ⁶⁸Ga-DOTA-TOC PET and ¹⁸F-DOPA PET showed a sensitivity of 100 %. Overall, in this patient cohort, ⁶⁸Ga-DOTA-TOC PET identified 28 lesions, anatomical imaging identified 26 lesions, and ¹⁸F-DOPA identified only 20 lesions. In this patient group, the overall sensitivity of ⁶⁸Ga-DOTA-TOC PET on a lesion basis was 100 % (McNemar p < 0.5) and that of ¹⁸F-DOPA was 76.9 % (McNemar p < 0.05).

Conclusion Our analysis in this relatively small patient cohort indicates that ⁶⁸Ga-DOTA-TOC PET may be superior to ¹⁸F-DOPA PET and even to the reference CT technique in providing particularly valuable information for pretherapeutic staging of paraganglioma and glomus tumors.

Disclosure: No significant relationships.

P089

Neuroendocrine Tumors Are a Common Finding in Patients with Neurofibromatosis Type 1

V. Marotta¹, M. del Prete¹, F. Marciello¹, V. Ramundo¹, R. Esposito¹, A. C. Carratù¹, C. de Luca di Roseto¹, A. Colao¹, A. Faggiano¹

Dept of Molecular and Clinical Endocrinology and Oncology, University of Naples Federico II, Italy

Introduction Neurofibromatosis type 1 (NF1) is an autosomal dominant inherited syndrome characterized by multi-organ involvement and cell proliferation as a consequence of decrease of tumor suppressor gene activity (NF1) and hyperactivation of proliferative and anti-apoptotic molecular mechanisms (mTOR pathway). For this reason, neuroendocrine tumors, which are known to be associated with mTOR pathway hyperactivation, are expected to occur frequently in NF1. This study aims to investigate the prevalence and characteristics of neuroendocrine tumours (NETs) in a cohort of patients with NF1.

Methods and Materials 21 patients affected with NF1 (14 females, 7 males, mean age 37.1 yrs, range 20–60) were included. Neuroendocrine hormone secretion was evaluated at different sites: thyroid C cells, adrenal and extra-adrenal chromaffin cells, pituitary, parathyroid, and diffuse neuroendocrine system. A radiological study was also performed to search for thyroid, parathyroid, pituitary, thorax, and abdominal lesions.

Results As a whole, 7 NETs of different type were found in 7 different patients, which is consistent with a rate of 33 %. In detail, 2 patients were affected with pituitary adenoma (non-functioning type), 2 patients with primary hyperparathyroidism (1 adenoma,

1 hyperplasia), 2 patients with medullary thyroid carcinoma, and 1 patient with pheochromocytoma.

Conclusion NF1 is characterized by an increased risk of developing NETs. Different types of NETs were included, not only pheochromocytoma but also pituitary, parathyroid, and C cell tumours, which are not known to be associated to NF1. A biochemical and radiological work-up for NETs is suggested to be included in the management of patients with NF1.

Disclosure: No significant relationships.

P090

Intraoperative Calcitonin Assay-Guided Surgery Achieves a 100-% Cure Rate in Patients with Medullary Thyroid Cancer

A. Faggiano¹, M. G. Chiofalo², F. Marciello¹, M. del Prete¹, V. Marotta¹, V. Ramundo¹, R. Esposito¹, C. de Luca di Roseto¹, A. C. Carratù¹, L. Pezzullo², A. Colao¹

¹Dept of Molecular and Clinical Endocrinology and Oncology, University of Naples Federico II; ²Surgery, National Cancer Institute, Naples, Italy

Total thyroidectomy plus adequate lymph node excision is the only curative approach for medullary thyroid cancer (MTC). However, about 20 % of MTC patients are not disease free after surgery due to persistent lymph node tumor tissue. In a previous study, we validated the use of intraoperative calcitonin (CT) measurements to predict disease outcome in patients operated for MTC. The aim of the current study was to apply this intraoperative procedure to increase the cure rate of surgery in patients with MTC. Since May 2010, all patients with a preoperative diagnosis of MTC, on the basis of a positive pentagastrin test, underwent total thyroidectomy and central lymph node dissection associated with intraoperative CT assay (CT measurements performed before surgical excision, at the time of anesthesia and manipulation, and 30 min after complete surgical excision): surgery was modulated on the basis of the intraoperative CT monitoring. 14 consecutive patients were included. Mean follow-up time ranged from 6–21 months. An intra-operative CT drop > 50 % 30 min after surgical resection was achieved in 11 patients (mean $\pm$ SEM 68 $\pm$ 3.5 %), while the CT drop after 30 min was 20 %, 27 %, and 42 % in the 3 other patients, respectively. These 3 patients were considered uncured and therefore submitted to lateral neck lymph node excision. All patients had complete clinical, hormonal, and radiological remission during the follow-up.

Disclosure: No significant relationships.

P091

Pancreatic and Adrenal Tumors in Patients with Genetic Syndromes

G. Poiana¹, M. Carsote¹, C. Ene¹, S. Radian¹, D. Terzea¹, D. Paun¹, D. Hortopan²

¹Endocrine; ²Imaging, I. Parhon, Romania

Introduction Genetic syndromes including neuroendocrine tumors represent challenging cases due to multiple synchronous tumors and severe prognosis.

Methods and Materials Case report data: type-1 neurofibromatosis-associated pheochromocytoma and 2 members of a family with von Hippel Lindau Syndrome.

Results A 42-year-old male patient was operated for duodenal somatostatinoma (Ki-67 10 %). He presented type-1 neurofibromatosis. Endocrine evaluations performed after the surgery showed chromogranin A and serotonin mildly increased. The computed tomography showed lymph nodes, a left adrenal tumor of 1.17 $\times$ 0.7 cm, 2 right adrenal tumors of 3.33 $\times$ 3.06 cm, of 4.58 $\times$ 3.66 cm. The metanephrines were 3 $\times$ above normal. Right adrenalectomy was recommended but the patient refused. He died soon after.

A 61-year-old male patient was operated for a brain tumor 2 decades ago. Two years ago he was diagnosed with 2 renal tumors of 7.7 $\times$ 6.6 cm and 5.3 $\times$ 5.4 cm (inoperable kidney cancer), a tumor at the pancreatic head of 5 $\times$ 3 cm, and multiple hepatic lymph nodes with carcinoid syndrome and jaundice. Cholecystojejunostomy was per-

formed. The hepatic biopsy: G1 NET. Computed tomography showed a left adrenal tumor of 1 cm and negative adrenal hypersecretion but high neuroendocrine markers. Therapy with octreotide was started.

A 33-year-old female is the daughter of the second patient. She associates VHL gene mutation. She presents multiple retinal angiomas, secondary ambliopia, a brain tumor that was recently removed, multiple medullar and cerebral hemangioblastomas. Two adrenal tumors were discovered with no adrenal hypersecretion, and a tumor of 1 cm at the pancreatic with negative neuroendocrine markers. Close follow-up is necessary.

Conclusion The genetic syndromes included a great variety of pancreatic and adrenal tumors, benign or malign, secretor or non-functional, solid or cystic. This multitude of phenotypes makes the diagnosis more difficult, thus imaging or functional imaging scans become essential.

Disclosure: No significant relationships.

P092

Aggressive Profile in a Case of Ovarian Teratoma-Associated Neuroendocrine Tumor

M. Carsote¹, C. Ene¹, D. Terzea², D. Paun¹, D. Hortopan³, C. Poiana⁴

¹Endocrine, I. Parhon; ²Pathology, UMPH Davila, Bucharest; ³Imaging, I. Parhon;

⁴Endocrinology, UMPH Davila, Bucharest, Romania

Introduction Neuroendocrine tumors developed from heterotopic neuroendocrine cells are rare but they associate an increased risk of an aggressive profile. Thus, their early detection might improve prognosis.

Methods and Materials We present the case of a female patient with an ovarian neuroendocrine tumor with atypical metastasis.

Results A 62-year-old female patient with a so far negative medical history was diagnosed in March 2011 with a right ovarian tumor of 7 $\times$ 8 cm, with a cystadenoma aspect. Double anexectomy with total hysterectomy was performed. The pathological report showed an ovarian teratoma with cartilages, bronchia, and a poorly differentiated neuroendocrine carcinoma. The IHC pointed 35–40 % positive for estrogen receptor (ER), negative progesterone receptor, +ve WT1, CROMO, SYN, CK20, negative inhibin, CK7, and a Ki-67 of 25–30 %. The serum neuroendocrine markers chromogranin A and neuronal specific enolase were 1.5 $\times$ above normal range. After 6 cycles of cisplatin and etoposid an abdominal (omental) tumor of 8 $\times$ 6 cm increased after 1 year. The new tumor was removed. The pathological report showed a poorly differentiated neuroendocrine carcinoma metastasis. ER expression was lost, while neuroendocrine features were steady, and Ki-67 increased to 65–70 %. Neuroendocrine markers were still high. New cycles of chemotherapy (carboplatin) were initiated associated with monthly octreotide LA 30 mg.

Conclusion Neuroendocrine tumors developing from an ovarian teratoma might be aggressive. The metastasis might not conserve the initial combination of hormonal receptors as estrogen or progesterone receptors, while dedifferentiation into a tumor with a higher Ki-67 is a rare but probable event.

Disclosure: No significant relationships.

P093

Delayed Diagnosis of MEN-1 in a Patient with an Inherited Bone Disease Challenges the Guidelines that Screening for MEN-1 in Patients with Primary Hyperparathyroidism Should Be Performed Only Below the Age of 30 Years

U. Feldt-Rasmussen¹, Å. K. Rasmussen², L. Friis-Hansen²

¹Medical Endocrinology; ²Molecular Genetics, National University Hospital, Copenhagen, Denmark

Introduction Current guidelines advise screening for MEN-1 in patients with primary hyperparathyroidism (pHPT) only below the age of 30 years.

Methods and Materials A 31-year-old female patient with osteogenesis imperfecta (OI) type 1 was referred for hypercalcaemia and suspicion of pHPT. She had serum Ca^{2+} 1.56 mmol/l, S-PTH 115 ng/ml (< 70); pHPT was diagnosed, without uptake on a parathyroid scintigraphy.

Results Exploratory neck surgery showed hyperplastic parathyroid glands; 3½ parathyroid gland + thymus were removed. The hyperparathyroidism was hypothesised to be due to abnormal calcium metabolism from OI (J Endocrinol Invest 1999). Years later a study on screening for mutations in the menin gene of all patients who had had pHPT was done, and she had a mutation (Trp341STOP). This incidental diagnosis of MEN-1 had 2 consequences:

- She had a full work-up for all MEN-1 organ manifestations, revealing no other affections at the time. Recently, she has had a doubling of chromogranin A, currently under investigation.
- Her 13-year-old daughter, found negative for OI, was shown to have the same menin gene mutation. She had mild pHPT already at that age. She was followed with annual biochemical measurements and due to increasing levels of serum calcium and fatigue, she was operated at 21 with a markedly positive effect on her general well-being.

Conclusion The patient had a bone disease, which could potentially explain the PHT; thus her concomitant mutation in the menin gene, shared by her daughter, was only discovered incidentally during a research project. It should therefore be underscored that all patients with pHPT below the age of 40 years (and not 30 years as in the current guidelines) should be screened for MEN-1 mutations independent of other concomitant diseases. Only thus can proper genetic counselling and disease management of patients and family members be performed.

Disclosure: No significant relationships.

P094

Confusing Genes: A Patient with MEN-2A and Cushing's Disease

A. Naziat¹, N. Karavitiaki¹, O. Ansorge², G. Sadler³, F. Gleeson⁴, T. Carnston⁵, B. Shine⁶, A. McCormack⁶, R. Thakker¹, A. B. Grossman¹

¹Dept of Endocrinology, Oxford Centre for Diabetes, Endocrinology and Metabolism; ²Pathology, Churchill Hospital; ³Endocrine Surgery; ⁴Radiology, John Radcliffe Hospital; ⁵MEN Genetics, University of Oxford, UK; ⁶Endocrinology, St Vincent's Hospital and Garvan Institute of Medical Research, Sydney, Australia

Introduction We report a case of MEN-2A with medullary thyroid carcinoma, pheochromocytoma, hyperparathyroidism, and Cushing's disease.

Methods and Materials A 68-year-old man had been diagnosed with pituitary-dependent Cushing's syndrome on inferior petrosal sinus sampling and underwent transsphenoidal adrenalectomy; although no tumour was found on histology, he achieved remission. He developed primary hyperparathyroidism (PHPT) which led to MEN-1 genetic analysis, but no mutation was identified. His Cushing's disease relapsed 15 years after the initial "cure" and was successfully re-operated, rendering him hypopituitary. However, he subsequently developed medullary thyroid cancer (MTC) with liver and bone metastases, and was also found to have bilateral pheochromocytomas. He was started on α - and β -adrenoceptor blockade immediately and underwent total thyroidectomy.

Results The tumour was positive on immunocytochemistry for calcitonin, but was negative for ACTH, suggesting that the MTC was not secreting ACTH ectopically. These new findings led to MEN-2 genetic testing and a characteristic RET-mutation was identified (cys 634Arg). Nuclear menin expression was preserved in the MTC and pituitary tissue, further excluding the possibility of MEN-1 mutation. His son had been confirmed to have RET-mutation with PHPT, MTC, and bilateral pheochromocytomas, but had no evidence of Cushing's disease.

Conclusion Cushing's disease is a well-established feature of MEN-1, but not MEN-2, and ACTH-dependent Cushing's syndrome in MEN-2 is usually secondary to ectopic production of ACTH by an MTC or pheochromocytoma. Rarely, an overlap between MEN-1

and MEN-2, and presence of both mutations in the same patient have been described in the literature. Only a single case of the combination of MTC, pheochromocytoma and Cushing's disease has been reported, and this was prior to establishment of the genetic basis of MEN-1 and MEN-2. Our case suggests that in patients with Cushing's disease who have other evidence of endocrine neoplasia, if MEN-1 is excluded then MEN-2 should be considered.

Disclosure: No significant relationships.

P095

Cushing's Syndrome Caused by PNET with Liver Metastases: A Rare Presentation

B. Altieri, E. Salomone, V. A. Sun, T. Schirò, L. di Pizio, A. Capozzi, M. C. Fabiano, G. P. Sorice, A. Giaccari, S. della Casa
Endocrinology and Metabolic Disease, Catholic University of Sacred Heart, Rome, Italy

Introduction Pancreatic neuroendocrine tumors (PNETs) are rare neoplasms representing 1–2 % of all pancreatic malignancies. Their clinical presentation depends on excessive hormonal secretion and action. Ectopic ACTH production by PNET is relatively rare. We report the case of a 43-year-old woman with Cushing's syndrome caused by a malignant unresectable PNET with liver metastases with positive ⁶⁸Ga-DOTA-NOC PET.

Methods and Materials The patient was referred to us for hirsutism, amenorrhea, muscle weakness, and edema of the bilateral lower extremities and face. She underwent computed tomography (CT) showing a 6-cm pancreatic mass associated with hepatic nodules. She was followed up 3 months later and hormonal evaluations were performed.

Results Laboratory examinations revealed a significant increase in plasma ACTH (260 pg/ml; range 10–55), cortisol (253 ng/ml; range 60–220), and urinary free cortisol (1815.2 mcg/24 h; range < 70.0). Cortisol levels did not suppress with low-dose (1 mg) and high-dose (8 mg) dexamethasone suppression test (281 ng/ml and 236 ng/ml, respectively). Therefore, we detected hypokalemia and elevated levels of serum gastrin (1976.0 pg/ml; range 30.0–115.0) and chromogranin A (2031.7 ng/ml; range 19.4–98.1). Histological examinations by needle biopsy of liver and pancreatic lesions indicated metastatic PNET ACTH-producing with cells positive for ACTH, synaptophysin and chromogranin A and a Ki-67 proliferation index was 5 %. For a functional study of NET, the patient underwent a gallium ⁶⁸DOTA-NOC PET/CT which showed an increased uptake of the tracer receptor at the level of pancreas and hepatic nodules.

Conclusion The patient underwent steroid-blocking ketoconazole therapy and somatostatin analogue (lanreotide 120 mg). A case of PNET associated with Cushing's syndrome is rare and to the best of our knowledge this is the first case ever described with detection of ectopic ACTH Cushing of pancreatic origin using PET with gallium peptides.

Disclosure: No significant relationships.

■ Genetics in Neuroendocrinology

P096

Two Single Nucleotide Polymorphisms (Q228K and Q307R) of the Aryl Hydrocarbon Receptor Interacting Protein (AIP) Gene Are Associated with a Predisposition to Familial Isolated Pituitary Adenomas but not Sporadic Somatotrophinomas in Turkish Patients

S. Yaman¹, Y. Oğre², F. Oğuz²

¹Endocrinology and Metabolic Diseases; ²Medical Biology, Faculty of Medicine, Istanbul University, Turkey

Introduction The majority of pituitary adenomas are sporadic but they occasionally occur in a familial setting. Recently, studies have

focused on the identification of AIP mutations in large international cohorts. The aim of this study was to investigate the effect of the AIP gene in the predisposition to familial isolated pituitary adenomas (FIPAs) and sporadic somatotrophinomas in our population.

Methods and Materials We studied 7 families with FIPAs, 47 sporadic acromegalic patients, and 70 healthy controls. The pituitary tumor types in these families were somatotrophinomas (n = 11), prolactinomas (n = 2), non-functional adenomas (n = 2), and corticotrophinoma (n = 1). Two patients with somatotrophinomas were excluded from the analysis. There were 14 patients in FIPA families (10 F/4 M; mean age at diagnosis 39 ± 13.6 years) and 47 with somatotrophinomas (25 F/22 M; mean age 35 ± 7.4 years). Genomic DNA was analysed from peripheral blood lymphocytes for mutations in the AIP gene by PCR amplification and full sequencing.

Results There was no difference in age and sex between FIPA patients and sporadic somatotrophinomas ($p = 0.261$ and $p = 0.226$, respectively). Sequencing of the AIP gene revealed 2 single nucleotide changes: c.682C>A (p.Q228K) and c.920A>G (p.Q307R) in FIPA patients. The genotype frequencies of these polymorphisms (78.6 % and 78.6 %, respectively) were significantly higher than in controls (10 % and 8.6 %, respectively; $p < 0.0001$). Coexistence of both polymorphisms was 57 % in FIPA patients. These 2 polymorphisms did not differ between sporadic somatotrophinomas and controls ($p > 0.05$). However, the presence of polymorphisms in sporadic somatotrophinomas showed a significant association with cavernous invasion and postoperative remission ($p = 0.025$ and $p = 0.031$, respectively).

Conclusion This is the first report of Q228K and Q307R SNPs leading to FIPAs in Turkish patients with relatively high age at diagnosis.

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Disclosure: No significant relationships.

P097

Gigantism and Prolactinoma in an AIP Mutation-Positive FIPA Family

N. Dalantaeva¹, L. Dzeranova¹, E. Pigarova¹, M. Korbonits², S. A. Prokofiev¹, I. Dedov¹

¹Neuroendocrinology, Endocrinology Research Centre, Moscow, Russian Federation; ²Endocrinology, William Harvey Research Institute, Bart's and The London School of Medicine, Queen Mary University of London, UK

Introduction The genetic aspects of familial isolated pituitary adenomas (FIPA) have been expanding rapidly in the last few years. Around 20 % of FIPA families have mutations in the aryl hydrocarbon receptor-interacting protein (AIP) gene and mutations have also been identified in young-onset pituitary adenoma patients without known family history.

Methods and Materials A 17-year-old male was admitted to the hospital with a chronic headache. Clinical studies revealed a large invasive pituitary adenoma, high GH and normal PRL levels. He had a rapid increase in height from the age of 14 and currently he is 202 cm. Radiotherapy was performed. Two years later transsphenoidal surgery was performed. Dopamine-agonist therapy (quinagolide, cabergoline) was initiated at 22 years. Combined sandostatin LAR 30 mg/m and cabergoline 1.5 mg/w was started at the age of 30 years (Table 3). IGF-1 and GH levels never normalised and tumour tissue is detectable in the pituitary fossa. The patient would probably benefit from GHR antagonist therapy and a second radiotherapy is considered.

Results The proband's sister was diagnosed with a microprolactinoma (age 18 years) which is successfully treated with caber-

Table 3. N. Dalantaeva et al. Treatment scheme and GH and IGF-1 levels of the patient described.

Age		GH (ng/ml)	IGF-1 (ng/ml)
17	Before treatment	192.2	–
22	Quinagolide, cabergoline	70	–
25	Quinagolide, cabergoline	49.9	794
27	20 mg S-LAR	37.2	518
29	30 mg S-LAR	37.8	497.7
35	30 mg S-LAR + 1.5 mg/w cabergoline	56.7	695.9

golide. We identified a truncating AIP mutation (p.Y261X) in the proband, sister, and unaffected father and uncle, while his daughter was AIP-negative. She developed a large aggressive meningioma and died of the disease at the age of 5 years despite surgery and chemotherapy.

Conclusion A germline AIP mutation caused very different disease characteristics in the 2 pituitary adenoma patients. The development of an aggressive meningioma in the proband's daughter raises the question of other genetic factors influencing tumour penetrance and behavior.

Disclosure: No significant relationships.

P098

A New Phenotype Associated with V84L Mutation in Von Hippel Lindau Disease

B. Altieri, G. Muscogiuri, T. Schirò, L. di Pizio, V. A. Sun, A. Capozzi, C. Cipolla, C. Pollicola, A. Pontecorvi, S. della Casa
Endocrinology and Metabolic Disease, Catholic University of Sacred Heart, Rome, Italy

Introduction The Von Hippel Lindau (VHL) disease is a rare autosomal disorder caused by point mutation or deletions in the VHL gene. It is associated with some specific VHL mutations. We describe the clinical findings in a 42-year-old man with a rare p.Val84Leu missense mutation.

Methods and Materials When he was 11 years old, he underwent left adrenalectomy for Pheo and 7 years later underwent right adrenalectomy. A 2-cm lesion of the pancreas was identified by screening CT when he was 35 years old. Subsequently, 2 liver lesions appeared and a needle biopsy indicated metastatic PNET. Because the octreoscan was positive, somatostatin analogue was started. In February 2009, the patient was subjected to cephalic duodenopancreatectomy with liver metastases dissection. One year later, 4 spinal hemangioblastomas were identified by MRI. In recent years, the patient underwent radiofrequency ablation and transarterial chemoembolization for unresectable liver metastases. The last CT showed disease progression, so the patient started everolimus. Ophthalmological screening for other VHL disease manifestations was negative. A genetic analysis was also performed.

Results Genetic testing revealed a VHL mutation: a G to C transition at nucleotide 250 (V84L). There was no family history of VHL disease or familial Pheo. A VHL analysis in the family showed that this variant was transmitted from the unaffected mother.

Conclusion More than 300 germline mutations have been identified that are involved in VHL disease. To our knowledge, the G > C transition at nucleotide 250 resulting in amino acid substitution p.Val84Leu is very rare. V84L mutation is associated with VHL type 2C characterized exclusively by Pheo. To the best of our knowledge, this is the first case that reports the association of complete VHL disease with p.Val84Leu mutation.

Disclosure: No significant relationships.

POSTER PRESENTATIONS

POSTER SESSION 2 – FRIDAY

■ Cushing's Syndrome

P099

Prevalence of Cushing's Syndrome in Uzbekistan, 2000–2012

Z. Khalimova, G. Narimova

Neuroendocrinology, Republican Specialized Scientific-Practical Medical Centre of Endocrinology, Tashkent, Uzbekistan

Introduction This study aimed to investigate the prevalence of Cushing's syndrome (CS) in Uzbekistan.

Methods and Materials Prospective analysis of frequency of hospitalised CS cases according to RSSPMCE data from 2000–2012. 143 patients with CS (53.1 % female aged 31.8 ± 2.5 years, 25.9 % male aged 29 ± 0.5 , and 21 % children and teenagers aged 13.4 ± 1.5 years) were studied. Age of CS manifestation was 27.2 years (ranging from 3–59 years) with accurate prevalence of cases among women (2:1).

Results CS was diagnosed within 6 months from the disease manifestation in 11.8 %, in 1–3 years in 74.1 %, and after 3 years in 11.2 %. Patients were distributed as follows: ACTH-dependent CS (87.4 %), ACTH-independent (7 %), ectopic CS (1.4 %) with primary source in lungs and liver, and iatrogenic CS in 4.2 % in patients with various chronic diseases (vulgar pemphigus, bronchial asthma, chronic bronchitis) due to long-term use of corticosteroids. Analysis of factors preceding the disease showed that 62.2 % patients had no obvious reasons, 4.2 % women attributed to pregnancy and delivery, 2.0 % patients to viral hepatitis, 3.5 % to catarrhal diseases and flu, 12.6 % to stress, 5.6 % to craniocerebral traumas, 5.6 % to various conditions, and 4.2 % to use of certain doses and duration of glucocorticoids use for various chronic diseases. Thus, at the time of diagnosis, carbohydrate exchange disturbances were registered in 38.5 %, osteopenia and osteoporosis in 59.4 %, arterial hypertension in 77.6 %, associated cardiovascular diseases in 34.3 %, and reproductive disorders in 55.9 %.

Conclusion Thus, CS is a severe neuroendocrine disease affecting young persons. Despite the clear clinics, its diagnosis is delayed for ≥ 1 year in 88 % of patients, which causes a high frequency of complications at the moment of diagnosis. All this gives a dares to diagnosis and treatment and defines necessity of its lifelong studying.

Disclosure: No significant relationships.

P100

Carbohydrate Metabolism in Newly Diagnosed Patients with Cushing's Syndrome (CS)

G. Narimova, Z. Khalimova

Neuroendocrinology, Republican Specialized Scientific-Practical Medical Centre of Endocrinology, Tashkent, Uzbekistan

Introduction CS characterised by endogenous excess of glucocorticoids is associated with various system complications including glucose metabolism disorders such as diabetes mellitus (DM; 20–50 %), impaired glucose tolerance (IGT), and, more rarely, by impaired fasting glycaemia (IFG).

Objective To study carbohydrate metabolism in newly revealed patients with CS.

Methods and Materials 143 patients with CS (53.1 % female aged 31.8 ± 2.5 years, 25.9 % male aged 29 ± 0.5 , 21 % children and teenagers aged 13.4 ± 1.5 years) were observed. Fasting and postprandial glycemia, OGTT, HbA_{1c}, glyceimic, and glucose in urea profiles were performed.

Results Various disorders of carbohydrate metabolism were revealed in 38.5 % patients, of them 32.7 % male and 67.3 % female. Fasting glycemia levels varied from 5.8–12.5 mmol/l (average 6.7 ± 1.6 mmol/l), postprandial glycemia was 10.8 ± 1.8 mmol/l. Average HbA_{1c} levels were 7.7 %. In 2.8 %, DM was revealed before CS diagnosis. Of newly diagnosed patients with CS, in 23.8 % DM of various degrees of severity, IGT in 20.3 %, and IFG in 3.5 % were revealed. Of 16 patients with IGT, 56.3 % progressed to DM. Progression of IGT to DM was caused by absence of remission in 6 and disease relapse in 3 patients. Taking into consideration that CS was diagnosed in 11.8 % of patients in 6 months after the onset of the disease, in 74.1 % in 1–3 years, and after 3 years in 11.2 % patients, we can declare that development of complications of carbohydrate metabolism disorders was caused by late diagnosis of CS, and timely diagnosis could possibly avoid these disturbances.

Conclusion At the time of diagnosis, $> 1/3$ patients with CS in Uzbekistan have carbohydrate metabolism disorders: IFG in 3.5 %, IGT in 20.3 %, and DM in 23.8 %. It has been proven that development of carbohydrate metabolism complications depends on remission of CS. Today, CS remains a diagnostic and therapeutic problem in neuroendocrinology.

Disclosure: No significant relationships.

P101

Cushing's Disease due to Aggressive Corticotroph Adenoma with Dissemination to the Right Pontocerebellar Angle (Clinical Case)

E. Karseladze¹, E. Marova¹, V. Azizyan², A. Karseladze³, I. Voronkova¹, A. Grigorev², L. Rozhinskaya¹, A. Bekyashev⁴

¹Neuroendocrinology; ²Neurosurgery, Federal State Budget Institution "Endocrinological Research Center"; ³Pathology; ⁴Neurosurgery, N. N. Blokhin Russian Cancer Research Center, Moscow, Russian Federation

Introduction Truly aggressive pituitary adenomas are uncommon, representing no more than 2–5 % of all pituitary tumors.

Methods and Materials We describe the clinical case of a 42-year-old man with aggressive pituitary ACTH-producing adenoma who initially presented with visual impairment and without signs of hypercortisolism.

Results After the patient underwent transcranial adenectomy he was diagnosed with Cushing's disease. The patient received conventional external pituitary radiotherapy with 50 Gy followed by short remission. Nevertheless, considering the presence of liquorrhea and elevated ACTH levels, 10 months after initial surgical intervention transnasal adenectomy was performed. Because of subsequent adrenal insufficiency, hydrocortisone was prescribed for 1 year. 18 months after transnasal adenectomy recurrent Cushing's disease was diagnosed by clinical signs and excessive 24-h urinary cortisol excretion (6958 nmol/24 h) and ACTH levels of 729 pg/ml (N-7-66). A tumor measuring $6.0 \times 3.5 \times 4.6$ cm of right pontocerebellar angle was detected. The removed petroclival tumor histologically was identical to corticotroph adenoma. The degree of atypia of tumor cells of our patient was the same as in non-invasive, clinically benign adenomas of the pituitary, and the level of expression of Ki-67 was low (< 5 %). Eleven months after complete removal of the tumor the patient had the recurrence of Cushing's disease.

Conclusion This clinical case is of interest for several reasons:

– Onset of the disease with visual field defect and no hormonal activity.

- Progression of hypercortisolism after transcranial adenomectomy and radiotherapy.
- The disease recurred 3 times during 5 years.
- Contradiction between aggressive behavior of the tumor and histologically benign appearance of its structure.

Disclosure: No significant relationships.

