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**Society for Endocrinology BES 2010**

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Plenary Lecturers’ Biographical Notes
Society for Endocrinology Dale Medal Lecture

S O’Rahilly, Institute of Metabolic Science, University of Cambridge, Cambridge, UK

Stephen O’Rahilly graduated in Medicine from University College Dublin in 1981. From 1982 to 1991 he undertook postgraduate clinical and research training in general medicine, diabetes and endocrinology in London, Oxford and Harvard. In 1991 he obtained a Wellcome Trust Senior Clinical Fellowship and established his laboratory at the University of Cambridge.

In 1996 he was appointed to the Chair of Metabolic Medicine and in 2002 to the Chair of Clinical Biochemistry and Medicine at the University of Cambridge. He is the Director of the University of Cambridge Metabolic Research Laboratories and the MRC Centre for Obesity and Related Metabolic disease at the Institute of Metabolic Science, Cambridge and is an honorary consultant physician at Addenbrooke’s Hospital where he is active as a clinician and teacher.

Prof. O’Rahilly’s research has been concerned with the elucidation of the basic causes of obesity and type 2 diabetes and the utilisation of this information to inform advances in prevention and treatment.

He is a Fellow of both the Academy of Medical Sciences and the Royal Society. He is currently Chairman of the Medical Research Society and of the MRC Translational Research Overview Group, and a member of the Council of the Society for Endocrinology.
Society for Endocrinology Transatlantic Medal Lecture

S Melmed, Cedars-Sinai Medical Center, Los Angeles, California, USA

Shlomo Melmed received his medical degree with distinction from the University of Cape Town, School of Medicine in 1970. At Cedars-Sinai since 1980, he is now Senior Vice-President for Academic Affairs, and Dean of the Medical Faculty. He is also Professor and Associate Dean of UCLA School of Medicine.

Dr Melmed is a Diplomate of the American Board of Internal Medicine, certified in Endocrinology and Metabolism; a fellow of the American College of Physicians and elected to Master of the ACP in 2010; an elected member of the Association of American Physicians and the American Society of Clinical Investigation. He is also the recipient of the Endocrinology Trust Medal of the Royal Society of Medicine; the Endocrine Society Clinical Investigator Award; and the Pituitary Society’s Lifetime Achievement Award.

Dr Melmed’s laboratory has been consistently funded by the National Institutes of Health (NIH) from 1980 to 2013, and he has trained over 60 physicians, scientists and graduate students who occupy leading positions in academic endocrinology worldwide. His research is devoted to molecular pathogenesis and treatment of pituitary tumors and growth factor regulation of anterior pituitary function. He has pioneered the discovery and application of novel treatments for endocrine tumors and is a recognized international authority on pituitary medicine.

Dr Melmed is acknowledged as a plenary speaker at multiple national and international meetings and is the author of over 250 peer-reviewed articles in prestigious publications. He co-edits Endocrinology: Basic and Clinical Principles, DeGroot and Jameson’s Textbook of Endocrinology, and Williams Textbook of Endocrinology; and is pituitary section author for Harrison’s Textbook of Medicine. He is the Editor-in-Chief of Pituitary, and is on the editorial board of Journal of Clinical Investigation and past Editor-in-Chief of Endocrinology.

Having served on the NIH Endocrinology Study Section, he has also chaired Special Endocrine Study Sections. He was President and founding member of the Pituitary Society, co-chairs its Program Committee, and was an elected member of the Endocrine Society Council. He was President of the International Society of Endocrinology and a member of the Executive Board Committee, Program Chair of the International Congress of Endocrinology, and a Director of the Association for the Accreditation of Human Research Protection Programs.
Society for Endocrinology European Medal Lecture

B Allolio, Department for Endocrinology and Diabetes, University of Wuerzburg, Wuerzburg, Germany

Bruno Allolio received his Bachelor of Physics Degree in 1974 and graduated in Medicine from the University of Cologne in 1975. He obtained his MD in 1977 and trained in General Medicine and Endocrinology at the University Hospital Cologne. He received basic science training under the auspices of John Landon at Bartholomew’s Hospital, London (1981–1982), and at the NIH, Bethesda (1989). He became Professor of Medicine and Head of the Department for Endocrinology and Diabetes at the University of Wuerzburg in 1992.

His interests include adrenal disorders with a current research focus on adrenocortical cancer, adrenal imaging and adrenal insufficiency. Further interests comprise pituitary adenomas, regulation of the HPA axis, bone disease, and hyponatraemia.

He has been a founding member of the European Network for the Study of Adrenal Tumours (ENSAT) and has served as a member of the Executive Committee of the European Society of Endocrinology (2004–2009) and as media officer in the Council of the German Society for Endocrinology.

Bruno Allolio has published more than 250 papers in international journals and more than 50 book chapters. He has served in the editorial boards of the European Journal of Endocrinology and the Journal of Clinical Endocrinology and Metabolism.
Society for Endocrinology Hoffenberg International Medal Lecture

T Yoshimura, Nagoya University, Nagoya, Japan

Prof. Takashi Yoshimura graduated from the Graduate School of Agriculture, Nagoya University in 1996. In 1995 he was awarded the JSPS Research Fellowship for young scientists and between 1996 and 1999 he worked as a Research Associate at the School of Agriculture, Nagoya University. At the same institution he was Assistant professor until 2005 and later became the Associate Professor, Graduate School of Bioagricultural Sciences, Nagoya University. He is currently a professor at the same institution and Director of the Avian Bioscience Research Center, Nagoya University.

Prof. Yoshimura was awarded the Young scientist award from the Japanese Society for Chronobiology in 2004 and in 2005 he received the Japan Prize of Agricultural Science for Young Scientist from the foundation of agricultural Sciences of Japan. In 2009 he received the JSPS prize and the Japanese Society of Animal Science Prize.
The British Thyroid Association Pitt-Rivers Lecture

A Weetman, University of Sheffield, Sheffield, UK

Tony Weetman has been the Sir Arthur Hall Professor of Medicine at the University of Sheffield, and Consultant Endocrinologist at the Sheffield Teaching Hospitals Trust, since 1991. He was Dean of the School of Medicine and Biomedical Sciences from 1999 to 2008 when he became Pro-Vice-Chancellor for the Faculty of Medicine, Dentistry and Health.

After graduating from the University of Newcastle-upon-Tyne in 1977, he had trained with Professor Reg Hall at the Welsh National School of Medicine, Dr Tony Fauci at the Laboratory of Immunoregulation, National Institutes of Health in Bethesda, USA and Prof. Sir Keith Peters at the Royal Postgraduate Medical School, London and the University of Cambridge.

Prof. Weetman is a Founder Fellow of the Academy of Medical Sciences, a former editor of Clinical Endocrinology, The British Medical Bulletin and Clinical and Experimental Immunology, and has served as an Associate Editor of Endocrine Reviews.

He received the Merck Prize of the European Thyroid Association in 2002 and gave the Bradshaw and Clinical Endocrinology Trust Lectures in 2006. He was President of the British Thyroid Association (2005–2008) and presently is Chair of the Medical Schools Council and a member of Council of the Royal College of Physicians of London.
Clinical Endocrinology Trust Visiting Professor Lecture

W F Young Jr, Mayo Clinic, Rochester, Minnesota, USA

Prof. William Young is Professor of Medicine at the Mayo Clinic College of Medicine and Vice-Chair of the Division of Endocrinology, Diabetes, Metabolism, and Nutrition. He received his MD degree from Michigan State University. Prof. Young trained in endocrinology and metabolism at Mayo Clinic, Rochester, Minnesota. He has been a member of the staff at Mayo Clinic since 1984.

Prof. Young is a member of The Endocrine Society, American Association of Clinical Endocrinologists, American Society of Hypertension, and Council of Science Editors. He is a senior editor for *Clinical Endocrinology* and editor of the *Mayo Clinic Endocrinology Update Newsletter*.

He is the recipient of multiple education awards. In 2003, he received the Distinguished Mayo Clinician Award (a distinction given to no more than 4 Mayo physicians annually).

Prof. Young’s clinical research focuses on primary aldosteronism and pheochromocytoma. He has published over 210 articles on endocrine hypertension and adrenal and pituitary disorders. He has presented at over 250 national and international meetings and he has been an invited visiting professor for more than 100 medical institutions.
Society for Endocrinology Medal Lecture

W Arlt, School of Clinical and Experimental Medicine, Centre for Endocrinology, Diabetes and Metabolism, University of Birmingham, Birmingham, UK

Prof. Wiebke Arlt received her undergraduate medical training at the University of Cologne in Germany. She obtained her MD degree in 1993 with distinction and trained in General Medicine and Endocrinology and Diabetes at the University Hospital Würzburg under the auspices of Prof. Bruno Allolio. This was followed by postdoctoral training in Molecular Endocrinology at the University of California in San Francisco and in 2001 she obtained a DSc. After moving to Birmingham in 2002 to work with Prof. Paul Stewart, she was awarded an MRC Senior Clinical Fellowship in 2004. Wiebke now is a Professor of Medicine and Head of the Centre for Endocrinology, Diabetes and Metabolism at the University of Birmingham and Honorary Consultant Endocrinologist at the University Hospital Birmingham and the Birmingham Women’s and Children’s Hospitals.

Prof. Arlt is a committed clinician scientist and her research focuses on the pre-receptor regulation of androgen action. Her achievements include DHEA replacement therapy in adrenal insufficiency and the identification of mutations in P450 oxidoreductase and PAPS synthase causing androgen excess.

She is a member of the Executive Committee of the European Society of Endocrinology and a steering committee member of the European Network for the Study of Adrenal Tumours (ENS@T) and the European Collaborative Project EuroDSD on disordered sex development.
Clinical Endocrinology Trust Lecture

P E Clayton, Endocrinology and Diabetes Group, Manchester Academic Health Sciences Centre, University of Manchester, Manchester, UK

Peter Clayton is Professor of Child Health and Paediatric Endocrinology in the Faculty of Medical and Human Sciences at the University of Manchester. He is an honorary consultant at the Royal Manchester Children’s Hospital and at the Christie Hospital and is the Director of the Greater Manchester, Lancashire and South Cumbria Medicines for Children Local Research Network (LRN).

He graduated from Manchester University Medical School in 1984 (Distinction in Paediatrics), having obtained a first class degree in Physiology and Pharmacology in 1981. He did his early paediatric training around Manchester before embarking on an academic career in Paediatric Endocrinology. His MD thesis was on ‘Growth Patterns after Neuroaxis Irradiation in Childhood’. He spent time at the University of Virginia, USA as an MRC Travelling Fellow in 1990/1.

His primary research interest is centred on understanding mechanisms leading to disordered growth and development. He has around 200 publications on clinical and basic science aspects of paediatric endocrinology. He is on his second term serving on the editorial board of Clinical Endocrinology, and has served on the editorial boards of Hormone Research and the European Journal of Endocrinology. He is currently on the councils of the Society for Endocrinology and the Growth hormone Research Society.
Plenary Lectures
The genetic component of quantitative metabolic traits is complex with a mixture of common alleles of small effect and rarer alleles of larger effect. We have principally focused on finding the latter through the study of extreme human phenotypes of obesity and insulin resistance, including lipodystrophy. By applying both candidate and hypothesis-free genetic approaches we have identified multiple different genetic variants that cause highly penetrant forms of these diseases. Some of these disorders, e.g. melanocortin 4 receptor deficiency are among the commonest human Mendelian disorders. All of these disorders have provided new knowledge regarding various aspects of the physiology of human energy balance and metabolism and functional studies as at a molecular and cellular level have provided valuable insights into structure-function relationships in key regulatory molecules. Genetic variants causing or predisposing to human obesity impact on appetite and satiety to a far greater extent than on metabolic rate or nutrient partitioning. These observations have led to a fundamental shift in thinking about the nature of the biological underpinnings of obesity of obesity. Rather than being a ‘metabolic’ disorder, obesity is essentially a heritable neurobehavioral trait, albeit one with adverse metabolic consequences. These discoveries have altered the way the severely obese child is evaluated clinically and, in the rare case of congenital leptin deficiency, have led to the institution of a dramatically effective, life saving therapy.

Society for Endocrinology Transatlantic Medal Lecture
PL2
Pathogenesis of pituitary adenomas
Shlomo Melmed
Cedars Sinai Medical Center, Los Angeles, California, USA.

Hormone-secreting anterior pituitary cells exhibit slow turnover rates. Subsequent postnatal alterations in pituitary size are determined throughout the lifespan by both extrinsic and intrinsic factors. Pituitary tumors account for ~15% of intracranial tumors and arise from differentiated anterior pituitary cell types, and are invariably benign. They may hyper-secrete hormones, or may be clinically silent. Although hypothalamic influences are permissive for adenoma growth, several lines of evidence point to an intrinsic pituitary cell defect giving rise to these tumors. Hereditary syndromes, including MEN1, Carney syndrome and AIP mutations account for a very small proportion of sporadic adenomas. Although LOH is well documented, identification of adenoma-specific suppressor gene loss has been largely elusive. Compound knockout transgenic models utilizing two or more gene deletions, or targeted knock-ins, have provided new insights for understanding the multi step progression of pituitary neoplasia. Several candidate activating genes and growth factors have been identified which mediate tumorigenesis by disrupting the cell cycle leading to aneuploidy and chromosomal imbalance. Curiously, despite these cellular aberrations pituitary adenomas are invariably benign. Cellular senescence is characterized by irreversible cell cycle arrest and constitutes a protective anti-proliferative response, which can be triggered by DNA damage, chromosomal instability and aneuploidy, loss of tumor suppressive signaling or oncogene activation. Cellular senescence may prevent cells from undergoing transformation in vitro, and in vivo senescence is an important protective mechanisms against malignant transformation. Pituitary tumors exhibit an intrinsic predisposition for senescence-associated molecular pathways and show cell-specific and tumor-specific protective mechanisms underlying the benign nature of GH-secreting or non-functioning tumors. These pathways can be bypassed in vitro, leading to rescue of the senescent phenotype. These mechanisms underlying pituitary trophic changes enable the role of the pituitary to maintain vital homeostatic functions.

We will review the current state of knowledge underlying the cascade of transformation of the normal pituitary through hyperplasia and ultimately adenoma formation, without progressing to malignancy. These observations provide an understanding of molecular mechanisms underlying the multistep pathogenesis of these adenomas and their benign propensity. These observations have enabled development of subcellular approaches for treating these common neoplastic disorders.

Society for Endocrinology Hoffmanberg International Medal Lecture
PL4
Molecular and endocrine mechanism of seasonal reproduction in birds and mammals
Takashi Yoshimura
Nagoya University, Nagoya, Japan.