P102

Hormonal and Morphological Features of ACTH-Ectopic Syndrome

E. Marova, S. Arapova, I. Voronkova, A. Lapshina, G. Kolesnikova, L. Rozhinskaya
Neuroendocrine, Endocrinological Research Centre, Moscow, Russian Federation

Aim To elucidate hormonal and morphological features of ACTH-producing neuroendocrine tumours (NET).

Methods and Materials The study included 38 patients (14 men, 24 women, mean age 42 ± 15.3 years) with ACTH ectopic syndrome (ACTH-ES). Duration of the disease was 2.89 ± 1.5 years. Plasma cortisol and ACTH rhythms, daily urinary cortisol levels, and salivary cortisol at 23:00 hours were measured; the desmopressin test was performed. MSCT was used to locate NET in the lungs, mediastinum, abdominal cavity, and adrenal glands. NET tissues were studied by histological and immunohistochemical (IHC) methods.

Results Plasma ACTH levels at 8:00 and 23:00 hours were 205.02 ± 57 and 256.4 ± 47.3 pg/ml, respectively; plasma cortisol 1593 ± 277.5 and 817 ± 135 nmol/l, urinary cortisol 1952.5 ± 408.5 nmol/24 hr; salivary cortisol (23:00) 48.7 ± 15 nmol/l. NET localization: lungs (n = 24), thymus (n = 5), pancreas (n = 2), kidney (n = 1), and appendix (n = 1). We failed to locate NET in 5 cases. Four patients presented with metastatic lesions in the adjacent organs. Relapses were documented in 4 patients 1 year after surgery, in 2 after 2 years and in 1 after 5 years. Histological studies revealed atypical (n = 11) and typical (n = 8) carcinoids, small-cell carcinoma (n = 3) in the lungs; thymic: atypical (n = 1) and typical (n = 1) carcinoids, and small (n = 1) and large-cell (n = 1) carcinomas; renal carcinoids (n = 1), and appendix (n = 1) carcinoma. IHC studies showed that 21 cases of ACTH-ES were due to ACTH-producing NET, 2 cases to CRH and 5 to ACTH+CRH. Ten cases related to polyhormonal NET activity.

Conclusion Tumours associated with ACTH-ES vary in terms of primary localization, hormonal and immune expression, histological features, and malignant potential.

Disclosure: No significant relationships.

P103

Hemostatic and Fibrinolytic Changes in Patients with Endogenous Hypercortisolism during Active Phase and in Disease Remission

Y. Khodakova, L. Rozhinskaya, S. Arapova
Neuroendocrinology and Osteopathy, Research Center for Endocrinology, Moscow, Russian Federation

Introduction Cushing's disease (CD) is associated with a persistent risk of cardiovascular complications, which were found to be 4× higher than in an age- and sex- matched population. The main cause is cardiovascular disease with thrombotic complications due to the activation of changes in the haemostatic and fibrinolytic systems with high levels of cortisol secretion.

Methods and Materials We studied 78 patients with active CD (group 1), 41 patients during 1 year of CD remission after successful surgical treatment (group 2), 20 patients served as controls (group 3). Prothrombin time (PT), thrombin time (TT), activated partial thromboplastin time (APTT), fibrinogen, tissue plasminogen activator (tPA) and inhibitor (PAI-1), D-dimer, factor VIII (F VIII), and von Willebrand factor (vWF) were investigated. Statistical analysis was performed using the Kruskal-Wallis criteria. Results are presented as median and 25th and 75th percentiles.

Results 87.3 % in group 1 had hypercoagulable state characterized by decreased levels of APTT in 24.4 %, increased levels of fibrinogen in 11.5 %, and D-dimer in 19.3%, endothelial dysfunction and hypofibrinolysis, which was characterized by increased levels of PAI-1 in 66.7 % and vWF in 11.5 %. Hypercoagulable syndrome persisted in 78 % patients of group 2. Significantly increased levels of fibrinogen in 42.3 %, D-dimer in 38.9%, and PAI-1 in 63.8 % were found.

Conclusion Our results suggest that patients with active CD have prothrombotic changes and endothelial dysfunction, which significantly increase the risk of thromboembolic complications compared with normal population. These disorders persist in patients with CD remission despite the normalization of cortisol secretion.

Disclosure: No significant relationships.

P104

Pregnancy in a Patient with Mild Cushing's Disease

S. Ciftci Dogansen¹, B. Alpaslan², B. Canbaz¹, S. Yaman¹

¹Division of Endocrinology and Metabolic Disease, Dept of Internal Medicine;

²Dept of Internal Medicine, Faculty of Medicine, Istanbul University, Turkey

Introduction Cushing's syndrome and pregnancy are highly uncommon since hypercortisolism usually causes amenorrhea, oligomenorrhea, and infertility due to the inhibition of gonadotropin secretion. Furthermore, ACTH-dependent Cushing's syndrome is a rare cause of hypercortisolism in pregnancy. A diagnosis of pathological hypercortisolism in pregnancy is often difficult because dynamic tests are difficult to interpret due to the physiological changes in the hypothalamo-pituitary-adrenal axis associated with pregnancy, and radiological diagnostics in pregnancy are limited.

Methods and Materials We report the case of a 27-year-old woman with Cushing's disease diagnosed in the 5th week of pregnancy. The patient, investigated for recurrent pregnancy loss, was referred to our endocrinology clinic when she was diagnosed with diabetes (blood glucose 13.4 mmol/l with an HbA_{1c} value of 8.1 %). On admission, she had mild cushingoid features. Diagnosis was confirmed by the finding of elevated urinary free cortisol levels with loss of normal circadian rhythm of serum cortisol. The mild symptoms of hypercortisolism did not lead to serious complications for the mother and the fetus, so only insulin treatment was introduced. Pregnancy was uneventful, and at 38 weeks of gestation, it was terminated by normal vaginal delivery and a healthy female infant weighing 2700 g was delivered. After delivery, contrast-enhanced pituitary MRI was taken on the persistence of her cushingoid features and an ACTH-secreting microadenoma was removed with transsphenoidal surgery.

Results Mild hypercortisolism can be well-tolerated without exacerbation during pregnancy, however, the mother and the fetus must be monitored closely and frequently for cardiovascular and metabolic parameters and treatment can be postponed until after delivery.

Conclusion Best outcome is achieved by a multidisciplinary approach consisting of endocrinology, obstetrics, anaesthesiology, and endocrine surgery specialists. Due to the rarity of the pathology, however, no generally accepted treatment guidelines are available. The decision for treatment depends on the severity of hypercortisolism and gestational age at time of diagnosis.

Disclosure: No significant relationships.

P105

Combination Therapy Ketoconazole + Cabergoline in Cushing's Disease

M. Barbot, N. Albiger, F. Ceccato, S. Koutroumpi, A. Daniele, F. Mantero, C. Scaroni
Endocrinology Unit, Dept of Medicine, University of Padova, Padua, Italy

Introduction Medical therapy represents a second-choice treatment in Cushing's disease (CD) due to the lack of drugs which target specifically the adenomatous tissue. The association of different drugs might yield better results with less side effects. This study

aimed to evaluate the effectiveness of ketoconazole + cabergoline and the reverse combination using urinary free cortisol (UFC) and late-night salivary cortisol (LNSC).

Methods and Materials Prospective analysis of 14 patients (f/m = 12/2; mean age 52, range 33–70 years) divided into 2 groups: 6 patients initially treated with cabergoline, then ketoconazole was added (group A); the other 8 patients received first ketoconazole alone and then with the addition of cabergoline (group B). Cabergoline was initiated at 0.5–1 mg/week and raised to a maximum of 3.5 mg/week. Ketoconazole treatment was started with 200 mg daily and increased to a maximum of 600 mg/day. Patients were compared with 14 age-matched patients in prolonged remission after effective neurosurgery for CD.

Results Combination therapy led to UFC normalization in 79 % of patients without differences between groups; another 2 patients achieved a significant reduction of UFC levels but without normalization; only one patient did not respond. LNSC decreased compared to baseline ($p = 0.015$) even though this parameter remained significantly higher than in control subjects: 6.60 ± 3.2 ng/ml vs 1.80 ± 0.93 ng/ml ($p < 0.0001$). All patients who normalize UFC reported improvement of clinical symptoms, especially those with lower LNSC levels.

Conclusion The association between cabergoline and ketoconazole is an effective second-line treatment with a satisfactory reduction of UFC levels. No differences were found between the 2 ways of treatment. Although a significant reduction in LNSC levels, this parameter remained increased in most patients and this phenomenon may represent a sign of inadequate disease control and disclose the possibility of future treatment escape.

Disclosure: No significant relationships.

P106

Long-Term Survival in Treated Patients with Cushing's Disease: Results of a 20-Year Markov Decision Model

X. Badia¹, A. Forsythe², G. Cummins³, M. Roset¹, J. Doyle⁴

¹Health Economics and Outcomes Research, IMS Health, Barcelona, Spain; ²Global Health Economics and Market Access, Novartis Pharmaceuticals Corp, East Hanover, NJ; ³Global Market Access and Commercialization, Quintiles Consulting, Hawthorne, NY; ⁴Mailman School of Public Health, Columbia University, New York, NY, USA

Introduction Limited information is available on the long-term survival of patients with Cushing's disease (CD) [1]. Survival comparisons following individual treatment strategies in CD have never been reported.

Methods and Materials A 20-year Markov decision model simulating a hypothetical cohort of 1000 40-year-old patients with CD was used to estimate the survival benefit of treatment vs no treatment and between individual treatment strategies. Transsphenoidal surgery (TSS) is the accepted first-line treatment and this model was specified using data for second-line treatments based on a retrospective chart audit, and published data for TSS, radiotherapy/radiosurgery (RAD), bilateral adrenalectomy (BLA) and available medical therapies (limited to multicenter, prospective trials with data > 12 months). Four initial second-line treatments were compared: pasireotide, TSS, RAD, and BLA. Non-responders could switch to subsequent therapies.

Results Survival rates began to diverge in year 4, 82–88 % for treated vs 78 % for untreated. At 10 years, the survival benefit was more pronounced: 69–79 % for treated vs 45 % for untreated. At 20 years, no untreated patients survived vs 52–67 % of treated. Survival among treated patients was estimated at 2 years: pasireotide 92 %, RAD 91 %, TSS 90 %, BLA 87 %; at 5 years: pasireotide 86 %, TSS 83 %, RAD 83 %, BLA 80 %; and at 20 years: pasireotide 67 %, TSS 64 %, RAD 63 %, BLA 52 %.

Conclusion In this model, the survival rate in untreated patients with CD was 45 % by year 10 and 0 % by year 20. For patients who failed first-line surgery, starting treatment with pasireotide is associated at 5 years with the highest rates of survival compared to the other second-line therapies; this marginal benefit appears to be con-

sistent over time. Real world data would be needed to confirm current findings in terms of clinical practice.

Reference:

1. Clayton RN, Raskauskiene D, Reulen RC, et al. Mortality and morbidity in Cushing's disease over 50 years in Stoke-on-Trent, UK: audit and meta-analysis of literature. *J Clin Endocrinol Metab* 2011; 96: 632–42.

Disclosure: No significant relationships.

P107

Burden of Cushing's Disease: A Retrospective Chart Audit

R. A. Feelders¹, A. Forsythe², V. Stemmer³, G. Cummins⁴, L. Gourgiotis⁵, J. Doyle⁶

¹Endocrine Section, Dept of Internal Medicine, Erasmus Medical Center, Rotterdam, The Netherlands; ²Oncology Global Health Economics and Market Access, Novartis Pharmaceuticals Corp, East Hanover, NJ, USA; ³Oncology Global Health Economics and Market Access, Novartis Pharma, Nürnberg, Germany; ⁴Global Market Access and Commercialization, Quintiles Consulting, Hawthorne, NY; ⁵Medical and Scientific Services, Quintiles, Durham, NC; ⁶Mailman School of Public Health, Columbia University, New York, NY, USA

Introduction Information on the socioeconomic burden of Cushing's disease (CD) is limited. This study focused on estimating the CD burden of illness (BOI), including the associated signs and symptoms, and investigating the relationship between urinary free cortisol (UFC) levels and BOI.

Methods and Materials A retrospective chart audit was conducted in January 2012 in Germany and France. 58 endocrinologists completed structured case report forms (CRF) on 215 patients with CD. Patient history, comorbidities, treatment, UFC values, office visits, and hospitalizations in 2011 were extracted from charts, and productivity loss was calculated. UFC values were means of quarterly laboratory measurements for each site, expressed as ULN-fold. Costs were determined based on published literature and local reference market data.

Results Mean age was 46 ± 0.9 years, 57 % female. Prior to 2011, 60 % had transsphenoidal surgery (TSS), 24 % radiotherapy/radiosurgery (RAD), 6 % bilateral adrenalectomy (BLA). In 2011, 48 % of patients had UFC $\leq 1 \times$ ULN, 39 % UFC > 1 to ≤ 2 ULN and 13 % UFC $> 2.0 \times$ ULN. Among patients who failed prior TSS, 67 % were treated with medical therapy (mitotane [15 %], metyrapone [15 %], ketoconazole [15 %], cabergoline [14 %], mifepristone [4 %], pasireotide [5 %], other oral therapies [6 %]) vs 27 % with second TSS, BLA or RAD. Patients with higher UFC had a greater prevalence of metabolic syndrome and its components, psychopathology and increased healthcare utilization compared to well-controlled patients (Table 4).

Table 4. R. A. Feelders et al. Prevalence of MS (and its components), psychopathology, and healthcare utilization depending on UFC.

	UFC < 1x ULN	UFC 1–2x ULN	UFC > 2x ULN
Prevalence of metabolic syndrome (% patients)	20	24	29
Prevalence of metabolic syndrome components (% patients)			
– Type-2 diabetes	25	38	36
– Hypertension	48	52	64
– Obesity	35	49	43
– Dyslipidemia	11	50	50
Prevalence of psychopathology (% patients)			
– Anxiety	8	14	43
– Depression	6	25	25
Resource utilization and costs (number of episodes per year)			
– Outpatient visits	8.9	9.2	19.7
– Days in hospital	2.2	4.0	7.7
– Medications utilized	2.4	3.5	3
Missed days from work/school (median)	0	10	30
Burden of illness	13,279 €	25,692 €	31,167 €

Conclusion In this retrospective study, higher urinary cortisol was associated with higher comorbidities, healthcare utilization, and costs. Effective urinary cortisol control may offer socioeconomic benefit. This hypothesis would need to be tested in a well-designed, randomized prospective study.

Disclosure: No significant relationships.

P108

Long-Term Efficacy of Pasireotide in a Patient with Cushing's Disease and Diabetes: Results in the Short Term Are not Always Predictive of Long-Term Response

L. Trementino, M. Cardinaletti, C. Concettoni, G. Marcelli, M. Boscaro, G. Arnaldi
Division of Endocrinology, Polytechnic University of Marche, Ancona, Italy

Introduction The management of Cushing's disease (CD) can be problematic, particularly when the disease persists following pituitary surgery.

Methods and Materials We report the case of a 55-year-old woman with CD that persisted after repeat transsphenoidal surgery. The patient had an overt phenotype with facial rubeosis, central obesity with supraclavicular fat accumulation, cervical fat pad, and proximal muscle weakness. She had received anti-hypertensive drugs and insulin for diabetes mellitus. Hormonal evaluations confirmed active hypercortisolism with absent cortisol rhythm, elevated midnight plasma cortisol (15.9 µg/dl) and increased 24-hour urinary free cortisol (UFC > 5× ULN) levels. After fulfilling inclusion criteria, the patient was randomized to pasireotide 900 µg bid in the phase-III study CSOM230B2305.

Results During the first 3 months of treatment, UFC levels decreased from baseline by almost 50 %. This was associated with improved clinical appearance (reduced facial rubeosis and supraclavicular fat accumulation; weight decrease of 7 %), although worsening of diabetes was also observed. However, UFC levels remained elevated (2.5× ULN) and the patient was considered to be a non-responder, therefore pasireotide dose was up-titrated to 1200 µg bid. From month 6 the patient had a progressive clinical and biochemical improvement, with resolution of hypertension, improvement of diabetes mellitus (with discontinuation of insulin and introduction of an oral hypoglycemic), and remission of all typical features of CD. Overall, her weight decreased by ~30 % from baseline. The patients' quality of life significantly improved, as did her sense of wellbeing. At month 12, UFC levels were normalized and cortisol rhythm restored. Pasireotide dose was progressively reduced to 300 µg bid. After 3 years the patient is still receiving pasireotide 300 µg bid with no loss of efficacy.

Conclusion In conclusion, short-term results are not always predictive of long-term response. In addition, pre-existing diabetes is not a contraindication to pasireotide treatment because the control of CD may outweigh any negative effects on glucose metabolism.

Disclosure: No significant relationships.

P109

Salivary Cortisol Is a Useful Tool to Assess the Immediate Response to Pasireotide in Patients with Cushing's Disease

M. Cardinaletti¹, L. Trementino¹, C. Concettoni¹, B. Polenta¹, M. Spinello², M. Boscaro¹, G. Arnaldi¹

¹Division of Endocrinology, Polytechnic University of Marche, Ancona; ²Novartis Pharma, Origgio, Italy

Introduction Pasireotide is a promising treatment option for patients with Cushing's disease (CD). The measurement of salivary cortisol is useful for diagnosing hypercortisolism and monitoring patients with CD following pituitary surgery. It may also be a better index of cortisol secretion than serum cortisol or urinary free cortisol (UFC). We investigated the value of salivary cortisol in monitoring short-term efficacy of pasireotide in patients with CD.

Methods and Materials Seven patients (5 females, 2 males; mean age 35.3 ± 7.4 years) received pasireotide 600 µg bid for 15 days in the phase-II study CSOM230B2208. Morning and midnight salivary cortisol, ACTH, and morning serum cortisol were assessed at baseline and after 1, 5, 12, and 15 days of treatment. UFC was determined at baseline and day 15.

Results On day 15, morning salivary cortisol had decreased in all patients; overall mean decrease from baseline was 70 % (27.7 ± 30.8 to 8.2 ± 7.7 mmol/l). Midnight salivary cortisol had decreased in 6 patients and normalized in 2; overall mean reduction from baseline was 50 % (27.2 ± 38.6 to 13.4 ± 15.4 mmol/l). Decreases in morning and midnight salivary cortisol were observed from day 1 (mean reduction from baseline of 34 % and 20 %, respectively) and persisted until day 15; the greatest decrease was on day 5 (mean reduction of 70 % and 58 %, respectively). At day 15, mean UFC had decreased from baseline by 65 % (1711 ± 1941 to 593 ± 360 mmol/24 h). UFC was normalized in one patient (14 %), who also had normalized midnight salivary cortisol, thereby restoring cortisol rhythm. Changes in ACTH and serum cortisol were similar to those of salivary cortisol.

Conclusion Pasireotide rapidly reduced and normalized salivary cortisol. Salivary cortisol may be a simple, non-invasive biomarker to assess immediate response to pasireotide in patients with CD, particularly to determine whether cortisol rhythm is normalized in patients with normalized UFC levels. More studies are necessary to confirm these preliminary results.

Disclosure: No significant relationships.

P110

Re-Evaluation of the 4-mg Intravenous Dexamethasone Suppression Test for Differentiation of Cushing's Disease from Pseudo-Cushing's Syndrome

M. Nouvel¹, M. Rabilloud², V. Raverot³, F. Subtil⁴, C. Pignard⁵, E. Jouanneau⁶, J. Vouillarmet⁶, C. Thivole⁶, F. Borson-Chazot¹, M. Pugeat¹, G. Raverot¹

¹Federation d'Endocrinologie du Pole Est; ²Service de Biostatistiques; ³Laboratoire d'Hormonologie; ⁴Service de Neurochirurgie; ⁵Service d'Endocrinologie, Hospices Civils de Lyon, Pierre Bénite, France

Introduction Differentiating Cushing's disease (CD) from pseudo-Cushing's syndrome (PCS) is one of the most challenging problems since no biological test is yet perfect. Almost 30 years ago, our group demonstrated the interest of 4-mg iv dexamethasone suppressing test (DST) to differentiate obese patients to CD.

Objective To reevaluate the diagnostic accuracy of the 4-mg iv DST in carefully selected patients with PCS and CD.

Methods and Materials Patients recruited from November 2008 to July 2011 were retrospectively studied. The criteria for PCS were: presence of clinical and biochemical (urinary free cortisol and/or 1 mg DST) features compatible with CS, normal pituitary MRI, and at least one year of clinical and biochemical follow-up. Diagnosis of CD was confirmed by pathology in 29 patients operated, 3 patients with abnormal MRI were medically treated. Patients underwent 4-mg dexamethasone infusion according to Abou-Samra et al (JCEM 1985). The diagnosis of CD is based on absence of ACTH and cortisol suppression at 8 am on day 2.

Results 68 patients (54 F/14 M), 32 with CD and 36 with PCS, were included. Age and BMI were similar between groups but hirsutism, proximal amyotrophy, and vascular weakness were significantly more frequent in the CD group ($p < 0.001$). Midnight plasma cortisol, 8:00 am cortisol, and ACTH after 4 mg DST were respectively associated with a 95.4-% (86.5–99), 90.5-% (81.8–96.7), and 98.4-% (92.1–99.6) diagnostic accuracy. 8:00 am ACTH levels on day 2 appear to be the best test to differentiate CD and PCS. ACTH threshold used in our endocrine units (10 ng/l) or the best ACTH threshold calculated (19.6 ng/l) are respectively associated with 100 % and 93.8 % of sensitivity; 80.6 % and 86.1 % of specificity.

Conclusion The 4-mg iv DST is an easy and accurate test to distinguish CD from PCS and deserves a place in the CD diagnosis assessment.

Disclosure: No significant relationships.

P111

Clinically Silent Corticotroph Macroadenomas

A. K. Uzum¹, G. Yenidunya¹, S. Ciftci Dogansen¹, B. Canbaz¹, B. Alpaslan², S. Yaman¹

¹Division of Endocrinology and Metabolism, Dept of Internal Medicine; ²Dept of Internal Medicine, Faculty of Medicine, Istanbul University, Turkey

Introduction Clinically silent corticotroph adenomas (SCAs) of the pituitary gland are tumors that stain for adrenocorticotrophic hormone (ACTH) but do not manifest with clinical or laboratory features of Cushing's disease (CD). Although these tumors are rare, they have a higher recurrence rate after surgery and radiotherapy. We describe 2 patients with clinically silent corticotroph macroadenomas who presented with local mass effects.

Methods and Materials A 57-year-old woman was admitted with complaints of headache and loss of vision in her left eye. Pituitary imaging (MRI) showed a 22-mm macroadenoma with cystic degeneration and suprasellar extension, compressing to optic chiasm. CD was diagnosed with dexamethasone suppression tests and no other hormonal hypersecretion was present. Visual field examination was compatible with bitemporal hemianopsia. After she underwent transphenoidal surgery, immunohistochemical staining showed strong positivity for ACTH and weak positivity for FSH and PRL. Ki-67 labelling index was 5 % and strong immunopositivity was detected for VEGF. Postoperatively gamma-knife radiotherapy was applied to the residual tumor. Control MRI after 4 years showed significant reduction of mass.

A 37-year-old man was admitted to the emergency room with sudden onset of headache, ptosis, ophthalmoplegia, diplopia, and partial loss of vision. MRI revealed a 23-mm macroadenoma with apoplexy. Both cortisol and ACTH levels were elevated (45 µg/dl and 254.5 pg/ml, respectively). Transsphenoidal operation was immediately performed and immunohistochemical evaluation showed 90-% ACTH positivity. Postoperatively, cortisol and ACTH decreased to normal levels and cortisol was suppressed (< 1.8 µg/dl) with overnight dexamethasone test. He has been in remission for 5 months without any signs of hormonal insufficiency.

Results We observed that clinically, SCAs can behave differently.

Conclusion Based on the literature, SCAs have more aggressive behaviour than non-functioning tumors and they should be followed up closely with pituitary imaging.

Disclosure: No significant relationships.

P112

Endoscopic Endonasal Pituitary Surgery for Cushing's Disease at the National Institute of Neurology and Neurosurgery in Mexico City

A. Dorantes-Argandar¹, B. García-Guzmán², M. Martínez-Moreno¹, J. Barges-Coll¹, J. L. Gómez-Amador¹, L. Portocarrero-Ortiz²

¹Neurosurgery/Skullbase Surgery; ²Neuroendocrinology, Instituto Nacional de Neurología y Neurocirugía MVS, Mexico City, Mexico

Introduction The endoscopic endonasal approach for the resection of ACTH-hypersecreting pituitary adenomas is minimally invasive and allows for more complete tumor removal with a reduced rate of complications. We present our first results in patients with Cushing's disease operated at our hospital since the introduction of the endoscopic technique in 2011.

Methods and Materials We reviewed data of 7 patients with Cushing's disease. All were treated by an endoscopic endonasal approach. Endocrinological remission was considered if there was cortisol suppression to < 1.8 µg/dl to a low-dose dexamethasone test and < 1.5× the upper normal limit of the 24-hour urinary free cortisol (UFC) on follow-up. Follow-up was obtained at 24 hours, 1, 3, 6, and 12 months post-surgery (mean 8 months).

Results Seven patients (6 female, 1 male) with an average age of 35 years (range 21–50) had radiologic evidence of pituitary lesions on preoperative MRI (3 macroadenomas, 4 microadenomas). Average

preoperative UFC was 654 µg/dl. Average preoperative basal cortisol was 24.8 µg/dl. Six patients (85.7 %) were considered to be in endocrinological remission at 3-month follow-up. One patient (16.6 %) with a microadenoma recurred at 7 month follow-up. In one patient (14.2 %), hypercortisolemia persisted after surgery. Both were treated with radiosurgery postoperatively. One patient (14.2 %) developed transient diabetes insipidus and a cerebrospinal fluid leak that resolved after lumbar drainage. One patient (14.2 %) required substitution with levothyroxine. Treatment failure was attributed to subtotal resection of a giant macroadenoma with bilateral cavernous sinus invasion.

Conclusion Our initial results showed that endoscopic endonasal pituitary surgery is a promising, safe, and effective treatment for ACTH-producing adenomas. Our remission rates and minimal complications are similar to those reported by more experienced surgeons using conventional microscopic or endoscopic techniques.

Disclosure: No significant relationships.

P113

Pituitary Corticotroph Microadenomas vs Macroadenomas Causing Cushing's Disease

K. Aydin, S. Dagdelen, T. Erbas

Dept of Endocrinology and Metabolism, Hacettepe University School of Medicine, Ankara, Turkey

Introduction Cushing's disease resulting from pituitary macroadenomas is a rare disorder. In this study, we aimed to analyse and compare clinical and biochemical features of macroadenomas and microadenomas causing Cushing's disease from the medical records of a tertiary referral center.

Methods and Materials Medical records of patients diagnosed with Cushing's disease between January 2000 and December 2010 were reviewed. Clinical and biochemical findings were compared between 2 groups according to pituitary tumor maximal diameter (microadenoma vs macroadenoma).

Results A total of 100 patients with Cushing's disease were included in the study. Clinical and biochemical features of patients in both groups are shown in Table 5. There was a statistically significant female preponderance in the microadenoma group (p = 0.007). Patients in the microadenoma group were younger than patients in the macroadenoma group (p = 0.001) and they were likely to be more cushingoid than the patients with macroadenoma (p < 0.001). Biochemical values of hypercortisolism were similar between the 2 groups, however, cure rates were lower in the macroadenoma group after surgery (80.0 vs 53.8 %). Immunohistochemical staining of pituitary tumor with ACTH, GH, TSH, LH, FSH, and prolactin were not different across groups.

Conclusion Pituitary macroadenoma causing Cushing's disease have similar biochemical features with Cushing's disease resulting from microadenoma. However, Cushing stigmata in macroadenoma were likely to be silent compared to patients with microadenoma.

Disclosure: No significant relationships.

Table 5. K. Aydin et al. Clinical and biochemical features of Cushing's disease resulting from microadenomas vs in macroadenomas.

	Microadenoma	Macroadenoma	p
n	65	35	–
Male/female	14/51	17/18	0.007
Age at diagnosis (a)	37.6 ± 11.9	46.9 ± 1.6	0.001
Cushing stigmata (n) (Present/absent)	58/7	11/24	< 0.001
ACTH (pg/ml)	62.4 (16.2–687.0)	76.1 (15.1–1250)	0.324
Basal cortisol (mcg/dl)	25.2 ± 13.7	20.8 ± 11.9	0.053
Cortisol after LDDST (mcg/dl)*	13.3 ± 11.2	11.5 ± 8.4	0.881
Urinary cortisol (mcg/day)	251 (50–2408)	285 (51–470)	0.801
Cure rate**	80.0 %	53.8 %	0.002

*Low-dose dexamethasone suppression test; **after exclusion of missing cases

P114

Cortisol Awakening Response (CAR) and Cortisol Circadian Rhythm in Healthy Subjects and Cushing's Syndrome

A. C. Moreira, S. L. Ruiz, P. C. L. Elias, M. Castro

School of Medicine of Ribeirao Preto, Sao Paulo University, Ribeirao Preto, Brazil

Introduction In healthy subjects (HS), morning awakening is associated with a burst of cortisol secretion, cortisol awakening response (CAR). CAR is a distinct property of the cortisol daily variation, with characteristics probably unrelated to those of cortisol circadian rhythm (CR). The absence of CR in Cushing's syndrome (CS) has been previously studied. The aim of this work was to study the CAR and the CR in patients with active CS, with CS after remission (CSR), and pseudo-Cushing (PS).

Methods and Materials We evaluated 19 HS, 10 PS, 10 CSR, and 10 active CS patients. Salivary cortisol (SF) samples were collected on 2 consecutive days. CR was determined at 08:00, 11:00, 17:00, 20:00, and 23:00 hours. CAR was obtained upon awakening the next morning at 15, 30, 45, and 60 min post-awakening. SF was determined by RIA, Kruskal-Wallis, and Wilcoxon-Mann-Whitney tests and Spearman correlations were used. Significance was assumed if $p < 0.05$.

Results SF levels (ng/dl; mean $\pm$ SEM) at 23:00 hours during CR studies were 194 ± 25 , 264 ± 56 , 381 ± 81 , and 1742 ± 266 in HS, PS, CSR, and CS, respectively ($p < 0.01$). SF CAR increased within the first 45 min after awakening ($p = 0.002$) in all groups. At 60 min, SF had decreased in all groups. The CAR absolute peak SF values were 2071 ± 217 , 2519 ± 161 , 2546 ± 253 , and 3057 ± 355 ng/dl ($p = 0.11$) and the relative increases after awakening (CARI%) were $154 \pm 25\%$, $240 \pm 59\%$, $179 \pm 67\%$, and $60 \pm 19\%$ in HS, PS, CSR, and CS, respectively ($p = 0.009$). There was a correlation between CARI% and the diurnal decrease in cortisol CR ($r = 0.31$; $p = 0.03$) and a negative correlation between CARI% and SF at time point zero ($r = -0.75$; $p < 0.01$).

Conclusion Our results demonstrate the presence of CAR in HS, PS, and CSR groups. In CS patients, CAR was attenuated. CAR could be blunted or masked by morning hypercortisolism. CR and CAR seems to be related components of the HPA axis.

Disclosure: No significant relationships.

P115

Outcome of Transsphenoidal Surgery for Cushing's Disease Dependent on Tumor Size: the Munich Experience

C. Dimopoulos, G. K. Stalla

Clinical Neuroendocrinology, Max-Planck-Institute of Psychiatry, Munich, Germany

Introduction Transsphenoidal surgery (TSS) currently represents the treatment of choice for Cushing's disease (CD). Depending on tumor size and extension, adenoma visibility on preoperative MRI, and neurosurgical expertise, remission rates after initial TSS range from 70–90 %, after second TSS between 50 and 70 %.

Methods and Materials In a retrospective, single-center analysis we assessed 51 patients with CD treated at our outpatient unit.

Results All patients underwent TSS as initial treatment for CD. Overall, 86 % of the patients were in remission after initial TSS. In the subset of microadenomas, a remission rate of 95 % was observed, followed by a lower remission rate in the group of macroadenomas (52 %). 45.4 % of the patients (40.5 % with microadenomas, 71.4 % with macroadenomas) experienced relapse after initial TSS. Mean time until relapse was 27.58 ± 26.00 months. 25.4 % of the patients underwent second TSS. Second TSS was carried out in the same neurosurgical center where initial TSS took place in 69.2 %. After second TSS, biochemical remission of the disease was documented in 31 % of the patients (33 % with microadenomas, 25 % with macroadenomas). 50 % of the patients (33.3 % with microadenomas, 100 % with macroadenomas) experienced a relapse after sec-

ond TSS. Mean time until second relapse was shorter (13.25 ± 16.76 months). 76.4 % of the patients underwent first TSS and 69.2 % underwent second TSS at a center of neurosurgical expertise. Both after first and second TSS recurrence rates in non-expert centers were higher, though not significant ($p > 0.05$).

Conclusion In our study, remission rates after first TSS were comparable, but after second TSS lower than in the literature. We observed higher recurrence rates both after first and after second TSS compared to the literature. Mean time to recurrence after second TSS was shorter. Patients who had been operated in non-expert centers experienced higher recurrence rates both after first and after second TSS, though this was not significant.

Disclosure: No significant relationships.

P116

Diagnosis and Complications of Cushing's Disease: Gender-Related Differences

M. Zilio, V. Camozzi, V. Daidone, P. de Lazzari, N. Albiger, S. Koutroumpi,

M. Barbot, F. Ceccato, L. Mazzai, F. Mantero, C. Scaroni

Endocrinology Unit, Dept of Medicine, University of Padova, Padua, Italy

Introduction Cushing's disease (CD) presents a remarkable preponderance in female gender, with a female-to-male ratio of 3–8:1. The aim of this study was to evaluate gender-related differences in the presentation of CD, as regards biochemical indices of hypercortisolism; sensitivity of diagnostic tests; clinical features, and complications of disease.

Methods and Materials We retrospectively studied 84 patients with CD, 67 women and 17 men (mean age: 42 years, range: 15–70), evaluated during active phase of disease. We compared male and female patients both as a whole and after dividing them according to age ($<$ or $>$ 50 years).

Results We observed no differences between male and female patients as regards age at diagnosis, disease duration, and BMI. Men, compared to women, presented higher urinary free cortisol values (median: 4.27 vs 2.35 times the upper limit of normality; $p < 0.001$) and ACTH values (median: 66.8 vs 43.2 ng/l; $p < 0.05$). As regards the tests performed to establish the diagnosis of CD, men presented a significantly lower ACTH response to DDAVP stimulation (responsive patients: 50 % vs 77 %; $p < 0.05$). The pituitary tumor itself was less easily visualized by pituitary MRI in males compared to females (50 % vs 79 %; $p < 0.05$). Furthermore, some complications of disease were more frequent or more severe in men, in particular hypokalemia (41 % vs 12 %; $p < 0.05$) and osteoporosis at the lumbar spine (59 % vs 20 %; $p < 0.01$), with a consequently higher risk of vertebral fractures. Among younger patients (≤ 50 years), males also presented a more severe degree of hypertension and a higher prevalence of carotid atherosclerotic plaques.

Conclusion Although CD is less frequent in male patients, men present with more florid clinical manifestations; this may imply more diagnostic difficulties. Gender differences are especially evident among patients < 50 years, with men more severely affected compared to women.

Disclosure: No significant relationships.

P117

Desmopressin-Dexamethasone Test for ACTH-Dependent Cushing's Syndrome

D. Romanholi¹, C. Lauretti², M. Cunha Neto³, V. Cescato³, N. Musolino³, D. Freire¹, M. A. Pereira¹, L. R. Salgado²

¹Endocrinology; ²General Internal Medicine Service; ³Neurosurgery Service, Hospital das Clínicas, Sao Paulo, Brazil

Introduction Patients with Cushing's disease (CD) present responses to CRH and desmopressin testing (DT) due to overexpression of CRH1 e V1b receptors, respectively, on tumoral corticotrophs. Normals (NC) also respond to the CRH test; however, only some present dose-dependent responses to DT. The CRH dexamethasone

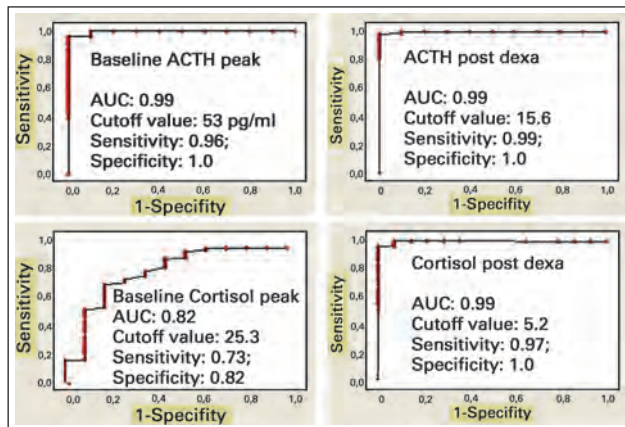


Figure 13. D. Romanholi et al. ACTH and cortisol responses at baseline and *post* dexamethasone test.

test has been used to differentiate pseudo-Cushing states from CD. Thus, authors studied the desmopressin test (10 µg iv bolus) before (baseline) and after 2 mg overnight dexamethasone (OVN) in 35 patients with surgically proven CD and 15 NC. When compared with the baseline test, the desmopressin-dexa test increased sensitivity (SE) and specificity (SP) of ACTH and cortisol responses; no responses were observed in NC.

Methods and Materials In this study, we repeated both tests performing an ROC analysis in 177 patients with (surgically proved) CD and 9 NC.

Results Baseline sensitivity and specificity for ACTH response (cut-off 53 pg/ml) were 96 % and 100 %; for cortisol response (cut-off 25.3 µg/dl), sensitivity and specificity were 73 % and 82 %, respectively. After overnight dexamethasone, sensitivity and specificity for ACTH (cut-off 15.6 pg/ml) were 99 % and 100 %, and for cortisol (cut-off 5.2 µg/dl) 97 % and 100 %, respectively (**Figure 13**).

Conclusion Desmopressin test after overnight dexamethasone increased both sensitivity and specificity for ACTH and cortisol responses in patients with CD, with the advantage of including a second diagnostic test in the same procedure.

Disclosure: No significant relationships.

P118

Alteration of Mental Status in Patients with Hypercortisolism

I. Komerduš, A. Dreval, A. Murzina, O. Nechaeva

Endocrinology, Moscow Regional Research Clinical Institute N. A. Vladimirsky, Russian Federation

Introduction Excessive glucocorticoid production caused alterations in all organs and systems, including mental health.

Aim To identify the relationship between severity of hypercortisolism and depression in Cushing's disease (CD) and ACTH ectopy (AES) before and after treatment.

Methods and Materials 15 patients (3 AES and 12 CD); 2 male, 13 female; 38.6 ± 10.5 years. The questionnaire of Center of Epidemiological Studies of USA-Depression (CES-D) was used to identify depression before and 12 months after treatment.

Results Depression was revealed in 40 % of all patients (in 25 % of CD and in 100 % AES). Serum cortisol levels (CD: 823 nmol/l [542–1222], AES: 1648 nmol/l [859–2087]) and UFC (CD: 787.3 nmol/d [271.8–1790.3], AES: 1261.3 nmol/d [853–1683.3]) did not differ between CD and AES patients ($p < 0.05$). ACTH levels in AES were higher than in CD (24.7 [21.6–45.5] and 11 pmol/l [5.1–32.4]), respectively ($p < 0.02$). Depression was positively correlated with ACTH levels ($r = 0.61$; $p = 0.01$). There was no correlation between presence of depression and serum cortisol, UFC, and potassium levels, as well as age of the patients prior to treatment. After

surgical treatment, adrenal insufficiency developed in 10 (83.3 %) CD patients. Hypercortisolism persisted in all AES patients and 2 CD patients. ACTH levels after treatment in CD (2.63 pmol/l [5.1–32.4]) and AES (22.1 pmol/l [21.6–45.5]; $p < 0.03$). Depression persisted in 25 % of CD patients and in 33 % AES patients. Depression was newly identified in 1 and became more severe in 2 CD patients. Depression persisted and was more severe in 1 out of 3 AES patients. There was a significantly positive correlation between ACTH level ($r = 0.5$; $p = 0.04$) and severity of depression after treatment.

Conclusion A significantly positive correlation between ACTH levels and presence of depression in patients before and after treatment was revealed. Worsening of depression in CD patients after successful treatment was probably related to the development of decompensated adrenal insufficiency.

Disclosure: No significant relationships.

P119

Transsphenoidal Surgery for ACTH-Dependent Cushing's Syndrome without Evident Pituitary Adenoma

W. Liebert¹, K. Michalek², J. Szymas³, R. Wasko², W. Paprzycki⁴, R. Kulakowski¹

¹Dept of Neurosurgery; ²Endocrinology, Metabolism and Internal Diseases; ³Dept of Pathology, ⁴Dept of Radiology, University of Medical Sciences, Poznan, Poland

Introduction Pituitary adenoma is the most common cause of ACTH-dependent Cushing's syndrome. Accurate preoperative imaging localization of microadenomas is important and correlated with surgical outcome. However, MR imaging can be negative in up to 40 %. In our study, we present the evaluation and management of these cases. Our aim was to assess the cure rate by transsphenoidal pituitary exploration.

Methods and Materials We analyzed a group of 12 patients (7 men, 5 women) with ACTH-dependent Cushing's syndrome. The diagnosis was based on a typical clinical course, biochemical, and MRI studies. In all patients, ectopic ACTH secretion was excluded. All patients underwent transsphenoidal microsurgery. The surface of the pituitary was examined thoroughly for the presence of a tumor. If no tumor was immediately visualized, a vertical paramedian incision was made, with the dissection proceeding in progressive lateral direction in an attempt to identify a tumor. If no tumor was identified the same maneuver was repeated on the contralateral side.

Results Clinical and biochemical cure was achieved in 8 (66.7 %) of 12 patients. Seven (58.3 %) patients had cortisol levels below normal range after operation. Histological examination revealed 7 basophilic adenomas. Out of the remaining 5 cases, 3 cases had focal corticotrophic hyperplasia, in 2 no tumor was identified.

Conclusion We conclude that a transsphenoidal exploration of the pituitary for Cushing's disease with negative MRI is an effective treatment in achieving endocrine remission.

Disclosure: No significant relationships.

P120

Gamma Knife Treatment for ACTH-Secreting Pituitary Adenomas

J. Jezková¹, J. Marek¹, V. Hana¹, M. Kršek¹, V. Vladyka², R. Liščák²

¹3rd Dept of Medicine, First Medical Faculty, Charles University; ²Dept of Stereotactic and Radiation Neurosurgery, Hospital Na Homolce, Prague, Czech Republic

Introduction Stereotactic radiosurgery using the Leksell gamma knife (LGK) is one of the treatment options for pituitary adenomas.

Methods and Materials We followed 27 patients with Cushing's disease and 14 patients with Nelson's syndrome treated with LGK irradiation. The mean follow-up period was 96 months. Pituitary function was monitored at 6-month intervals *post* irradiation.

Results When assessing the success of treatment, the normalization of 24-h free urinary cortisol was achieved in 40.7 %, 51.8 %, 77.3 %, and 86.7 % of patients with Cushing's disease within 2, 3, 5, and 8

years after LGK irradiation. Irradiation stopped growth of all adenomas and caused tumour shrinkage in 86.7 %. Among the patients with Nelson's syndrome, normal levels of ACTH were reached only in one patient. ACTH levels of another 6 patients were approaching the upper levels of normal limits. After LGK irradiation, the adenoma stopped growing or decreased in size in all but one patient. Hypopituitarism developed in 11.1 % of patients with Cushing's disease and 16.7 % of patients with Nelson's syndrome. Hypopituitarism did not develop in patients irradiated with a mean dose to the pituitary < 15 Gy.

Conclusion LGK is an integral part of the treatment approach in ACTH-secreting pituitary adenomas. Until the effect of irradiation is evident and hormonal production is normalized, hormonal production must be suppressed pharmacologically. Hypopituitarism after LGK can be avoided by keeping the mean radiation dose to pituitary < 15 Gy and to distal infundibulum < 17 Gy.

Disclosure: No significant relationships.

P121

Hemochrome in Patients with Cushing's Syndrome (CS)

A. Cozzolino, C. Simeoli, M. de Leo, D. Iacuanelli, A. Colao, R. Pivonello
Dept of Clinical and Molecular Endocrinology and Oncology, University of Naples Federico II, Italy

Introduction Glucocorticoids (GC) are well-known to have a stimulatory effect on neutrophil count and an inhibitory effect on other categories of leukocytes. On the other hand, the effect on erythropoiesis has not been completely clarified despite some evidence of stimulation in the count of erythroid series. The aim of our study was to evaluate hemochrome in patients with endogenous GC excess, namely CS.

Methods and Materials 58 patients with CS (19 M/39 F; 6–73 yrs; 51 pituitary CS, 4 adrenal CS, 3 ectopic CS) and 58 healthy gender- and age-matched controls entered the study. Hemochrome was evaluated in both patients and controls. In patients, hemochrome was correlated with the hormonal pattern.

Results Medium corpuscular volume (MCV; $p = 0.000$), hemoglobin (Hb; $p = 0.004$), hematocrit (HCT; $p = 0.000$), and mean corpuscular hemoglobin (MCH; $p = 0.005$) were significantly higher in patients than controls. A significantly higher number of white blood cells (WBC; $p = 0.000$) was also observed in patients than in controls, associated with a significantly higher count of neutrophils ($p = 0.000$) and lower count of eosinophils ($p = 0.000$) as well as lymphocytes ($p = 0.006$). Moreover, a significantly higher prevalence of macrocytosis (17.2 % vs 0 %; $p = 0.002$), leukocytosis (24.1 % vs 3.3 %; $p = 0.002$) and neutrophilia (36.4 % vs 5 %; $p = 0.000$) was found in patients than in controls. These parameters seemed to be not correlated with serum iron, ferritin, transferrin, folate, and vitamin B₁₂ levels as well as serum and urinary cortisol levels or disease duration.

Conclusion In conclusion, CS seems to affect hemochrome parameters, inducing an increase in erythrocyte volume and hemoglobin content. CS also determines increase in neutrophil count, commonly inducing neutrophil leukocytosis associated with an inhibition of eosinophils and lymphocytes. These results suggest that peculiar hemochrome alterations could be useful as markers of CS, if confirmed in a larger cohort of patients.

Disclosure: No significant relationships.

P122

Visceral Adiposity Index (VAI) in Cushing's Disease (CD): Evaluation of Gender Differences and Correlation with Metabolic Syndrome (MS)

C. Simeoli¹, M. de Leo¹, A. Cozzolino¹, D. Iacuanelli¹, M. C. Amato², C. Giordano², A. Colao¹, R. Pivonello¹

¹Dept of Clinical and Molecular Endocrinology and Oncology, University of Naples Federico II; ²Section of Endocrinology and Metabolic Disease, Dibimis, University of Palermo, Italy

Introduction CD is frequently associated with visceral obesity and MS, which displays peculiar features and is associated with cardio-metabolic risk in patients with CD either during active disease or after disease remission. The aim of the current study was to evaluate VAI, a new and reliable indicator of adipose tissue function and distribution, in patients with active and cured CD, and to evaluate the association of this index with patients' gender as well as with the prevalence and severity of MS.

Methods and Materials 20 patients (13 F/7 M, 10–66 years) and 40 age-, gender- and BMI-matched controls were retrospectively enrolled in the study. VAI and MS prevalence and severity were evaluated during active disease and 3–10 years after remission. VAI was calculated according to Amato et al, whereas the prevalence and severity of MS was estimated in line with NCEP and IDF definitions.

Results VAI was significantly higher ($p < 0.05$) in patients with active disease than in controls. In patients with active disease, VAI was higher in women than in men ($p = 0.008$), in patients with (50 %) than those without (50 %) MS ($p < 0.05$) as well as in patients fulfilling ≥ 4 criteria than in those fulfilling 3 criteria of MS ($p < 0.05$). After remission, VAI significantly decreased ($p = 0.023$) compared to active disease, becoming similar to that of controls. However, it remained higher in patients with (10 %) than in those without (90 %) MS ($p = 0.021$), but appeared similar in patients and controls.

Conclusion The results of the current study demonstrate that active CD patients are associated with higher VAI compared to cured patients and controls, with higher values in women than in men. VAI correlated with prevalence and severity of MS, suggesting that it could be an easy and useful tool for the assessment of adipose dysfunction associated with the cardio-metabolic risk in CD patients.

Disclosure: No significant relationships.

■ Somatostatin, Dopamine, and Their Analogues

P123

Water Extract of Saffron Diminishes Stress-Induced Dopamine-Related Behavior in Rats

F. Sadeghi Mahallati¹, Z. Fatahi², H. Sahraei³, A. H. Rohani⁴, A. Eidi⁵, Z. Hooshmandi⁶

¹Dept of Physiology, Neuroscience Research Center, Faculty of Medicine, Shahid Beheshti University of Medical Sciences; ²Science and Research Branch;

³Sanandaj Branch, Islamic Azad University; ⁴Applied Neuroscience Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran

Introduction In this research, the effects of water extract of saffron on blood corticosterone levels and the dopamine-related behaviors (rearing and locomotion) induced by foot shock stress in male Wistar rats was studied.

Methods and Materials Animals after surgery and amygdala cannulation received water extract or saline before stress induction. The remaining animals received intraperitoneal injection of the drugs. Stress was induced by a communication box electroshock apparatus for 7 days. Blood corticosterone levels were evaluated on days 1 and 7. Animal behavior (rearing and locomotion) was recorded by video camera.

Results The result showed that stress elevated corticosterone plasma concentration in the control group, which was statistically significant to water extract-treated groups. On the other hand, intra-amygdala administration of the water extract had no effect on plasma

corticosterone level. Our results on dopamine-related behaviors revealed that intraperitoneal administration of saffron water extract increased the number of rearing, with respect to the control group. Intra-amygdala administration of the water extract failed to increase the number of rearing. With regard with locomotion, ip administration of water extract increased locomotion. Intra-amygdala administration of water extract failed to increase the number of locomotion.

Conclusion Our experiments showed that saffron water extract can interact with the effects of stress on dopamine-related behaviors as measured by locomotion and rearing. The interaction was in opposite direction of stress effect and revealed that stress-induced behavioral functions may be suppressed by saffron extract and also the site of this action is outside the amygdala.

Disclosure: No significant relationships.

P124

Ethanollic Extract of Saffron and its Constituent Crocin Diminishes Psychological Stress-Induced Dopamine-Related Behavior in Rats

M. Ghasemi¹, S. Shekarforoush², Z. Fatahi³, Z. Hooshmandi⁴, A. Haeri Rohani⁵, A. Eid⁶, H. Sahraei⁶

¹Neuroscience Research Center and Dept of Physiology, Faculty of Medicine, Shahid Beheshti; ²Physiology, Islamic Azad University, Arsanjan Branch; ³Science and Research Branch, Islamic Azad University; ⁴Sanandaj Branch, Islamic Azad University, Tehran; ⁵Dept of Physiology and Biophysics and Neuroscience; ⁶Baqiyatallah University of Medical Sciences, Tehran, Iran

Aim In the present study, the effects of saffron ethanollic extract and its constituent, crocin, on the dopamine-related behaviors (sniffing, rearing, coping, and locomotion) and blood corticosterone levels, food intake, weight gain, and anorexic time induced by foot shock stress in male Wistar rats (W: 250–300 g) were examined.

Methods and Materials Stress applied over 7 consecutive days. The electroshock characters were as follows: 0.1 mA, 60 Hz, which conducted for 10 sec. Animal coping, rearing, sniffing, and locomotion were recorded by a video camera.

Results Results showed that stress cannot elevate corticosterone plasma concentration in the extract- and crocin- (1, 5, 10 mg/kg) treated groups. Animal weight gain and anorexic time also reduced in the experimental group. In addition, rearing, sniffing, and locomotion were increased whereas coping time was decreased. Intra-amygdala administration of the extract and crocin (1, 5, 10 µg/rat) had no effect on plasma corticosterone levels, animal weight gain, and anorexic time.

Conclusion Our experiments showed that saffron ethanollic extract and crocin can interact with the effects of stress on dopamine-related behaviors as measured by rearing, sniffing, and locomotion, as well as the coping time. Moreover, the site of this action seems to be outside the amygdala.

Disclosure: No significant relationships.

P125

SSTR2 and -5 Are Highly Expressed in Pancreatic Neuroendocrine Tumor Samples by Immunohistochemistry Using Monoclonal Antibodies against SSTR Ligand-Binding Domains

V. Rossi¹, S. Staibano², M. Mascolo², G. Ilardi², G. Bellastella¹, D. Visconti¹, G. de Rosa², A. Bellastella¹, A. A. Sinisi¹

¹Scienze Cardiotoraciche e Respiratorie, Second University of Napoli; ²Scienze Morfologiche, University of Naples Federico II, Italy

Introduction Pancreatic neuroendocrine tumors (PNET) are rare functioning or non-functioning tumors arising from islet cells, which express variable somatostatin receptor (sstr) subtypes 1–5. Classic SST analogue targeting sstr2 have been used with limited efficacy. The new SST analogues (SOM230, BIM) bind also sstr1 and -5 and may result more efficiently in the control of PNET-secre-

tory activity or progression. The immunodetection of these sstr subtypes may be of value in predicting the efficacy of new ligands.

Aim To evaluate by immunohistochemistry the expression of sstrs 1, 2, 3, and 5 on PNET, using a panel of new MoAbs against the sstr-binding domain (Y-SSTR1, 2, 3, 5).

Methods and Materials We used tissue sections from 15 low-differentiated PNET and, as control, sections from normal tissues. After incubation with primary antibodies a standard streptavidin-biotin-labeled peroxidase technique was applied. The degree of immunostaining (IS) was evaluated semi-quantitatively according to an arbitrary scale.

Results In normal tissues, islets were strongly positive for all 4 sstrs. Variable focal-specific positivity was found in PNET specimens. A high or intermediate grade IS for sstr5 was found in 9 (60 %), and for sstr2 in 12 (80 %) out of 15 tumors; low- or intermediate-grade IS for sstr1 and 3 was found in all samples.

Conclusion MoAbs Y-SSTR 1, 2, 3, and 5 showed a strong ability to react with different sstrs in histological sections, allowing for the visualization, localization, and semi-quantitative expression of all sstrs in the pancreatic normal and malignant tissues. Y-sstr MoAbs produced a sensitive and specific reaction with a constant behavior in the time. Thus, these MoAbs identifying the binding sites on the cell membrane represent a valid tool to select the SST-analogue in PNET medical treatment.

Disclosure: No significant relationships.

P126

Regulation of Somatostatin Receptor Type 2 (SSTR2) by the Transcriptional Cofactor ZAC1

M. Theodoropoulou¹, A. Barlier², M. M. Pertuit², M. Buchfelder³, G. K. Stalla¹

¹Endocrinology, Max Planck Institute of Psychiatry, Munich, Germany;

²Laboratoire de Biologie Moléculaire, CHU Conception, Marseille, France;

³Neurosurgical Clinic, University of Erlangen-Nürnberg, Erlangen, Germany

Introduction Somatostatin analogs (SSA) transduce their effects upon binding to SSTR2 and, in part, through the transcriptional cofactor ZAC1. Low SSTR2 and ZAC1 levels were independently shown to correlate with poor treatment response. The present study aims to examine SSTR2 and ZAC1 expression in the same acromegalic pituitary adenomas and their putative correlation.

Methods and Materials SSTR2 and ZAC1 mRNA were quantified by real-time RT-PCR in pituitary adenomas from 17 acromegalic patients (10 males, mean age 45 ± 11; not treated preoperatively). Statistical significance was determined as non-parametric Spearman test. Somatotrophinoma GH3 cells were used to determine the effect of Zac1 on Sstr2.

Results SSTR2 correlated positively with ZAC1 transcript in untreated acromegalic tumors (p < 0.04). Considering that these patients did not receive SSA before surgery the correlation is not due to ZAC1 up-regulation down-streams to activated SSTR2. This prompted us to examine whether ZAC1 regulates SSTR2 gene transcription. Zac1 inhibition by siRNA in GH3 somatotrophinoma cells suppressed Sstr2 expression. In contrast, increased Zac1 expression after treatment with the PI3K inhibitors LY294002 and wortmannin was accompanied by increased Sstr2 expression. Rat up-stream Sstr2 promoter bears high homology to the human SSTR2 which depends on the transcription factor Sp1. Zac1 increased Sp1 expression, indicating that it may act by regulating Sp1 availability and binding on the Sstr2 promoter.

Conclusion These preliminary data show that ZAC1 enhances SSTR2 gene transcription in somatotrophinoma cells.

Disclosure: No significant relationships.

Table 6. D. Miljic et al. Patient characteristics

Patient	Sex	Age	Diagnosis	VTE	Comorbidities
1	Male	27	NFPA	DVT+PE	Obesity, hypopituitarism
2	Male	20	Craniopharyngeoma	DVT	Obesity, sleep apnea, hypopituitarism
3	Male	50	Craniopharyngeoma	DVT	Hydrocephalus, hypopituitarism
4	Female	23	Craniopharyngeoma	DVT	Hypopituitarism, obesity

■ Diabetes Insipidus and Water Homeostasis

P127

Adipsic Diabetes Insipidus (DI) and Venous Thromboembolism (VTE): Report of 4 Cases

D. Miljic¹, P. Miljic², M. Petakov¹, M. Doknic¹, S. Pekic¹, M. Stojanovic¹, V. Popovic¹¹Neuroendocrinology, Clinic of Endocrinology; ²Unit for Hemostasis, Clinic of Hematology, Clinical Center of Serbia, Faculty of Medicine, University of Belgrade, Serbia

Introduction Although diabetes insipidus is a common complication of pituitary surgery, adipsic DI is very rare. It can occur after transcranial surgery for craniopharyngeoma, suprasellar pituitary adenoma, and anterior-communicating artery aneurysm but also with head injury, toluene exposure, and developmental disorders. Various complications like obesity, sleep apnea, thermo-regulatory disorders, seizures, and VTE have been described in these patients but published data are scarce due to the rarity of the disorder. Morbidity and mortality data have been published as single case reports with only one large series of 13 patients (4 with VTE) suggesting increased risk for VTE in patients with adipsic DI.

Methods and Materials Our observational retrospective study found 4 patients with adipsic DI, all with VTE, who were treated at our department in the past decade.

Results Patient management required repeated prolonged hospitalizations and careful management of water balance. In 3 patients, VTE occurred within 3 months of pituitary surgery. Two patients died. Two patients had severe urinary tract infections as the precipitating factor. All patients were replaced with hydrocortisone, l-thyroxine, and DDAVP. Sex steroids were omitted in the perioperative period. A patient with sleep apnea was not replaced with testosterone (Table 6).

Conclusion Awareness of VTE and increased morbidity and mortality is needed in patients with adipsic DI since some potentially fatal comorbidities can thereby be prevented. Surgery, immobilization, obesity, infection, hemoconcentration, and coagulation changes induced by DDAVP treatment (increases in FVIII and VWF) may contribute to the pathogenesis of VTE and prolonged hospitalization. Thrombo-prophylactic treatment after pituitary surgery and during episodes of hypernatremia is warranted.

Disclosure: No significant relationships.

P128

Wolfram Syndrome without Optic Atrophy

G. Oruk, A. Gorgel, M. Bahceci, D. Dolek

Endocrinology, Katip Celebi University, Izmir Atatürk Training and Research Hospital, Izmir, Turkey

Introduction The main features of Wolfram syndrome are diabetes mellitus, diabetes insipidus, sensorineural deafness, and optic atrophy. It is a progressive neurodegenerative disorder in which patients present with non-autoimmune and non-HLA-linked diabetes mellitus associated with optic atrophy in the first decade, diabetes insipidus and sensorineural deafness in the second decade, renal tract abnormalities early in the third decade and multiple neurological abnormalities early in the fourth decade. Usually, Wolfram pa-

tients die from central respiratory failure as a result of brain stem atrophy in their third or fourth decade.

Methods and Materials A 50-year-old female patient was admitted to the outpatient clinic with symptoms of polyuria, polydipsia, unstable walk, and hearing loss. Her history revealed diabetes mellitus for 8, hearing loss for 26 years, and thyroidectomy 20 years ago. She was receiving intensive insulin treatment for diabetes mellitus and was using a hearing device for the hearing loss.