Animals living outside the tropics use changes in daylength to adapt to seasonal changes in environment, but the molecular and endocrine mechanisms underlying seasonal reproduction are not fully understood. The Japanese quail is a robust model for the study of these mechanisms because of its rapid and dramatic response to changes in photoperiod. In the previous study, we have demonstrated that local thyroid hormone catabolism within the mediobasal hypothalamus (MBH) by thyroid hormone-activating enzyme (type 2 deiodinase: DIO2) regulates the seasonal reproduction. Rapid induction of DIO2 gene expression in the ependymal cells (EC) lining ventrolateral walls of third ventricle of the MBH is the earliest event yet recorded in the photoperiodic signal transduction pathway. To address the identity of the photoperiodic transduction pathway, we have dissected the molecular dynamics of gene expression regulating photoreduced thyroid hormone metabolism using a chicken high density oligonucleotide microarray. We identified two waves of gene expression. The first was initiated ~14 h after dawn of the first long day and included increased TSH β subunit expression in the pars tuberalis of the pituitary gland; the second occurred ~4 h later in the EC and included increased DIO2 expression. TSH receptor was found in the EC of the MBH and intracerebroventricular administration of TSH to short day quail stimulated gonadal growth, and expression of DIO2. This TSH induced expression of DIO2 was shown to be mediated through a thyrotropin receptor-cAMP signalling pathway by the promoter analysis. Increased pars tuberalis TSH therefore appeared to trigger long day photoinduced seasonal breeding. In addition, we have also demonstrated the involvement of TSH signalling pathway in mammals by using the TSH receptor null mice.
The British Thyroid Association Pitt-Rivers Lecture PL5
Thyroid associations
A Westman
Sheffield, UK.

It is a clinical commonplace that many other autoimmune conditions are associated with Hashimoto’s thyroiditis and Graves’ disease, but the breadth of these associations makes it clear that the classically defined spectrum of autoimmune diseases, ranging from organ-to-non-organ-specific, does not in fact exist. All of these associations originate primarily in shared genetic predisposition. As well as HLA alleles, polymorphisms in CTLA-4, TTP22 and CD25 are common to many autoimmune diseases, begging the question of why single autoimmune diseases occur. In some cases, this may be due to additional genetic factors: for instance, polymorphisms in the TNFRI gene predispose to Graves’ disease rather than Hashimoto’s thyroiditis. Recently, a multinational consortium has identified several novel genetic polymorphisms associated with vitiligo and it will be important to determine if these too turn out to be disease-specific. In other cases, environmental factors have a clear role in determining organ-specificity, such as the strong association between smoking and ophthalmopathy, and the sudden recent rise in the prevalence of Hashimoto’s thyroiditis.

A further non-genetic mechanism to explain disease associations is the sharing of autoantigens, most clearly seen with the association between ophthalmopathy and thyroid disease. Nonetheless, characterisation of autoantigens involved in this association has been slow and in other disorders commonly found with thyroid autoimmunity we still don’t know the autoantigenic basis: vitiligo is only infrequently associated with melanocyte autoantibodies (against tyrosinase, TRP1 and MCHR) and an absence of consistent ovarian autoantibodies is also a feature of premature ovarian failure, raising the question of whether these conditions have a more complex aetiology. Thyroid disease associations are clinically important, and developments in protein screening technologies mean that we will soon be in a position to screen for multiple autoantibodies in arrays, and thus able to offer more accurate disease predictions.

Partly supported by the Clinical Endocrinology Trust.

Clinical Endocrinology Trust Visiting Professor Lecture PL6
Endocrine hypertension: then and now
William Young
Mayo Clinic, Rochester, Minnesota, USA.

The evaluation and treatment of pheochromocytoma and primary aldosteronism have evolved dramatically since these two forms of endocrine hypertension were first detected and treated in 1926 and 1944, respectively. We will review the challenges that surrounded the management of the prismatic cases of these two disorders and the advances that have occurred since the initial descriptions. For example, the biochemical testing for pheochromocytomas has progressed from histamine-stimulation tests to phenolamine suppression tests, radio-immunoeassays for catecholamines, clonidine suppression tests, fluorometric assays for total metanephrines and vanillylmandelic acid, high pressure liquid chromatography for fractionated catecholamines and metanephrines, and, most recently, tandem mass spectrometry assays for plasma and urine fractionated metanephrines. Localization of catecholamine-secreting tumors has progressed from exploratory laparotomy to intravenous urograms, body computed tomography, 131I-metadobenzyguanidine (MIBG) scintigraphy, body magnetic resonance imaging, 123I-MIBG scintigraphy with single photon emission computed tomographic (SPECT) images, and positron emission tomography (PET) scanning with 18F-fluorodeoxyglucose (FDG) or 18F-hydroxyephedrine or 6-(18F)fluorohydramine. Approximately 15-20% of patients with catecholamine-secreting tumors have disease-causing germline mutations. Genetic testing is now available for at least six forms of hereditary catecholamine-secreting tumors. The biochemical testing for primary aldosteronism has progressed from bioassays to detect mineralocorticid effect to radioimmunoeassays for aldosterone, enzyme assays for plasma renin activity, renin mass measurement assays, and tandem mass spectrometry assays for aldosterone. Localization of aldosterone-producing adenaomas has progressed from adenal venous sampling to iodochloridostic scintigraphy to posture stimulation test, body computed tomography, measurements of aldosterone precursors (e.g. 18-hydroxycorticosterone), and back to adrenal venous sampling. Genetic testing is now available for the rare glucocorticoid remediable aldosteronism. The surgical management for adrenal-dependent hypertension has also advanced from open laparotomy (five night hospital stay and 4-6 weeks recovery) to laparoscopic adrenalectomy (one night hospital stay and 1 week recovery).

With these remarkable advances in the diagnosis and treatment of adrenal-dependent hypertension that have occurred over the past five decades, one can only wonder what medical advances await this field in the coming years.

Society for Endocrinology BES 2010, Manchester, UK

Society for Endocrinology Medal Lecture PL7
The hitchhiker’s guide to the steroid galaxy
Wicke Arlt
School of Clinical and Experimental Medicine, Centre for Endocrinology, Diabetes and Metabolism (CEDAM), University of Birmingham, Birmingham, UK.

Androgen excess is a key feature of the polycystic ovary syndrome (PCOS), which affects 5–10% of the female population and comes with a significantly increased metabolic risk. Rare disorders often teach us important lessons about common disease and this is highlighted by two novel variants of congenital adrenal hyperplasia we recently identified. In 1985, a patient with combined deficiencies of 17-hydroxylase and 21-hydroxylase was reported. However, the encoding genes were normal. We identified the molecular basis of this disorder, inactivating mutations in P450 oxidoreductase (POR), the mandatory electron donor for 17-hydroxylase and 21-hydroxylase. Uniquely, both affected 46,XX and 46,XY individuals present with disordered sex development. Male underandrologisation is a logical consequence of 17-hydroxylase deficiency and subsequent lack of synthesis of DHEA, the crucial precursor of human sex steroid synthesis. However, virilisation in affected girls was an apparently contradictory finding, which we now have shown to be explained by the existence of an alternative pathway towards active androgens, with important implications for normal and disordered human fetal development. DHEA is efficiently converted to active androgens or alternatively to its sulphate ester, DHEAS. We have shown that DHEA and DHEAS are not interconverted freely and continuously as previously assumed; thus, the conversion of DHEA to DHEAS represents a permanent inactivation Impairment of DHEA sulfation will therefore result in an increased flux of DHEA towards androgen synthesis. We have analysed a girl presenting with premature pubic hair development, followed by the onset of acne, hirsutism, oligo- and eventually amenorrhoea, i.e. hallmark features of early-onset PCOS, increasingly frequent in young girls due to the increase in obesity rates. However, in this girl DHEAS levels were undetectable whereas DHEA and active androgens were high, suggesting a defect in DHEA sulfation. We have identified the underlying molecular defect, highlighting novel candidate genes for PCOS.

Clinical Endocrinology Trust Lecture PL8
Ubiquitination: the ‘Kiss of Death’ for human growth
Peter E Clayton, Dan Hanson, Philip Murray, Amit Sud & Graeme Black
University of Manchester, Manchester, UK.

Ubiquitination (Ub) is the process that controls the level and activity of cellular proteins. Mono-ubiquitination of a protein alters its function, while poly-ubiquitination targets a protein for degradation (as the ‘kiss of death’).

Endocrine Abstracts (2010) Vol 21
Alterations in the Ub system are associated with a wide range of disease, e.g. cancer, neurological diseases and viral infection. Disorders of growth where the phenotype is primarily short stature are usually caused by disruption of the GH–IGF system or by mutations in genes controlling bone growth. However a murine knock-out of Cullin 7, a component of the E3 ligase of the Ub system, results in significant fetal growth restriction and embryonic lethality. We have now identified mutations in CUL7 in families with 3-M syndrome, a growth disorder with reduced but proportionate fetal and postnatal growth restriction, indicating that this component of the Ub system is essential to growth. We now show that families with an apparently identical 3-M phenotype can have mutations in OBSL1, a cytoskeletal adaptor protein expressed in cardiac and skeletal muscle, and hitherto not implicated in either growth or the Ub system. CUL7 and OBSL1 appear to act in a common pathway, as knock-out of OBSL1 leads to down-regulation of CUL7, knock-out of CUL7 leads to down-regulation of OBSL1 and co-immunoprecipitation experiments demonstrate that the two proteins physically associate. Children with 3-M syndrome respond poorly to GH treatment: in fibroblasts with a CUL7 mutation, GH signalling through Stat5 and Mapk appears normal, but Akt activation by IGF1 is markedly reduced. These data indicate that the 3-M syndrome is genetically heterogeneous, that disordered ubiquitination with CUL7 mutations causes a primary growth disorder, that OBSL1 is a novel component in this system, and that IGF1 signalling is disrupted. This model growth disorder is providing new avenues to explore in the control of human growth.

Generously supported by the Clinical Endocrinology Trust
Symposia
Novel mechanistic insights into thyroid diseases

§1.1 Human mutations causing hypothyroidism
Jose Moreno
Madrid, Spain.

Hypothyroidism is the most frequent innate endocrine disorder, reaching a prevalence of 1 in 1200 newborns. Defects causing hypothyroidism occur at any level of the hypothalamus-pituitary-thyroid axis, but also at peripheral tissues, where alterations of intracellular transport, deiodination or nuclear action of thyroid hormone have been described in humans.

In recent decades, molecular research found evidence that hypothyroidism is a genetic disease. Defects in more than 20 genes have been associated with different types of hypothyroidism. However, environmental conditions like iodine deficiency also cause hypothyroidism. Indeed, iodine shortage may influence the phenotype of genetically-determined hypothyroidism, especially that of partial dyshormonogenesises. Furthermore, the deficiency of iodothyronine deiodinase (DEHAL1), the enzyme that recycles iodide within the thyroid, exemplifies the close genetic-environmental interplay in the expression of hypothyroidism.

Congenital hypothyroidism (CH) can be permanent or transient. Recently, we learned that transient CH is not only originated by immune or iatrogenic factors, but rather that it is also a genetic disease, caused by haplinsufficiency of DUOX2, the main component of the thyroidal H2O2-generation system.

All known cases of human genetic hypothyroidism are monogenic diseases inherited in classical Mendelian fashion, but they only represent a minority among CH population. The majority of CH cases are sporadic, and do not follow Mendelian inheritance. Moreover, monozygotic twins are discordant for CH. This calls for a paradigm change when understanding the pathogenesis of hypothyroidism, including that, in certain instances, it may be a multigenic disease. This hypothesis was proven correct in Pax8 and Tif11 double heterozygous KO mice, but is not shown in humans. Other non-Mendelian mechanisms that could be involved are the occurrence of (early) spontaneous mutations together with germline defects in a "two-hit" model and alterations of the epigenetic modification of DNA and histones, and both represent attractive challenges for future translational research of hypothyroidism.

§1.2 Genetic mechanisms defining the response to thyroid hormone replacement
V Parker 1,2
1University of Bristol, Bristol, UK; 2University of Western Australia, Perth, Western Australia, Australia.

The adequacy of thyroid hormone replacement in hypothyroid subjects has long been debated. A proportion of subjects on thyroxine report not achieving their pre-disease level of well-being and evidence from community studies suggest impaired well-being in subjects on thyroxine compared to the general population. Our work on the large HUNT 2 cohort from Norway confirms this and furthermore revealed that subjects on thyroxine have a different relationship between TSH and well-being. Despite this, ten randomised controlled studies (and meta-analysis) of combination T4/T3 replacement compared to T4 alone did not confirm findings from an initial study which showed a benefit of combination therapy.

We explored a genetic basis for these findings in light of greater understanding of the human genome and common variation within it. These methods have already shown us that single nucleotide changes in genes may be associated with serum levels, for example a SNP in the deiodinase 1 gene (DIO1) and one in the phosphodiesterase 8B gene (PDE8B) have been shown to be associated with T4/T3 and TSH levels respectively. Although they have only a small effect on hormone levels, they are important findings as they further our understanding of thyroid physiology and can be used to confirm associations via Mendelian Randomisation. There is also evidence of an effect of these polymorphisms beyond serum hormone levels, with SNPs in DIO2 increasing risk for osteoarthritis.

Therefore, we investigated the effect of common variation in the three deiodinase genes on response to combination T4/T3 versus T4 only therapy in the largest trial – WATTS. We found that subjects with a polymorphism in DIO2 had impaired well-being at baseline on T4 and furthermore improved significantly when put on combination therapy. Whilst this result requires replication, it suggests that there may be a proportion of the population on thyroxine who required combination therapy.

§1.3 The clinical spectrum of Pendred syndrome
P Kopp
Northwestern University, Chicago, Illinois, USA.

Pendred syndrome (PS) is an autosomal, recessive disorder characterized by sensorineural deafness, goiter, and a positive perchlorate test. PS, one of the most common forms of syndromic deafness, is caused by biallelic mutations in the SLC26A4 gene, which encodes the anion transporter pendrin. Functionally, pendrin can serve as an exchanger of several anions including chloride, bicarbonate and iodide. Pendrin is expressed in the inner ear, the thyroid and the kidney.

The phenotypic expression of goiter and deafness is variable. Some individuals develop very large goiters, others present with no thyroid enlargement. This variability is probably influenced by the nutritional iodide intake. Under conditions of normal iodide intake, patients with PS are usually euthyroid, but if the iodide supply is scarce, overt hypothyroidism may develop. Sensorineural hearing loss, usually profound prelingual deafness, is the hallmark of PS. High-resolution imaging of the inner ear reveals malformations of the vestibular aqueduct, and the endolympathic duct and sac in nearly 100% of individuals with PS. In the absence of a goiter, the (familial) finding of an enlarged vestibular aqueduct (EVA) may point to the non-syndromic form of deafness caused by biallelic SLC26A4 gene mutations.

In the inner ear, pendrin is involved in chloride/bicarbonate exchange and the maintenance of the endocochlear potential. In thyroid follicular cells, pendrin is inserted into the apical membrane. Functional studies suggest that pendrin may be involved in the efflux of iodide into the follicular lumen. Inactivating mutations of pendrin impair the efflux of iodide, which may explain the partial organisation defect. Alternatively, pendrin could be of importance in maintaining an optimal follicular pH necessary for theorganisation of iodide. In the kidney pendrin acts as a chloride-bicarbonate exchanger in beta-intercalated cells of the cortical collecting duct, a subpopulation of cells that mediate bicarbonate secretion.

§1.4 Novel mechanisms regulating the sodium iodide symporter: implications for thyroid tumourigenesis
Chris McCabe
University of Birmingham, Birmingham, UK.

The treatment of thyroid cancer has not changed substantially for several decades, in contrast to most other tumour types. Total thyroidectomy and administration of ablative radioiodine remain the cornerstones of the therapeutic regimen, which is associated with a good 5-year survival rate. Nonetheless, thyroid cancer treatment is not perfect, and several hurdles remain to be overcome. Radioiodine ablation of differentiated thyroid cancers and their metastases utilises the ability of the thyroid to accumulate iodide. Treatment is therefore dependent on the expression and function of the sodium iodide symporter (NIS), which transports iodide into thyroid follicular cells for thyroid hormone biosynthesis. However, thyroid cancers, NIS expression levels are variable and the avidity of the thyroid for radioiodine can be reduced by transcriptional and post-translational effects on NIS expression. Whilst the mechanisms which diminish NIS activity in thyroid cancer are not fully understood, we have recently elucidated two discrete routes by which PTTG binding factor (PBF) represses iodide uptake through the down-regulation of NIS function. PBF is a protein described so far in fewer than 10 publications, but which exhibits a growing multifunctionality in endocrine tumourigenesis. In the thyroid, PBF is over-expressed in tumours compared to normals, and expression correlates with early tumour recurrence. In both primary human thyroid cultures and rat thyroid FRTL-5 cells, PBF significantly represses activity of the human NIS promoter and inhibits iodide uptake. More recently, we demonstrated that PBF binds NIS in vitro in GST pull-down and co-immunoprecipitation experiments. Transient PBF over-expression is associated with a significant reduction in plasma membrane-associated NIS, and a concurrent repression of iodide uptake. Further targeted transgenic over-expression of PBF in mouse thyroids results in reduced NIS mRNA and protein expression, and profound goitre formation. Thus PBF represents a gene which exhibits both transcriptional and post-translational repression of NIS expression and function. Given that effective radioiodine uptake remains critical to the management of differentiated thyroid cancer and its metastases, PBF represents a novel therapeutic target, particularly in the treatment of radioiodine-resistant tumours.
New endocrinology of bone

S2.1

Insight into bone metabolism from human experiments of nature

Miep Helrich
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In the past 20 years or so careful observations in human and rodent diseases of bone metabolism have greatly helped to understand these diseases, but also uncover important pathways in bone metabolism. Such pathways have led to successful drug discovery programmes and some of the latest drugs to be licensed for use in common bone diseases have directly been the result of understanding such ‘experiments of nature’. An example is the targeting of cathepsin K as an anti-resorptive treatment, a target that was chosen after the gene CTSK was found to be involved in the rare disease pseudohypoparathyroidism in which osteoclasts cannot degrade bone collagen.

In this presentation I will first outline some of the key cellular pathways involved in bone formation and bone degradation and remodelling. I will then describe some of the rare diseases that have provided insights in these pathways. I will focus specifically on diseases caused by osteoclast dysfunction as we have most information in those conditions. I will discuss the various types of osteopetrosis, high bone mass disorders caused largely by defects in osteoclast function (TCIRG1, CLC7, OSTM1, CA2, PLEKHM1), but also, in some cases, by defects in osteoclast formation (RANK, RANKL).

I will then discuss some recent findings in the diseases classed as ‘Paget-like diseases of bone’, a group of conditions in which bone turnover is increased and in which mutations in QSTM1, VCP and RANK have been identified. It has become clear that protein degradation pathways may be affected in these diseases. Finally, I will briefly discuss the rare, but important high bone mass conditions caused by mutations in LR5P5 and SOST. The discovery of these genes has led to a rapid translation of basic science to clinical applications and offers hope for treatment of common low bone mass disorders such as osteoporosis.

S2.2

PPARG osteoporosis and diabetes mellitus

Clifford Rosen
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The relationship between bone and fat has been the subject of intense investigations over the last decade. In particular the concept that adipocytes and osteoblasts arise from the same precursor has led to significant advances in our understanding of the bone marrow milieu and the in vivo responsiveness of the skeleton to nutritional, genetic and environmental stimuli. PPAR-γ lies at the center of a mesenchymal network that ultimately determines the fate of marrow stem cells and the function of adipocytes. Although PPAR-γ2 activation is essential for adipocyte differentiation, and in some cases prevents osteogenic development, this transcription factor is context specific and developmentally sensitive. Its role in brown adipogenesis and its relationship to skeletal acquisition will be reviewed. In addition, the phenotypic characterization of the skeleton in genome ‘gain’ or ‘loss of function’ polymorphisms or mutations is remarkable and provides insights into the inter-relationship of fat to bone. Finally, we recently identified a chaperone protein that escorts PPARG into the nucleus and may link circadian rhythms to adipose and skeletal differentiation. Studies of anti-diabetic agents that enhance PPAR-γ activation but cause bone loss suggest there are multiple pathways by which this nuclear transcription factor can affect metabolism of bone cells. Deletion of PPARG in stromal cells using the α2 promoter results in a provocative skeletal phenotype. Future studies are aimed at determining how hyperglycemia affects the skeleton, particularly through PPAR-γ and the role of altered circadian pathways in the syndromes of night time eating disorders, osteoporosis and obesity.

S2.3

Ghrelin and ghrelin signaling in bone metabolism

Hans van Leeuwen
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GH levels decline with aging and this is thought to contribute to the age-related decrease in bone mass. However, data on the effects of GH replacement on osteoporosis are contradictory. The potential therapeutic benefits depend on the dose, frequency and duration of treatment. It is for this reason that treatments with GH secretagogues (GHS), which stimulate pulsatile release of GH as occurs in normal physiology, have been postulated to be a more successful therapeutic approach. GHS are a class of synthetic compounds that can stimulate GH secretion both in vivo and in vitro. Both peptidyl and non-peptidyl GHS’ have been characterized and differ in their effects on bone formation and resorption. In 1996 the GH secretagogue receptor (GHS-R) was discovered, on which a few years later the natural ligand ghrelin was identified. Stomach-derived ghrelin stimulates GH release by activating the GHS-R in the pituitary and hypothalamus. GH secretagogue activity is, however, not ghrelin’s major activity since mice deficient for ghrelin or its receptor are not dwarves. Moreover, ghrelin exerts metabolic effects by regulating appetite, feeding and energy homeostasis through central interaction with the satiety hormone leptin. Like the GHS, ghrelin may affect bone metabolism through its effects on GH secretion. However, infusion of ghrelin into GH-deficient rats increased their bone mineral density (BMD). The concentrations of ghrelin used did not evoke a rise in body weight and food intake excluding possible indirect effects on BMD of ghrelin through its orexigenic activity. This study indicated direct effects on bone cells, which was corroborated by studies on isolated osteoblasts. The recent developments on ghrelin in bone metabolism and bone cell function will be discussed.

S2.4

FGF23, osteoblasts and phosphate homeostasis

B Fraser

Abstract unavailable.

S3.1

Embryonic stem cells: derivation, propagation and therapeutic potential

S Minger

Abstract unavailable.

S3.2

Pluripotent stem cells in the testis: restoring fertility

Stefan Schlatt
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Spermatogonial stem cells open novel strategies for derivation of pluripotent cell lineages and fertility preservation in boys and men exposed to gonadotoxic therapies. The testicular stem cells are descendants of the primordial germ cells. They are the only germline cells remaining mitotically active in adulthood. Their highly specialized task is the preservation of DNA integrity in the germline. In contrast to all other stem cells they do not serve any beneficial functions for the individual male organism but act as an integral part of the germline preserving the DNA integrity of the species. Species-specific differences must be considered when spermatogonia are considered for development of new technologies. In rodents, very few stem cells undergo an enormous premeiotic expansion while in primates numerous A spermatogonia undergo limited premeiotic divisions. Such differences in expansion as well as potential differences in the associated stem cell niches have consequences for in vitro and in vivo strategies. In recent years in vitro procedures have revealed the derivation of pluripotent cells from testicular stem cells in a variety of species, including man. Also, approaches for in vitro maturation of spermatogonia into sperm are developed. Germ cell transplantation and testicular grafting are available as exciting experimental strategies, which may also have clinical potential. However, human applications must still be regarded as experimental and many aspects of the novel procedures need to be optimized prior to a safe and efficient use.
A longstanding unsettled question is whether pancreatic β-cells originate from exocrine duct cells. We have now used a genetic labeling approach to fate map embryonic and adult pancreatic duct cells. We employed a BAC genomic clone containing the Hnf1b locus to generate a transgenic mouse line that expresses a hormone-inducible form of Cre in pancreatic ducts. We verified that in this model Cre is specifically expressed throughout the pancreatic duct epithelium, in virtually all cells expressing Hnf1b, and showed that Cre-induced recombination of a reporter locus is strictly hormone-dependent. We subsequently performed lineage tracing analysis of Hnf1b+ duct cells of the developing and adult pancreas. We show that Hnf1b+ cells of the early branching pancreas contribute to acinar, duct, and endocrine lineages. Hnf1b+ cells thereafter form the embryonic duct epithelium that gives rise to both ductal and endocrine lineages, but not to acinar cells. By the end of gestation, however, the fate of Hnf1b+ duct cells is restricted. We provide compelling evidence that the differentiated duct epithelium does not make a significant contribution to acinar or endocrine cells during neonatal growth, during a 6-month observation period, or during growth of β-cells that follows the ligation of the pancreatic duct or the ablation of β-cells followed by EGF and gastrin treatment. Thus, once the ductal epithelium differentiates it has a restricted plasticity, even under regenerative settings.

Glucocorticoids (GCs) are the most commonly used anti-inflammatory and immunosuppressive drugs, their efficacy seems to be caused by the interference of the ligand-activated glucocorticoid receptor (GR) with many pro-inflammatory pathways via different mechanisms. The ubiquitous expression of the GR is a prerequisite of this efficacy. The main draw back of GCs in many cell types. DUSP1 dephosphorylates and inactivates mitogen-activated protein kinases (MAPKs) that are required for expression of pro-inflammatory gene expression. Such compounds (selective GR modulators or SGRMs) are predicted to retain anti-inflammatory effects but have fewer side effects. However, GCs can also upregulate several anti-inflammatory mediators. For example, expression of the dual specificity phosphatase DUSP1 is increased by GCs in many cell types. DUSP1 dephosphorylates and inactivates mitogen-activated protein kinases (MAPKs) that are required for expression of pro-inflammatory gene products. Hence the induction of DUSP1 is a mechanism for restraining inflammatory responses, and DUSP1+ mouse macrophages are partially insensitive to anti-inflammatory effects of GCs. GC regulation of DUSP1 gene expression is unusual. Several consensus GR binding sites are conserved between mouse and human DUSP1 loci up to 29 kb upstream of the transcription start site, but these sites are differentially employed by the two organisms. The mouse DUSP1 gene is regulated via an element at −29 kb, the human DUSP1 gene via elements at −4.6 and −1.3 kb. Unusually, transcripitional induction is independent of GR dimerisation in both species. Induction of DUSP1 by GC is highly sensitive to cell density, whereas other GC responses are unaffected by cell density. Perhaps reflecting the unusual control of the DUSP1 gene, it can be induced by two recently described SGRMs. We discuss the possibility that both conventional GCs and SGRMs exert anti-inflammatory effects both by transrepression and by upregulation of anti-inflammatory mediators like DUSP1.
of GCs, however, is due to their potential to induce undesired effects, in particular upon high doses and prolonged usage. Introduction of topical GCs, that act locally, in the treatment of e.g. inflammatory skin diseases led to a reduction of systemic adverse effects. Nevertheless, undesirable cutaneous effects such as skin atrophy persist from the use of topical GCs. Therefore, a high medical need exists for drugs as effective as GCs but with a reduced side effect profile. Glucocorticoid function by binding to and activating the GR, which positively or negatively regulates the expression of specific genes. Several experiments suggest that negative regulation of gene expression (transrepression) by the GR accounts for its anti-inflammatory action. This occurs through direct or indirect binding of the receptor to pro-inflammatory transcription factors that are already bound to their regulatory sites. The positive action of the receptor (transactivation) occurs through homodimer binding of the ligand receptor complex to discrete nucleotide sequences and this contributes to some of the adverse effects of the hormone. Glucocorticoid receptor ligands that promote the negative regulatory action of the receptor with reduced positive regulatory function should therefore show an improved therapeutic index. Consequently, our goal has been to identify GR ligands, which preferentially induce transrepression with little transactivation only.

Here, we show a representative of a novel class of compounds, selective glucocorticoid receptor agonists (SEGRAs), with a dissociation of transactivation from transrepression activity in vitro and in vivo. These non-steroidal GR-agonists represent promising new structures with improved effect/side effect profile.

Vitamin D and calcium signalling in the immune system

S5.1 Vitamin D and calcium signalling in autoimmune disease
Chantal Matthieu
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Vitamin D has become an important player beyond bone and calcium metabolism. Receptors for 1,25(OH)2D3 or calcitriol as well as the machinery to produce 1,25(OH)2D3 locally are present all over the immune system. Regulation of the enzyme responsible for the final activation of 1,25-dihydroxy vitamin D3 (CYP27B1) is however completely different in immune cells than in kidney cells. Particular immunomodulating effects of 1,25(OH)2D3 are observed on dendritic cells, the central cell in the immune system, determining the balance between tolerance and autoimmunity.

Type 1 diabetes is an autoimmune disease where the insulin producing cell of the pancreas, the β-cell, is under attack. Vitamin D not only has effects on the immune system, but also affects the behaviour of the β-cell in a way that will alter its fate. Vitamin D deficiency dramatically affects disease presentation in an animal models of type 1 diabetes, whereas NOD mice lacking the vitamin D receptor have an unaltered diabetes presentation. Thus, vitamin D and its structural analogues may provide tools for the prevention or cure of type 1 diabetes.

Anti-bacterial action of vitamin D
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Immunomodulatory properties of vitamin D have been recognized for more than 25 years. However, until recently it was unclear whether these actions of contributed to normal immune function. Two new developments have helped to clarify this. Firstly, our perspective on what constitutes normal vitamin D status has changed considerably, with the introduction of the term “vitamin D insufficiency” to describe individuals with inadequate (but not rachitic) serum levels of 25-hydroxy vitamin D (25(OH)D), the main circulating form of vitamin D. This has led to the conclusion that millions of people worldwide have impaired 25OHD status. Secondly, a series of studies by our group and others have shown that macrophage antibacterial innate immune responses are associated with enhanced conversion of 25OHD to active 1,25-dihydroxy vitamin D (1,25(OH)2D), and elevated expression of the vitamin D receptor (VDR). This intracrine response to vitamin D provides a flexible system for handling pathogens such as *Mycobacterium tuberculosis*: local synthesis of 1,25(OH)2D promotes synthesis of antimicrobial proteins and enhances key autophagy events required for killing of pathogens. In addition, intracrine metabolism of vitamin D promotes feedback regulation of Toll-like receptor signaling and facilitates communication with the adaptive immune system. These macrophage actions of vitamin D are characterized by localized synthesis of 1,25(OH)2D via the enzyme 1α-hydroxylase (CYP27B1), a reaction which is primarily dependent on the availability of substrate for CYP27B1, namely 25OHD. Thus, innate immune responses to vitamin D will be strongly influenced by the vitamin D status of individuals, and may be compromised in those with vitamin D insufficiency. The implications of this new perspective on the interaction between vitamin D and the immune system will be discussed with particular reference to the mechanisms involved in regulating vitamin D metabolism and the impact on human health.