Results Physical examination was normal except for ataxia and bilateral sensorineural hearing loss. During follow-up, it was noticed that urine output was 14 liters/day. A water deprivation test revealed findings compatible with central diabetes insipidus. Her symptoms improved with desmopressin. Renal, hepatic, thyroid functions, and other blood tests were normal. First findings in our patient were hearing loss, diabetes mellitus, diabetes insipidus, and neurological findings that had occurred consecutively. Fundus examination did not reveal optic atrophy or diabetic retinopathy. Only 5 cases of Wolfram syndrome are reported in the literature without optic atrophy.

Conclusion Because the condition is rare and clinical findings of the case have presented without optic atrophy and she has completed 50 years of her life without life-threatening problems, the case warranted a report. It can be concluded that patients with Wolfram should be followed all their lives, with good follow-up and treatment, quality of life of the Wolfram patients can be improved.

Disclosure: No significant relationships.

P129

Gestational Diabetes Insipidus: A Rare Complication

I. Bilbao, M. Aranburu, C. Garcia, A. Yoldi, N. Egaña, M. Alvarez Coca, M. Goena
Endocrinology, Hospital Universitario Donostia, San Sebastian, Spain

Introduction Diabetes insipidus (DI) is defined as the passage of large volumes (> 3 l/24 h) of diluted urine (< 300 mOsmol/kg), this can occur due to circulating arginine vasopressin (ADH) deficiency, renal resistance to vasopressin, or primary polydipsia in which the excess of fluid intake leads to a deficit in ADH production. During pregnancy, ADH clearance is increased due to placental vasopressinase activity and may cause gestational DI or may aggravate a previously subclinical DI.

Methods and Results We present the case of a 36-year-old woman who underwent surgery for a macroprolactinoma at the age of 19 due to poor response to dopamine agonists. No postoperative hormone deficits were found, the water deprivation test was negative, and a low dose of cabergoline was required to control prolactin levels and residual tumour size. She got pregnant at the age of 36 and cabergoline was stopped during pregnancy. She was referred in the 30th week with symptoms of polydipsia and polyuria, treatment with dDAVP was commenced soon after the diagnosis of DI was made with a good response. Delivery was induced in the 37th week after the patient developed edema of the lower limbs and AST/ALT elevation. Her symptoms resolved in the 4th week of puerperium.

Conclusion Pregnancy is a condition that may unmask previously subclinical DI, therefore a close follow-up is recommended in patients who might be at risk of developing this rare condition.

Disclosure: No significant relationships.

P130

Water Consumption Is Increased and Vasopressin Secretion Is Diminished after Centrally Administered Orexin in Rats

G. K. Kis¹, L. Daruka¹, A. H. Molnár², K. Rákosi³, J. Gardi⁴, F. László², F. A. László¹, C. Varga¹

¹Dept of Physiology, Anatomy and Neuroscience, Faculty of Science and Informatics; ²Institute of Physical Education and Sport Sciences, Faculty of Juhász Gyula Teachers Training College; ³Dept of Medical Chemistry, Faculty of Medicine; ⁴First Dept of Internal Medicine, University of Szeged, Hungary

Introduction Water intake and vasopressin (VP) secretion were studied in rats after orexin-A and orexin-B were administered intracerebroventricularly (icv).

Methods and Materials Different doses of orexins were administered (10–30–90 µg/10 µl) through a guide cannula (implanted into the right lateral ventricle of male Wistar rats [180–230 g]) under anaesthesia. Water consumption was measured during 6 hours. As the 30 µg/10 µl dose of orexin-A proved most efficient, this dose of orexin-A was administered subsequently. A VP level elevation was induced by histamine (10 mg/kg) or 2.5 % NaCl (10 ml/kg) administered intraperitoneally. The orexin-1 receptor (OX₁R) antagonist (SB 408124) was injected simultaneously with orexin-A in the similar dose of the neuropeptide. Plasma VP levels were measured by RIA.

Results 10 µg/10 µl icv orexin-A or -B did not increase water intake. Increased water consumption was observed after administration of 30 µg/10 µl orexin-A. There were no changes in basal VP secretion after the administration of different doses of the orexins. A significant increase in vasopressin concentration was detected following histamine, and a moderate VP level enhancement was detected after 2.5 % NaCl administration. Icv orexin-A (30 µg/10 µl) reduced the increased VP level induced by histamine or hyperosmosis. The inhibitory effects were prevented by SB 408124.

Conclusion (1) Orexin-A or orexin-B increased water consumption. After orexin-A administration, the induced polydipsia was more pronounced. SB 408124 decreased polydipsia significantly. (2) Histamine or hyperosmotic VP release enhancement was reduced by icv orexin administration. This inhibition was not observed following SB 408124 administration. (3) Our results suggest that the effect of orexin-A on water consumption or diminution of the histamine and osmosis-induced VP level increase is mediated by the OX₁R. Sponsored by the Hungarian Government and EC (SR0P-4.2.2-08/1-2008-0006 and SR0P-4.2.1/B-09/1/KONV-2010-0005).

Disclosure: No significant relationships.

P131

Copeptin as a Diagnostic Marker in the Management of Neurosurgical Patients with Disturbance of Water Homeostasis

B. Winzeler¹, N. Nigro¹, C. Zweifel², B. Arici³, C. Kelly², L. Mariani², S. Berkman⁴, H. Landolt⁴, M. Katar⁵, P. Schuetz³, B. Mueller³, M. Christ-Crain¹

¹Dept of Endocrinology, Diabetology and Metabolism; ²Neurosurgery, University Hospital of Basel; ³Endocrinology; ⁴Neurosurgery, Kantonsspital Aarau, Switzerland; ⁵Neurology, Columbia University, New York, NY, USA

Introduction Disorders of water balance caused by disturbances in arginine vasopressin (AVP) secretion remain a common cause of morbidity among patients undergoing pituitary surgery. Levels of AVP might contribute to a straightforward diagnosis, although its measurement is cumbersome. Copeptin, the stable C-terminal glycopeptide of the AVP prohormone, is a reliable surrogate of plasma AVP. Our aim is to elucidate whether copeptin may become a useful marker in the diagnostic approach of water and electrolyte disturbances after neurosurgery.

Methods and Materials From the preoperative day to the day of discharge, patients undergoing transsphenoidal or transcranial pituitary surgery were daily monitored for clinical items (ie, vital signs

and balance of fluids), routine laboratory parameters, and copeptin. Tumour-specific features and the intraoperative manipulation of the neurohypophysis were also recorded.

Results We present 12 patients (mean age 53 years, 58.3 % females) of an ongoing study with hormone-inactive (n = 4) and hormone-secreting pituitary adenomas (n = 3), meningiomas (n = 2), and Rathke's cleft cysts (n = 3). Nine patients showed a normal postoperative course, diabetes insipidus (DI) occurred in 3 patients within 36 hours after surgery. In patients with an uneventful postoperative course, surgery induced a significant copeptin release (p = 0.008), with an increase from 4.2 pmol/l (± 2.3) preoperatively to 12.2 pmol/l (± 9.2) postoperatively (measured within 24 hours after surgery). Copeptin levels in patients with postoperative DI decreased in all 3 patients from 3.85 pmol/l (± 0.85) preoperatively to 2.33 pmol/l (± 0.35) after surgery. The mean difference in copeptin levels from pre- to postoperative in patients with uneventful course was +4.1 vs -1.76 in patients with DI (p = 0.009).

Conclusion Copeptin may become a novel tool in the early goal-directed management of patients with disturbance of neuroendocrine homeostasis after pituitary surgery.

Disclosure: No significant relationships.

P132

Predictors of Water and Electrolyte Disturbances in Patients Undergoing Transsphenoidal Surgery for Pituitary Adenomas

D. Mikhaylova¹, L. Dzeranova¹, E. Pigarova¹, L. Rozhinskaya¹, A. Grigoriev², V. Azizyan², O. Ivashenko²

¹Endocrinology; ²Neurosurgery, Research Centre for Endocrinology, Moscow, Russian Federation

Introduction Neurosurgery is a frequently used treatment for pituitary adenomas and in this study the authors aimed to evaluate predictors of water and electrolyte disturbances in patients undergoing endoscopic, endonasal transsphenoidal surgery for pituitary adenomas.

Methods and Materials The study included 152 patients undergoing endoscopic endonasal transsphenoidal surgery for pituitary adenomas (118 women, 34 men) aged 18–65 years (median 40 [31–52]). 75 patients with Cushing's disease, 61 patients with acromegaly, 4 patients with prolactinomas, 7 patients with non-functioning pituitary adenomas, 1 patient with thyrotropinoma, 1 patient with Nelson's syndrome, and 3 patients with plurihormonal secreting adenomas were examined. Patients were monitored for balance of fluids, serum electrolytes, plasma, and urine osmolality. 119 patients were brought into remission after surgery, 28 patients were re-operated.

Results Postoperative water and electrolyte disturbances occurred in 67 patients: in 33 patients as diabetes insipidus, in 31 as transient disorders, in 3 patients as 3-phased disturbances. Water and electrolyte disturbances occurred more frequently in patients with intraoperative pituitary injuries (p = 0.007). No significant differences in postoperative water and frequency of electrolyte disturbances were found when comparing patients with macro- or microadenomas (p = 0.4), primary or re-operated patients (p = 0.3). Comparing patients brought into remission after surgery or not (p = 0.2) and patients with postoperative hypopituitarism or not (p = 0.4) no significant differences were found, either. Patients with corticotropinomas suffered from postoperative water and electrolyte disturbances more frequently compared to patients with somatotropinomas (p = 0.004) but patients with growth hormone-secreting pituitary adenomas more frequently suffered from postoperative water and electrolyte disturbances as compared with patients with prolactinomas and non-functioning pituitary adenomas (p = 0.02).

Conclusion ACTH-secreting pituitary adenoma or intraoperative pituitary injuries can be considered as predictors of water and electrolyte disturbances. Size of pituitary adenoma, re-operation, remission of disease, and postoperative hypopituitarism exert no influence upon postoperative water and electrolyte disturbances.

Disclosure: No significant relationships.

P133

Frequency of Sodium and Water Disturbances in Neurosurgical Patients at the National Institute of Neurology and Neurosurgery in Mexico City

B. García-Guzmán, A. Balderrama-Soto, L. Portocarrero-Ortiz
Neuroendocrinology, Instituto Nacional de Neurología y Neurocirugía MVS,
Mexico City, Mexico

Introduction Sodium and water postsurgical disturbances are frequent in neurosurgical patients. They occur as a consequence of overproduction or absence of antidiuretic hormone that condition syndromes of inappropriate secretion of antidiuretic hormone (SIADH) and central diabetes insipidus (CDI). These derangements are associated with the appearance, exacerbation, or precipitation of neurological symptoms and an increase in morbidity and mortality.

Methods and Materials We conducted a prospective, observational, and longitudinal study during the period of January to March 2012 on 330 consecutive neurosurgical patients and evaluated them during the postsurgical period for the appearance of sodium and water disturbances.

Results Patients (168 male, 162 female) had a mean age of 45.9 years (range 16–78 years) and were divided according to the following diagnostics: neuroepithelial tumors (n = 132; 40 %), meningiomas (n = 42; 17.3 %), sellar-region tumors (n = 54; 16.3 %), subarachnoid hemorrhage (n = 51; 15.45 %), medullary syndrome (n = 15; 4.54 %), diverse neurological diseases (n = 12; 3.63 %), and cranial nerve tumors (n = 9; 2.72 %) (Table 7). 54 patients (16.36 %) presented sodium and water disturbances (hyponatremia n = 31, 57 %; hyponatremia n = 23, 42 %). The main causes for hyponatremia (mean sodium 153.3 mmol/l) were transitory CDI (50 %), hyponatremia associated with mannitol and diuretic administration (31 %), adipsic diabetes insipidus (9.5 %), and hyperglycemic hyperosmolar state (9.5 %). The main causes for hyponatremia (mean sodium 128 mmol/l) were SIADH (95 %) and hyponatremia secondary to desmopressin administration (5 %). All the disturbances presented within the first 48 postsurgical hours.

Conclusion The main sodium and water disturbances at our institution were associated with sellar-region tumors, predominantly craniopharyngiomas. They all presented within the first 48 postsurgical hours.

Disclosure: No significant relationships.

Table 7. B. García-Guzmán et al. Results of the assessment of sodium and water disturbances.

Diagnostics	Sodium and water disturbance	Group frequency
Craniopharyngioma	CDI	4/9; 44 %
Non-functional pituitary adenoma	CDI	8/39; 20.5 %
	SIADH	10/39; 25.6 %
	Adipsic diabetes insipidus	3/39; 7.69 %
Subarachnoid hemorrhage	Hyponatremia associated with mannitol	6/51; 41.7 %
Neuroepithelial tumors	SIADH	3/51; 5.8 %
	Hyponatremia associated with mannitol	4/132; 3.03 %
	SIADH	4/132; 3.03 %
Meningioma	CDI	3/132; 2.27 %
	SIADH	5/42; 11.9 %
Diverse neurological diseases	Hyponatremia secondary to desmopressin administration	1/42; 2.38 %
	Hyperglycemic hyperosmolar state	3/12; 25 %

Table 8. E. Cagiltay et al. Laboratory test results of the presented patient.

Time	Urine volume (ml)	Urine osmolality (mosm/l)	Urine density	Serum osmolality (mosm/l)	Body mass (kg)
0	550	139	1005	314	82
1	350	151	1005		81
Desmopressin injected					
2	280	220	1010	315	81
3	90	431	1010		80

P134

A Rare Cause of Diabetes Insipidus: Familial Neurohypophysial Diabetes Insipidus (FNDI)

E. Cagiltay, L. Ozsari, M. Alis, S. Caglayan, A. Yonem, M. E. Onde
Dept of Endocrinology and Metabolism, Gata Haydarpasa Teaching Hospital,
Istanbul, Turkey

Introduction Diabetes insipidus also has a genetical basis.

Methods and Materials A 31-year-old male patient was referred to our clinic with symptoms of polyuria and polydipsia starting from childhood. His initial evaluation revealed that his mother, his brother, and his 6-year-old son also had symptoms of polyuria and polydipsia. His urine density and osmolality were lower than normal. His anterior hypophyseal tests were normal. He was observed drinking 10–12 liters of water/day. His water deprivation test and desmopressin test results were in accordance with complete central diabetes insipidus (Table 8). His dynamical hypophyseal imaging revealed a neurohypophysis with intact localisation, size, and signal intensity. His post-contrast dynamical imaging revealed an adenohypophysial 2 × 4 mm image consistent with microadenoma at the posterior left half of the gland. The patient was diagnosed as having familial neurohypophysial diabetes insipidus (FNDI) and genetic counselling was advised. Unfortunately, he was lost to follow-up. With desmopressin treatment, his clinical and laboratory findings recovered.

Results See Table 8.

Conclusion FNDI is a rare disorder stemming from an autosomal dominant mutation in the vasopressin gene. Usually, the mutation involves DNA sequences in the neurophysin or signal peptide region of the precursor gene rather than the region encoding vasopressin itself. These mutations eventually cause the cell death of vasopressin-producing neurons. Recently, a novel mutation analysis showed a c.322G > T (p.?[?]p.Glu108X) in exon 2 of the AVP-NP11 gene in a different family. The documentation of this kind of families and their possible novel mutations will help us better understand the pathophysiology of this disease.

Disclosure: No significant relationships.

Neuropeptides

P135

In Vitro Hypothalamic Neuropeptides and Neurotransmitter Receptor Alteration by 2,3,7,8-Tetrachlorodibenzo-P-Dioxin (TCDD)

K. Solak, F. Wijnolts, B. Blaauw, M. van den Berg, M. van Duursen
Toxicology, Institute for Risk Assessment Sciences (IRAS), Utrecht, The Netherlands

Introduction 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) is a highly persistent environmental pollutant known to induce a broad spectrum of toxic responses, primarily via aryl hydrocarbon receptor- (AhR-) mediated pathways. Despite poor brain tissue penetration, TCDD accumulates in high concentrations in the hypothalamus in vivo causing dose-dependent Cyp1a1 mRNA up-regulation, Ca²⁺_{intracellular} changes, oxidative stress, and alterations in neurotransmitter concentrations or their turnover rates. TCDD-induced effects have been implicated in diseases such as wasting syndrome.

Despite *in vivo* data no predictive *in vitro* model to study TCDD-mediated mechanisms of action on hypothalamus exists.

Methods and Materials We describe the multitask properties of the novel rat hypothalamic GnV-3 cell line. We focused on AhR-mediated effects to investigate TCDD action on most abundant neurotransmitter receptors within the hypothalamus. Changes in $\text{Ca}^{2+}_{\text{intracellular}}$ in GnV-3 cells were investigated using FURA-2. Moreover, mRNA expression of the orexigenic peptides ghrelin and neuropeptide was assessed by qRT-PCR. Finally, TCDD-dependent changes in $\text{cAMP}_{\text{intracellular}}$ concentration were examined.

Results The qRT-PCR and Western blot data demonstrated the presence of AhR on mRNA and protein levels, respectively. AhR immunostaining revealed AhR protein translocation from cytosol to the nucleus after 1 h of 10 nM TCDD treatment. NMDA, kainite, and AMPA glutamate-type receptor mRNA levels were elevated showing a significant increase after 4 h and 24 h, 6 h and 24 h and 6 h for Grin1, Grik2, and AMPA1, respectively. Expression of 5-HT_{2C} receptor mRNA was down- and up-regulated after 2 h and 24 h, respectively. GABAa2 receptor mRNA was up-regulated after 24 h by TCDD. Neuropeptide Y mRNA level was significantly elevated after 4 h and 6 h. TCDD significantly down-regulated cAMP and elevated $\text{Ca}^{2+}_{\text{intracellular}}$ levels.

Conclusion The obtained *in vitro* data indicate that the GnV-3 cells may be used in mechanistic studies on hypothalamic effects by dioxin-like compounds.

Disclosure: No significant relationships.

P136

Diabetes Effects on Met-Enkephalin Synthesis in Piglet Brains

K. Pierzchala-Koziec, J. Zubel, E. Oclon

Dept of Animal Physiology and Endocrinology, University of Agriculture, Krakow, Poland

Introduction Endogenous opioid peptides, such as Met-enkephalin, are involved in the regulation of many physiological processes. Opioids affect memory, pain feeling, reward, and motivation system, circulatory system, immune processes, and metabolism. The aim of the study was to determine the effects of hyperglycemia induced by inflammation or immunosuppression on the synthesis, hydrolysis, and concentration of Met-enkephalin in different brain areas.

Methods and Materials The study was conducted on piglets (n = 18, body weight ~10 kg, female) divided into a control and 2 experimental groups: (1) diabetes mellitus type 1 (DM1) induced by streptozotocin injections and (2) diabetes mellitus type 2 (DM2) induced by corticosteroid injections. Fragments of the hippocampus and striatum were taken and directed to the estimation of proenkephalin mRNA expression by hybridization *in situ* and to the native and cryptic Met-enkephalin levels determination by RIA. Cryptic Met-enkephalin was obtained by enzymatic processing from the proenkephalin sources.

Results Expression of proenkephalin mRNA was higher in the hippocampus than in the striatum of control animals. DM1 and DM2 decreased the synthesis of proenkephalin in the hippocampus and increased it in the striatum (by 50 and 25 %, respectively; $p < 0.01$). Similarly, the level of cryptic Met-enkephalin was significantly decreased in both brain fragments by DM1 as well as by DM2 ($p < 0.01$). On the other hand, native Met-enkephalin was increased in both brain areas by DM1 (by 25 and 33 %, respectively; $p < 0.01$) and decreased by DM2 (by 49 and 11 %, respectively). It must be pointed out that Met-enkephalin responses to hyperglycemia were stronger in the hippocampus than in the striatum of tested piglets.

Conclusion (1) Diabetes differentially affected the synthesis and processing of Met-enkephalin in the hippocampus and striatum of piglets. (2) Significant changes of native and cryptic Met-enkephalins in the brain indicate involvement of endogenous opioid peptides in the regulation of carbohydrate metabolism, both in inflammation and immunosuppression situations.

Disclosure: No significant relationships. Supported by Grant NCBiR 12006406.

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Hypothalamic Ghrelin Activity during Acute and Chronic Inflammation in Piglets

J. Zubel, K. Pierzchala-Koziec, E. Oclon

Dept of Animal Physiology and Endocrinology, University of Agriculture, Krakow, Poland

Introduction Ghrelin is mainly synthesized in the stomach but also distributed in the central tissues. There is growing evidence suggesting that the development of obesity-related disturbances may be closely associated with hormonal dysregulation and chronic inflammation. The present study was performed to examine the effects of acute and chronic inflammations on hypothalamic ghrelin activity.

Methods and Materials The experiment was carried out on 24 piglets at the age of 10 weeks and 15 kg bodyweight (bw). Animals (females) were kept under standard conditions and divided into 4 experimental groups: (1) control, (2) obese (chronic inflammation), (3) acute inflammation, and (4) obese with acute inflammation. Piglets from groups 1 and 3 were fed with a commercial feed whereas those in groups 2 and 4 were fed with a high-calorie diet to develop obesity. To induce acute inflammation animals received ip injection of streptozotocin (100 mg/kg bw) once. 24 hours after injection the hypothalamus was quickly removed and directed to measure mRNA of ghrelin and its receptor by quantitative PCR analysis. Additionally, fragments of the hypothalamus were taken for radioimmunological ghrelin estimation of concentration and secretion.

Results The expression of ghrelin mRNA and receptors were significantly changed during acute and chronic inflammation. A significant difference in the concentration of ghrelin was observed between groups with acute (rise by 76 %) and chronic inflammations (decrease by 43 %) compared to control piglets (18.97 ± 1.4 pg/mg). Also inflammatory processes changed *in vitro* ghrelin release from the hypothalamus.

Conclusion The obtained results indicate that ghrelin activity in the hypothalamus strongly depends on the type of inflammation.

Disclosure: No significant relationships. Supported by grants # NN 311 227 138 and NR 12 006406.

P138

Melanin-Concentrating Hormone Receptor 1 mRNA Expression in the Mammary Gland of the Rat

D. Batagello¹, H. Costa², R. Silva², J. Silva², C. Lotfi², L. Sita², E. Bevilacqua³, J. C. Bittencourt²

¹Experimental Psychology, Center for Neuroscience and Behavior; ²Anatomy;

³Dept of Cell Biology and Development, University of Sao Paulo, Brazil

Introduction In the rat, melanin-concentrating hormone (MCH) mRNA, as well as MCH and neuropeptide glutamic acid-isoleucine (NEI) peptides, are primarily located in the neurons of 2 central nervous system (CNS) regions: the lateral hypothalamic and the incerto-hypothalamic areas. However, novel sites of MCH mRNA expression and immunoreactivity during the final portion of the lactation period (from days 19–21) have been identified in other hypothalamic nuclei, such as the ventral part of the medial preoptic area (MPOAv). Two different MCH receptors have been described in the CNS: MCHR1 (in rats) and MCHR2 (in rats and humans). However, there are no descriptions of MCHR1 expression in the mammary glands of non-lactating or lactating dams. Moreover, it has been described that serum MCH concentration is slightly increased at lactation day 12. The objective of this project is to detect MCHR1 mRNA expression in the mammary glands of lactating and non-lactating rats, correlating MCH mRNA expression in the MPOAv with the consequent MCHR1 mRNA expression in the mammary gland, thereby increasing the knowledge regarding the control of maternal behavior (MB).

Methods and Materials To study the possible involvement of MCH in MB, we used *in situ* hybridization to detect MCHR1 mRNA expression in mammary gland tissue from 4 groups of rats: virgins (control) and dams at lactation days 5, 12, and 19, respectively.

Results We detected MCHR1 mRNA in the mammary glands of dams in the lactation day 19 group and comparable expression in non-lactating dams, both expressions in the stroma, although a differential expression was found in the lactiferous duct of dams of lactation day 19.

Conclusion To our knowledge, these are novel findings. Our preliminary data suggest that MCHR1 mRNA expression in mammary gland tissue participates in the late stages of lactation.

Disclosure: No significant relationships.

■ Neuroimmunendocrinology

P139

Adipokine Profiles in Acute Ischemic Stroke

B. Baranowska¹, M. Grudniak², J. Kochanowski², A. Baranowska-Bik³, E. Wolinska-Witort¹, W. Bik¹

¹Dept of Neuroendocrinology, Medical Centre for Postgraduate Education; ²Dept of Neurology, Warsaw Medical University; ³Dept of Endocrinology, Medical Centre for Postgraduate Education, Warsaw, Poland

Introduction The metabolic syndrome is an important risk factor for stroke. The disturbances in adipokine release may be connected with metabolic syndrome. We aimed to evaluate the relationship between changes in adipokine profiles and metabolic as well as pro-inflammatory markers in patients with acute ischemic stroke.

Methods and Materials Material consisted of 52 women aged 60–85 years with acute ischemic stroke and 72 aged-matched control women. Stroke was defined according to the NIHSS (National Institutes of Health Stroke Scale) and was confirmed with brain CT or MRI scan. Serum concentrations of leptin, total adiponectin, and its isoforms (high molecular weight [HMW], middle molecular weight [MMW], low molecular weight [LMW]), resistin and TNF- α were measured within 24 hours of the first symptoms of stroke and 10 days after the acute phase. Clinical and biochemical data concerning metabolic disturbances were collected. Insulin resistance was estimated using the homeostasis model assessment method (HOMA).

Results The incidence of type-2 diabetes, hypertension, and obesity was higher in patients with stroke. In patients with stroke, leptin, resistin, and TNF- α levels were significantly higher ($p < 0.01$, $p < 0.01$, and $p < 0.001$, respectively), and total adiponectin and MMW levels were lower ($p < 0.05$, $p < 0.05$) as compared with controls. Positive correlations between leptin and BMI, HOMA, LDL cholesterol, and TNF- α were found. Negative correlations between total adiponectin, HMW, and HOMA, triglycerides were observed. HMW adiponectin and resistin levels correlated with severity of stroke evaluated according to the NIHSS scale.

Conclusion The changes in adipokine profiles were observed in patients with acute ischemic stroke. The relationship between adipokines and metabolic disturbances as well as with proinflammatory markers was found in patients with acute ischemic stroke. HMW adiponectin and resistin may be important markers in the evaluation of stroke severity. This work was supported by grant MNiSW 5484/B/P01/2011/40

Disclosure: No significant relationships.

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Galanin Receptor 3 Is Mediating Important Functions in Polymorphonuclear Neutrophils

S. Schmidhuber¹, A. Lang¹, S. Brunner¹, J. MacDougall², F. Locker¹, B. Brodowicz¹, R. Lang³, B. Kofler¹

¹Laura Bassi Centre of Expertise Therapep, Research Program for Receptor Biochemistry and Tumor Metabolism, Dept of Pediatrics, Paracelsus Medical University, Salzburg, Austria; ²Dept of Physiology and Biophysics, University of Calgary, Canada; ³Dept of Dermatology, Paracelsus Medical University, Salzburg, Austria

Introduction Galanin is a bioactive neuropeptide that participates in the recruitment of polymorphonuclear neutrophils (PMNs) during acute inflammatory processes. The galanin receptor (GALR) sub-types (GALR1, GALR2, and GALR3) involved, however, are unclear. Consequently, we aimed to determine GALR messenger RNA (mRNA) expression in PMNs, and to ascertain if these receptors are involved in PMN functions.

Methods and Materials PMNs were obtained from freshly isolated venous blood. GALR expression in PMNs was quantified by polymerase chain reaction amplification of reverse-transcribed RNA (RT-PCR) and by Western blot analysis. Expression of beta-2-integrin CD11b was analyzed by flow cytometry. Enzymatic activity of myeloperoxidase (MPO) was measured by a colorimetric assay and gelatinase-secretion (MMP-9) was determined by zymography. Leukocyte rolling and adhesion in rats were determined by intravital microscopy of the synovial microcirculation.

Results Using RT-PCR we were able to show that GalR2 and GalR3 receptors are found to be expressed in human resting PMNs. Additionally, immunoblotting using specific GalR2/3 antibodies confirmed that mRNAs detected by RT-PCR were translated into proteins. Treatment of PMNs with galanin increased the release of MPO, boosted MMP-9 activity and enhanced the expression of CD11b, markers of degranulation of secondary and azurophil granules, respectively, which are all secreted during cell activation. To identify the nature of the receptor involved in galanin-mediated PMN activation, we used the specific GALR3 antagonist SNAP-37889. We found that SNAP-37889 treatment significantly reduced MPO and MMP-9 secretion. In addition, a decrease of galanin-mediated CD11 expression was observed. In vivo, we found that galanin-mediated PMN recruitment, rolling and adhesion to the blood vessel wall in inflamed rat knee joints is GALR3 dependent.

Conclusion Taken together, we provide evidence that SNAP-37889 is a valuable tool for elucidating pharmacological effects of GALR3 antagonism in inflammatory disorders.

Disclosure: No significant relationships.

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Alarin as a Novel Regulator of Polymorphonuclear Neutrophils

A. Lang¹, S. Schmidhuber¹, F. Locker¹, S. Wintersteller², R. Lang¹, B. Kofler¹

¹Research Program for Receptor Biochemistry and Tumor Metabolism, Dept of Pediatrics; ²Dept of Pediatrics, Laura Bassi Centre of Expertise "Therapep", Paracelsus Medical University, Salzburg, Austria

Introduction Alarin, a recently discovered bioactive neuropeptide of the galanin-peptide family, has been shown to inhibit neurogenic inflammation in the skin. Therefore, we aimed to elucidate if this peptide is also involved in the recruitment and physiology of polymorphonuclear leukocytes/neutrophils (PMNs).

Methods and Materials PMNs were obtained from freshly isolated venous blood. Adherence of PMNs was specified by a methylene blue assay and expression of beta-2-integrin CD11b was analyzed by flow cytometry. Enzymatic activity of myeloperoxidase (MPO) was measured by a colorimetric assay. Release of lactoferrin was determined by ELISA and gelatinase-secretion (MMP-9) was determined by zymography. Cell-based label-free technology was used to measure cellular response of alarin in PMNs manifested through dynamic mass redistribution (DMR) within the cells.

Results Treatment of PMNs with alarin results in significant changes in adherence. In accordance with these findings, we were able to demonstrate that the surface expression of CD11b, which is activated during cellular activation and adhesion, is up-regulated already 10 minutes after alarin treatment. Using this assay, we could demonstrate that full-length alarin (1–25) has the most potent effects on CD11b up-regulation when comparing different fragments of alarin. Moreover, alarin exposure increases the release of MPO, which is a marker of degranulation of azurophil/primary granules that occurs during PMN activation. In addition, we found that alarin boosts lactoferrin and MMP 9 activity, indicating that it can mobilize specific secondary (lactoferrin) and gelatinase granules, which are secreted during cell activation. Using a cell-based label-free technology, we could demonstrate that these effects of alarin on PMNs are most probably mediated via an alarin-specific receptor.

Conclusion Taken together, we have identified and characterized alarin as another regulator for human PMN activity, implying possible functions in acute inflammatory responses.

Disclosure: No significant relationships.

■ Central and Peripheral Control of Neuroendocrine Systems

P142

B-Type Natriuretic Peptide Increases Circulating Cortisol Concentrations and Adrenergic Activity: A Randomised Placebo-Controlled Cross-Over Study in Healthy Men

G. Grimm¹, M. Res², M. Ried², B. Heinisch³, A. Luger², M. Clodi², G. Vila²

¹Dept of Medical and Chemical Laboratory Diagnostics; ²Division of Endocrinology and Metabolism; ³Division of Gastroenterology, Dept of Internal Medicine III Medical University of Vienna, Austria

Introduction B-type natriuretic peptide (BNP) released from the heart in response to volume overload is widely used in clinical practice as a diagnostic and prognostic biomarker for heart failure. BNP induces vasodilatation and increases natriuresis and diuresis, thereby releasing the preload. We recently described that BNP exerts anorexic effects in men by reducing circulating concentrations of the gut hormone ghrelin.

Methods and Materials This study evaluates the acute effects of BNP on other hormones: pituitary-adrenal axis, plasma catecholamines, thyroid hormones, and prolactin. Ten healthy male volunteers were recruited in a randomised placebo-controlled cross-over study, receiving once placebo and once an intravenous infusion of 3.0 pmol/kg/min human BNP-32 during 4 hours. Circulating levels of hormones were measured hourly.

Results BNP administration increased plasma BNP concentrations, which remained constant at between 400 and 500 pg/ml during the final 2 hours of the study. Patients remained in supine position throughout the study and there were no differences in blood pressure between placebo and BNP sessions. BNP prevented the decrease in cortisol over time leading to elevated cortisol concentrations ($p = 0.022$). There were no significant changes in GH and prolactin levels. BNP increased circulating adrenaline and noradrenaline concentrations ($p = 0.018$ and $p = 0.036$, respectively). The thyroid and parathyroid hormone axis, as well as prolactin were not affected by BNP administration.

Conclusion In summary, we show that BNP administration directly increases circulating cortisol and catecholamine concentrations in healthy men, also in the absence of changes in blood pressure and inflammatory markers. These novel cross-talks within the human endocrine system highlight hitherto unknown mechanisms underlying the pathophysiology of altered HPA axis activity and increased adrenergic activity accompanying heart failure, and could provide the basis for novel therapeutic strategies.

Disclosure: No significant relationships.

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Type-1 Cannabinoid Receptor Mediates Changes in Messenger RNA Expression in the Hypothalamus during Water Deprivation in Rats

S. Ruginski, F. Vecchiato, E. Uchoa, L. Elias, J. Antunes-Rodrigues

Physiology, School of Medicine of Ribeirão Preto, University of São Paulo, Ribeirão Preto, Brazil

Introduction Water deprivation (WD) induces a well-known hyperosmolality-mediated anorexic effect. Considering that the type-1 cannabinoid receptor (CB1R) has been implicated in the central control of food intake, the aim of the present study was to investigate the effects of the CB1R antagonism on mRNA expression for neuropeptides regulating both fluid and energy homeostasis.

Methods and Materials For this purpose, after habituation, male Wistar rats were water-deprived for 24 hours and intraperitoneally injected with vehicle (5 % ETOH in 0.9 % NaCl) or AM251 (CB1R antagonist, 3 mg/kg/1.5 ml). 90 minutes after injection animals were euthanized by decapitation and the brains were quickly removed and stored until mRNA extraction and semi-quantitative determination by real-time PCR.

Results The results demonstrate that the paraventricular nucleus (PVN) of WD rats shows increased mRNA expression for vasopressin (AVP), this effect being potentiated by AM251. In the arcuate nucleus (ARC), the relative expression of Agouti-related protein (AgRP) mRNA was significantly increased by WD and potentiated in AM251+WD animals. The expression of cocaine- and amphetamine-regulated transcript (CART) mRNA in the PVN and ARC, as well as the expression of neuropeptide Y (NPY) mRNA in the ARC were only increased in WD rats injected with AM251. On the other hand, the relative mRNA expression for corticotrophin-releasing factor (CRF) in the PVN and proopiomelanocortin (POMC) in the ARC was significantly enhanced in AM251-treated animals irrespective of their hydration state. The expression of orexin-A and melanin-concentrating hormone (MCH) mRNAs was not altered by AM251 or WD in the lateral hypothalamic area (LHA).

Conclusion Collectively, these results indicate that the CB1R is likely to modulate central anorexic and orexigenic pathways integrated in the PVN and ARC, which produce coordinated behavioral responses regarding food and fluid consumption.

Disclosure: No significant relationships.

■ Neuroendocrine Regulation of Adrenal Function

P144

Acute Effect of Fludrocortisone on Basal and HCRH-Stimulated Hypothalamic-Pituitary-Adrenal (HPA) Axis in Humans

I. Karamouzis¹, R. Berardelli¹, E. Marinazzo¹, V. D'Angelo¹, D. Zinna¹, R. Giordano², E. Ghigo¹, E. Arvat¹

¹Division of Endocrinology, Diabetology and Metabolism, Dept of Internal Medicine; ²Dept of Clinical and Biological Science, University of Turin, Italy

Introduction Mineralocorticoid receptors (MR) in the hippocampus play an important role in the control of the hypothalamic-pituitary-adrenal axis (HPA), mediating the “proactive” feedback of glucocorticoids. Fludrocortisone (FC), a potent MR agonist, has been shown to decrease HPA activity through a mechanism placed at the hippocampal level. In order to clarify the effects of MR agonism on HPA function in humans, we studied the effects of FC in a dose-related manner on both basal and CRH-stimulated HPA axis during the quiescent phase.

Methods and Materials Eight young women were studied. ACTH, cortisol, and aldosterone levels were evaluated every 15', from 16:00–20:00 hours, in several randomized sessions: (1) placebo po + placebo iv, (2) 0.3 mg FC po + placebo, (3) 0.1 mg FC + placebo,

(4) 0.075 mg FC + placebo, (5) 0.05 mg FC + placebo, (6) placebo + hCRH (2.0 µg/kg iv bolus), (7) 0.3 mg FC + hCRH, (8) 0.1 mg FC + hCRH, (9) 0.075 mg FC + hCRH, and (10) 0.05 mg FC + hCRH.

Results FC induced a dose-related trend toward a further decrease of the ACTH and cortisol levels, while it showed a significant and dose-dependent inhibition of the hormonal response to hCRH ($p < 0.05$ for the doses of 0.3, 0.1, and 0.075 mg). Conversely, 0.05 mg FC did not modify the CRH-stimulatory effect on both ACTH and cortisol secretion. Aldosterone levels were not modified by FC administration.

Conclusion Fludrocortisone inhibits corticotrope and adrenal responses to hCRH in humans in a dose-dependent manner. The 0.075-mg FC seems the lowest active while 0.05 mg the first neutral dose on HPA activity. These data suggest a possible hypophysial MR-mediated inhibiting effect of FC, although its pituitary glucocorticoid-mediated effect cannot be excluded. The interplay between fludrocortisone and hypophysial glucocorticoid receptors needs to be clarified in order to better define the clinical consequences of hormonal replacement therapy of patients with primary adrenal insufficiency.

Disclosure: No significant relationships.

■ Hypopituitarism

P145

Growth Hormone Deficiency and Neuroendocrine Disorders in Adults with Different Pituitary Tumors

Y. Urmanova, M. Shakirova

Neuroendocrinology, Scientific-Practical Center of Endocrinology, Tashkent, Uzbekistan

Introduction Growth hormone deficiency in patients with pituitary tumors is a present-day problem in neuroendocrinology. The quality of life of persons with adult growth hormone deficiency (AGHD) became low because of different complications, first of all the metabolic syndrome, emotional problems, cognitive and different psychological disorders.

Methods and Materials We examined 40 patients with different pituitary tumors (mean age 42.7 years) to assess their neuroendocrine status dynamics. Examination included hormonal assessment, perimetry, assessment of neurological status, examination of the sellar area by means of computer and magnetic resonance tomography was performed in all patients.

Results Microadenomas (< 10 mm) were found in 6 patients, mezo-adenomas (11–15 mm) in 7, 9 persons had macroadenomas (> 15 mm) and 18 patients giant adenomas (> 2 cm). Many patients have inactive pituitary adenomas ($n = 31$), prolactinoma ($n = 3$), corticotropinoma ($n = 2$), astrocytoma ($n = 1$), germinoma ($n = 1$), meningioma ($n = 1$), and craniopharyngioma ($n = 1$). Visual abnormalities, such as vision acuteness reduction and vision field narrowing, were observed in 40 patients (100 %), in 6 patients amaurosis was diagnosed, panhypopituitarism was found in 2 persons (5 %), hypopituitarism in 35 (87.5 %) patients with STI, primary infertility ($n = 4$; 10 %), impotence ($n = 1$; 2.5 %), and secondary amenorrhea ($n = 10$; 25 %). General brain symptoms were present, such as headache ($n = 24$; 60 %), decrease of memory ($n = 21$; 52.5 %), dyslopia ($n = 1$; 2.5 %), ptosis ($n = 1$; 2.5 %). Hypopituitarism, that is, reduction in the levels of pituitary tropic hormones, such as STH, LH, and FSH, was found in 77 %, large pituitary tumors with supra-, para-, and infrasellar growth being registered in the patients.

Conclusion Parallel to the decrease in other pituitary hormones STH level reduction and decrease of cognitive functions is typical for patients with pituitary tumors

Disclosure: No significant relationships.

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Clinical Efficacy of Jintropin, a Gene-Engineered Growth Hormone, in the Treatment of Somatotrophic Deficiency in Children and Adolescents

S. Ismailov¹, B. Nazarova¹, Y. Urmanova², F. Suleymanova¹, R. Zakirov

¹Pediatric Endocrinology, Center of Endocrinology, Tashkent; ²Endocrinology, Tashkent Pediatric Medical Institute, Uzbekistan

Aim To study clinical efficacy of jintropin in the treatment of somatotrophic deficiency in children and adolescents.

Methods and Materials In the course of an open study, we examined 26 outpatient children and adolescents aged 4–18 years referred to the our Center of Endocrinology. Somatotrophic deficiency was diagnosed in 20 patients to be treated with a subcutaneously administered dose of 0.033 mg/kg/d of 0.08–0.1 IU/kg/d of jintropin (Eurofarm), a clinically tested medication, every day for 6 months before sleep.

Results Patients were divided into 2 groups: (1) 20 patients with somatotrophic deficiency (SD) and (2) 6 patients with an idiopathic low stature (ILS). SD was diagnosed by blood STH after clonidine test (0.15 mg/m² body surface), STH levels of 0.5 ± 0.1 ng/ml, 0.9 ± 0.2 ng/ml, 1.7 ± 0.5 ng/ml, 2.3 ± 0.5 ng/ml, and 1.4 ± 0.4 ng/ml registered at 0, 30, 60, 90, and 120 minutes of test, respectively. Median peak STH in the stimulation tests was not above 1.4 ng/ml (range: 0.05–9.5 ng/ml). Treatment with jintropin results in sustaining of normal body proportions. In 6 months of therapy mean growth velocity was 9.4 ± 0.4 cm/year (6.6 ± 11.6 cm/year), the absolute mean 6-month growth increment being 6.1 ± 0.2 cm. Levels of IGF-1 and urinary GH ($p < 0.05$) was found confidently increased in 3 months of therapy, these levels and the one of IGFSB-3 ($p < 0.001$) being registered appropriately increasing in 6 months.

Conclusion Assessment of changes in anthropometric parameters following treatment with jintropin showed growth tempo normalization. Mean 6-month growth velocity was 9.4 ± 0.4 cm/year with the absolute growth increment being 6.1 ± 0.2 cm.

Disclosure: No significant relationships.

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Pregnancies and Live Births in a Large Cohort of Patients on Growth Hormone Replacement Therapy (GHRT)

G. Vilà¹, A. Akerblad², F. Lundgren², B. M. Biller³, V. Hana⁴, E. H. Nielsen⁵, M. Riedl⁶, M. Koltowska-Haggstrom², S. M. Webb⁶, A. Luger¹

¹Division of Endocrinology and Metabolism, Dept of Internal Medicine III, Medical University of Vienna, Austria; ²Pfizer Specialty Care, Pfizer Inc, Sollentuna, Sweden; ³Neuroendocrine Unit, Massachusetts General Hospital and Harvard Medical School, Boston, USA; ⁴3rd Dept of Medicine, First Medical Faculty, Charles University, Prague, Czech Republic; ⁵Dept of Endocrinology, Aalborg Hospital, Aarhus University Hospital, Aalborg, Denmark; ⁶Endocrinology/Medicine Depts and Ciberer 747, Hospital Sant Pau, Universitat Autònoma de Barcelona, Spain

Introduction GHRT has not been formally approved for use during conception and pregnancy in patients who are on GHRT for growth hormone deficiency (GHD). There are few data and no guidelines on whether to continue or withdraw GHRT during conception and pregnancy.

Methods and Materials We searched for pregnancy reports in the KIMS (Pfizer International Metabolic Database), a pharmacoepidemiological study of adults with GHD, among 4651 women aged 15–50 years (followed for 19,348 patient years) and 7438 men aged > 16 years (followed for 36,074 patient years).

Results A total of 151 pregnancies were reported, of which 136 were in 114 female patients (aged 22–41 years) and 15 were in partners of male patients (men's age 26–68 years). Five percent of women and one man discontinued GHRT before conception. Ovulation induction or assisted reproduction treatments were used in 65 % of women. GHRT use in pregnant women was as follows: continuation in 23 %, withdrawal in 66 %, and dose reduction in 1.5 %.

Within the cohort that stopped GH during pregnancy, 59 % gave birth to healthy babies, 12 % reported fetal loss, and outcome is not reported in 29 %. Within the cohort that continued GH during pregnancy, 45 % gave birth to healthy babies, 35 % reported fetal loss, and outcome is not reported in 20 %. To date, birth of 92 healthy children has been confirmed, 21 of them from twin pregnancies, 3 from a triplet pregnancy.

Conclusion We provide the first demographic data on pregnancy rates in a large cohort of patients receiving GHRT. It appears that in the clinical practice setting, nearly all patients taking GH replacement continue treatment during the time when they seek fertility, and 1/4 continue it during pregnancy. The collection of missing data on pregnancy outcomes is ongoing.

Disclosure: No significant relationships.

P148

Endocrine Alterations in Patients 3 Months after Severe Brain Injury

D. Marina¹, M. Klose¹, A. Liebach², L. Westergaard², A. Nordenbo², U. Feldt-Rasmussen¹

¹Medical Endocrinology, Rigshospitalet, Copenhagen; ²Center for Traumatic Brain Injuries and Neurorehabilitation, Hvidovre Hospital, Hvidovre, Denmark

Introduction Recent research has suggested that different types of brain injuries (BI) should be classified as conditions related to a high risk in developing acute or chronic hypopituitarism.

Aim To assess the occurrence of hypopituitarism 3 months after BI in patients attending long-term neurorehabilitation.

Methods and Materials 151 consecutive BI patients (103 men [68 %], mean 44.5 years [SD 17.7]) referred to neurorehabilitation in the period from 01/2007 to 01/2011; BI was caused by head trauma (n = 109; 72 %), aneurysmal subarachnoid haemorrhage (n = 14; 9 %); intracerebral haemorrhage (n = 10; 7 %), anoxia (n = 8; 5 %), and others (n = 10; 7 %). All patients had pituitary endocrine evaluation 3.5 months ($\pm$ 1.5) after injury. The diagnosis of hormone insufficiencies was based on the evaluation of pituitary and peripheral hormone levels in relation to local age-related reference ranges. A 250 microg synacthen test was performed in 140 (93 %) patients.

Results Overall, hormone alterations were observed in 90/151 (60 %) patients. Hypogonadotrophic hypogonadism was recorded in 22 men (22 %) and 11 women (23 %), secondary hypothyroidism was recorded in 16 patients (11 %), 70 patients (48 %) had hyperprolactinaemia while one patient had hypoprolactinaemia. One patient had peak cortisol of 491 mmol/l (cut-off < 500 mmol/l). Neither hypogonadotrophic hypogonadism nor secondary hypothyroidism was significantly related to etiology, time from BI to endocrine testing, treatment with antiepileptic drugs, or hyperprolactinaemia.

Conclusion A substantial number of severely brain-injured patients had hormonal alterations after approximately 3 months follow-up. The importance of this finding for the further rehabilitation process and the long-term consequences, however, remains to be proven in a longer follow-up of this particular patient group.

Disclosure: No significant relationships.

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Assessment of Glucocorticoid Therapy with Salivary Cortisol in Secondary Adrenal Insufficiency

F. Ceccato, N. Albiger, M. Barbot, A. Daniele, Z. Marialuisa, S. Ferasin, R. Silvia, S. Koutroumpi, G. Occhi, F. Mantero, C. Scaroni
Dept of Medicine Dimed, University of Padova, Padua, Italy

Introduction Appropriate glucocorticoid replacement therapy in adrenal insufficiency (AI) is crucial, given the risks of chronic under- or overtreatment, particularly in patients on multiple medications. Salivary sampling allows for non-invasive, stress-free cortisol

measurement. Our aim was to determine whether salivary cortisol measurement is helpful in assessing the adequacy of glucocorticoid therapy with cortisone acetate (CA) twice daily in patients with secondary AI.

Methods and Materials A prospective cohort study at the Endocrinology Unit of the Padua University Hospital, Italy. Six samples of salivary cortisol were collected from 28 patients with secondary AI on CA treatment and from 36 healthy volunteers at fixed times of the day, and used to calculate levels at each time-point and the area under the curve (AUC) across the different sampling times.

Results Salivary cortisol median levels were lower in patients than controls in the morning (1.54 vs 7.44 ng/ml; $p < 0.0001$) but no differences were found in the afternoon or at night before resting. Median salivary cortisol levels were higher in patients immediately following CA administration (11.52 vs 5.14 ng/ml; $p < 0.001$ and 5.50 vs 3.28 ng/ml; $p < 0.05$). Ten patients showed an AUC above the 97.5th percentile of controls, without clinical signs of hypercortisolism, and salivary cortisol levels 90 minutes after each dose of CA predict the AUC ($R^2 = 0.94$; $p < 0.0001$ for the first dose and $R^2 = 0.75$; $p < 0.0001$ for the second dose). All patients had GH-deficiency: those taking GH therapy (n = 17) had similar cortisol levels to healthy controls, while untreated patients had higher levels.

Conclusion Two salivary cortisol determinations, able to predict the daily AUC, may allow assessment of the adequacy of glucocorticoid replacement therapy in secondary AI and for identifying cases of over- or undertreatment.

Disclosure: No significant relationships.

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Changes in Blood Levels of Some Adipose- and Bone-Related Proteins in Patients with Growth Hormone Deficiency of Different Origins

S. A. Mucha, A. Siejka, J. Komorowski
Clinical Endocrinology, Medical University of Lodz, Poland

Introduction Growth hormone deficiency (GHD) has negative effects on metabolic profile leading to an increased cardiovascular mortality and morbidity. The purpose of the study was to investigate the relationship between body weight, IGF-1 levels, and adipose tissue hormones as well as bone-related proteins in patients with untreated GHD of different origins.

Methods and Materials We studied 44 patients with adult-onset (AO) GHD (14 women, 30 men), 12 with childhood onset (CO) GHD (5 women, 7 men), and 11 control subjects without GHD (4 women, 7 men). In the blood of patients with GHD and controls, serum concentrations of specific proteins, such as leptin, adiponectin, resistin, visfatin, and osteoprotegerin (ELISA) were measured.

Results As expected, the lowest IGF-1 concentrations were observed in a CO-GHD group. BMI was significantly higher in the AO- and CO-GHD groups ($p < 0.05$). We found higher levels of leptin in men with AO-GHD than in males without GHD. We also showed significantly higher concentrations of osteoprotegerin in the group of all patients with AO-GHD than in patients with CO-GHD. Moreover, negative correlation between adiponectin and Body Mass Index (BMI) in all patients with CO-GHD was observed, as well as the negative correlation between serum leptin and adiponectin in male patients with CO-GHD.

Conclusion The obtained results allow for a better understanding of metabolic disorders and causes of complications in patients with growth hormone deficiency. Further studies are needed to elucidate the role of adipose and bone-related markers in the increased risk of cardiovascular and bone complications in patients with GHD.

Disclosure: No significant relationships.

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Prevalence of Hypovitaminosis D in Adult Patients with Hypopituitarism

C. di Somma, M. C. Savanelli, L. Vuolo, E. Scarano, V. Brunelli, M. Rubino, A. Colao
Dept of Molecular and Clinical Endocrinology and Oncology, University of Naples Federico II, Italy

Introduction Italy is considered a country with a high prevalence of 25 (OH) vitamin D deficiency. Changes in vitamin D serum levels were linked with the onset and progression of several diseases, including osteoporosis and cardiovascular disease. Hypopituitary patients show several comorbidities like cardiovascular disease and osteoporosis.

Aim To evaluate the prevalence of vitamin D deficiency in hypopituitary patients.

Methods and Materials 57 patients were enrolled (27 M/30 F), 24 with hypopituitarism and GH deficiency (GHD) and 33 non-GHD patients with hypopituitarism, aged between 30 and 80 years, and 90 controls (age-, sex-, and BMI-matched). In all subjects we evaluated serum levels of vitamin D, PTH, serum, and urinary Ca and P. 25 (OH) Vitamin D levels were considered deficient if < 10 ng/ml, insufficient if 10–30 ng/ml, and normal if > 30 ng/ml.

Results 25 (OH) Vitamin D levels were lower in patients than in controls (21.7 ± 10 ng/ml vs 31.4 ± 12 ; $p < 0.01$). The levels of PTH, calcium, and phosphorus did not differ between patients and controls. Six patients (11 %) had vitamin D deficiency, 40 (70 %) had insufficient levels of vitamin D, and 11 patients (19 %) had normal levels of vitamin D. There were no differences in both levels of vitamin D and in the prevalence of hypovitaminosis D between the 2 groups of patients ($p = ns$). In addition, vitamin D levels were inversely related to age ($p < 0.05$).

Conclusion Hypopituitary patients show vitamin D levels lower than controls, so in these patients, vitamin D deficiency has to represent an additional risk factor for the hypopituitary comorbidities such as cardiovascular disease and osteoporosis. It would therefore be advisable to ensure vitamin D supplementation in all patients with inadequate levels.

Disclosure: No significant relationships.

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Pituitary Stalk Interruption Syndrome with Multiple Hormone Deficiencies

E. Kiliç Kan, G. Cengiz Ecemis, H. Atmaca, B. Çolak, A. Atmaca
Endocrinology and Metabolism, 19 Mayıs Üniversitesi Tıp Fakültesi Endokrinoloji Bilim Dalı Atakum, Samsun, Turkey

Introduction Pituitary stalk interruption syndrome (PSIS) is a congenital abnormality of the pituitary. It is rarely seen as a cause of pituitary deficiency. It is usually characterized by a triad of a very thin or interrupted pituitary stalk, an ectopic (or absent) posterior pituitary (EPP), and hypoplasia or aplasia of the anterior pituitary visible on magnetic resonance imaging (MRI). Usually, such patients present with short stature and are misdiagnosed as idiopathic pituitary deficiency. We present 3 PSIS patients with multiple pituitary hormone deficiencies and discuss their clinical manifestation.

Methods and Materials Data of 3 patients with short stature retrospectively analyzed for clinical, laboratory, and imaging features. We present 3 PSIS patients diagnosed with clinical and MRI findings.

Results Three male patients, mean age 35.3 years. They were admitted for short stature at the ages of 7, 12, and 20. All of them had multiple pituitary deficiencies and were treated with levothyroxine, prednisolone, sex hormone, and one patient with GH therapy. In one patient breech delivery history was detected. They all had small adenohypophysis, one patient had ectopic neurohypophysis, one patient had not visible infundibulum, and 2 patients had $1/3$ visible pituitary stalk on MRI.

Conclusion The most remarkable clinical manifestation of patients with PSIS was growth retardation. Non-visualization of pituitary

stalk and hypoplasia of the anterior hypophysis on MRI may indicate adenohypophyseal dysfunction. Therefore, management not only includes hormonal replacement but also close follow-up to monitor other hormonal deficiencies, especially if they initially present with isolated GH deficiency.

Disclosure: No significant relationships.

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Secondary Adrenal Insufficiency Patients Report Greater Impact of Adrenal Insufficiency than Primary Patients. A Worldwide Patient Survey

M. Forss¹, G. Batcheller¹, S. Skrtic², G. Johannsson²

¹Duocort Pharma, Helsingborg; ²Dept of Endocrinology, Institute of Medicine, University of Gothenburg, Sweden

Introduction The aim was to survey patients with adrenal insufficiency (AI) to document current practice in glucocorticoid replacement therapy, and assess self-perceived health status and outcomes.

Methods and Materials Patients were recruited via patient organizations to respond anonymously to a web-based survey (data on age and gender not collected). The survey was open for 3 months in 2008.

Results Responders were 1245 patients with AI. Of these, 84 % had primary AI, 11 % had secondary AI, and 5 % were unsure. Hydrocortisone was used by the majority of responders (75 %). Median dosing was 20 mg in both primary and secondary respondents. Compared to secondary AI patients, a TID dosing regimen (34 % vs 21 %) and weight or body surface area-adjusted dosing (37 % vs 11 %) were both more common in primary AI patients. A larger proportion of secondary AI perceived their AI to affect their QoL than those with primary AI (87 % vs 60 %). The extent of QoL impairment was reported to be higher in secondary AI. A larger proportion of secondary patients (87 % vs 61 %) described fatigue during the day as a problem, with greater impact on daily activities than reported by primary patients. A smaller proportion of secondary AI patients consider themselves fit to work (39 %) compared to primary AI (65 %). A larger proportion of secondary AI patients reported part-time work and more absenteeism.

Conclusion Secondary AI patients report greater QoL impairment, more fatigue during the day, and less ability to work than primary AI patients. The median dosing used in primary and secondary AI patients was the same. These data suggest, from the patients' perspective, a need for improvement in glucocorticoid replacement strategies, especially for secondary AI patients.

Disclosure: No significant relationships.

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Hypertension Impairs Potential Benefits of GH Replacement Therapy on Left Ventricular Remodeling in Hypopituitary Patients

L. Curtò¹, C. de Gregorio², F. Marini¹, A. Recupero², S. Coglitore², F. Trimarchi¹, S. Cannavò¹

¹Section of Endocrinology; ²Section of Cardiology, Dept of Medicine and Pharmacology, University of Messina, Italy

Introduction Data on the adaptation of left ventricular (LV) mass to systemic hypertension (SH) in patients with growth hormone deficiency (GHD) are limited. We assessed LV geometric remodeling (LVGR) and function in GHD patients with and without SH, before and after long-term GH replacement therapy (GHRT).

Methods and Materials 51 patients (29 F), mean age 45 ± 15 years, underwent transthoracic echocardiogram at diagnosis (T0) and after 50 ± 26 months (T1) of GHRT. Patients were divided into 2 groups based on SH presence (group A: $n = 15$; 29 %) or absence (group B: $n = 36$; 71 %). Absolute and indexed LV mass (LVM and LVMi), LVGR, and function were assessed, compared with 24 control subjects (15 F).

Results Optimal GH replacement dose was reached in about 90 % of patients. At T1, IGF-1 levels increased from 96 ± 50 to 180 ± 60 ng/ml (+85 %; $p < 0.000001$), from 105 ± 60 to 174 ± 32 ng/ml in group A (+68 %; $p < 0.0005$) and from 92 ± 46 to 183 ± 68 in group B (+91 %; $p < 0.000001$). At T0, LV chamber size and function were not significantly different between patients and controls, except for midwall fractional shortening (MFS), lower in GHD ($p < 0.001$). LVM and LVMi were higher in group A than group B and controls. At T0, LV hypertrophy (LVH) was present in 27 % of patients (group A: 40 %, group B: 22 %) and 4 % controls ($p = 0.05$). At T1, the LVH rate increased to 60 % in group A ($p = 0.05$ vs T0) and decreased to 19 % in group B ($p = \text{ns}$ vs T0) ($p = 0.01$, group A vs group B). A significant MFS decrease was only seen in group A (-7.2 %; $p < 0.05$ vs group B and T1 vs T0).

Conclusion SH impairs efficacy of GHRH on LV remodelling. Coexistence of LVH and depressed MFS (< 18 %) might permit precocious identification of patients with higher CV risk, thus requiring more careful blood pressure monitoring in hypertensive GHD patients.

Disclosure: No significant relationships.

P155

Estrogen Therapy Effects on Different Hormonal Changes in Women with Hypopituitarism

I. Illovskaya¹, V. Zektser², N. Goncharov³, G. Melnichenko⁴, I. Dedov⁵

¹Therapeutic Endocrinology, Moscow Regional Research & Clinical Institute;

²Chronomedicine, 1st Moscow State Medical University N. B. I. M. Sechenov;

³Biochemical Endocrinology, National Endocrinology Research Center, Moscow;

⁴Clinical Endocrinology; ⁵General Director, National Research Center for Endocrinology, Moscow, Russian Federation

Introduction Patients with hypopituitarism usually need complex hormonal therapy for different types of deficiency. We investigated changes of androgens, free T4, and prolactin levels during treatment with sex steroids in women with central hypogonadism.