Vitamin D as a risk factor for multiple sclerosis
Brenda Banwell
The Hospital for Sick Children, Toronto, Ontario, Canada.

Multiple sclerosis (MS) is an inflammatory and neurodegenerative disease of the central nervous system. Genetic and environmental risk factors are likely operative in MS pathobiology. MS prevalence varies from very low rates in peri-equatorial regions, to over 100/100,000 population in temperate regions distant from the equator; prompting consideration of limited sunlight and vitamin D insufficiency as potential risk factors for MS. In a large study of MS patients living in temperate countries, significantly fewer were born in November and significantly more in May, suggesting that seasonally-related environmental risk factors may already even be operative per- or perinatally. Several retrospective case-control studies have shown that greater sun exposure during childhood and adolescence was associated with a reduced risk of adult-onset MS. Individuals reporting oral ingestion of vitamin D supplements, or ingestion of a diet rich in vitamin D-containing foods also have a lower MS risk. The increasing recognition of MS in children and adolescents has prompted study of vitamin D status in children with confirmed MS, and in children who have experienced a first demyelinating event (50% of whom will ultimately be diagnosed with MS). In our national pediatric demyelination study in Canada, low serum 25-hydroxy vitamin D (25(OH)D) concentrations at the time of a first demyelinating attack predict MS risk- with a 20% reduction in risk for every 10 nmol/l increase in 25(OH)D concentration. These data, the available literature, and potential biological and therapeutic considerations will be presented.

Vitamin D and regulatory T cells in allergy and asthma
Catherine Hawrylowicz
Guys Hospital, London, UK.

Some but not all epidemiologic studies suggest an association between vitamin D deficiency and an increased incidence of asthma symptoms. Similar associations have been made with respiratory infections, e.g. influenza, tuberculosis. We are interested in the wider role of vitamin D in maintaining pulmonary health through the induction of both anti-microbial and regulatory pathways. We, and others, propose these are critical to controlling infections in the airways, whilst limiting inflammatory responses that damage lung tissue and impair gaseous exchange. Regulatory T cell (Treg) populations of interest in allergic and asthmatic disease include CD4+Foxp3 + T and IL-10-secreting T regulatory cells. These cells are thought to play an important role in maintaining immune homeostasis in health, including in the respiratory environment and their function is believed to be impaired in patients. Considerable interest exists in therapeutic strategies aimed at inducing or boosting T regulatory cell activity in patients in order to provide improved, long-lasting and safer treatments for allergic and asthmatic disease. Our studies are investigating the capacity of 1α 25dihydroxyvitamin D3, alone or in concert with glucocorticoids, to promote both IL-10 secreting regulatory T cells and Foxp3 + Treg cells.

Fit or fat? Mechanisms regulating our metabolic fate
C Palmer
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Monogenic obesity disorders and mouse models of obesity have overwhelmingly pointed to hyperphagia as the predominant genetic force in promoting obesity, a
was therefore no surprise when the most robust common variant associated with obesity from GWAS studies was associated with increased energy intake. We and other groups have shown that variants in the FTO gene are not associated with increased food intake per se, but rather display a preference for energy dense foods resulting in an increased caloric intake. This leads to an increase in BMI that is readily detectable in children as young as four years of age. This increase in BMI is largely due to an increase in whole body fat mass, and specific fat depots are not selectively involved in this phenotype. Mice deleted for the FTO locus display hyperphagia, however increased metabolism leads to a leaner phenotype, whereas in the humans increased metabolism is also observed in the hyperphagic minor allele carriers, but this is clearly not sufficient to prevent obesity in these individuals. This increased BMI from early childhood to adulthood leads to an increased risk of type 2 diabetes, dyslipidemia, hypertension and finally results in premature death through cardiovascular disease. These observations represent a clear mendelian randomisation experiment confirming the powerful causal role of poor dietary habits and obesity in driving premature morbidity and mortality being observed in many populations around the world.

**S6.2**
The phenotype of the FTO knockout mouse
J Bruning

Abstract unavailable.

**S6.3**
Gut-brain circuits controlling glucose homeostasis
T Lam

Abstract unavailable.

**S6.4**
Mechanisms of serotonin regulating food intake: the role of the melanocortin pathway
Lora Heisler & Daniel Lam
University of Cambridge, Cambridge, UK.

The central serotonin (5-hydroxytryptamine, 5-HT) system is an established modulator of energy balance. Therefore, it is unsurprising that former (e.g. t-fenfluramine), current (e.g. sibutramine), and drug discovery (e.g. lorcaserin) obesity treatments target serotonin pathways to affect food intake and body weight. Pharmacological and genetic research implicates the Gq-coupled serotonin 2C receptor (5-HT2CR) and the Gi-coupled serotonin 1B receptor (5-HT1BR) specifically in these effects. We sought to clarify how serotonin in general, and the 5-HT2CRs and 5-HT1BRs in particular, modulate ingestive behavior. Through a combination of functional neuroanatomy, genetic, feeding, and electrophysiology studies in rodents, we found that 5-HT2CR and 5-HT1BR agonists require melanocortin pathways to exert their effects on appetite. Specifically, we observed that serotonin and 5-HT2CR agonists activate neurons expressing the endogenous anorectic melanocortin agonist proopiomelanocortin (POMC)/α-melanocyte stimulating hormone (α-MSH) and that serotonin and 5-HT1BR agonists inhibit the activity of neurons expressing the endogenous orexigenic melanocortin antagonist agouti-related peptide (AgRP) in the arcuate nucleus of the hypothalamus. We further verified the necessity for 5-HT2CRs expressed on POMC neurons in serotonin’s effects on energy balance using a genetic mouse line in which 5-HT2CRs are exclusively expressed in POMC neurons. Exclusive expression of 5-HT2CR on POMC neurons abolished the hyperphagia, obesity, and attenuated anorectic responses to serotonin drugs evident in the complete 5-HT2CR knockout, illustrating that 5-HT2CRs specifically on POMC neurons underlie the effects of 5-HT2CR agonists on energy balance. In the brain, α-MSH and AgRP compete for action at the melanocortin 3 (MC3) and melanocortin 4 (MC4) receptors. To further clarify the pathway through which serotonin influences appetite, we examined whether pharmacological blockade or genetic inactivation of the MC3Rs or MC4Rs abolishes t-fenfluramine, 5-HT2CR and/or 5-HT1BR agonist hypophagia. We observed that activation of the MC4Rs, but not the MC3Rs, is required for t-fenfluramine, 5-HT2CR and 5-HT1BR agonists to influence feeding. A model is presented in which activation of the melanocortin system is downstream of serotonin and is necessary to produce the complete anorectic effect of serotoninergic compounds.

Dilemmas in managing gender identity problems in adolescence

**S7.1**
Dilemmas in managing gender identity problems in adolescence: psychological issue
Peggy Cohen-Kettenis
VU University Medical Centre, Amsterdam, The Netherlands.

Treatment of gender dysphoric individuals, has in medicine nearly always met with a great deal of skepticism. In recent years, changes in the clinical management of adolescents have been made by clinicians in Europe and North America. In their clinics, puberty is suppressed in an early pubertal stage and/or at certain ages. The clinicians have come to this decision for various reasons: 1) the adverse effects of a relatively late treatment start on the adolescents’ psychological functioning, and on their social their intellectual development; 2) the unsatisfactory physical results of late treatment and 3) the unfavourable post-operative outcome, in terms of psychological functioning and quality of life, related to a late rather than an early start of treatment. Pubertal suppression allows adolescents to make the decision to start cross-sex hormone treatment under less stressful conditions than when they have to do so, while fully aware of and distressed by their unwanted physical masculinization or feminisation. Concerns, however also exist regarding the ability of adolescents to decide on pubertal suppression and the safety of this intervention. We will discuss both psychological pros and cons of pubertal suppression.

**S7.2**
Dilemmas in managing gender identity problems in adolescence: a medical overview
Iuean Hughes
Cambridge, UK.

The Endocrine Society has published guidelines on the endocrine treatment of transsexual persons (J Clin Endocrinol Metab 2009 94 5312–54). For adolescents with gender identity disorder (GID), the guideline recommends that suppression of pubertal hormones start when girls and boys exhibit physical changes of puberty (confirmed by pubertal levels of estradiol and testosterone, respectively), but no earlier than Tanner stages 2-3. In practice, this means assessing the onset of puberty in girls by evidence of breast ‘budding’ and in boys, an increase in testis volume from 3 to 4 ml. Increase in height velocity predestines constitutional signs of puberty in girls but such auxological data is seldom available. The age of onset of puberty in normals ranges from 8 to 13 and 10–13 years in girls and boys, respectively. Furthermore, puberty is probably starting earlier now, especially in girls. Sex steroid levels increase according to Tanner stages but for random measurements in individual subjects, these would not confirm the physical changes. The debate about early versus late suppression of puberty in GID must recognise the variability in puberty onset and tempo and the imprecision of gonadal steroids as biological markers. Focus should be on predicting the irreversible, late physical changes as GnRH analogues can halt and even reverse early changes. For MTF transsexuals, voice ‘breaking’ and mandibular growth are irreversible and disturbing long-term. However, these are late events occurring between stages 3 and 4. The advocates of early versus late puberty suppression may find there is common ground once the characteristics of normal puberty are considered in a practical context.

Role of the circadian clock in endocrinology

**S8.1**
Molecular basis of the circadian clock: regulation of endocrine rhythms
Michael Hastings
MRC Laboratory of Molecular Biology, Cambridge, UK.

Circadian rhythms are cycles of behaviour and physiology that persist with a period of approximately (circa) one day (-dian) when an individual is held in temporal isolation. They permeate all levels of biological activity, from gene expression and hormone secretion to cognitive ability. These daily rhythms are co-ordinated by a hypothalamic pacemaker, the suprachiasmatic nuclei (SCN). Individual SCN neurons are competent circadian pacemakers, synchronised to solar time by retinal innervation. Their intrinsic oscillator consists of a series of inter-linked, autoregulatory transcriptional/post-translational feedback loops.
incorporating Per and Cry genes. Mutations that alter the rate of transcription of these genes or the stability of Per and Cry proteins affect clock speed, shortening or lengthening the period of dependent behavioural, metabolic and molecular circadian cycles. Molecular time-keeping in SCN neurons is synchronised and sustained by inter-neuronal signals mediated by the neuropeptide vasoactive intestinal peptide and its Vpα2 receptor. A molecular clock mechanism comparable to that of the SCN is present in most major organ systems. These tissue clocks are synchronised by endocrine, autonomic and behavioural cues that are dependent on the SCN. In turn they drive the circadian expression of local transcriptomes and proteomes, thereby co-ordinating circadian metabolism and physiology. Rhythmic glucocorticoid signalling is a potent internal synchroniser of local clocks. The role of local, SCN-synchronised clocks in controlling vital processes (including xenobiotic detoxification, cell division and nutrient metabolism) is essential to health. Disturbances to circadian timing arising from modern working schedules are becoming recognised as an increasingly relevant factor in major systemic illness. In neurodegenerative conditions, such as Alzheimer’s disease and Huntington’s disease, breakdown of the circadian regulation over sleep and wakefulness is a major determinant of institutionalisation. Moreover, the newly identified molecular components of circadian control systems provide novel avenues for therapeutic intervention in systemic disease.

S8.2

Circadian clocks in adipose tissue
Jeffrey Gimble, Xingyu Wu, Gang Yu & Elizabeth Floyd
Pennington Biomedical Research Center, Baton Rouge, Louisiana, USA.

There is a growing body of literature indicating that circadian mechanisms regulate metabolism in adipose tissues. Genes encoding the core circadian regulatory proteins (CCRP) display a robust oscillatory expression profile in murine adipose tissue depots, as well as the brain, liver, and heart. Temporally restricted food access as well as photic stimuli can entrain the CCRP expression. In murine models, deletion or mutation of the CCRP genes, clock and PPAR gamma coactivator 1 (PGC1α), have been associated with increased risk of obesity and/or abnormal metabolic function. Transcriptomic analyses using classical algorithms demonstrate that >20% of the expressed genes in these tissues exhibit circadian rhythmicity. These in vivo findings have been extended to in vitro models. The expression profile of CCRP encoding genes can be synchronized in primary cultures of undifferentiated and adipocyte-differentiated human adipose-derived stem cells by dexamethasone. Similar observations relating to the synchronization of CCRP genes have been made in murine 3T3-L1 pre-adipocytes. In addition, siRNA suppression of the CCRP Bmal1 inhibited adipogenesis in the 3T3-L1 model. A survey of human adipose tissue mRNAs has correlated the expression of the CCRP encoding genes with adipogenic markers. In light of epidemiological evidence linking circadian dysregulation to the incidence of obesity, hypertension, cardiac disease, and the metabolic syndrome, there is a need for further understanding of the relationship between chronobiology and adipose biology.

S8.3

The adrenal clock: impact on adrenal steroidogenesis
Silke Kiessling, Gregor Eichele & Henrik Oster
Max Planck Institute for Biophysical Chemistry, Gottingen, Germany.

In mammals, a master pacemaker residing in the hypothalamic suprachiasmatic nuclei (SCN) and subordinate clocks found throughout the body coordinate circadian rhythms of behavior and physiology. One prominent physiological rhythm is the release of glucocorticoids (GCs) by the adrenal gland, brought about by the rhythmic activation of the hypothalamus–pituitary–adrenal (HPA) axis and the secretion of ACTH from the pituitary. In humans, blood GC levels peak in the early morning while nocturnal rodents show highest secretion rates in the evening, coinciding with the beginning of the active phase in both species. We have characterized a peripheral circadian clock residing in the outer cortical layers of the murine adrenal. Rhythmic activation of several core clock genes and of hundreds of clock controlled genes was detected, indicating a role of the adrenal clock in regulating gluco- and mineral corticoid biogenesis. In mice with a genetic disruption of the circadian system, hpa axis regulation is defective. Organ culture and tissue transplantation experiments suggest that the adrenal clock gates GC production in response to ACTH. These data are supported by transcriptome analyses, identifying a number of clock-controlled regulators of the steroidogenic machinery.

In vivo the adrenal circadian clock can be directly – and independently of the SCN pacemaker – entrained by light, and drive GC release rhythms in the absence of a rhythmic ACTH signal. When internal synchrony is disrupted, e.g. by repetitive night shifts or after long distance travels (jet lag), the adrenal clock regulates the adaptation of physiological and behavioral rhythms. Pharmacological manipulation of GC rhythms modifies behavioral re-entrainment during jet lag and, thus, might provide a new target for the alleviation of the numerous physiological and psychological symptoms classified as the jet lag syndrome.

S8.4

Human clock gene polymorphisms and metabolic syndrome
E Scott

Abstract unavailable.

Mechanisms and management of ovarian failure

S9.1

Genetic mechanisms underlying ovarian insufficiency
Ken McEleavey
Institut Pasteur, Paris, France.

Primary ovarian insufficiency (POI) describes a continuum of impaired ovarian function that includes both primary amenorrhea and premature menopause. A significant minority of the cases are familial suggesting a genetic basis for POI. Several genetic causes of syndromic and non-syndromic forms of POI have been identified. Syndromic forms are associated with monosomy X, rearrangements of Xq and mutations in the APEXCD, FOXL2, EIF2B, ATM and GALT genes. In non-syndromic rare forms, mutations of the FSH and LH receptor genes as well as mutations involving BMP15, FSHL and NOBOX have been described. These account for a small minority of POI cases and the clinical relevance of some of the mutations remains unclear. NR5A1, which encodes the steroidogenic factor-1 protein, is a key transcriptional regulator of genes involved in many reproductive processes. Mutations in NR5A1 were initially described in individuals with adrenal insufficiency and subsequently in cases of 46,XY disorders of sex development (DSD) with no evidence of adrenal failure. We initially identified a variety of NR5A1 mutations in families with cases of 46,XY DSD and 46,XX POI. The mutant proteins show altered transcription activity on target gonadal promoters. We have also identified mutations in apparently sporadic cases of POI. Several of these mutations appear to be inherited and may be present at lower frequencies in specific populations indicating possible founder origins. Some sporadic cases also have somatic anomalies. Therefore, in the case of NR5A1 mutations, the distinction between syndromic and non-syndromic forms of POI may be blurred. Our understanding of the role of NR5A1 in gonad development and function is further compounded by the spectrum of gonadal phenotypes that can be associated with the same mutation. Several new phenotypes linked to NR5A1 mutations, which were originally identified in individuals with POI, will be presented.

S9.2

Mechanisms regulating ovarian reserve
Kate Hardy
London, UK.

The population of follicles within the mammalian ovary declines steadily with age. The majority of follicles in the ovary are at the primordial (resting) stage of development, each consisting of a small oocyte surrounded by a single layer of flattened granulosa cells (GCs). The rate at which follicles enter the growing phase, involving oocyte growth and GC shape change and proliferation, is a major determinant of the rate at which the reserve of primordial follicles is depleted. Deviations in the rate at which follicles initiate growth will have a significant bearing on fertility, the age of the menopause and disorders of ovulation in women, such as primary ovarian failure and polycystic ovary syndrome (PCOS). Studies of follicle development in mice lacking specific genes, and in ovaries or pieces of ovary cultured under different conditions in vitro, suggest that early follicle development is mainly regulated by local growth factor signals. However, while a number of growth factors have been implicated in early follicle development, particularly members of the transforming growth factor-β family.
superfamily, the source and identity of the factor or factors that signal a follicle to start growing remain unclear. We have recently developed an alternative strategy for identifying the source and action (stimulatory or inhibitory) of local signals by analysing the spatial distribution of primordial and growing follicles within the ovary. These studies have provided insight into the local ovarian signals that determine whether a follicle is quiescent or starts to grow and provide us with important leads in the investigation of candidate growth factor pathways. Such studies will provide a better understanding of, and treatments for, ovarian disorders.

S9.3
Can ovarian failure be predicted?
Frank Broekmans
University Medical Centre Utrecht, Heidelberglaan, The Netherlands.

Several reasons exist for wishing to have advance notice of the timing of menopause. Since menopause relates strongly to the occurrence of natural infertility some 10 years earlier, long-term prediction of menopause may help women to timely start attempts to have children. If premature or early menopause could be predicted, preventive measures could be taken in order to minimise the related long-term health risks. Incipient ovarian failure in terms of the current inability to create a viable ongoing pregnancy could be another issue that has been the subject of numerous studies, especially directed in the field of assisted reproduction.

The process of ovarian ageing consist of the gradual decline in number and quality of the remaining follicles and oocytes in both ovaries at a given age. Decline in follicle numbers dictates the occurrence of irregular cycles and menopause, while quality decay of the oocytes results in decreasing fertility, defined as the capacity to conceive and give birth to a child. There is substantial individual variation in the onset of menopause, varying roughly between 40 and 60 years, with a mean age of 51. Along the same pattern, the rate of decline in fertility may vary considerably between women of the same age. These notions underline the need for tests (ovarian reserve tests, ORTs) that describe future and current ovarian reserve status. Among these tests are calendar age, family history, AMH, poor response in IVF, basal FSH, antral follicle count and genetic variation for long-term prediction. From recent long-term follow up studies, only AMH has shown to provide some individual value in the prediction of time to menopause. The application of genetic profiles has so far been hampered by inconsistent findings and only very small effects of the associated variation on age at menopause.

Assessment of ovarian reserve status in ART patients has demonstrated to be inadequate when pregnancy prospects are concerned. From recent individual patient data analysis it has been demonstrated that ORTs do not add to the prediction based on female age alone. Response to ovarian hyperstimulation has appeared to be highly predictable, especially when AMH and the AFC are applied. The management options for predicted poor and high responders are still under study and debate. The combination of poor response in a first ART cycle and an abnormal AMH or AFC test result may identify a group of patients that have such a poor prognosis that further treatment may better be refused.

S9.4
Clinical management of ovarian insufficiency
M Davies

Abstract unavailable.
Clinical Management Workshops

Generously supported by Clinical Endocrinology
Long-term consequences of endocrine diseases

CM1.1
Subclinical thyroid disease
Jayne Franklyn
University of Birmingham, Birmingham, UK.

Subclinical hypothyroidism is characterised by low serum TSH with normal free $T_3$ and free $T_4$. Subclinical hypothyroidism may reflect Graves’ disease or toxic nodular hyperthyroidism, and is found in up to 5% of the over 60’s. In addition, about 20% of patients who are taking T4 therapy have low serum TSH, i.e. biochemical evidence for over-treatment. In those not prescribed T4 it is important to exclude other causes of low TSH, especially non-thyroidal illness and drug therapies. The potential risks of subclinical hyperthyroidism are cardiovascular and osteoporosis. Effects on cardiac function are well documented, including increased risk of AF and vascular mortality. Bone mineral density may be reduced, especially in post-menopausal women, and there is evidence of an effect on fracture risk. There are no studies of intervention with clinical endpoints, although some evidence of improvement in BMD. Guidelines suggest consideration of treatment in the elderly, those with subclinical hyperthyroidism proven to be Graves’ disease or toxic nodular goitre, especially in those with AF or vascular disease.

Subclinical hypothyroidism is high serum TSH with normal free $T_4$. If a standard cut-off for serum TSH of 4.5 mU/l is adopted, the prevalence is ~10% of the over 60’s and rises with age. Subclinical hypothyroidism is also found in 25% of those taking T4. Possible associations include hyperlipidaemia (and vascular risk), impaired cognitive function, and impaired well-being. Evidence suggests that subclinical hyperthyroidism has a finite rate of progression to overt thyroid dysfunction (although rate is variable) and there is weak association with total and LDL cholesterol. Epidemiological studies have revealed conflicting evidence regarding the risk of vascular end points but there may be an association with IHD. In contrast, there is a lack of association of subclinical hypothyroidism with changes in cognitive function, neuropsychiatric symptoms or well-being. Most studies of intervention with T4 show minor effects on lipids and minimal/no effect on cognitive function or symptoms. The main indication for treatment of subclinical hypothyroidism is therefore risk of progression to overt disease. Guidelines suggest consideration of T4 in those with TSH >10 mU/l, but not in those with lower TSH. An exception to this is pregnancy in which even mild subclinical hypothyroidism should be treated because of a possible association of maternal thyroid dysfunction with effects on neurodevelopment in offspring.

CM1.2
Mild primary hyperparathyroidism
Peter Selby
Manchester University, Manchester, UK.

Primary hyperparathyroidism (PHP) is one of the most common endocrine diseases; it is frequently found in asymptomatic patients when there is some doubt as to the appropriate choice of surgery (PTX), medical therapy or watchful waiting. These decisions are generally based on the consensus guidelines produced by the NIH however there is concern that these reflect established medical/surgical practice in the USA as much as clinical evidence. What evidence that does exist is frequently scanty and where present contradictory. In order to develop evidence based guidance it is necessary to understand the effects of PHP: Renal stones were once seen as the hallmark of primary hyperparathyroidism but are now seen less frequently; meta-analysis of reports of the effect of primary hyperparathyroidism indicates that although their frequency is reduced by surgery this still remains above that expected for the population suggesting that these may occur in patients with PHP who also have other predisposing conditions for stone disease.

Reduced bone density is generally accepted to occur in patients with PHP. The effect of this on fractures is less clearly demonstrated as is the effect of PTX on fracture rates.

Although there is reasonable consensus on the associating between PHP and hypertension there is little consistency in the association between other manifestations of vascular disease and PHP. Neuropsychiatric and other non-specific symptoms are frequently associated with PHP but are common in the general population and no definite association has been demonstrated. The effect of PTX on these symptoms is contradictory. If evidence based guidance for the management of PHP is to be developed it is important to both understand the natural history of the condition and to the effects of treatment, both medical and surgical. This will require an adequately powered RCT comparing PTX to medical therapy to placebo. Such a study has frequently been discussed but still needs to be undertaken.

CM1.3
Long term consequences of Cushing’s syndrome
Susan Webb, E Resmini, M J Barahona, A Santos & J Ybarra
III-B Sant Pau and Department of Endocrinology/Medicine, Hospital Sant Pau, UAB and Centro de Investigacion Biomédica en Red de Enfermedades Raras (CIBER-ER, Unidad 747), ISCHII, Pare Claret 167, 08025 Barcelona, Spain.

Endogenous hypercortisolism and chronic glucocorticoid (GC) therapy reduce bone mass, increase central fat mass, alter adipokines and enhance cardiovascular risk. Surgery (pituitary, adrenal or for ectopic ACTH) can control hypercortisolism in 90% of patients in experienced hands, and is often followed by inhibition of the adrenal axis, requiring substitution therapy with GC for months or years. We have been interested in learning on long-term outcome of ‘cured’ CS patients, since recent evidence suggests that specific morbidity is not always reversible. Duration of both exogenous GC replacement therapy after successful surgery and endogenous hypercortisolism both negatively affect bone mineral density (BMD) in women in long-term remission after successful therapy for CS, when compared to controls; furthermore, an unfavorable lipid profile is seen in these cured CS.

Persistent accumulation of central fat not only in active hypercortisolism but also in ‘cured’ patients, with an unfavorable adipokine profile (low adiponectin, elevated plasma sTNF-R1 and IL-6), leading to a state of low-grade inflammation have been found. This ‘inflammatory state’ may determine vascular damage, atherosclerosis and cardiovascular disease in patients with long-term cured CS. Moreover, hypercortisolism affects behavior, mood, neural activity, memory and other functions of the central nervous system. Brain volume reduction appears to be partly reversible when hypercortisolism is controlled; psychopathology (most common depression) is highly prevalent at baseline improves 1 year after treatment, but often with residual symptoms; cognition and impaired health-related quality of life (HRQoL) do not appear to normalize after endocrine cure, strongly suggesting that all these changes are not fully reversible.

In conclusion, having suffered CS even if hypercortisolism is controlled confers a prematurity increase in cardiovascular risk and impaired HRQoL; clinical management of these patients should be particularly careful in identifying these long-term consequences of chronic hypercortisolism, to improve prognosis and offer the patient a realistic expectation with respect to the results of treatment.

CM1.4
Addisons disease
Eystein Husebye
University of Bergen, Bergen, Norway.

Primary adrenal insufficiency is often a consequence of autoimmune destruction of the adrenal cortex. Mortality is increased primarily due to acute adrenal crises, especially among patients diagnosed at young age. In the long-term more than 50% of the patients will develop other autoimmune diseases or manifestations, particularly autoimmune thyroid disease. Despite seemingly adequate treatment, accumulating evidence document reduced subjective health status, particularly general health and vitality, and physical functioning in women. Working ability is also reduced. However, sexual function in women seems to be normal despite lack of androgens.

Replacement therapy with glucocorticoids and mineralocorticoids is standard, but we lack effect parameters to guide treatment. Thus there is risk for long-term metabolic consequences on glucose regulation, cardiovascular function, and bone.

There is a need to determine the long-term consequences of the replacement therapy, and to provide individualised and more physiological treatment. Slow-release preparations of hydrocortisone may become the mainstay therapy, and continuous subcutaneous hydrocortisone infusion an alternative for selected patients.

Endocrine incidentalomas: what to do with lumps and bumps

CM2.1
Thyroid
Mark Vanderpump
Royal Free Hampstead NHS Trust, London, UK.

The aetiology of thyroid nodules is due to the interaction between genetic and environmental factors. Thyroid nodules are common. Epidemiological studies...
suggest that 1% of men and 5% of women have thyroid nodules detected clinically and that the frequency increases with age and in iodine-deficient populations. With the increasing use of sensitive imaging techniques, an increasing proportion of thyroid nodules are detected incidentally. Up to 50% of nodules > 1 cm detected by ultrasound are undetected by clinical examination. Autopsy and prospective ultrasound studies in the US detected asymptomatic nodules in 50 and 67% respectively. Many nodules are detected because of their size or anterior position in the neck, or the skill of the physician performing the examination but most thyroid nodules will not be clinically recognised. Although thyroid nodules are common, thyroid cancers are rare. The annual incidence quoted of all thyroid cancer ranges between 1 and 10 per 100,000 population in most countries and is two to four times as frequent in women as men. Recently reports have suggested an increase in thyroid cancer incidence which is only partly explained by an increased detection of small papillary thyroid cancers. Clinically silent papillary microcarcinomas (diameter < 1 cm) have been reported in up to 36% of adults at post-mortem in population-based studies. A comparison of these papillary microcarcinomas incidence rates in autopsy studies with the incidence rates for clinically apparent papillary carcinomas strongly suggests that most papillary microcarcinomas will not lead to clinically apparent thyroid carcinomas. Therefore the challenge in the management of nodular thyroid disease is to identify those few nodules that are malignant when the vast majority of thyroid nodules, which are so common in the population, are benign. This requires a rational evidence-based strategy for the differential diagnosis, risk stratification, treatment and follow-up.

CM2.2
Pituitary incidentalomas
Miles Levy
Department of Endocrinology, Leicester Royal Infirmary, Leicester, UK.

Background
Incidentally discovered pituitary lesions are a common problem in the endocrinology clinic due to increased access to detailed brain imaging. The challenge for endocrinologists is to determine which pituitary lesions are clinically significant and which are truly incidental. Pituitary microadenomas have been found at autopsy in 1.5–27% of subjects, whilst population-based radiological studies report small pituitary lesions in ~10%. It is difficult to produce consensus guidelines on this topic because the term ‘incidentaloma’ is subjective and dependent upon clinicians’ pre-scan clinical suspicion of pituitary disease. The subject is further complicated by the possibility of sub-clinical pituitary hyper- and hypo-function, and the confounding variables that may affect the reliability of pituitary imaging.

Content of session
The wide range of approaches to the investigation and management of pituitary incidentalomas is highlighted by a recent survey completed by 214 consultants (76%) and registrars (34%) prepared specifically for this forum. A series of questions based on a previous survey were posed regarding the investigation and management of two theoretical case scenarios (Case 1: incidental microadenoma, Case 2: incidental macroadenoma). The format of this session will be a presentation of the survey results in the context of an up-to-date review of the recent literature, followed by an interactive discussion.

CM2.3
Adrenal lumps & bumps
Paul Stewart
University of Birmingham, Birmingham, UK.