Methods and Materials We examined 55 women (mean age 25 [21–32] years) with acquired hypopituitarism after neurosurgery ($n = 48$; 87.3 %) and primary “empty” sella turcica ($n = 7$; 12.7 %); duration of hypopituitarism 6.3 (5–8) years. 30 had hypogonadism and hypothyroidism, 15 hypogonadism, hypothyroidism and hypocortisolism; 10 hypogonadism, hypothyroidism, hypocortisolism and GH insufficiency. Before the study all of them took L-thyroxine (50–100 µg/day), 25 women took hydrocortisone (5–15 mg/day), neither sex steroids nor GH replacement. Free T4 levels were within reference range in all women (12.1 [10.7–13.5] pmol/l, range 9–20); 10 women had hyperprolactinemia before but now they had normal prolactin levels without dopamine agonists (209 [88–418] mU/l, range 90–540). Androgen levels were decreased: total T 0.1 (0.1–0.2) nmol/l (range 0.7–2.7), DHEA-S 128 (83–850) nmol/l (range 1150–9500). They started receiving 2 mg 17β-estradiol (14 days) and 2 mg 17β-estradiol + 10 mg dydrogesterone (14 days) in a cyclic regimen, duration of treatment 4.6 (3.2–5.6) years.

Results After 3 months of sex steroid therapy 15/55 (27.3 %) patients demonstrated decrease of free T4 levels < 9 pmol/l, L-thyroxine dose was increased by 25–50 µg. During the observation free T4 levels were stable. Prolactin level elevation up to 1200–3000 mU/l was observed in 3/10 patients with previous hyperprolactinemia. Two of them needed additional intake of dopamine agonists. During therapy patients with secondary adrenal insufficiency did not need any changes of the hydrocortisone therapy. Total T levels did not change considerably 0.1 (0.08–0.3) nmol/l. DHEA-S levels increased: 270 (94–1357) nmol/l ($p < 0.05$).

Conclusion Increase of L-thyroxine dose could be demanded during sex steroid therapy in women with hypopituitarism. Prolactin levels should be monitored in women with previous hyperprolactinemia. Estradiol + dydrogesterone therapy did not worsen androgen deficiency.

Disclosure: No significant relationships.

P156

Hypopituitarism Presenting with Neuropsychiatric Symptoms and Hyponatremia

A. K. Uzum¹, G. Yenidunya¹, M. Altinkaynak², S. Ciftci Dogansen¹, B. Canbaz¹, S. Yarmar¹

¹Division of Endocrinology and Metabolism, Dept of Internal Medicine; ²Dept of Internal Medicine, Faculty of Medicine, Istanbul University, Turkey

Introduction Hypopituitarism can present with mental changes including depression, delusion, and psychosis.

Methods and Materials We present 2 patients with Sheehan's syndrome manifested with mental symptoms and hyponatremia.

Case 1: A 36-year-old woman had a delivery complicated with abnormal vaginal bleeding necessitating massive transfusion 4 months ago. Blurry vision and diplopia had developed on day 15 *post partum*, accompanied by weakness and fatigue. She was hospitalized at the psychiatry department with postpartum depression. Laboratory assessments showed hyponatremia, anterior pituitary insufficiency, and increased FT4 with suppressed TSH levels. Thyroid autoantibodies were positive. After labor she was amenorrheic and unable to lactate. We transferred her to our clinic with Sheehan's syndrome complicated with postpartum thyroiditis and initiated glucocorticoid replacement immediately. Sella MRI indicated partial empty sella. Central hypothyroidism developed during follow-up. L-thyroxine and estrogen/progesterone replacement were added subsequently.

Case 2: A 53-year-old woman was hospitalized at the reanimation unit with pneumonia complicated by hypotension, hyponatremia, and adult respiratory distress syndrome. She was given antibiotherapy with high-dose glucocorticoids for septic shock and was extubated within a few days. Hypopituitarism was suspected as the patient had a pale and apathetic appearance, lethargy, and absence of axillary and pubic hair. The diagnosis was supported by low anterior pituitary hormone levels. Prednisolon 5 mg/day and subsequently L-thyroxine replacement were initiated. In her past medical history, she had had 8 deliveries and had been amenorrheic since her last delivery. She had been receiving antidepressant drugs, admitted to emergency rooms with hypotension and hyponatremia necessitating saline infusions several times.

Results Combination of hyponatremia and hypocortisolism in a woman with a past history of massive hemorrhage at delivery should alert the physician for hypopituitarism.

Conclusion Despite the fact that the number of complicated deliveries have been decreasing over the years, Sheehan's syndrome is still not a rare diagnosis.

Disclosure: No significant relationships.

Basic and Animal Studies

P157

Perinatal Exposure of Rats to Calcium Channel Blockers Modifies Neuroendocrine Functions and Reproductive Behavior

A. Reznikov, N. Nosenko, L. Tarasenko, P. Sinitsyn, A. Lymareva

Dept of Endocrinology of Reproduction and Adaptation, V. P. Komissarenko Institute of Endocrinology and Metabolism, Kiev, Ukraine

Introduction The mechanisms of sex-associated development of behavioral and neuroendocrine phenotypes are poorly understood. In this work, early and long-term neuroendocrine and behavioral effects of calcium channel blockers administered in pre- and early postnatal life were studied.

Methods and Materials Pregnant Wistar rats were fed daily with nimodipine (20 mg/kg bodyweight [bw]/day) from gestational days 15–21. The descendants aged 5 or 10 days, 3, 6, or 8 months were taken for the study. Other female and male animals were given verapamil (subcutaneously in a dose of 0.5 mg/kg bw/day) on postnatal days 3–7 and explored on the same days as descendants. Hypo-

thalamic-pituitary-adrenal axis, reproductive behavior, testosterone metabolism, and electrophoretic profile of soluble proteins in the brain discrete areas were studied.

Results Prenatal nimodipine resulted in enhancement of testosterone conversion to dihydrotestosterone both in the preoptic area (POA) and medial basal hypothalamus (MBH) of 10-day-old females and in the POA of males, while verapamil induced an increase of testosterone transformation to estradiol in MBH in females. A modifying effect of both blockers on the soluble proteins in POA and MBH was found in 5- and 10-day-old offspring, reflecting disorders in sexual brain differentiation. Nimodipine treatment caused the feminization of sexual behavior in males and masculinization in females, while verapamil resulted in defeminization in females. A decrease of basal blood plasma corticosterone level in males caused by nimodipine, prolongation of adrenocortical reaction to noradrenaline infusion into the 3rd brain ventricle of males and females exposed to calcium channel blockers were found in adults.

Conclusion The findings of the study demonstrate the essential role of calcium signalling in the early developmental programming of neuroendocrine control of reproduction and adaptation.

Disclosure: No significant relationships.

P158

Influence of Dehydroepiandrosterone (DHEA) on Behavior in Old Non-Human Primates

N. Goncharov, A. Dzhokua, G. Katsiya, Z. Kulova, V. Barkaya
Biochemical Endocrinology, Endocrinology Research Center, Moscow, Russian Federation

Introduction At present, there is a lack of satisfactory understanding of how DHEA affects cognition and nervous system function. DHEA produced dose-dependent response. Intermediate dose-enhanced memory and the highest doses had no effect. Aim of the present study was to evaluate effects of long-term DHEA administration of physiological doses on the higher brain activity (HBA) in rhesus macaques (RM) at the limits of their biological age.

Methods and Materials The study included 9 male RM aged 24–30 years. Five of them were given im injections of DHEA (1 mg thrice weekly for 3 months). Four control monkeys were administered the vehicle alone. Cortisol, testosterone, and free thyroxine were measured by chemiluminescent immunoassay. HBA was studied by classical motor-food conditioning (to estimate long-term memory) and by determining delayed response time (a measure of short-term memory). RM had to respond to a positive signal (1000 Hz) and no bar-pressing was required in response to a negative signal (400 Hz). RM were free to move in the cage during the experiment. Their behaviour was tested before, within 1, 2, and 3 months of DHEA administration and 3 months after its termination.

Results HBA increased of all 5 RM within 1, 2, and 3 months after the beginning of DHEA administration. Both long- and short-term memory stably improved while response time decreased from 5 to 1–1.5 sec. The behavior of RM changed radically from passive to enhanced motor activity and food motivation. DHEA administration caused a rise in testosterone and free thyroxine levels. These effects of DHEA persisted as long as 3 months after DHEA treatment.

Conclusion Administration of physiological doses of DHEA to old RM induced a stable increase of HBA with harmonization of excitation and inhibition processes.

Disclosure: No significant relationships.

P159

Immunohistomorphometric Study of GH Cells after Treatment with Genistein in an Andropause Animal Model

V. Milosevic¹, S. Trifunovic¹, D. Bijelic¹, F. Percinic-Popovska², N. Nestorovic¹, M. Sekulic¹, V. Ajdzanovic¹
¹Dept of Cytology, Institute for Biological Research "Sinisa Stankovic", University of Belgrade, Serbia; ²Dept of Histology and Embryology, Faculty of Veterinary Medicine, SS Cyril and Methodius University, Skopje, Macedonia

Introduction Andropause, the culminating phase of ageing in males, implies the decline of growth hormone (GH)/insulin-like growth factor-1 (IGF-1) axis, which is responsible for somatic development. On the other hand, the soybean phytoestrogen genistein is increasingly consumed as an alternative therapeutic for age-related diseases, namely cardiovascular conditions, cancer, and osteoporosis. The aim of our study was to examine the effects of genistein on the immunohistomorphometric features of GH cells in an andropause animal model.

Methods and Materials 16-month-old Wistar rats were divided into sham-operated (SO), orchidectomized (Orx), and genistein-treated orchidectomized (Orx + G) groups. Genistein (30 mg/kg/day) was administered subcutaneously for 3 weeks, while the SO and Orx groups received the vehicle alone. GH cells were identified by the peroxidase-antiperoxidase (PAP) immunohistochemical procedure.

Results In the Orx + G group, GH cell volumes as well as the relative volume densities were significantly ($p < 0.05$) decreased by 18 % and by 62 %, respectively, in comparison with the same parameters of SO animals. Compared to Orx animals, the cell volumes and relative volume densities of GH cells in the Orx + G group were also significantly ($p < 0.05$) decreased by 14 % and by 65 %, respectively.

Conclusion It can be concluded that chronically applied genistein suppresses the immunohistomorphometric parameters of pituitary GH cells in an andropause animal model.

Disclosure: No significant relationships.

P160

Combination Effects of High-Fat Diet and Acute Food-Shock Stress on Carbohydrate Metabolism

J. Ghalami, H. Zardooz, F. Rostamkhani, Z. Zardooz
Neuroscience Research Center and Dept of Physiology, Faculty of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran

Introduction High-fat diet and/or stress may be the cause of metabolic disorders such as metabolic syndrome and diabetes. In this study, the possible interaction of acute food-shock stress and high-fat diet (45 % cow intra-abdominal fat) on carbohydrate metabolism was investigated. Glucose tolerance and plasma insulin and corticosterone levels were evaluated in male Wistar rats.

Methods and Materials Animals were divided into 2 groups of normal and high-fat diets. The animals of the high-fat diet group received high-fat food for 4 weeks before the beginning of the experiments. Each diet group was subdivided into control and stressed groups. Stress was induced by a communication box for 1 hour. Blood sampling was done by using the retroorbital puncture method at the beginning and the end of the trials. The oral glucose tolerance test (OGTT) was performed after 16-h fasting.

Results High intra-abdominal cow fat diet decreased basal plasma glucose levels while it did not affect basal plasma insulin levels in the fasted rats. Food-shock stress increased plasma insulin concentrations only in the fasted normal-diet animals. Plasma glucose concentration increased 15 min after OGTT only in the normal diet stressed group whereas plasma insulin levels increased in the stressed groups of both diets. In both diet groups food-shock stress increased plasma corticosterone concentration compared to before stress induction. Finally, no significant interaction between the diet type and the response to stress was observed.

Conclusion The data of the present study has shown that the high-fat diet used in this study does not only deteriorate the effect of stress on carbohydrate metabolism but also may have a protective role against metabolic impairments induced by the acute food-shock stress.

Disclosure: No significant relationships.

P161

Isolated Islet Insulin Secretion in Response to Psychological Stress and High-Fat Diet

H. Zardooz¹, J. Ghalami¹, F. Rostamkhan²

¹Neuroscience Research Center and Dept of Physiology, Faculty of Medicine, Shahid Beheshti University of Medical Sciences; ²Dept of Biology, Shahr-e-rey Branch, Islamic Azad University, Tehran, Iran

Introduction One of the most important issues in modern societies is the prevalence of metabolic disorders induced by diet and stress. In this study, the effect of a high-fat (45 % cow intra-abdominal fat) diet on glucose-stimulated insulin secretion from isolated islets in the presence of acute psychological stress was examined in male Wistar rats.

Methods and Materials The animals were divided into high-fat and normal diet groups and each group was further divided into stress and control subgroups. The high-fat diet group consumed high-fat food for 30 days before the beginning of the experiment. Psychological stress was induced by a communication box for 1 hour. Blood samples were taken by using the retroorbital puncture method before and immediately after stress exposure, then plasma corticosterone levels were determined. 24 hours after the stress session, the animals were dissected for islet isolation and glucose-stimulated insulin secretion was studied statically at different glucose concentrations (5.6, 8.3, 16.7 mM).

Results In both diet groups, a mild increase of plasma corticosterone levels was observed immediately after stress exposure. Psychological stress decreased insulin secretion from isolated islets in response to 8.3 mM glucose concentration in normal diet rats and in response to 5.6 and 8.3 mM glucose concentrations in the high-fat diet animals. Statistical analysis did not show any significant interaction between stress and high-fat diet in response to different glucose concentrations.

Conclusion The results of the present study showed that cow intra-abdominal fat (45 %) administered for 30 days did not affect glucose-induced insulin secretion from isolated islets alone or in the presence of acute psychological stress.

Disclosure: No significant relationships.

P162

Effect of Social Isolation and Conspecific Presence on Behavior, Neuroendocrine Stress Hormones, and Stress-Related Gene Expression in Postnatal Pigs

E. Kanitz¹, T. Hameister¹, A. Tuchscherer², M. Tuchscherer³, B. Puppe³

¹Behavioural Physiology; ²Genetics and Biometry; ³Behavioural Physiology, Leibniz Institute for Farm Animal Biology, Dummerstorf, Germany

Introduction It is known that the presence of a significant social partner can alleviate the behavioral and physiological responses to stressful stimuli. This study investigated whether social support in postnatal pigs by familiar or unfamiliar conspecifics could buffer behavioral and neuroendocrine stress responses of a 4-h social isolation.

Methods and Materials Piglets were classified into 4 treatment groups (control without isolation, isolation alone, isolation with familiar or unfamiliar piglets) and examined at 7, 21, or 35 days of age. Behavioral responses were analyzed in repeated open-field/novel-object (OF/NO) tests, and the mRNA expression of glucocorticoid receptor (GR), mineralocorticoid receptor (MR), 11 β -hydroxysteroid dehydrogenase 1 and 2 (11 β -HSD1 and 11 β -HSD2) was quantified by real-time RT-PCR in stress-related brain regions.

Results Piglets isolated alone were more excited in the OF/NO test and showed higher ACTH and cortisol concentrations compared to controls. Furthermore, these piglets also displayed a lower MR/GR ratio and a higher 11 β -HSD2 mRNA expression in different brain regions. Social support during isolation reduced behavioral activity in the OF/NO test and diminished the release of stress hormones compared to piglets isolated alone. Moreover, the imbalance of MR/GR mRNA expression was reversed, and 11 β -HSD2 mRNA expression was not affected in socially supported piglets. With respect to the level of familiarity, the behavioral and neuroendocrine responses of piglets isolated with a familiar conspecific were more comparable with that of controls. There was no age-dependent effect of social support on analyzed parameters.

Conclusion In conclusion, social support can buffer the negative consequences of psychosocial stress in pigs indicated by diminished arousal and neuroendocrine stress response. These results should be considered in livestock practices with respect to improved welfare and emotional experience.

Disclosure: No significant relationships.

P163

From the Rat to the Beta Cell: A Fast and Effective Technique of Separation of Langerhans Islets and Direct Purification of Pancreatic Beta Cells

G. Tamagno¹, S. Vigolo², M. Olivieri², C. Martin², E. de Carlo²

¹Dept of Endocrinology & Diabetes Mellitus, St Vincent's University Hospital, University College Dublin, Ireland; ²Dept of Medical and Surgical Sciences, 3rd Medical Clinic, University of Padua, Italy

Introduction Isolated Langerhans islets represent a useful model for the study of the endocrine pancreas. The possibility to purify pancreatic beta cells from a mixed Langerhans islet cell population may lead towards a dedicated focus on beta cell research. We describe an effective and rapid immunomagnetic technique for the direct purification of beta cells from isolated Langerhans islets of the rat.

Methods and Materials After sacrificing the rat, the Langerhans islets were separated by ductal injection of the pancreas with collagenase, altered to a mixed Langerhans islet cell population, and incubated with conditioned immunomagnetic beads targeted to the beta cell surface. The beads were previously coated with a specific antibody against the surface of the beta cell, namely K14D10. The suspension of mixed Langerhans islet cells and immunomagnetic K14D10-conditioned beads was pelleted by a magnetic particle concentrator to isolate the bead-bound cells, which were finally suspended in a culture medium.

Results Purified cells were immunoreactive for insulin and no glucagon-positive cells were detected at immunocytochemistry. RT-PCR confirmed the purification of the pancreatic beta cells.

Conclusion This immunomagnetic technique allows a rapid, effective, and consistent purification of beta cells from isolated Langerhans islets in a direct manner by conditioning the immunomagnetic beads only. This technique is easy, fast, and reproducible. It promises to be a reliable method for providing purified beta cells for in vitro research.

Disclosure: No significant relationships.

P164

8-Prenylningenin Modulates Proliferation and BCL-2 Expression in GH3 Cells

B. Voellger¹, R. Rupa¹, E. Kirches², C. Mawrin², R. Firsching¹

¹Klinik für Neurochirurgie; ²Institut für Neuropathologie, Otto-von-Guericke-Universität, Magdeburg, Germany

Introduction 8-Prenylningenin (8-PN), a phytoestrogen discovered in hops, is known to modulate proliferation in various tumour cells. This study aims to analyze the impact of 8-PN on the proliferation and expression of apoptosis modulators in GH3 pituitary adenoma cells of the rat in vitro.

Methods and Materials 10⁶ GH3 cells per well were cultivated in 6-well plates for 4–48 hours with 0.5–50 μ M 8-PN. Ethanol served as solvent for 8-PN. Medium and medium-containing ethanol served as controls. Cells were counted twice using a hemocytometer. Relative expressions of B-cell lymphoma-2 protein (BCL-2) and BCL-2-associated X protein (BAX) as compared to beta 2-microglobulin (B2MG) were assessed in 2 cell passages using quantitative real-time polymerase chain reaction (QRT-PCR). Statistical analysis was conducted using NeoOffice and the R software package. Pairwise T served as statistical test. $P < 0.05$ was supposed to be statistically significant.

Results After 48 hours of incubation, we counted 1.24×10^6 cells per well (CPW) in the control medium, 1.36×10^6 CPW in the ethanol control, 1.54×10^6 CPW in 0.5 μ M 8-PN, 1.37×10^6 CPW in 5 μ M 8-PN and 1.11×10^6 CPW in 50 μ M 8-PN. Cell counts were significantly decreased at 50 μ M 8-PN as compared to the ethanol control ($p = 0.0475$), 0.5 μ M 8-PN ($p = 0.0062$) and 5 μ M 8-PN ($p = 0.0418$). After 4 hours of incubation, a significant peak in BCL-2 expression was detected in cells treated with 0.5 μ M 8-PN as compared to the ethanol control ($p = 0.031$) and 50 μ M 8-PN ($p = 0.031$). Significant changes in BAX expression could not be found. Changes in the BAX/BCL-2 ratio did not reach statistical significance ($p = 0.064$ and above).

Conclusion In GH3 cells, 8-PN induces an early significant peak in BCL-2 expression and increases proliferation at 0.5 μ M, whereas it exerts a significant anti-proliferative effect at 50 μ M.

Disclosure: No significant relationships.

P165

Total and High-Molecular Weight (HMW) Adiponectin Levels in Cardiac Tissue in a Rat Model of Myocardial Infarction

M. Kalisz¹, E. Wolinska-Witort¹, M. Maczewski², U. Mackiewicz², W. Bik¹, B. Baranowska¹, M. Chmielowska¹, L. Martynska¹, E. Wasilewska-Dziubinska¹
¹Dept of Clinical Neuroendocrinology; ²Dept of Clinical Physiology, Medical Centre of Postgraduate Education, Warsaw, Poland

Introduction Adiponectin is an adipose-derived cytokine that circulates in serum as a trimer, hexamer, and HMW form. Adiponectin is also synthesized by cardiomyocytes and may play a potentially important role in the regulation of cardiac functions. It has been reported that adiponectin accumulates in the heart following ischemic damage primarily as the result of leakage from the vascular compartment. Recently, BNP (B-type natriuretic peptide) a marker of cardiac dysfunction, which is synthesized in the ventricular myocardium, has been identified as a potential regulator of adiponectin production.

Methods and Materials Cardiac tissue samples were obtained from 2 groups of rats: group 1 was SHAM-operated ($n = 9$) and group 2 included animals 2 months after surgical induction of myocardial infarction ($n = 9$). The heart was divided into right and left ventricles and homogenized in lysis buffer. Total and HMW rat adiponectin levels were determined with ELISA kit. The BNP mRNA was detected by real-time PCR in the left ventricular tissue.

Results Two months after myocardial infarction the level of total adiponectin increased in the left ventricle compared with the SHAM-operated group. Adiponectin levels in the right ventricle in both groups of rats were similar. Equally, myocardial infarction had no significant effect on HMW adiponectin levels in the left and right ventricles. The BNP mRNA levels in the left ventricular tissue were 2.5 $\times$ higher in rats after myocardial infarction than in SHAM-operated animals.

Conclusion Accumulation of total adiponectin in the left ventricle after myocardial infarction could be a consequence of ischemic damage. No significant change in HMW adiponectin levels may suggest that after myocardial infarction other adiponectin isoform (MMW and/or LMW) concentrations increase.

Disclosure: No significant relationships.

P166

Cabergoline Modifies Cardiac Development and Behavior in Zebrafish Larvae

N. S. Eltz¹, L. R. Nery¹, C. S. Hackmann¹, L. Duarte¹, M. R. M. Vianna¹, M. A. Czepielewski²

¹Zelab and Laboratório de Biologia do Desenvolvimento do Sistema Nervoso, Faculdade de Biociências PUCRS; ²PPG Endocrinologia, Faculdade de Medicina UFRGS, Porto Alegre, Brazil

Introduction Cabergoline (CAB), a dopaminergic D₂ agonist, has been used for many years in the treatment of hyperprolactinemia, prolactinomas, acromegaly, and Parkinson's disease. Most recently, it has been indicated for Cushing's disease and Nelson's syndrome. Long-term use of CAB has been associated with side effects including cardiac disturbances in adults. In patients with hyperprolactinemia, the use of CAB for fertility improvement and during pregnancy remains controversial.

Methods and Materials In this study, wild-type zebrafish embryos were exposed to CAB at 0.01, 0.1, 1.0, and 10.0 mg/l for the initial 48 hpf when toxicity, cardiac cell apoptosis, heart rate, and abnormalities were evaluated. At 10 dpf these parameters were re-evaluated and a general behavioral screen performed. All experiments were made in triplicates ($n = 10$), approved by the institutional animal ethics committee and compared using ANOVA followed by Tukey *post hoc*.

Results Evaluation of embryotoxicity showed no effects on survival and hatching efficiency, but a delayed eclosion rate and lack of coloration was observed in the 10.0-mg/l-treated embryos. CAB at 1.0 and 10.0 mg/l caused cardiac edemas in 34.5 % and 54.5 % of treated embryos, respectively. Heart rate was decreased in 10.0-mg/l CAB-treated animals at 48 hpf, but returned to control levels at 10 dpf. Apoptotic cell death on heart tissue was evaluated using acridine orange and was also increased in the 1.0-mg/l- and 10.0-mg/l-treated animals at 48 hpf and normal at 10 dpf.

Conclusion Despite the lack of persistent effects on heart rate and apoptosis, animals treated with the 2 higher doses of CAB spent significantly more time mobile when evaluated at 10 dpf. These results demonstrate a deleterious effect of CAB on cardiac development of zebrafish and a persistent effect on behavior which underlying molecular mechanisms need to be investigated. Data gathered here could contribute to clarify the effects of CAB found in human heart.

Disclosure: Financial support from CNPq, FAPERGS, and PUCRS.

P167

Effect of Octreotide (OCT) and MTOR Inhibitors (MTORi) on Cell Proliferation in Hepatocellular Carcinoma (HCC) Cell Lines

C. Pivonello¹, G. Vitale², A. di Samo³, F. Izzo⁴, M. Negri¹, M. Samataro¹, G. Cuomo¹, A. Colao¹, L. J. Hofland⁵, R. Pivonello¹

¹Dept of Clinical and Molecular Endocrinology and Oncology, University of Naples Federico II; ²Scienze Mediche, Istituto Auxologico Italiano IRCCS, Milan; ³Infectious Diseases and Interventional Ultrasound Unit, D. Cotugno Hospital, Naples; ⁴Surgical Oncology, Pascale National Cancer Institute, Naples, Italy; ⁵Division Endocrinology, Internal Medicine, Erasmus Medical Center, Rotterdam, The Netherlands

Introduction HCC has been described to present neuroendocrine features, although controversial data have been reported on the effect of somatostatin analogues (SA) on tumor proliferation either in vivo or in vitro. The aim of the study was to evaluate the expression levels of somatostatin receptors (SSTR) as well as mTOR, p70S6k, and 4eBP1, and to investigate the in vitro effect of SA and mTORi, used alone and in combination, on cell proliferation in 2 HCC cell lines, HepG2 and HuH-7.

Methods and Materials mTOR, p70S6k, 4eBP1, and SSTR expression were assessed by RT-qPCR, whereas SSTR expression and localization were also evaluated by western blot (WB) and immunocytochemistry (ICC). Cell proliferation was tested using a DNA assay.

Results SSTR1, -2, and -5 as well as mTOR, p70S6k, and 4eBP1 were expressed in both cell lines. In HuH-7, SSTR2 and SSTR5 were localized heterogeneously on cell membrane and in cytoplasm. In HepG2, SSTR5 was mainly localized in cytoplasm whereas SSTR2 was detected by WB but not by ICC. The SA OCT showed no significant inhibitory effect in both cell lines. BIM23244 (specific for SSTR2 and -5) and BIM23926 (specific for SSTR1) induced a significant inhibitory effect (18.2 %; $p = 0.02$, and 16.4 %; $p = 0.00$) in HepG2 and HuH-7, respectively. Treatment with mTORi (rapamycin [RAP], everolimus [EVE], temsirolimus [TEM]) resulted in dose-dependent growth inhibition in both cell lines. Co-treatment with OCT (10-8M) and RAP did not show any synergistic or additive effect if RAP was used at 10-8M, but when RAP was used at 10-12M, the co-administration of OCT reverted its anti-proliferative effect in both cell lines up-regulating pERK1/2.

Conclusion These results provide evidence of SSTR expression and mTOR pattern and demonstrated that mTORi induce a growth inhibitory effect in HCC cell lines, although this effect could be, in specific conditions, reverted by SA.

Disclosure: No significant relationships.

P168

Effect of the Co-Administration of MTOR Inhibitors (MTORi) with Octreotide (OCT) or Cabergoline (CAB) on Cell Proliferation in a Human Bronchial Carcinoid Cell Line (NCI-H727)

M. Samataro¹, C. Pivonello¹, M. Negri¹, G. Cuomo¹, F. Cariati¹, A. Faggiano¹, A. Colao¹, R. Pivonello²

¹Dept of Clinical and Molecular Endocrinology and Oncology, University of Naples Federico II, Italy

Introduction mTOR pathway plays a pivotal role on proliferative signalling in neuroendocrine tumors (NET). mTORC1 inhibition is generally followed by an inhibition of cell proliferation, however, accompanied by feedback activation of Akt, probably the key activator of the phenomenon of escape. Somatostatin receptor type-2 (SSTR2) and dopamine receptor type-2 (DR2) are expressed in NETs and their activation induces cell growth inhibition. The aim of current study was to investigate whether the SSTR2 agonist octreotide and DR2 agonist cabergoline inhibit the Akt pathway potentiating the antiproliferative effect of mTORi in the H727 cell line.

Methods and Materials SSTR, DR, and mTOR pathway component (mTOR, 4eBP1, and p70S6K) expression were assessed by RT-qPCR and by immunofluorescence. Cell viability was evaluated by MTT assay. The mTORi rapamycin (RAP) and everolimus (EVE) were tested at escalating doses, as well as CAB and OCT, evaluating cell proliferation after 3, 6, and 9 days. WB was performed to assess intracellular signaling.

Results SSTR2, DR2, and mTOR pathway are expressed in the H727 cell line. RAP and EVE induced a significant time- and dose-dependent inhibition of cell proliferation. 40 % and 60 % of growth inhibition was observed after 3 days of treatment with CAB (10-6M) and OCT (10-6M), respectively, while a slight dose-dependent inhibition was observed after 6 days. Co-treatment of RAP (10-8M) with CAB or OCT (at both concentrations 10-8M and 10-6M) induced a slight and not significant (10 %) cell inhibition of proliferation compared to RAP alone. RAP induced pAkt activation. OCT did not change pAkt expression whereas CAB (10-6M) induced pAkt inhibition, although this effect was reverted when CAB was combined with RAP.

Conclusion mTORi are able to significantly inhibit cell proliferation in H727. CAB and OCT do not show a significant additive anti-proliferative effect and although CAB (10-6M) inhibits pAkt this effect does not overcome pAkt activation induced by RAP.

Disclosure: No significant relationships.