The widespread use of abdominal CT/MRI has resulted in a new and common diagnosis for the clinical endocrinologist – the management of patients with adrenal incidentalomas. Defined as an adrenal mass discovered incidentally in the work-up or treatment of clinical conditions not related to suspicion of adrenal disease, incidentalomas cover a spectrum of underlying adrenal pathologies with a common pathway of discovery. Because of the risk of malignancy, they raise uncertainty, confusion and concern in doctors and patients alike and consume significant resource. This presentation will define the scale of the problem, discuss diagnostic challenges as they relate to functionality of the tumours and ascertaining whether the lesions are benign or malignant. It will review the natural history and suggested follow-up and treatment of patients based on published NIH clinical guidelines, but will also suggest that such guidelines perhaps over-inflate the real risk of malignancy and a more ‘risk-averse’ approach to management is now required.

Finally, new biomarker research based on analyzing the urinary steroid metabolome may improve the diagnosis and follow-up of such cases; in-house data will be presented.

CM2.4
Pancreas
B Harrison
Abstract unavailable.

CM3.1
Prenatal diagnosis and treatment of CAH
S Lajic
Abstract unavailable.

CM3.2
Testicular adrenal rest tissue and male fertility in CAH
Hedi Claahsen-van der Grinten1, Barto Otten2, Fred Sweep3 & Ad Hermus2
1Department of Pediatric Endocrinology, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands; 2Department of Endocrinology, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands; 3Department of Chemical Endocrinology, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands.

Infertility is a serious problem in female as well in male CAH patients. The most important cause of male infertility in CAH patients is the presence of testicular adrenal rest tumors (TART). The reported prevalence in adult CAH patients is up to 96%. TART have no malignant features but because of their typical localization near the mediastinum testis, longstanding compression of the seminiferous tubules may lead to obstructive azoospermia and irreversible damage of testicular tissue. TART contain adrenal specific enzymes and produce adrenal specific hormones suggesting that these tumors consist of adrenal-like cells, which may nestle in the developing testis in the early embryonal period. The presence at the mRNA level of ACTH and angiotensin II (AB) receptors in these tumors support this hypothesis. Therefore, it is thought that elevated ACTH and AB levels as in poor hormonal control contribute to tumor growth.

Furthermore, adrenal rest cells may already be stimulated in utero when ACTH levels are elevated. The prevalence of TART in infancy is not yet studied in detail but in CAH children above 5 years old the prevalence of TART is up to 24%. Probably this prevalence is underestimated because very small tumors are only detectable by scrotal ultrasound, not routinely performed in all centers. Treatment options of TART are still controversial. In some patients intensifying glucocorticoid therapy will lead to reduction of the tumor size by suppression of ACTH secretion. However, high doses of glucocorticoids may have serious side effects. Tissue sparing surgery may be performed before irreversible damage is present. However, it is controversial whether tissue-sparing surgery can be performed without causing additional gonadal damage.

References

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CM3.3
The adult with CAH: results of the UK congenital adrenal hyperplasia, adult study executive (CaHASE)
R J Ross, D S Willis, S H Wild, N Krone, E J Doherty, T S Han, P V Carroll, G S Conway, D A Rees, R H Stimson, B R Walker, J C Connell & W Arlt
University of Sheffield, Sheffield, UK.

Background
No treatment guidelines exist for CAH adults. To address this we have undertaken a cross-sectional analysis of health status.

Patients and methods
Three hundred and eighty patients from 17 tertiary centres were contacted: 203 (53%) consented (138 f, 65 m, median age 34 (range 18–69) years). One hundred and sixty-five had classic and 34 non-classic CAH. The UK prevalence is 3591 adults >18 years therefore the capture rate was 5.7%. Results were compared to Health Survey for England (HSE) data.

Results
Glucocorticoids (GC) consisted of hydrocortisone (25%), prednisolone (44%), dexamethasone (19%), cortisone acetate (0.5%) or combination (10%): 1.5%
CM3.4
Quality of life in CAH: does it differ from adrenal insufficiency?
Stefanie Nahner
Wurzburg, Germany.

Quality of life (QoL) is significantly impaired in patients with primary and secondary adrenal insufficiency (AI) irrespective of the glucocorticoid dosing regimen or the currently available glucocorticoid preparation. The impairment of QoL has been particularly attributed to the unphysiological mode of glucocorticoid replacement, which only roughly resembles the physiological profile. In both congenital adrenal hyperplasia (CAH) and Addison’s disease, failure in cortisol synthesis results in increased pituitary ACTH release. Different from other AI patients, CAH patients suffer from ACTH driven adrenal androgen excess which may result in disordered sex development, virilisation, impaired fertility, and short stature. Furthermore, glucocorticoid doses in CAH are usually higher and replacement regimens are more complex than in isolated AI. This may further impact QoL in CAH.

To date only little data from small studies is available on QoL in patients with CAH with conflicting results. Most studies demonstrate impairment of QoL and psychosocial wellbeing, mainly in women. In an own analysis, patients with 21-hydroxylase deficiency from Germany and the UK were assessed with the SF-36, GBB-24 and HADS questionnaire and compared to patients with primary AI of other origin. German patients with classic 21-hydroxylase deficiency (21-OHD) showed significant impairment in three out of five GBB-24 scores, but neither impaired SF-36 nor HADS scores, whereas scores in UK CAH patients were significantly impaired for most dimensions. Primary AI patients showed impairment in more dimensions of the applied tests compared to German CAH patients. Particularly in women significant differences were observed with better subjective QoL in CAH patients.

This data suggests that QoL in classic 21-OHD is impaired compared to healthy controls but appears to be better than in primary AI. Differences between groups suggest effects unrelated to hormone replacement therapy and furthermore significant influence of quality of patient care on QoL outcome.

The management of thyroid cancer
CM4.1
Investigation of thyroid cancer from thyroid lump to surgical referral
Petros Peros
Royal Victoria Infirmary, Newcastle upon Tyne, UK.

The introduction of fine needle aspiration biopsy revolutionised the investigation of thyroid nodules. Several decades on, diagnosis relies largely on the same technique. Meanwhile clinicians are faced with an epidemic of incidentally discovered thyroid nodules and an increasingly ‘informed’ public frequently misled by unreliable sources. Molecular diagnostics and new imaging techniques are in the horizon, but unlikely to influence clinical practice in the next few years. The time between discovery of a nodule and surgical referral is usually marked by anxiety and uncertainty. How clinicians handle patients during this phase impacts on the rest of their journey. More focus on managing the person rather than investigating the nodule is essential for improving the experience of thyroid cancer patients.

CM4.2
Surgical treatment of thyroid cancer
Fausto Palazzo*1
1Hammersmith Hospital, London, UK; 2Imperial College, London, UK.

Thyroid cancer is the commonest endocrine cancer. It is increasingly diagnosed both due to the diagnosis of previously undiagnosed subclinical lesions as well as due to a lesser true increase in incidence. Surgery is the only treatment modality that can cure thyroid cancer and has a key role in the multidisciplinary treatment of the malignancy. The combination of the rarity of the disease and broad spectrum of clinical outcomes has hindered the accumulation of level 1 evidence in the management of thyroid cancer with inevitable controversies regarding the surgical and adjuvant treatment of all types of thyroid cancer and its variants. The controversies and growing consensus towards an optimal extent of thyroid surgery and the role of lymph node dissection tailored to the disease type and risk group are discussed. The data regarding the value of surgical specialisation on thyroid surgery are also reviewed.

CM4.3
Radioiodine treatment and long-term follow-up of differentiated thyroid cancer
Furio Pacini
Section of Endocrinology and Metabolism, Department of Internal Medicine, Endocrinology and Metabolism and Biochemistry, University of Siena, Siena, Italy.

After total thyroidectomy, patients with differentiated thyroid cancer are treated with *131I* activities aimed at ablating any remnant thyroid tissue and potential microscopic residual tumor. This procedure decreases the risk of locoregional recurrence and facilitates the long-term follow-up. In addition the high activity of *131I* allows obtaining a highly sensitive post-therapeutic WBS. Radiodine ablation is recommended in high-risk patients and in low-risk patients, while there is no indication in very low-risk patients (those with unifocal T1 tumors, <1 cm in size, with favorable histology, no extrathyroidal extension and lymph node metastases). Effective thyroid ablation requires stimulation by TSH. The method of choice for preparation to perform a radioiodine ablation is based on the administration of recombinant human TSH (rhTSH) while the patient is on levo-thyroxine (L-T4) therapy. Recent multicenter and prospective studies have demonstrated that this preparation is highly effective and safe and that the rate of successful ablation is similar to that obtained with L-T4 withdrawal when using 3700 MBq (100 mCi) or 1850 MBq (50 mCi) of *131I*. Based on these results the use of rhTSH has been approved in Europe by the European Medicine Agency (EMEA) and in USA by the FDA.
After thyroid ablation, aim of follow-up is the early discovery and treatment of persistent or recurrent disease. Two to three months after initial treatment thyroid function tests (FT₃, FT₄, TSH) should be obtained to check the adequacy of L-T₄ suppressive therapy. At 6 to 12 months the follow-up is aimed to ascertain whether the patient is free of disease. This follow-up is based on physical examination, neck ultrasound, rhtTSH stimulated serum Tg measurement with or without diagnostic WBS. At this time most (nearly 80%) of the patients will belong to the low-risk categories and will disclose normal neck ultrasound and undetectable (<1.0 ng/ml) stimulated serum Tg in the absence of serum Tg antibodies. Diagnostic WBS does not add any clinical information in this setting and may be omitted. These patients may be considered in complete remission and their rate of subsequent recurrence is very low (<1.0% at 10 years). Patients in remission may be shifted from suppressive to replacement L-T₄ therapy, with the goal of maintaining a serum TSH level within the normal range. The subsequent follow-up of these patients should be based on yearly physical examination, serum Tg measurement on replacement L-T₄ and neck ultrasound.

The few patients with evidence of persistent disease, or with detectable levels of serum Tg increasing with time, require imaging techniques for the localization of disease and appropriate treatment, including therapeutic doses of ¹³¹I. Included in this category are the 5–10% of DTC patients that presented with local or distant metastases at diagnosis and an additional 5–10% that develop recurrent disease during follow-up. When appropriately treated, 2/3 of those patients with local disease and 1/3 of those with distant disease may achieve complete remission.

Treatment of loco-regional disease is based on the combination of surgery and radioiodine therapy. External beam radiotherapy may be indicated when complete surgical excision is not possible or when there is no significant radioiodine uptake in the tumor. Distant metastases are more successfully cured if they take up radioiodine, are of small size located in the lungs (not visible at X-rays). Lung macro-nodules may benefit from radioiodine therapy but the definitive cure rate is very low. Bone metastases have the worst prognosis even when aggressively treated by combination of radioiodine therapy and external beam radiotherapy. Whenever radioiodine therapy is not effective and the disease progress, enrollment of the patients in experimental trials with tyrosine kinase inhibitor should be the treatment of choice.

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**CM4.4**

**Novel therapies for recurrent thyroid cancer**

J Fagin

Abstract unavailable.
Nurse Session
Neuroendocrinology & case presentations

N1.1

Abstract unavailable.

N1.2
MEN-1 case presentation
Christine Gibson
Manchester Royal Infirmary, Manchester, UK.

A patient from a known family with MEN-1 was referred to the endocrine team in 1995 for endocrine screening. At that time she was well although she had hyperprolactinaemia which was attributed to macroprolactin isoforms. Subsequently she was found to have an occipital meningioma which was removed in 2002, but unfortunately developed post-operative partial seizures. In 2006 she developed a non-functioning adenoma in the tail of the pancreas which was successfully removed by distal pancreatectomy. She remained well following this and continued to attend for regular review. In 2009 however, she complained of episodic confusion. Although it was thought these might be epileptiform, she was subjected to a 48 h fast and rapidly developed symptomatic hypoglycaemia with inappropriately high plasma insulin levels. A 2 cm insulinoma was located in the head of the pancreas by CT scanning and she underwent a modified Whipple’s procedure with resolution of her symptoms. The psychological issues surrounding the decision to proceed to a second pancreatectomy operation were difficult and challenging for both the patient and her family, due to the death of a close family member after a similar problem.

N1.3

Abstract unavailable.

N1.4
Has Carcinoid syndrome given me more than the average number of ears?
Ian White
London, UK.

In 1996, in Monte Carlo’s famous Loewes Hotel, I went from sudden crippling gastric pain to continuous flushing in the space of <24 h, on my return to the UK, I was diagnosed with Carcinoid syndrome. Based on freely available information on the Web at that time, in my mind my life expectancy was going to be 6 years from the point of diagnosis. This session will discuss the impact of a ‘skewed’ sample on the following 6 years of my life and more generally what living with this condition has been like since then. I will also present the regime I have developed to co-exist with my tumors. Oh I do have two ears – slightly more than the average – you just can’t trust statistics!

Acromegaly – psychological aspects & case presentations

N2.1

Abstract unavailable.

N2.2

Abstract unavailable.

N2.3
A psychological aspect of acromegaly
Shashana Shalet
Salford Royal Hospitals NHS Foundation Trust, Salford, UK.

A 39-year-old male was diagnosed with a growth hormone and prolactin secreting pituitary macroadenoma. He was treated with transphenoidal subtotal resection 2006 and radiotherapy 2007, but despite this he has persistent biochemically active disease as well as partial anterior hypopituitarism. In 2007 he was due to participate in a Somavert trial but whilst being reviewed he admitted to having auditory and visual hallucinations for about 10 years. His visual hallucinations were only at the edges of his visual field defect and he had auditory hallucinations, sometimes for full days. He was reviewed by the psychiatrists and started on treatment – quetiapine, which has vastly improved his auditory symptoms. He was subsequently treated with Somavert (outside the trial) and cabergoline, which he tolerates well.

The conclusion drawn by the consultant psychiatrist was that the tumour, which was pressing on the right temporal lobe, could have been the cause for the auditory hallucinations while the contact between the tumour and the anterior visual pathways are thought to be the cause of the visual hallucinations. Commonly it is found that traditional, or atypical, antipsychotic medication has a significant effect on increasing prolactin production due to the dopamine blocking action so consideration of treatment for this patient was important. Quetiapine was thought to be a good choice of antipsychotic as it has little influence on prolactin production and has very little trophic effect on the gland.

N2.4
McCune–Albright syndrome - case presentation
Katherine Powell
Norfolk and Norwich University Hospital, Norwich, UK.

I would like to present the case of a 66-year-old gentleman with McCune-Albright syndrome. This unusual condition consists of three main features; namely, polyostotic fibrous dysplasia, café au lait patches on the skin and hormonal abnormalities, including acromegaly. The presentation will look briefly at these three elements and the investigations leading up to diagnosis. However, the main focus will be on the psychological impact illness has had on this patient and his slow recovery from debilitating depression. Investigations into this patient’s condition began only when he was admitted to hospital with a suspected pulmonary embolus. At that time, a sharp-eyed health professional in the Accident and Emergency Department noticed that his features were acromegalic; a term hitherto unknown to the patient. For several months prior to this, the patient had been experiencing a gradual lowering of his mood, together with an increasingly overwhelming exhaustion. As he explained, he managed to continue to work, but was so tired he could do absolutely nothing else. His fatigue was so extreme, that some nights he was too weary to get up from his armchair to go to bed. This poor quality of life led to unhealthy coping mechanisms, such as comfort eating. The inevitable weight gain further reduced his capacity for exercise and the inexorable downward spiral in both physical and mental well-being began. With regular support from both the endocrine consult and specialist nurse the patient eventually reached a turning point. He began to lose weight, ultimately losing 30% of his body weight, became a volunteer at the hospital and started to rebuild a social life. Since then, he has blossomed and his self-esteem restored. In the light of this, I will look at the role of the endocrine nurse in the ongoing treatment and monitoring of this condition and discuss the importance of holistic care of patients with lifelong endocrine conditions.
Clinical Debate
D1

This house believes that androgen replacement therapy should be offered to every hypogonadal woman

Susan Davis
Monash University, Melbourne, Australia.

‘Androgen replacement therapy’ is a term loosely used in the medical and lay literature, however in the context of this discussion it will be limited to the use of testosterone as a pharmacotherapy. Furthermore, whether the term ‘hypogonadal’ includes naturally menopausal women can also be considered contentious, as the postmenopausal ovary is an ongoing source of sex steroids and thus in the strictest terms such women are not necessarily hypogonadal. Indeed, the point at which a woman becomes ‘hypogonadal’ in terms of testosterone production is not clear, as the levels of the pre-androgens, DHEA, DHEAS and androstenedione decline with age, as does testosterone1, the bioavailability of testosterone is strongly governed by the circulating level of sex hormone binding globulin and not all women with low levels of the pre-androgens or free testosterone will be symptomatic2. Furthermore the production of androgens is not limited to the ovary with the adrenals being a major source of androgens throughout life.

The main clinical benefit of testosterone therapy has been seen for the management of hypoactive sexual desire disorder (HSDD) in both premenopausal and postmenopausal women, with and without concurrent oestrogen therapy3,4. Other potential benefits include favourable effects on mood and wellbeing, bone density with recent data also suggesting favourable effects on cognitive performance. It is hence the responsibility to ascertain whether their female patients have symptoms that may be alleviated with testosterone therapy and offering the women the options of further assessment and possibly management with testosterone therapy.


D2

This house believes that androgen replacement therapy, to get things going, should be offered to every hypogonadal woman

Stephen Franks
Imperial College London, London, UK.

‘This house believes that androgen replacement therapy, to get things going, should be offered to every hypogonadal woman’.

In opposing this motion, I shall review the advantages and disadvantages of androgen replacement therapy and draw attention to the folly of offering treatment to every hypogonadal woman.
Young Endocrinologists Session
Alternative careers for basic and clinical scientists

YE1.1
The ‘darksie’ – pharmaceutical industry for endocrinologists
John Porter
Pfizer, Surrey, UK.

For most trainees there is a well-worn path to a consultant post in the NHS. There are however alternatives to this career path – either temporarily or long term. I am one of a number of endocrinologists working in the pharmaceutical industry as medical advisers, clinical research physicians, and medical directors. I will describe my pathway from training in paediatric endocrinology to the industry. I will consider some of the challenges and differences from working in the NHS. I will look at what could be learnt from a temporary stint in the industry, and whether this is possible. I will also look at the future of industry/NHS collaboration and the challenge of revalidating outside the orthodox career pathway.

YE1.2
Clinical biochemistry: one foot in the lab
Susan Knox
Department of Clinical Biochemistry, Glasgow Royal Infirmary, Glasgow, UK.

Clinical biochemistry is a branch of laboratory medicine in which chemical and biochemical methods are used to detect changes in the composition of body fluids (e.g. blood) as a result of disease. The results of biochemical tests may be of use in screening for disease, diagnosis, monitoring treatment and assessing prognosis. This presentation will focus on the role of the clinical biochemist, who works as part of a team including chemical pathologists and biomedical scientists, in providing a hospital biochemistry service.

Biochemistry provides high quality analytical results on patient samples. Many departments (e.g. A&E) are dependent on a rapid service measuring kidney function or testing for heart disease for example. Tests required in large numbers are analysed on automated equipment. Many specialist analyses are performed manually requiring scientists with technical expertise. The biochemist has a major role in service provision including liaison with clinical staff – advising on relevant analyses and interpreting results, development of new techniques, audit, teaching and research.

There is a structured career pathway within the NHS. Many entrants to the profession will already have obtained a PhD. Approved training programmes are available in the UK and often include completion of an MSc in clinical biochemistry. After gaining wide experience, trainees can apply to become health professionals council (HPC) registered. Registered scientist posts generally involve developing an area of expertise in paediatric biochemistry, toxicology or endocrinology for example.

Most biochemists obtain higher professional qualifications such as FRCPath. More senior positions often involve taking charge of specialist sections in the department.

The work of a biochemist is interesting, varied and rewarding. It is a career suited to anyone with an interest in science and medicine and provides a unique opportunity to combine practical skills, clinical liaison, management and research.

YE1.3
Scientific communication – medical communication agencies
J Netteship

Abstract unavailable.

YE1.4
Government agencies – scientific roles in the civil service
Natalie Thatcher
Food Standards Agency, London, UK.

The Food Standards Agency (FSA) is an independent government department set up to protect the public’s health and consumer interests in relation to food. The Agency has a vision of ‘safe food and healthy eating for all’. The work of a scientist, I my case a toxicologist, at the FSA is varied and requires us to develop a number of skills. Eating is not risk free and it is very important for the safety of the public to assess and minimise that risk to an acceptable level, and to offer advice to help the public make lifestyle choices based on the best possible evidence. It is here that the work of the FSA toxicologists and scientists are key to that of the agency, whether we are dealing with incidents relating to food safety (e.g. melamine in imported milk powder) or the day-to-day decisions upon which policy is based. In the Chemical Risk Assessment Unit we advise colleagues internally and within other government departments and form the secretariat for the independent Committee on Toxicity who provide toxicology advice to the FSA and Department of Health on matters concerning the toxicity of chemicals.

There is also direct access and interaction with the public in response to the many enquiries submitted.

In addition to providing advice we are responsible for the FSA risk assessment research programme. The aim of this programme is to identify, quantify and establish an evidence based risk assessment process in order to ensure the safety of chemicals in food. Examples of projects within this programme include the recently completed work investigating the effects of caffeine consumption in pregnant women, which led to a revision of FSA policy and a change in advice to the public; work looking at the health effects of mixtures of additives in foods; and ongoing work investigating the effects of phytoestrogens on bone metabolism in post-menopausal women.
Special Interest Group Sessions
Low endogenous testosterone levels in men have been associated with a more adverse cardiovascular disease risk factor profile including lower HDL-cholesterol and higher blood glucose levels, and increased risk of cardiovascular disease. However, it is uncertain whether low testosterone levels may precede disease onset, or are a result of preexisting disease. Prospective studies may help clarify this relationship.

The EPIC Norfolk study reported that in 11,606 men aged 40–79 years first seen in 1993–1997 and followed up to 2003, endogenous testosterone levels at baseline were inversely related to mortality due to all causes (925 events) cardiovascular disease and cancer. Odds ratios (95% CI) for mortality for increasing quartiles of endogenous testosterone compared with the lowest quartile were: 0.75 (0.55–1.00), 0.62 (0.45–0.84), and 0.59 (0.49–0.85) respectively (P<0.001) independent of age, blood pressure, lipids, smoking, diabetes and other hormone levels. An increase of 6 nmol/l serum testosterone (1 s.d.) was associated with a 0.81 (0.71–0.92, P<0.01) odds ratio for mortality. Inverse relationships were also observed for cardiovascular disease and cancer deaths.

The Rancho Bernardo study reported that in 794 men aged 50–91 followed up from baseline in 1984–1987 to 2004, in whom 538 deaths occurred. Men with total testosterone in the lowest quartile were 40% (HR 1.40, 95% CI 1.14–1.71) more likely to die than those with higher levels, independent of age, adiposity and lifestyle as well as lipids, blood pressure and other metabolic covariates. In this study low testosterone also predicted increased risk of cardiovascular and respiratory disease mortality.

These two prospective population studies in UK and US indicate that low testosterone levels are predictive of subsequent mortality over a long period in apparently healthy men and independently of known risk factors. Whether testosterone supplementation may improve health outcomes in men remains to be tested in clinical trials.

Testosterone deficiency in metabolic syndrome
F Hayes
St Vincents University Hospital, Dublin, Ireland.

Epidemiologic studies have associated low testosterone levels with increased cardiovascular mortality in men, the mechanism of which is unclear. However, low testosterone levels have been linked to the development of cardiovascular risk factors including metabolic syndrome, regardless of the definition employed. This relationship has been observed in epidemiologic studies of community-dwelling men as well as in men with prostate cancer treated with androgen-deprivation therapy.

Insulin resistance plays a key role in the development of metabolic syndrome and testosterone levels are positively correlated with insulin sensitivity in men. It is likely that, at least part of, the relationship between low testosterone and metabolic syndrome is explained by the increase in central body fat associated with hypogonadism due to increased lipoprotein lipase activity, increased triglyceride uptake and decreased lipolysis. However, the fact that the relationship between low testosterone levels and metabolic syndrome is strong even in lean individuals, and that short-term induction of hypogonadism also adversely affects insulin sensitivity, suggests that factors other than body composition may also be implicated. Other potential mechanisms by which low testosterone levels may promote insulin resistance include an increase in the level of pro-inflammatory cytokines and the induction of mitochondrial dysfunction.

To date, limited data are available on the impact of testosterone supplementation on metabolic syndrome. A non-placebo-controlled study of hypogonadal men showed a significant reduction in waist circumference, total cholesterol and triglycerides after 12 months testosterone therapy. However, the demonstration that increasing insulin resistance is associated with decreased testosterone secretion from Leydig cells and that weight loss increases testosterone levels in obese men suggests that the relationship between low testosterone levels and metabolic syndrome, is in fact, bidirectional.

Thus, larger placebo-controlled trials are required to establish the causal nature of the testosterone deficiency associated with metabolic syndrome in men and the potential metabolic benefits of androgen replacement therapy.

Late onset hypogonadism
F Wu
Abstract unavailable.

Assay problems and treatment decisions: when and how to treat?
Herman Behre
Halle, Germany.

Owing to the difficulties in proper diagnosis of male hypogonadism, there is an ongoing debate on treatment needs of these patients. The comparison of commonly quoted thresholds of serum total testosterone concentrations for considering testosterone replacement therapy surprisingly reveals different thresholds in different European countries. The lower limit of ‘normal’ serum testosterone is 10 nmol/l in Germany, 7.5 nmol/l in France, 7.5–8 nmol/l in the UK, and ~9 nmol/l in Spain. The emerging question is: why are different thresholds of serum testosterone considered as treatment indication for hypogonadism? Is a testosterone laboratory value of 8.5 nmol/l of different relevance for a patient in Germany compared to a patient in France? For proper laboratory diagnosis of testosterone deficiency some pre-analytical and analytical aspects should be considered. One important pre-analytical aspect is the existence of a significant circadian rhythm of testosterone. It should be noted that the recommended ranges for normal testosterone levels reflect morning levels only. An important analytical aspect is the accuracy and precision of different laboratory assays for testosterone measurement. Depending on the assay system applied values for testosterone levels of exactly the same blood sample may be 7 or 13 nmol/l. The application of different assays might be one explanation for different testosterone thresholds used as indication for treatment of hypogonadal patients by different physicians.

In addition, it has been demonstrated that different thresholds of testosterone concentrations exist for distinctive clinical symptoms of hypogonadism. Hypogonadal patients treated with testosterone show significant inter-individual variation of the association of testosterone levels with clinical symptoms of testosterone deficiency, whereas this association seems to be comparably constant for an individual patient. The decision on the best modality for testosterone treatment should at least in part be based on the accurately measured testosterone level of the individual patient.

*Growth hormone deficiency: a silent epidemic?*
Mark Sherlock
University of Birmingham, Birmingham, UK.

Pituitary adenomas/ tumours remain the commonest cause of growth hormone deficiency (GHD; indeed recent studies from the UK and Belgium have reported higher than previously estimated prevalence of clinically significant pituitary adenomas). However, the aetiologic spectrum of pituitary dysfunction has changed in recent years with endocrinologists increasingly reviewing patients with pituitary dysfunction secondary to radiation injury, traumatic brain injury and subarachnoid haemorrhage. With the improving survival rate for patients with tumours of the head, neck and central nervous system pituitary dysfunction secondary to cranial irradiation more frequent (the GH-IGF1 axis is the most sensitive to radiation injury). This is also the case for survivors of childhood cancers in particular those treated with cranial and total body irradiation. A number of factors are key predictors of risk of hypopituitarism, the dose and site of irradiation, age of patient and duration since radiation.

Two other conditions, which have been associated with GHD and dysfunction of other pituitary axis are traumatic brain injury and subarachnoid haemorrhage. Impaired quality of life and functional capacity is described commonly following both cranial irradiation and traumatic brain injury and the associated GHD and other axis deficiency may have a role to play in these abnormalities. GHD has been postulated as being an explanation for some of these symptoms, however to date there is a paucity of data regarding the impact of hormone replacement on quality of life and functional capacity in these patients. This symposium will highlight the changing aetiology of pituitary dysfunction and the challenges in diagnosis and treatment of these patients.
SIG2.2
Diagnostic difficulties of GH deficiency
R Murray
Leeds Teaching Hospitals NHS Trust, Leeds, UK.

In adults, the absence of a biological marker equivalent to height in children, means the diagnosis of GHD relies exclusively on biochemical tests. Confirmation of GHD when suspected involves use of GH stimulation tests, serum levels of GH-dependent peptides, and 24 h GH profiles. GH stimulation tests have become the mainstay for diagnosis of adult GHD, which superficially appears to be relatively simple. GHD in the adult is accepted as a peak GH response of < 3 μg/l to the ITT. This value provides good separation of GHD adults from normal subjects, however, is arbitrary. The peak GH response to all GH stimulation tests is significantly impaired by increases in fat mass. A gender dichotomy further complicates interpretation of GH stimulation tests; the peak response to the ITT is greater in males, whereas the peak GH response to the GST is greater in females.

A number of clinical scenarios present a further degree of complexity to the diagnosis of GH status. Given the effect of fat mass on the peak response to stimulation tests, does one diagnose GHD in obese individuals who have received a putative insult to the hypothalamic-pituitary axis? In patients who have received cranial irradiation for tumours distant to the h-p axis a differential response to the ITT and GHRL-arginine test/AST is observed. Which of these tests is the more reliable in defining GH status of these individuals? In patients with GH-secreting tumours, GHD is most likely to occur in those who have received XRT. In these individuals the relationship between GH and IGF1 is perturbed, with IGF1 levels being relatively more preserved than predicted from prevailing GH levels. Is a patient with treated acromegaly GHD if there is failure to respond to stimulation tests, but IGF1 SDS is in the upper half of the normative range?

SIG2.3
GH: old hormone, new developments: therapeutic dilemmas
Ulla Feldt-Rasmussen
Department of Medical Endocrinology, National University Hospital, Copenhagen University, Copenhagen, Denmark.