Neuroendocrinology of Appetite and Metabolism

P169

Cyclic Estradiol Treatment Improves Leptin Sensitivity in Diet-Induced Obese Ovariectomized Mice

M. Khaksari Haddad¹, P. Enriori², W. Chen², S. Litwak²

¹Physiology, Kerman Medical Faculty, Iran; ²Dept of Physiology, Faculty of Medicine, Monash University and Monash Obesity and Diabetes Institute, Melbourne, Australia

Introduction The male brain is relatively less sensitive to leptin than the female brain. To understand the underlying mechanism, we tested the hypothesis that sensitivity to leptin in ovariectomized (OVX) mice on a high-fat diet could be restored by estrogen supplementation.

Methods and Materials OVX B6D2 mice were fed either a regular or high-fat diet until they were 15 weeks old. Afterwards they were divided into 3 groups, one group received 2 µg estradiol benzoate for the last 4 weeks and the 2 other groups were administered its oil vehicle (vehicle and pair body weight groups) subcutaneously in a 4-day cyclic regimen. Finally, leptin or aCSF was injected into the third ventricle, and food intake, body weight, and presence of pSTAT3 in the arcuate nucleus (ARC) were measured for 48 h.

Results OVX mice on a high-fat diet increased food intake and body weight and developed extreme obesity compared to those on a regular diet. All these alterations were reversed by a cyclic regimen of estradiol treatment. Administering estradiol to OVX mice on a high-fat diet increased leptin sensitivity and changed body fat distribution, the effect was significant, particularly with respect to body weight, the decrease in food intake, body weight, and the increase in pSTAT3 in ARC was significant and was pronounced in the estradiol treatment group.

Conclusion These data indicate that estrogen acts within the brain to increase leptin sensitivity, and increased signaling of leptin may partially mediate an estradiol-induced inhibitory effect on feeding.

Disclosure: No significant relationships.

P170

Oxytocin Projections to the Nucleus of the Solitary Tract Mediate Increased Meal-Related Satiety Responses after Adrenalectomy

E. Uchoa¹, D. Zahm², B. Borges¹, R. Rorato¹, J. Antunes-Rodrigues¹, L. Elias¹

¹Dept of Physiology, University of Sao Paulo, School of Medicine of Ribeirao Preto, Ribeirao Preto, Brazil; ²Dept of Physiological and Pharmacological Science, Saint Louis University, MO, USA

Introduction Hypophagia induced by adrenalectomy is associated with increased neuronal activation of the nucleus of the solitary tract (NTS) induced by feeding. Oxytocin (OT) neurons in the paraventricular nucleus of the hypothalamus project to the NTS and participate in the hypophagia in response to ADX. This study evaluated the effect of adrenalectomy on the density of OT fibers in the NTS and the intracerebroventricular (icv) pretreatment with OT-receptor antagonist, vasotocin (OVT), on the activation of NTS neurons after refeeding in adrenalectomized (ADX) rats.

Methods and Materials Male Sprague-Dawley or Wistar rats (250–300 g, $n = 4-9$ /group), subjected to ADX or sham surgery, had an icv cannula placed in the lateral ventricle. Half of ADX animals received corticosterone in the drinking water (B: 25 mg/l, ADX+B). Animals were fasted for 16 hours and they were pretreated with icv injection of OVT (5 µg/5 µl) or vehicle (0.9 % NaCl/5 µl). 15 minutes after the injection, animals were re-fed for 2 h or kept fasted, thereafter they were anesthetized and transcardiacally perfused for brain tissue collection.

Results ADX animals showed higher density of OT fibers in the NTS than sham and ADX+B groups. In vehicle-pretreated animals, refeeding increased the numbers of Fos-immunoreactive and Fos/

TH-immunoreactive neurons in the NTS in sham, ADX and ADX+B groups, with a higher number of these neurons in the ADX group. Compared with fasting, refeeding also increased the activation of NTS neurons in OVT-pretreated sham, ADX and ADX+B animals. OVT pretreatment reversed the increased activation of NTS neurons after refeeding in the ADX group, with no effects on sham and ADX+B groups.

Conclusion These data demonstrated that OT projections to the NTS are enhanced after ADX and OT-receptor antagonist reverses the increased activation of NTS induced by feeding in ADX animals, indicating that OT projections to the NTS contribute to the higher satiety-related responses, reducing the meal size induced by adrenalectomy.

Disclosure: No significant relationships.

P171

Pathophysiological and Morphological Changes in the Endocrine Pancreas of P27-Deficient (MENX) Rats

T. Wiedemann¹, M. Irmeler², M. Bielohuby², M. Bidlingmaier², N. Pellegata¹

¹Pathology; ²Institut für Experimentelle Genetik, Helmholtz-Zentrum München;

³Medizinische Klinik und Poliklinik IV, Klinikum der Universität München, Munich, Germany

Introduction MENX is a spontaneous multiple endocrine neoplasia syndrome in the rat. It is caused by a recessively inherited germline mutation of the Cdkn1b (p27) gene. Affected rats develop neuroendocrine tumors and pancreatic beta-cell hyperplasia within their first year of life.

Methods and Materials Since MENX rats develop pancreatic beta-cell hyperplasia our main focus was to analyze the physiological behavior of these rats by oral glucose tolerance test (oGTT). Furthermore, we performed histological and immunohistochemical analysis of the endocrine pancreas. In addition, gene expression levels were determined by Affimetrix Gene ST Arrays using cDNA of microdissected hyperplastic pancreatic islets of MENX and age-matched wild-type rats.

Results Immunohistochemical stainings of the endocrine pancreas revealed insulin and glucagon expression in MENX-affected rats similarly to wild-type rats. Interestingly, preliminary oGTT results demonstrated a gender-dependent, glucose-induced hyperinsulinemia and hypoglycemia present in MENX-affected rats. Expression profiling of hyperplastic pancreatic islets, using a cut-off of > 1.5-fold difference, identified 387 up- and 198 down-regulated genes, compared to wild-type pancreatic islet tissue. Preliminary analysis showed that among the significantly dysregulated genes many belong to the gene ontology category “system development” and “digestion”.

Conclusion MENX-affected rats develop pancreatic beta-cell hyperplasia and reveal a gender-specific impaired insulin secretion but not synthesis. Our initial results suggest that MENX rats are a suitable animal model to improve the understanding of beta-cell proliferation and insulin secretion.

Disclosure: No significant relationships.

P172

Neonatal Events Modify the Response to Ghrelin in Adulthood in a Model of Obese Female Rats

M. Garrido Novelle¹, M. J. Vázquez Villar², K. da Boit Martinello², M. Tena Sempere³, C. Diéguez²

¹Cimis-Ciberobn; ²Cimis, University of Santiago de Compostela; ³Dept of Cell Biology, Physiology, and Immunology, University of Córdoba, Spain

Introduction It is currently accepted that ambient, non-genetic factors influence perinatal development and evoke structural and functional changes that may persist throughout life. Overfeeding and androgenization after birth are 2 of these key factors that could result in “metabolic imprinting” of neural circuits early in life and

therefore increase the body weight homeostatic “set-point”, stimulate appetite, and result in obesity.

Methods and Materials We used female Sprague-Dawley rats that were overfed by modifying the litter size or prenatally androgenized by subcutaneous injection of 1.25 mg testosterone propionate in olive oil (T-1500; Sigma) on one day. Rats were acutely administered with ghrelin (5 µg/5 µl; Bachem) or vehicle (5 µl saline) by intracerebroventricular injection in the lateral ventricle and food intake was measured at 2 hours. This treatment was after weaning, on day 24, or in adulthood, on day 90. We used 8–10 rats/group.

Results We observed the important orexigenic effect of ghrelin regardless of the factor to which the animals were subjected at early developmental stages, and this effect remains at later stages. This response increased significantly when the animals were subjected to overfeeding or perinatal androgenization in both young and adult animals. Androgenization increases food intake alone, but this effect was only observed at day 24 but not in 90-day-old animals.

Conclusion The action of nutrition and perinatal hormones may have important consequences in programming the development and organization of hypothalamic circuits that regulate body weight and energy balance. An increased response to ghrelin could explain the obese phenotype presenting the animals with a modified perinatal environment.

Disclosure: No significant relationships.

■ Neuroendocrinology of Reproduction

P173

Significance of Risk Factors in Puberty Retardation in Boys

Y. Urmanova¹, U. Mavlonov²

¹Endocrinology, Tashkent Pediatric Medical Institute; ²Endocrinology, Bukhara Medical Institute, Uzbekistan

Introduction This work was initiated to study the role of risk factors in puberty retardation in boys.

Methods and Materials We examined 54 boys with a constitutional form of puberty retardation (CPR) (mean age 13.8 years). All examinees underwent general clinical examination as well as biochemical and hormonal investigations (levels of GH, LH, FSH, free testosterone, prolactin etc); their bone age as well as anthropometric parameters were assessed.

Results Vertigo (32 %), fatigue (30 %), headaches (29 %), general weakness (28 %), memory reduction (25 %), irritability (24 %), and sleep disorders (17.4 %) were among the most frequent complaints of the patients. As to the degree of development disorder manifestations, growth and puberty retardation growth, puberty and speech development retardation growth, puberty, speech and psychomotor retardation were found in 17 %, 4.3 %, and 1.06 % of examinees, respectively. According to findings the boys had reached the first stage of sexual development by Tanner although the third would have been normal. Mean testicular volume was 4.98 ± 3.63 ml. As to the mean hormone levels, free testosterone was 1.21 nmol/l; LH, FSH and TSH being 2.7, 3.4 and 2.3 mIU/l, respectively. Levels of GH, cortisol, and triiodothyronine were 2.3, 106.5 and 1.7 ng/ml, respectively, thyroxine being 96.9 nmol/l. Mean height was 125.7 cm (SDS > -2). Thus, a delay in the activation of the hypothalamic-hypophyseal-gonadal system could be observed. Among risk factors in boys with a constitutional form of puberty retardation a predominance of familial low stature (n = 45; 84 %), perinatal trauma (n = 23; 42.5 %), craniocerebral injury (n = 19; 35.1 %), mother's complicated pregnancy (n = 17; 31.4 %), BMI > 30 (n = 16; 29.6 %), frequent stresses (n = 15; 28 %), smoking (n = 5; 9.2 %) and frequent chills (n = 5; 9.2 %) was observed.

Conclusion (1) A constitutional form of puberty retardation in boys is characterized by a diversity of clinical manifestations requiring further detailed research. (2) Low stature, craniocerebral injuries, perinatal trauma, BMI > 30, and stresses have been found to be frequent risk factors in the boys.

Disclosure: No significant relationships.

P174

Metabolic Syndrome as a Complication of Inactive Pituitary Adenoma in an 18-Year-Old Patient

Y. Urmanova, I. Nabieva

Endocrinology, Tashkent Pediatric Medical Institute, Uzbekistan

Aim To describe a case of metabolic syndrome (MS) in an 18-year-old male patient with inactive pituitary adenoma.

Methods and Materials An 18-year-old patient complained of headaches and fatigue with blood pressure > 150/100 mmHg, weight gain within the previous 3–4 months, and potency reduction. According to his medical history, the patient had been feeling sick for the previous 6 months with an appearance of the above symptoms, weight gain in particular. The patient underwent general clinical examination and biochemical investigations, including measurement of lipid profile and oral glucose tolerance test. To make hormonal assessment levels of STH, LH, FSH as well as IGF-1, prolactin and free testosterone were measured, neurovisualization being conducted by MRT of the pituitary.

Results Blood pressure was 150/100 mmHg, weight 100 kg, height 180 cm, BMI 33.3 kg/m². Biochemical analysis showed high levels of LDL, triglycerides and cholesterol as well as reduction in levels of HDL and impaired glucose tolerance (fasting and 2-h blood glucose being 5.3 and 8.1 mmol/l, respectively). Hormonal investigation showed reduction in basal levels of STH (0.3 ng/ml), LH (1.2 mIU/l, FSH (2.1 mIU/l), IGF-1 (98 nmol/l), and free testosterone (10.3 nmol/l). Pituitary MRT demonstrated presence of a 5 × 8 mm microadenoma of endolaterosellar localization. The investigations underlie clinical diagnosis of pituitary microadenoma complicated with hypopituitarism (insufficiency of STH, IGF-1, LH, and FSH), secondary hypogonadism and metabolic syndrome (arterial hypertension, dyslipidemia, first-degree obesity, and insulin resistance).

Conclusion (1) STH reduction causing MS onset and progression is concomitant to the pituitary microadenoma of endolaterosellar localization. (2) Due to androgen deficiency the clinical picture is burdened by secondary hypogonadism. (3) Metabolic syndrome is among early manifestations in young patients with inactive pituitary adenomas.

Disclosure: No significant relationships.

P175

Structure of Puberty Retardation Risk Factors in Male Adolescents

Y. Urmanova¹, U. Mavlonov²¹Endocrinology, Tashkent Pediatric Medical Institute; ²Endocrinology, Bukhara Medical Institute, Uzbekistan

Introduction The work was initiated to analyze risk factors of puberty retardation in male adolescents.

Methods and Materials We examined 97 male adolescents (mean age 13.8 years) and divided them into 3 groups: 54 patients with a constitutional form of puberty retardation, 18 boys with Turkish-saddle volume formations, and 25 examinees with empty Turkish-saddle syndrome were included. All examinees underwent general clinical examination as well as biochemical and hormonal investigations (levels of GH, LH, FSH, testosterone, prolactin, TSH, etc); roentgenologic examination, including Turkish-saddle CT and MRI and hand roentgenography, was performed in 18 patients. Anthropometric examination (target height, percentile, growth velocity, height and weight SDS) was performed according to the height-weight Tanner-Whitehouse charts, sexual development stage being assessed by Tanner. Karyotyping was performed in all examinees.

Results Familial low stature (n = 45; 84 %), perinatal trauma (n = 23; 42.5 %), craniocerebral injury (n = 19; 35.1 %), mother's complicated pregnancy (n = 17; 31.4 %), BMI > 30 (n = 16; 29.6 %), frequent stresses (n = 15; 28 %), smoking (n = 5; 9.2 %), and frequent chills (n = 5; 9.2 %) were registered as the most frequent risk factors in boys with a constitutional form of puberty retardation. Puberty

retardation in patients with Turkish-saddle volume formations was most frequently manifested in craniocerebral injury (35 %), perinatal trauma (25 %), frequent stresses (18 %), and smoking (5 %). In the third group, craniocerebral injury (35 %), mother's complicated pregnancy (32 %), perinatal trauma (31 %), and frequent chills (16 %) were identified as predominant risk factors.

Conclusion (1) Various risk factors of puberty retardation have been identified. (2) Puberty retardation risk factors should be thoroughly registered.

Disclosure: No significant relationships.

P176

Structure of Endocrine Infertility in Women with Inactive Pituitary Adenomas

K. Nasirova

Neuroendocrinology, Republican Specialized Scientific-Practical Medical Centre of Endocrinology, Tashkent, Uzbekistan

Introduction We studied the structure and features of clinical forms of endocrine infertility in women with inactive pituitary adenomas (IPAs).

Subjects 46 women with IPA and EI aged 17–35 years, middle age 25.37 ± 0.79. Control group: 20 healthy women.

Methods Clinical and hormonal tests, ophthalmoscopy, folliculometry, measurement of basal temperature (BT), CT/MRI of hypothalamic-pituitary area.

Results Menstrual cycle disorders (MCD) (84.8 %), headaches (67.4 %), lactorrhea (65.2 %) prevailed in IPA with infertility. 21 women had primary infertility (45.6 %) and 25 secondary infertility (54.3 %). Cases of polycystic ovary (PCO) were found in 27 (58.7 %) patients. FM revealed anovulatory cycles in 87 %, follicle persistence in 30 %, and its atresia in 70 %. BT results: diphasic thermograms were revealed only in 4 (8.6 %) patients, shortening of hyperthermal phase in 6 (13 %), and in 36 women (82.6 %) the curve was monophasic. Prolactin levels were normal in 34 (73.9 %) patients and increased in 12 women (26.1 %). TSH varied from 0.2–7.8 mIU/l and on the average, was 0.73 ± 0.08 mIU/l; LH levels were on average 14.28 ± 1.12 mIU/l, which is significantly 2× higher compared to the control values of 6 ± 0.31 mIU/l (p < 0.05); FSH values varied from 1.5–13.6 mIU/l (average 5.53 ± 0.44 mIU/l); estradiol was decreased to 39.1 ± 4.2 ng/ml. Progesterone levels varied from 0.1–2.8 pg/ml (average 0.63 ± 0.05 pg/ml); testosterone levels varied from 0.1–1.2 nmol/l and on the average were 0.87 ± 0.03 nmol/l, which was significantly higher (p < 0.05) compared to controls.

Conclusion Genesis of infertility in IPA depends on tumor size and consists of hypothalamic disorders of gonadotropin secretion promoting development of atresia (70 %) and persistence of a follicle (30 %), in most cases caused by PCO (54.3 %), dysgonadotropinemia and depending on tumor size.

Disclosure: No significant relationships.

P177

Induction of Fertility in Women with Inactive Pituitary Adenomas

K. Nasirova

Neuroendocrinology, Republican Specialized Scientific-Practical Medical Centre of Endocrinology, Tashkent, Uzbekistan

Research Objective To develop an algorithm of fertility induction in women with inactive pituitary adenomas (IPA) and endocrine infertility (EI).

Methods and Materials 46 women with IPA and EI: 36 (78.2 %) micro- and 10 (21.8 %) macroadenomas. Average age 25.37. LH, FSH, Prl, cortisol, estradiol, progesterone, and testosterone levels on days 7, 14, and 21 of the menstrual cycle; visual-field and eye bottom examination, CT/MRI of hypothalamic-pituitary area in dynamics.

Results Ultrasound of ovaries and uterus revealed hypoplasia of the uterus degree 1–2 in 36.9 %, degree 3 in 4.3 %, and chronic adnexitis in 5.6 %. Polycystic ovaries (PCO) were found in 58.7 %. 26 % had hyperprolactinemia, 45.6 % dysgonadotropinemia, 26 % FSH secretion disorder, 73.2 % hypoestrogenia, and 61 % hyperandrogenia. Women were divided into 3 groups: group 1 had normoprolactinemic dysgonadotropinemia with clinics of PCO (n = 21; 45.6 %). These patients received metformin 500 1 tab tid + yarina 1 tab/day within 6 months + dopamine agonists (DA). 42.8 % became pregnant, among them in 1 (4.7 %) case was a miscarriage in week 11, 8 women delivered (38 %). Group 2: 13 women with normogonadotropic ovarian insufficiency (OI) (28.2 %) received cyclic hormonal replacement therapy for 3–6 months. 61.5 % became pregnant, all of them delivered, but in 1 (7.6 %) case stillbirth took place. Group 3 consisted of 12 (26 %) patients with elevated Prl and low gonadotropin levels. All received DA within 1.5 years. These patients underwent induction of ovulation by clomides in combination with chorionic gonadotropin. As a result, 58.3 % became pregnant, 1 (8.3 %) had a miscarriage in week 5–6, and in 50 % pregnancy was matured by delivery.

Conclusion As a result of the long-term medical-diagnostic procedures, 52.2 % women became pregnant, 47.8 % of them delivered, 47.8 %, basically, patients with secondary PCO, probably, required high-tech techniques of fertility induction, such as IVF, etc.

Disclosure: No significant relationships.

P178

Effect of Hyperprolactinemia on the Reproductive Function of Females with Empty Sella

D. Artikova

Endocrinology, Tashkent Medical Academy, Uzbekistan

Introduction The aim of the study was to assess a relationship between prolactinemia and the condition of the reproductive system in females with empty sella.

Methods and Materials 154 females with empty sella, mean age 37.6 ± 0.93 years, were investigated. Empty sella was diagnosed based on MRI findings. Obstetric and gynecological history as well as hormonal background were studied.

Results Hyperprolactinemia was revealed in 39 (25.3 %) patients with empty sella with clinically manifested lactorrhea, in 17 (46.3 %) with menstrual cycle disorders. Hyperprolactinemia with menstrual disorders was associated with a decrease of gonadotropic hormones and an increase of thyroid-stimulating hormone. In females with hyperprolactinemia without menstrual disorders no other hormonal changes were noted. Of 13 (8.4 %) females with empty sella applied for infertility, hyperprolactinemia was observed in 9 (69.0 %), with 5 (57.0 %) being isolated, combination of hyperprolactinemia and oligomenorrhea in 2 (22.0 %), hyperprolactinemia and amenorrhea in 2 (22.0 %) patients. Oligomenorrhea was noted in 3 (23.0 %) patients, 1 (8.0 %) patient had no menstrual disorders and lactorrhea. Study of blood hormones showed hyperprolactinemia (28.2–50.3 ng/ml). Other hormones were not changed.

Conclusion Lactorrhea associated with disorders of menstrual function was due to an increase of TSH levels. In 69 %, infertility of females with empty sella was related to hyperprolactinemia.

Disclosure: No significant relationships.

P179

Physiological Aspects of Salsolinol Action at the Hypothalamic-Pituitary Level in Lactating Sheep

T. Misztal, E. Dobek, M. Hasiec, K. Gorski, K. Romanowicz

Dept of Endocrinology, The Kielanowski Institute of Animal Physiology and Nutrition, Jablonna, Poland

Introduction Increased prolactin secretion, suppressed stress reaction, and reproductive behavior in lactating females result in changes in the regulatory mechanisms at the hypothalamic-pituitary level. In

recent years, we have shown that high concentrations of salsolinol (a derivative of dopamine) occurs within the extracellular spaces of the mediobasal hypothalamus/median eminence (MBH/ME) in lactating sheep and that this compound is responsible for the stimulation of prolactin secretion in response to suckling stimulus.

Methods and Materials We examined an involvement of salsolinol in the regulation of both the hypothalamic-pituitary-adrenal and hypothalamic-pituitary-gonadal axis activity in lactating sheep (5th week of lactation), in which the intracerebroventricular infusions of the antagonizing compound (1MeDIQ) were performed. Simultaneously, the push-pull perfusion of the MBH/ME was made to study corticoliberin (CRH) and gonadoliberin (GnRH) release and plasma samples were collected from the jugular vein to determine changes in LH and ACTH concentrations.

Results Blocking of salsolinol action within the central nervous system in lactating sheep caused an increase in the hypothalamic perfusate CRH concentration, compared to control animals ($p < 0.056$). In turn, plasma ACTH concentration in the 1-MeDIQ-treated group was significantly ($p < 0.001$) higher than in controls. On the contrary, a significant ($p < 0.001$) decrease in the hypothalamic perfusate GnRH concentration was noted in sheep infused with 1-MeDIQ in comparison with controls. Treatment with 1-MeDIQ also significantly decreased ($p < 0.001$) LH concentration in blood plasma. This was reflected by the significantly ($p < 0.05$) lower amplitude of LH pulses compared to control animals.

Conclusion In conclusion, salsolinol may be involved in the regulatory mechanisms of both the CRH/ACTH and GnRH/LH release in lactating sheep. Although it suppresses the corticotropic axis, surprisingly its action seems to be stimulatory for the gonadotropic one. These phenomena require further investigation.

Disclosure: No significant relationships. This research was supported by grant # N N311 082037 (MNiSW, Poland).

P180

Spatial Differences in the Expression of VEGF-A Isoforms in the Ovine Pituitary Gland during Breeding Season: Implications for the Control of Vascular Permeability

J. Castle-Miller¹, S. Malone¹, C. Pickworth¹, D. Bates², D. Tortonesi¹

¹Centre of Comparative and Clinical Anatomy; ²Microvascular Research Laboratories, University of Bristol, UK

Introduction The vasculature of the ovine pituitary gland is composed of fenestrated portal vessels originating from the superior hypophyseal arteries, which supply both the pars tuberalis (PT) and the pars distalis (PD), and the non-fenestrated inferior hypophyseal arteries supplying the PD. A finely tuned balance between vascular endothelial growth factor-A (VEGF-A) isoforms, in particular VEGF₁₆₅ (pro-permeability) and VEGF_{165b} (anti-permeability), has been shown to govern endothelial fenestrations and vascular permeability. In the rodent pituitary, VEGF-A is produced by folliculostellate cells, whose morphology has been shown to be melatonin-dependent. In this study, we investigated the differential expression of VEGF-A isoforms in the melatonin receptor-rich PT and PD of the ovine pituitary during the breeding season.

Methods and Materials Protein and mRNA extracted from the pituitaries of ewes was subjected to ELISA and RT-PCR, respectively.

Results VEGF-A ($p = 0.04$), and VEGF_{165b} ($p = 0.02$) protein expression was higher in the PD when compared to the PT, a result specific to post-pubertal animals ($p = 0.04$). VEGF₁₆₅ and VEGF_{165b} mRNA expression was observed exclusively in the PD ($n = 7$), whereas VEGF₁₂₀ was detected in the PT ($n = 7$). Using immunohistochemistry, VEGF-A and VEGF_{165b} expression observed in the PD, was localised to cells with the morphological appearance of folliculostellate cells.

Conclusion These results provide evidence for the expression of VEGF in the ovine pituitary during the breeding season, indicating a spatial difference in isoform expression between the PD and PT.

Expression of VEGF₁₆₅/VEGF_{165b} in the PD may be involved in the differential regulation of vascular fenestrations and thus portal vasculature permeability. Although the expression of pituitary VEGF during the non-breeding season remains to be determined, this potential mechanism of permeability control may have important implications on reproductive function, in relation to the delivery of cellular products to the systemic circulation.

Disclosure: No significant relationships.

P181

Chronic Cold Stress during the Prenatal Period Permanently Damages Ovary Function and Hypothalamic Neurotransmitters of the Progeny

R. Barra, C. Araya, H. Lara

Biochemistry and Molecular Biology, Faculty of Chemistry and Pharmaceutical Sciences, University of Chile, Santiago de Chile, Chile

Introduction Stress is particularly harmful when exposure occurs early in development. We found that chronic cold stress in adult rats activates ovarian sympathetic innervation and develops polycystic ovary (PCO) conditions. PCO syndrome is the most frequent ovarian pathology during reproductive years in women and most of the characteristic cases of PCOS originate during early development in human and are expressed either before or during puberty, suggesting a condition derived from *in utero* exposure to neural or metabolic-derived insults.

Objective To study the effects of prenatal stress in the development of ovary function and hypothalamic neurotransmitters of the progeny.

Methods and Materials Pregnant rats exposed to cold stress daily during pregnancy at 4 °C (from 10:00–13:00 hours). Progeny was studied at puberty for estrous cycling activity and ovulatory capacity. We also determined in medium basal hypothalamus (MBH) the changes in hypothalamic neurotransmitters involved in GnRH secretion.

Results Prenatally stressed rats presented lower numbers of pre-ovulatory follicles and estradiol plasma levels at prepubertal age. This was correlated with delayed puberty and slower capacity to start cycling regularly. In fact, regular (4-day) estrous cycling activity was delayed by 3 weeks after puberty compared with 4-day cycles in control rats. Adult rats presented important changes in MBH monoamine turnover. Both serotonin and dopamine turnover rates were decreased to 50 % in the hypothalamus of stressed rats as compared with control.

Conclusion The exposure to a sympathetic stress while *in utero* produced permanent modifications in rats and involves peripheral changes in ovarian follicular development and in the hypothalamic neuronal control of GnRH secretion, being the first evidence showing that sympathetic nerve activation in the mother strongly affects reproductive performance of the progeny.

Disclosure: No significant relationships. Supported by Fondecyt 1090036.

P182

Disturbed Eating Behavior and Its Influences on the Quality of Life in Polycystic Ovary Syndrome

A. Painold¹, P. Milz², H. P. Kapfhammer¹, T. Pieber³, E. Lerchbaum³, B. Obermayer-Pietsch³

¹Dept of Psychiatry, Medical University of Graz, Austria; ²University Hospital of Psychiatry, The Key Institute for Brain-Mind Research, Zurich, Switzerland; ³Division of Endocrinology and Metabolism, Dept of Internal Medicine, Medical University of Graz, Austria

Introduction Disturbed eating behavior and even eating disorders are reported problems in patients with polycystic ovary syndrome (PCOS). Increased appetite, weight gain, and obesity strongly affect their quality of life. This study investigated the relationship between

food craving (FC), quality of life, hormone levels, and metabolic parameters in PCOS patients.

Methods and Materials 164 patients completed a Food Craving Inventory (FCI) and the Polycystic Ovary Syndrome Questionnaire (PCOSQ) on quality of life in PCOS. Body weight, height, glucose and insulin levels were obtained. Food craving subscales were built and a sum score and food category-specific subscores were calculated. Pearson correlations evaluated statistical relationships between variables. Significant correlations ($p < 0.05$) are reported.

Results The most frequently craved single food item was chocolate, followed by pasta. Across categories, craving was strongest for sweets and carbohydrates. General FC (particularly craving for fat and carbohydrates) was associated with increased weight, Body Mass Index, and waist-to-hip ratio. FC was also associated with a decreased quality of life in PCOS. Furthermore, the higher the subjective stress because of being overweight, the higher the overall food craving score as well as the scores for fat, fast food, and carbohydrate craving. General FC was associated with increased insulin and glucose levels. The higher the cortisol and testosterone levels and the free androgen index, the higher the FC scores for fat.

Conclusion Stronger FC in PCOS is associated with decreased quality of life. FC, weight gain, and metabolic problems seem to be a coherent burden in PCOS. Disturbed eating behavior may represent compensation for the clinical features in PCOS and psychological treatment may be helpful and necessary. The FCI itself may be a useful tool to define disturbed eating patterns in PCOS. Clinical applications of the FCI may include nutritional planning in weight loss treatment and measuring changes in FC over the course of the disease.

Disclosure: No significant relationships.

P183

Influence of Orexin A on GNRH-Stimulated Secretion of LH of Immature and Mature Female Rats in the Pituitary Primary Culture

L. Martynska, E. Wolinska-Witort, M. Chmielowska, M. Kalisz, W. Bik, E. Wasilewska-Dziubinska, B. Baranowska

Dept of Clinical Neuroendocrinology, Medical Centre of Postgraduate Education, Warsaw, Poland

Introduction Orexin A (OXA) shows broad activity in the central nervous system and peripheral tissues. It regulates appetite, sleep-wake rhythm, and secretion of pituitary hormones. OXA acts through 2 types of receptors, Ox1R and Ox2R, whose presence was also demonstrated in the pituitary. The aim of this study was to investigate the direct effect of OXA on GnRH-stimulated LH secretion from pituitary cells of sexually immature and mature female rats.

Methods and Materials Pituitaries for *in vitro* studies were collected from female rats: sexually immature (IM) and sexually mature ovariectomized (M/OVX) and M/OVX after subcutaneous estradiol supplementation (M/OVX+E₂). Pituitary cells in primary cultures (5×10^5 cells/well) were exposed to 1-hour action of GnRH (1 nM or 1000 nM) and OXA in concentrations 0.1 nM and 100 nM. Cells cultured without GnRH and OXA were control groups. The cultured media were collected after incubation and the concentration of LH was measured with RIA method.

Results It was found that GnRH increased LH secretion by 75 % ($p \leq 0.01$) from IM pituitary cells and by 67 % ($p \leq 0.01$) from the pituitary cells of M/OVX+E₂ in comparison to basic secretion. Orexin A did not alter GnRH-stimulated LH secretion in the IM group, it decreased the secretion level by 39 % (100 nM GnRH; $p \leq 0.01$) in group M/OVX+E₂ and by 35 % (1 nM GnRH; $p \leq 0.01$) in group M/OVX and increased secretion by 85 % (100 nM GnRH; $p \leq 0.05$) in group M/OVX in comparison to control groups.

Conclusion Results show that orexin A may modify the sensitivity of gonadotrophic cells to GnRH, and its effect depends on the maturity and estrogen status of female rats. This study was supported by CMKP projects no. 501-2-1-27-19/06 and 501-1-31-22-11.

Disclosure: No significant relationships.

P184

Mating Regulates Mitogen-Activated Protein Kinase (MAPK) Phosphorylation Associated with Estradiol in the Rat Pituitary Gland

P. Reuquén¹, M. Oróstica², P. Orihuela²

¹Biología, Universidad Andres Bello; ²Biología, Universidad de Santiago de Chile, Santiago de Chile, Chile

Introduction Mating regulates several physiological changes in the female reproductive tract, which are potentially required for successful pregnancy. Furthermore, mating changes the estradiol (E₂) signaling pathway in the rat oviduct from a non-genomic to a genomic mode. However, the effects of mating on other organs distant to the reproductive tract are not well-determined. One or more of 3 mating-associated components could be the relevant signal: sensory stimulation, seminal fluid, and sperm cells. We analyzed whether one of the components of mating, such as sensory stimulation, could regulate the signaling pathway of MAPK in the rat pituitary gland. In addition, the effect of cervical stimulation on the E₂-induced MAPK activity decrease was investigated.

Methods and Materials Rats in the midnight of estrus were pseudopregnant by cervical stimulation (pseudopregnant) with a rod glass or kept isolated (control) and 0, 3, 6, 12, or 24 h later pituitary gland was excised and processed by western blot or immunohistochemistry to determine the level and cellular localization of phosphorylated MAPK or total MAPK. Other rats were pseudopregnant or kept isolated and 12 h later E₂ was injected to determine the phosphorylation of MAPK in the pituitary gland after 1 h after E₂ treatment.

Results Phosphorylation of MAPK was decreased 6–12 h in the control groups while their levels remained constant following pseudopregnancy. However, localization of phosphorylated MAPK was not affected. Furthermore, E₂ decreased the phosphorylation of MAPK although pseudopregnancy blocked this effect of E₂.

Conclusion We conclude that sensory stimulation of the cervical area modifies MAPK signaling and its E₂ response in the pituitary gland, highlighting a hitherto unsuspected early, strong, and broad influence of mating beyond of the reproductive tract.

Disclosure: No significant relationships. Supported by Fondecyt 1110662 and FBO807.

P185

Fipronil Prenatal Exposition Promotes an Endocrine Disrupt Effect in Adult Rats' Offspring

M. S. Berto Udo¹, T. M. Sandini¹, H. Souza Spinoza², M. M. Bernardi³

¹Clinical and Toxicological Analyses; ²Pathology Dept, University of São Paulo;

³Pharmacology, Universidade Paulista, São Paulo, Brazil

Introduction Fipronil is a broad-action insecticide used in plague. It blocks the chloride channels coupled with GABA receptors. Moreover, GABAergic inhibitor agents have been described as substances that are able to alter the endocrine system. Thus, the present study proposed to assess fipronil action on sexual behavior of female and male rats' offspring exposed in the intrauterine period.

Methods and Materials 40 female rats were mated and when pregnant they received different treatment (0.1, 1.0 and 10.0 mg/kg of fipronil or water by gavage; n = 10/group) from the 6th until the 20th pregnancy day. Offspring were evaluated as adults for exploratory activity in the open field, motor coordination in elevated bar, and sexual parameters: estrus cycle for females and sexual behavior for both sexes. Female sexual behavior was assessed by lordoses coefficient and male sexual behavior was assessed by the following parameters: first mount and intromission latencies, number of mounts and intromissions before ejaculation, ejaculation latency, first post-ejaculatory mount and intromission latencies. Copulatory efficiency was also calculated.

Results Results showed that the exploratory activity of experimental male offspring, but not in female, was increased and no effects on motor coordination were observed in both sexes. The male

offspring exposure to the lower dose had a reduction of sexual motivation indicated by the increase on the first mount and first intromission latencies but they did not have alterations on the copulatory efficiency. Female offspring prenatally treated with 0.1 mg/kg of fipronil showed an increase on estrous length phase and decrease on diestrus, and an irregular estrus cycle.

Conclusion Thus, it is possible to conclude that prenatal exposure to fipronil induced neuroendocrine impairment in adult male and female offspring, mainly in male sexual behavior and in female estrus cycle, suggesting an endocrine disrupt effect.

Disclosure: No significant relationships.

P186

CT Scan of the Anterior Skull Base in Kallmann Syndrome Reveals Specific Ethmoid Abnormalities

L. Maione¹, S. Benadjaoud¹, F. Benoudiba¹, R. Pivonello², C. Eloit¹, D. Ducreux¹, P. Chanson¹, J. Young¹

¹APHP Endocrinology and Reproductive Diseases, Paris Sud University, Le Kremlin-Bicêtre, France; ²Endocrinology and Clinical Oncology, University of Naples Federico II, Italy

Introduction Kallmann syndrome (KS) is a developmental disease defined by the association between congenital hypogonadotropic hypogonadism (CHH) and olfactory system impairment. MRI usually detects rhinencephalic and olfactory abnormalities but this technique is not efficient enough to study bone structures. Our objective was to search for specific bone abnormalities in KS anterior skull base on CT scan.

Methods and Materials 37 KS patients were compared to 15 normosmic CHH and 30 controls in a prospective case-control study. All patients and controls underwent a high-resolution CT scan in bone windows with axial, coronal, and sagittal reconstructions. Olfactory fossa (OF) height, width, surface, medio-lateral and foveo-lateral angles were measured. Cribriform plate foramina were counted bilaterally.

Results KS patients present the absence of olfactory fossa and a flattening of the ethmoid floor compared to nCHH or controls. Olfactory fossa height, width, and surface were all significantly shorter in KS patients than in nCHH and controls (p < 0.0001). KS subjects also presented wider MLA and FLA angles than nCHH and controls (p < 0.0001). Cribriform plate foramina were present in KS patients as in nCHH and controls.

Conclusion Severe olfactory fossa hypoplasia is a specific hallmark in Kallmann patients. Olfactory fossa height < 3.6 mm at CT scan is a useful diagnostic tool to discriminate KS from nCHH. Normal cribriform plate foramina in Kallmann patients suggests that olfactory structure integrity is not mandatory for their formation and maintenance, unraveling original aspects on neuronal migration and concomitant bone formation in Kallmann syndrome.

Disclosure: No significant relationships.

P187

Reduced Sensitivity of the Adeno-Hypophysis-Testes Axis in Ageing Roosters

A. Rosenstrauch

Life Sciences, Achva Academic College, Shikmim, Israel

Introduction Domestic roosters reach sexual maturity at about 25 weeks of age and peak fertility at about 37 weeks. Fertility declines drastically at about 70 weeks due to a lower concentration of ejaculated spermatozoa, which is accompanied by lower plasma testosterone and LH concentrations. To examine the endocrine control of spermiogenesis, we studied the sensitivity of the adeno-hypophysis-testes axis by in vitro cross-incubations between high- and low-fertile roosters.

Methods and Materials Ten adeno-hypophyses each of high- (37-week-old) and low- (67-week-old) fertile roosters were pre-in-

cubated separately in absence (control) and presence of avian GnRH for 60 min. Testes of both high- and low-fertile roosters were then incubated separately in adeno-hypophysis pre-incubation media (30, 60, and 120 min incubation) and the levels of testosterone per g tissue released into the media were determined by RIA.

Results Response to high-fertile adeno-hypophysis media (pre-incubated with GnRH): testes of high-fertile roosters showed an increase of 360 % in testosterone release after 30 min. In addition, the low-fertile testes reached only an increase of 300 % in testosterone release after prolonged incubation of 60 and 120 min. Response to low-fertile adeno-hypophysis media (pre-incubated with GnRH): the low-fertile testes reached only 110 % above control levels in testosterone levels and did not show any change in 30, 60, and 120 min while the high-fertile testes reached 120 % above controls after 30 min and 240 % above controls at 60 and 120 min. These results indicate that increased age causes a reduction in the sensitivity of the testes to adeno-hypophysis stimulation.

Conclusion It is concluded that in aged roosters there is a reduced responsiveness by both the adeno-hypophysis and testes which disrupts hormonal control of spermiogenesis and leads to low fertility.

Disclosure: No significant relationships.

Other

P188

Median Age of Onset of Puberty in Healthy Uzbek Boys of Tashkent City

G. Rakhimova, K. Gilyazetdinov, A. Akbarov
Children, Republican Specialized Scientific-Practical Medical Centre of Endocrinology, Tashkent, Uzbekistan

Introduction Age of onset of puberty is much lower today than in the past century due to the stability of socio-economic conditions, improved quality of life, and overall health of the population.

Methods and Materials We examined 818 healthy boys aged 7–18 years, the study was conducted between 2009 and 2011. Boys were examined in 3 urban districts of Tashkent City (Mirzo-Ulugbek, Shakhantaur, Yunus-Abad). Testicular volume was evaluated with a Prader orchidometer. Age of onset of puberty was defined as the age at attainment of testicular volume ≥ 4 ml.

Results We revealed that the earliest age of onset of puberty was at 9.0 years (3.3 %). All healthy boys had testicular volume > 4 ml by 14 years. The data suggest for healthy Uzbek boys an upper reference value of the age of onset of puberty. Thereby, the diagnosis of delayed puberty may be established at 14 years, which generally coincides with the international guidelines for the diagnosis of delayed sexual development. Median age of onset of puberty for Uzbek boys of Tashkent City was 11.9 (95%-CI: 11.1–12.7), this data agrees with Great Britain (11.9) and Byelorussia (11.9), but differs from the USA (11.5) and the Netherlands (11.5).

Conclusion (1) The earliest age of onset of puberty in healthy Uzbek boys of Tashkent City was 9.0 years and 14 years The ultimate one. (2) Median age of the onset of puberty for Uzbek boys of Tashkent City was 11.9 years. (3) We have defined criteria for the diagnosis of delayed puberty at 14 years.

Disclosure: No significant relationships.

P189

Risk Factors for the Development of Empty-Sella Syndrome

D. Artikova
Endocrinology, Tashkent Medical Academy, Uzbekistan

Introduction The aim of the study was to make an integral and prognostic assessment of probable risk indices of primary and secondary empty-sella development.

Methods and Materials 183 patients, 154 males/29 females, mean age 37.9 ± 0.86 years (females 37.6 ± 0.93 , males 39.0 ± 2.29) were studied. The diagnosis was established based on MRI findings (Magnetom Open Viva, 0.2 Tesla, Siemens). The hormonal background was examined. Factor analysis was performed by likelihood ratio.

Results In primary empty sella a higher risk was noted in females > 40 , with obesity, brain trauma, history of > 3 pregnancies, clinical hypothyroidism, and TSH high levels. Secondary empty sella was more common in patients treated for microadenoma, macroadenoma with dopamine agonists, with a history of up to 3 pregnancies, high levels of TSH, normal body weight, age 30–40, and in males.

Conclusion Based on the findings it is possible to determine the likelihood risk rate of empty sella, to predict the dynamics of the disease, and carry out prevention at early stages.

Disclosure: No significant relationships.

P190

Sex Steroids and Aquaporin4 in Brain Injury Caused by Trauma in Female Rats

N. Shahrokhi, M. Khaksari Hadad, N. Adeli
Physiology Research Center, School of Medicine, Kerman University of Medical Sciences, Iran

Introduction Brain damage induced by trauma is the most common cause of death. In the present study, the role of different doses of sex steroids was investigated on aquaporin4 (AQP4) protein concentration changes in the brain tissue of ovariectomized (OVX) rats after traumatic brain injury (TBI).

Methods and Materials In this study, 140 female rats N-MRI were divided into 7 groups with each group consisting of 2 subgroups. Sham group, ovariectomized sham group, other TBI groups including vehicle, physiologic and pharmacologic estrogen doses ($33.3 \mu\text{g/kg}$, 1 mg/kg), and progesterone doses (1.7 mg/kg , 8 mg/kg) group. TBI was induced by Marmarou method, 30 minutes after TBI hormones were injected ip. Parameters such as water content and AQP4 concentration were assessed 24 hours after TBI and the Evans blue content was determined 5 hours after TBI.

Results Concentration expression of AQP4 ($p < 0.01$), water content ($p < 0.01$), and disruption of the blood-brain barrier ($p < 0.001$) were significantly lower in different doses of estrogen compared to the vehicle group. The brain-water content was significantly lower in different doses of progesterone compared to the vehicle group ($p < 0.001$) but there is no significant effect on AQP4 expression. The brain Evans blue content in physiological doses of progesterone was significantly lower than in the vehicle group but it was higher in pharmacologic dose of progesterone ($p < 0.001$).

Conclusion We concluded that physiologic and pharmacologic doses of estrogen reduced AQP4 expression in OVX rats after TBI.

Disclosure: No significant relationships.

P191

Endocrine Alterations after Aneurysmal Subarachnoid Haemorrhage

L. Portocarrero-Ortiz¹, Y. O. Piquet-Uscanga², A. Balderrama-Soto¹, A. Arauz Góngora²

¹Neuroendocrinology; ²Neurology, Instituto Nacional de Neurología y Neurocirugía M. V. S., Distrito Federal, Mexico

Introduction Aneurysmal subarachnoid haemorrhage (SAH) is associated with changes in the hormonal profile. The prevalence of hypopituitarism has been reported between 38 % and 45 %. the non-thyroidal illness syndrome (NTIS) was reported in 40 %.

Methods and Materials From June 2010 to May 2011, 83 patients were hospitalized with spontaneous SAH. Endocrine evaluation was performed in the first 72 hours post SAH and followed for 6 months.

Table 9. L. Portacarrero-Ortiz et al. Endocrine alterations in patients with SAH.

		72 h	1 w	2 w	3 w	1 m	3 m	6 m
Gonadal axis	Testosterone < 200 ng/dl or low E ₂ , LH, and FSH	9 (10.8%)	16 (19.2 %)	17 (20.5 %)	14 (16.8 %)	6 (7.2 %)	1 (1.2 %)	0
Thyroid axis	Low FT3 Low T4L and TSH	21 (25 %) 1 (1.2 %)	32 (38.5 %) 2 (2.4 %)	31 (37.3%) 0	16 (19.2 %) 0	9 (10.8 %) 0	4 (4.8 %) 0	3 (3.6 %) 0
Adrenal axis	Cortisol < 3 µg/dl Cortisol 3–10 µg/dl	5 (6 %) 6 (7.2 %)	2 (2.4 %) 2 (2.4 %)	2 (2.4 %) 2 (2.4 %)	1 (1.2 %) 1 (1.2 %)	1 (1.2 %) 1 (1.2 %)	0 0	0 0
Hyperprolactinemia	Prolactin > 25 ng/ml	4 (4.8 %)	8 (9.6 %)	7 (8.4 %)	3 (3.6 %)	4 (4.8 %)	2 (2.4 %)	1 (1.2 %)

The severity of haemorrhage was evaluated by Fisher Scale and Hunt Hess Scale. The disability was evaluated by Rankin Scale.

Results 24 (29 %) men and 59 (71 %) women with a mean age of 48 ± 16.2 years. The score in Fisher Scale was 0 in 8 (10 %) patients, 1 in 12 (15 %), 2 in 21 (25 %), and 3 in 42 (50 %) patients. The score in Hunt Hess Scale was 1 in 8 (10 %) patients, 2 in 33 (41 %), 3 in 24 (33 %) patients, 4 in 12 (16 %), and 5 in 3 (4 %) patients. The most frequent endocrine alteration was NTIS, followed of hypogonadotropic hypogonadism (**Table 9**). Bleeding post SAH presented in 9.6 % and hydrocephalus and vasospasms in 30 %. 21 (21.6 %) patients died. We did not find a correlation between endocrine alteration and complications ($p = 0.62$), mortality ($p = 0.53$), and disability ($p = 0.35$).

Conclusion Endocrine alterations are frequent in SAH, there was no correlation between severity of haemorrhage and alterations in the hormone profile. Patients had hormonal recuperation which did not influence their prognosis.

Disclosure: No significant relationships.

P192

Cinacalcet in Patients with Primary Hyperparathyroidism (PHPT): Comparison between Sporadic and MEN-1 PHPT

M. del Prete, V. Marotta, V. Ramundo, F. Marciello, C. de Luca di Roseto, A. C. Carratù, R. Esposito, A. Colao, A. Faggiano
Dept of Molecular and Clinical Endocrinology and Oncology, University of Naples Federico II, Italy

PHPT is a common endocrine disease characterized by hypercalcemia and parathyroid adenoma/hyperplasia. Approximately 5 % of PHPT are associated with an inherited disease. Multiple endocrine neoplasia (MEN) type 1 is the commonest cause of inherited PHPT. The main therapeutic approach for PHPT is surgery, while therapy with cinacalcet is indicated in patients who refuse or have contraindications for surgery. The aim of this study was to compare the effectiveness of cinacalcet in patients with sporadic and MEN-1-related PHPT. 30 patients were enrolled: 15 of them had sporadic PHPT (6 M, 9 F, mean age 67) and 15 MEN-1 PHPT (5 M, 10 F, mean age 41). Serum concentrations of PTH, calcium, and phosphorus were evaluated before and 6 and 12 months after starting cinacalcet. Serum calcium and PTH concentrations significantly decreased in both groups ($p < 0.01$). There were no significant differences between sporadic and MEN-1 PHPT in the rate of calcium and PTH concentration decrease after 6 and 12 months of therapy. At 12-month follow-up, the dose of cinacalcet required to normalize calcemia in sporadic PHPT was 30 mg/day in 10 patients and 60 mg in 5 other patients, while in MEN-1 PHPT the dose of cinacalcet was 30 mg in 4 patients, 60 mg in 9, and 90 mg in another 2 patients. Cinacalcet is effective to control hypercalcemia both in sporadic and MEN-1 PHPT. Contrary to what was expected, MEN-1 PHPT required a higher dose of cinacalcet to normalize serum calcium than sporadic PHPT. This might be explained by the fact that in MEN-1 PHPT all parathyroid tissue is affected.

Disclosure: No significant relationships.

P193

Ectopic Craniopharyngioma. A Case Report

L. A. Ortega-Porcayo¹, A. Balderrama-Soto², J. A. Ponce-Gómez¹, J. L. Gómez-Amador¹, L. Portocarrero-Ortiz²
¹Neurosurgery; ²Neuroendocrinology, Instituto Nacional de Neurología y Neurocirugía M. V. S., Distrito Federal, Mexico

Introduction Craniopharyngiomas account for 1.2–4.6 % of all intracranial tumors. They arise from squamous epithelial remnants of the obliterated craniopharyngeal canal, which represents the path taken by Rathke's pouch from the oropharynx to the floor of the third ventricle. Rare cases have been reported in ectopic locations including the third ventricle, nasopharynx, sphenoid bone, cerebellopontine angle, corpus callosum, and pineal region.

Methods and Materials A 35-year-old female, with a 1-year history of pulsatile and intermittent headache is presented. 15 days before she was admitted to the neurosurgery department she started with intense headache, accompanied by nausea and vomiting. A few days later she presented micropsias and macropsias.

Results MRI (**Figure 14**) showed an extraaxial lesion in the middle and anterior cranial fossa of the left side. The tumor was cystic and heterogeneous, with well-defined borders and irregular shape with calcifications. It was hypointense in T2 and hyperintense in T2 with partial contrast enhancement of the solid portion. We performed a frontal-lateral endoscopic approach. A dense greenish-yellow fluid was aspirated and we resected a small solid portion of a brown tumor with calcifications. Histology showed necrosis and calcifications, phantom cells and keratin, inflammation and hemosiderophages, keratinized squamous epithelium and stellate epithelial cells. Diagnosis: adamantinomatous craniopharyngioma.

Conclusion Craniopharyngiomas account for 1 % of all intracranial tumors in adults. The craniopharyngeal canal extends from the floor of the sella to the vomer and may rarely give rise to primary ectopic craniopharyngiomas, in unusual cases there can be abnormal migration of Rathke's pouch. Secondary ectopic craniopharyngiomas are a post-operative complication by implantation or dissemination by cerebrospinal fluid.

Disclosure: No significant relationships.



Figure 14. L. A. Ortega-Porcayo et al. MRI of the patient described.

P194

Expression of Clock Genes in Rat Cumulus-Oocyte Complexes: Preliminary ResultsL. A. Coelho¹, R. Peres², F. Amara², J. Cipolla-Neto²¹Faculty of Animal Science and Food Engineering, University of São Paulo, Pirassununga; ²Physiology and Biophysics, Institute of Biomedical Sciences, University of São Paulo, Brazil

Introduction Clock genes appear to be involved in reproductive processes. mRNA expression of circadian genes was detected in mice, cattle, and rabbit oocytes and preimplantation embryos. In spite of some clock genes being present in rat ovaries, *in situ* hybridization and immunohistochemistry studies have indicated no expression in rat oocytes and no information regarding cumulus cells has been reported. The aim of this study was to investigate the expression of clock genes (Bmal1, Cry1, Cry2, Per1, Per2) at different meiotic cell cycle stages in cumulus-oocyte complexes (COCs) from 27-day-old female rats.

Methods and Materials To obtain germinal vesicle (GV) immature COCs from ovarian follicles, rats were treated with 20 IU equine chorionic gonadotropin (eCG) for induction of follicular development and killed by euthanasia 48 h later. To obtain COCs at MII oocyte stage from oviducts, rats were injected with 20 IU eCG and 48 h later with 20 IU human chorionic gonadotropin (hCG), and then sacrificed after 14–16 h. The oocytes in COCs were separated from their cumulus cells by repeated pipetting through a narrow-bore pipette in culture medium.

Results Quantitative real-time PCR analysis revealed the presence of transcripts of all genes studied in both oocytes and cumulus cells from immature (GV) and mature (MII) COCs. At both maturational stages, copy numbers for bmal1, cry1, per1, and per2 in oocytes were significantly higher than in cumulus cells. No difference between oocytes and cumulus cells in cry2 content was observed.

Conclusion Results confirm the presence of clockwork genes in rat cumulus-oocyte complexes and suggest their involvement in the meiotic cellular cycle. Further studies are necessary to investigate the functional involvement of the pineal gland in daily clock gene expression of rat immature and mature cumulus-oocytes complexes. Research supported by FAPESP.

Disclosure: No significant relationships.

P195

Resolution of Type-2 Diabetes Following IFN- α Therapy for Chronic Hepatitis C

K. Doo-Man, P. Ju Ri

Dept of Internal Medicine, Hallym University College of Medicine, Seoul, Korea

Introduction IFN- α is widely used for the treatment for chronic hepatitis C virus which causes many cases of liver cirrhosis. Patients with hepatitis C virus (HCV) infection have a significantly increased prevalence of type-2 diabetes compared to controls (hepatitis B virus-infected patients). Most of the earlier literature describes diabetes as developing in the course of interferon- α therapy for a variety of diseases. We report on a patient whose type-2 diabetes resolved during IFN- α therapy for HCV infection.

Methods and Materials A 57-year-old man presented with fatigue, polyuria, and polydipsia. He was newly diagnosed with type-2 diabetes. During the hospital stay, he was also diagnosed with chronic hepatitis. Serological studies for chronic hepatitis demonstrated presence of hepatitis C, type 2a/2c genotype. He was started on subcutaneous insulin and IFN- α .

Results After 24 weeks of treatment with IFN- α , HCV polymerase chain reaction was negative and diabetes resolved despite an increase in his body weight.

Conclusion Previous HCV infection markedly increased the prevalence of type-2 diabetes, regardless of the presence of liver cirrhosis. Non-diabetic HCV patients have insulin resistance and specific

defects in the insulin-signaling pathway. The specific mechanisms by which HCV leads to type-2 diabetes are not fully understood, but it seems that an increase of insulin resistance associated with both steatosis and the overproduction of pro-inflammatory cytokines could play a crucial role. Although it is unclear whether the resolution of diabetes in this case occurred as an effect of IFN- α or as a result of becoming HCV-RNA-negative, it has suggested a great deal about the role of IFN- α and HCV infection in the pathogenesis of diabetes.

Disclosure: No significant relationships.

P196

Prostate Cancer (PCa) and Insulin Resistance (IR): Effect of Neoadjuvant Androgen Deprivation Therapy (ADT) on Insulin Sensitivity in Patients with PCaR. Pivonello¹, P. Vitale¹, R. di Domenico², M. Galdiero¹, F. Io Calzo¹, F. Ciarati¹, S. Perdonà², A. Colao¹¹Dept of Molecular and Clinical Endocrinology and Oncology, University of Naples Federico II; ²Urology Section, National Tumor Institute, G. Pascale Foundation, Naples, Italy

Introduction ADT is one of the main tools in the medical treatment of PCa. The aim of the present study was to evaluate the effect of short-term neoadjuvant ADT on insulin sensitivity in patients with PCa.

Methods and Materials 19 patients with PCa (age 58–74, mean: 66.2 ± 4.1 yrs) were enrolled in the study: 9 patients were treated with ADT (cyproterone acetate, 400 mg/die) for 3 months whereas no treatment was given to the remaining 10 patients. The evaluation of anthropometric, metabolic, and hormonal parameters as well as IR indexes was performed in all patients at baseline and after 3 months.

Results At baseline, none of the patients had diabetes or impaired fasting glucose (IFG) except for one (11 %) included in the group of treated patients had impaired glucose tolerance (IGT); according to proposed HOMA-1 cut-off value for IR (> 2.6), 6 (60 %) untreated patients and 6 (66 %) treated patients had IR. No significant difference in anthropometric, metabolic, and hormonal parameters was found between the 2 groups at baseline. After 3 months, in patients treated with ADT, HOMA-1 ($p = 0.01$), HOMA-2 ($p = 0.01$), Quicky ($p = 0.01$), McAuley ($p = 0.01$), fasting insulin ($p = 0.01$), post-glucose load insulin AUC ($p = 0.01$) were significantly increased, whereas testosterone ($p = 0.008$) and 17β -oestradiol ($p = 0.01$) were significantly decreased; one (11 %) patient developed IGT, and IR was diagnosed in 3 (33 %) additional patients ($\chi^2 = 3.95$; $p = 0.047$). Conversely, no significant changes were found in the group of untreated patients after 3 months. No additional patient developed IR or IGT in the group of untreated patients.

Conclusion The preliminary results of the current study demonstrate for the first time with the use of neoadjuvant treatment that ADT is able to impair or worsen insulin sensitivity and glucose metabolism in patients with PCa.

Disclosure: No significant relationships.

P197

Once-Weekly, CTP-Modified HGH (MOD-4023) Is Effective in Growth Hormone-Deficient Adults: A Phase-II, Dose and Frequency Finding StudyL. Amitzi¹, V. Popovic², M. Goth³, P. Vanuga⁴, J. Payer⁵, M. Pfeifer⁶, M. Bidlingmaier⁷, E. Fima⁸¹Nes Ziona, Israel; ²Belgrade, Serbia; ³Budapest, Hungary; ⁴Lubochna, Slovakia; ⁵Bratislava, Slovakia; ⁶Ljubljana, Slovenia; ⁷Munich, Germany; ⁸University of Haifa, Carmel Medical Center, Haifa, Israel

Introduction Growth hormone (GH) replacement therapy currently requires daily injections, which may cause poor compliance and distress for patients. CTP-modified hGH (MOD-4023) is being developed for once-weekly administration in growth hormone-deficient (GHD) adults and children. The present study evaluated the

safety, pharmacokinetics (PK), and pharmacodynamics (PD) of MOD-4023 in GHD adults.

Methods and Materials 39 normalized GHDA patients currently treated with daily GH were randomized and switched to 3 dose levels of once-weekly MOD-4023 to evaluate safety and PK/PD profile (30 %, 45 %, or 100 % of each patient's cumulative weekly hGH dose). The study comprised 2 stages. Stage I included an optimization period and 4 weeks of once-weekly subcutaneously administered MOD-4023. Stage II is an optional 16 week extension period of once weekly MOD-4023 administration to collect further safety information and confirm the results obtained in stage I. Here we present the results of stage I.

Results MOD-4023 was well-tolerated and a dose-dependent response of IGF-1 concentration was demonstrated. In most patients,

IGF-1 levels were maintained within ± 2 SDs during the 4 weeks, without exceeding +2 SDs at peak levels. In 2 cohorts (45 % and 100 %) the mean IGF-1 values were comparable to those obtained with daily hGH at steady state. The adverse effects reported were consistent with known hGH-related side effects and were mostly mild. MOD-4023 was not immunogenic.

Conclusion Once-weekly repeated doses of long-acting MOD-4023 were safe and well-tolerated in adult GHD patients. IGF-1 levels were maintained within the normal range in most MOD-4023-treated patients for the entire 4 weeks of treatment.

Based on the positive results of stage I, an estimated target dose range for a phase-III study has been established and the 16-week extension study was initiated to further confirm the estimated dose range and to provide additional safety information.

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