GH is now well recognised as therapeutic modality for treatment of adult patients with documented GH deficiency, and long-term experience is being gathered. This has, however, revealed also several therapeutic dilemmas, which clinicians managing these patients need to be aware of. Some of these examples are: What to do in case of brain tumours/meningeomas? What to do in patients with concomitant diabetes mellitus with and without complications? How do we handle the patient with previous acromegaly? And how do we manage the patient with recurrent pituitary adenomas? The presentation will be based on patient cases as illustration and documentation of available evidence from current literature and own experience.

SIG2.4
Future issues for GH replacement in adults
Verda Popovic
University Clinical Center, Belgrade, Serbia.

Growth hormone (GH) deficient adults present with a wide spectrum of clinical presentations commonly called GHD syndrome. Detection of GHD is important especially as successful GH replacement therapy ameliorates many symptoms of the GHD syndrome and is accompanied by a significant improvement in quality of life (QoL) in GHD patients. GH therapy has been available for adults in the last twenty years and the results from large pharmacological epidemiological follow up under GH treatment, show a decrease in morbidity and mortality.

One of the future issues for GH replacement is changing the administration schedule from daily to weekly or monthly. GH is replaced by daily injections which in the long term may compromise patient’s compliance (assessment of frequency of missed GH doses) and persistence (continuing GH therapy without interruption). Many factors may be associated with low compliance estimated by low insulin-like growth factor 1 (IGF1) levels at the end of each year of follow up. A few studies have evaluated patient’s relationship to compliance and the need for improvement was realized. Several approaches have been taken to develop long acting forms of GH. The long acting GH molecule has been obtained by different approaches (binding of polyethylene glycol (PEG) with rGH or rGH is embedded in a matrix of sodium hyaluronate and lecitin). Each of the preparations has been tested in experimental model and clinical trials are ongoing. The extended GH half life result in increased in IGF1 and the extended duration if the IGF1 increase which allowed less frequent GH administration (weekly, every 14 days or monthly). During clinical trial with pegylated GH molecules, lipostrophy occurred at the injection-site and the study had to be terminated. On the other hand phase III, double blind, randomized, placebo controlled multicenter study with rGH embedded in sodium hyaluronate and lecitin was successfully completed.

Another future issue is the use of EMEA approved biosimilars of GH which still do not have enough data providing safety and efficacy, including immunogenicity studies and clinical trials. In some European countries biosimilar GH is already widely used and reimbursed.

PCOS Special Interest Group Session

SIG3.1
Androgen activation by 5α-reductase in patients with PCOS
Jeremy Tomlinson
University of Birmingham, Birmingham, UK.

Polycystic ovary syndrome (PCOS) is one of the most prevalent conditions facing the clinical endocrinologist, affecting 5–10% of all women. The condition is characterized in part, by clinical and / or biochemical androgen excess. Despite its prevalence, the molecular mechanisms that contribute to its pathogenesis remain relatively poorly understood. Whilst androgen excess forms part of the diagnostic criteria, the source of the androgen excess is unclear, and ovarian, adrenal and adipose tissue have all been implicated.

The 5α-reductases (5αR) are a series of enzymes that are able to activate testosterone to dihydrotestosterone and three separate isoforms have been identified. Type 1 (5αR1) is located in skin hair follicles and liver, whereas type 2 (5αR2) is highly expressed in the prostate in men, liver and urogenital epithelium. Both isoforms are expressed in the granulosa cells of the ovary. Recently, type 3 (5αR3) has been identified, although is pattern of expression and biological function remain to be determined. Importantly, in addition to activating androgens, these enzymes are also crucial in clearing and inactivating cortisol and this has been implicated in the pathogenesis of PCOS.

5αR activity is increased in patients with PCOS compared with BMI-matched controls indicative of enhanced androgen activation. Furthermore, 5αR inhibitors including Finasteride (selective 5αR2 inhibitor) have been shown to be efficacious in treating symptomatic hyperandrogenism. Finally, increased 5αR activity has also been linked to the metabolic phenotype associated with PCOS, in particular, insulin resistance and obesity. Whilst it is possible that this observation may relate solely to the generation of potent androgens, the contribution of enhanced glucocorticoid clearance to the metabolic phenotype needs to be considered.

SIG3.2
Abstract unavailable.

SIG3.3
Abstract unavailable.
Polycystic ovary syndrome (PCOS) is the commonest cause of anovulatory infertility, menstrual disturbances and hirsutism. In its classic form the presentation is of amenorrhoea or oligomenorrhoea associated with clinical and/or biochemical evidence of hyperandrogenism. However, it is clear that the spectrum of presenting symptoms of women with polycystic ovaries is wide, including anovulation without hirsutism (androgen levels are usually raised) and hirsutism with regular cycles. This has been recognised in the form of a revised diagnostic criteria for PCOS resulting from the joint ESHRE/ASRM consensus conference, held in Rotterdam in 2003. The typical gonadotrophin profile is elevated serum levels of LH with normal or slightly low FSH. PCOS is also associated with a metabolic disturbance in which the central abnormalities appear to be hyperinsulinaemia and insulin resistance. Women with PCOS are relatively hyperinsulinaemic and insulin resistant when compared with weight-matched controls; 20–45% of obese PCO subjects have impaired glucose tolerance. Although hyperinsulinaemia and insulin resistance must be regarded as characteristic features of anovulatory women with PCOS, these metabolic abnormalities are not found to the same degree in equally hyperandrogenaemic women with PCO who have regular menstrual cycles. The diagnosis of PCOS is made principally on clinical grounds, supported by ultrasonography and by a small number of biochemical investigations, including (when appropriate) estimation of serum testosterone, LH and FSH. The choice of investigations in women with PCOS depends primarily on the mode of presentation. Because of the high risk of impaired glucose tolerance or frank diabetes, anovulatory women with PCOS should have a glucose tolerance test. It is not necessary to measure circulating insulin levels.
Systems Biology Session

Generously supported by the
Journal of Endocrinology
How to access funds and revolutionise your research

SB1.1
System biology: an overview (BBSRC strategy and funding streams)
Colin Miles
Swindon, UK.

The presentation will provide an introduction to systems biology research and describe the support provided by the UK research councils for research and training in systems biology since 2004/5.

SB1.2
Integration of live cell imaging and mathematical modelling: how to use systems biology to revolutionise your research
M White
University of Liverpool, Liverpool, UK.

The advent of high throughput techniques and genome sequencing has brought about a new era in the availability of biological data. This has created a need for new approaches for data integration and to interpret complex biological data sets. A cell cannot be considered as using a set of independent signalling pathways. Instead, it is now clear that signalling proteins are integrated into a complex network. The human brain cannot handle this complexity. At the same time, we need to take a more quantitative approach to the analysis of how such systems work. Many of the experimental techniques that we use are poorly quantitative and are not easily integrated together. Systems biology seeks to solve some of these problems by applying multi-disciplinary experimental and theoretical approaches to analyse and understand biological systems. Both the experimental and theoretical approaches need to be appropriate for the task. It is important to be able to analyse enough components in order to be able to understand the function of the whole system. Research scientists need to be trained to communicate with people from other disciplines. I will discuss how these hurdles may be overcome. My talk will use examples from analysis of the NF-xB system and prolactin gene expression. Nuclear factor xB (NF-xB) regulates cellular stress responses and the immune response to infection. We used an integrated live cell imaging and mathematical approach to analyse this system. We found that NF-xB activation results in oscillations in nuclear NF-xB abundance. We also found that the timing of the oscillations regulates downstream gene expression. I will show how the use of modelling has made unexpected insights into how this system works. Finally, I will go on to discuss new approaches for the analysis of transcriptional variation in live cells.

SB1.3
Mathematical modelling and endocrine research
Julian Davis
Manchester University, Manchester, UK.

Endocrine systems are dynamically changing and complex systems. Quantitative measurements of hormones over time became possible with the development of sensitive immunoassays, and it rapidly became clear that hormone release is often pulsatile. The timing of ultradian hormone pulses and circadian variation turns out to be a central characteristic of many systems, and this has required increasing use of mathematical analysis. Regulation of gene expression has traditionally been analysed using RNA and protein preparations derived from large numbers of cells. However these types of analyses using ‘averaged’ data from cell lines can give an impression that all cells behave similarly and uniformly in space and time, but this is clearly not the case in real life. We have studied the regulation of pituitary gene promoter activity using the luciferase reporter gene—the luminescence signal can be readily measured in cell lysates using a luminometer, but advances in luminescence microscopy allow measurements from individual living cells over time. Surprisingly, promoter activity in single cells fluctuates dramatically, and we have utilized mathematical modelling to analyse the characteristics of the cycles of gene expression among individual cells and propose hypotheses about how they might be generated. While endocrine cells display individual intrinsic rhythmic properties, in a tissue they must be co-ordinated in space and time to achieve the synchronized secretion of a large pulse of hormone over a short period. Some of this coordination may be explained by neural influences, but some appears to be due to the autonomous behaviour of intact tissues. Analysis of this coordination requires more detailed spatio-temporal mathematical modelling than before, and this will hold the next challenge. ‘Systems biology’ has involved interactions between two very distinct specialties, which brings the reward of new ways of seeing biological phenomena, and lead to new hypotheses about how endocrine systems operate.

SB1.4
Applications of systems biology for the endocrinologist
A Vidal-Puig
Abstract unavailable.

SB1.5
Systems biology: a funder’s perspective
Tom Foulkes
London, UK.

The MRC currently operates a ‘highlight notice’ in systems biology for medicine. Highlight notices are requests for applications through the normal response mode route – they represent a priority but do not have a specific budget attached. This notice is of considerable relevance to endocrinology research, as will be discussed at the March meeting. MRC-funded endocrinology researchers are already beginning to use systems approaches with some success, but there is an opportunity for these approaches to be used more widely in the field. More details on the highlight notice can be found at http://www.mrc.ac.uk/Fundingopportunities/Highlightnotices/index.htm
Senior Endocrinologists Session
Will we ever discover the mechanism of hormone action?
J Tata
MRC National Institute for Medical Research, London, UK.

Although scientists have been seeking the mechanism of action of hormones for over 100 years, which is often before the identity and structures of many hormones were known, we are still unable to say definitively in molecular or structural terms how any hormone exerts a given physiological action. This failure is largely due to the fact that most investigations are technology driven and not hypothesis based. At the same time, two important features of hormones and their actions, namely evolutionary and historical, have often been ignored. Most hormones have been structurally highly conserved during evolution, yet their physiological actions vary enormously in different organisms or from one tissue to another in the same species. Thanks to the rapid advances in technologies based on gene cloning and DNA sequencing in the last 30 years, we know much about receptors, which are a key element of hormone action. Most hormone receptors are located in the cell membrane or nucleus and are cellular homologues of the oncogenes c-erbB or c-erbA. These studies, have established that receptors are highly conserved through evolution, but, again, they do not explain the widely different physiological actions of a given hormone. A historical overview based on a time-line of the progression of new technologies over the last 80 years – from physiological models to biochemical interactions to molecular and structural biology – reveals that our understanding of hormone action is derivatives of newly evolving techniques. We are now at a stage where focusing on immediate post-receptor events in the hormonal target cell might well bring us closer to understanding how any individual hormone brings about a particular physiological action.

Sleep, light and hormones in Antarctica
Josephine Arendt
University of Surrey, Guildford, UK.

Light of sufficient intensity and suitable spectral composition is the main factor which maintains a 24 h period in human circadian rhythms. At the British Antarctic Survey base of Halley (75°S) the sun does not rise for 3 months in winter and does not set for 3 months in summer. In this isolated environment most subjects remain synchronised to the 24 h day however the melatonin rhythm and sleep are delayed in winter compared to summer. Summer timing can be restored with a skeleton photoperiod of standard bright white light. Decrements in several other sleep parameters (efficiency, latency, duration, quality) are seen in winter. The introduction of extra light (Philips Bright Light Devices), sufficient to increase daily maximum/average light exposure in winter from 570/30 lux to 2000/70 lux is associated with benefits for sleep and there is some evidence that blue enriched light is more effective than standard white light. The majority of base members adapt to night shift within a week, assessed by the melatonin rhythm, in contrast to night shift workers in temperate zones who rarely adapt completely. Their sleep improves during night work and in winter they have problems returning to day work, evidenced by free-run or circadian desynchrony. Tinted light treatment also addresses this problem. Post-prandial response to meals is compromised during periods of night shift desynchrony with evidence of insulin resistance, and elevated triglycerides, risk factors for heart disease. These observations suggest that sub-optimal light conditions are deleterious to health. In high latitudes in winter, short photoperiods together with an interior workplace are associated with a lack of bright natural light. Workstations may have no windows and outdoor activity during daylight hours can be difficult if not undesirable in poor weather conditions. Thus data from Polar regions may well have applicability in such situations.

Is the placenta just a parasitic endocrine organ?
P Lowry

Abstract unavailable.

Are salivary hormone measurements a waste of time?
P Wood

Abstract unavailable.

La Mujer Barbuda by Ribera, 1631: a clinical conundrum
M Tunbridge
University of Oxford, Oxford, UK.

The picture showing a markedly virilised central figure with an infant at the breast and a shadowy figure in the background was painted in 1631 by Jose Ribera, a Spaniard living in Naples. It was commissioned by Ribera’s patron, the Duke of Alcalia. The painting is of a real named person. The provenance of the painting is well known and through the Duke’s family inheritance it returned to Spain and is to be seen in the Museo Fondacion Duque de Lerma in Toledo. It can also be found on Google by searching for Spanish painters then Ribera (1591–1652) and his works by title. The case for a possible diagnosis of an underlying medical condition, which could explain all the features evident in the painting will be made. A differential diagnosis and the relevant possible investigations of someone presenting with similar problems in the 21st century will be sought from the audience. The manner in which such a person might have been treated in the 17th and subsequent centuries will be compared to the way in which gender issues are managed today.

Animated enchantment: an investigation of the enduring popularity of Walt Disney’s first feature films
Howard Jacobs
Queen Mary, University of London, London, UK.

In this talk, which will include part my submission for a PhD in Film Studies in the University of London, I explore some of the reasons for the widespread and enduring popularity of Disney’s first feature-length animated films (Snow White and The Seven Dwarfs (1937), Pinocchio (1940), Dumbo (1941) and Bambi (1942)). Despite their old fashioned style, the first two have recently been releasé – in 70th anniversary editions in Blu-Ray format – for yet another generation to enjoy. The latter two are often shown on television. I use psychoanalytical concepts developed by Melanie Klein to explore the content of the films and those of D W Winnicott to explore the visual fascination of the animated form Disney gave them. The models have been selected to provide a framework for exploring the spectator’s response to the films rather than to probe deeper understanding of their protagonists – i.e. no attempt is made to ‘psychoanalyse’ the characters in the film, rather their narrative and structure is explored in an attempt to better understand the basis for our enjoyment of them. Using this approach, one can uncover the social, psychological and the unconscious minds of their viewers, both children and adults. The talk will be illustrated by film clips and stills.
Oral Communications
Diabetes and metabolism

OC1.1 IGFI receptor and insulin receptor stoichiometry is a critical determinant of nitric oxide bioavailability
Afroz Abbas, Hema Viswambharan, Helen Imrie, Adil Rajwani, Stephen Wheatcroft, Peter Grant & Mark Kearney
University of Leeds, Leeds, West Yorkshire, UK.

Introduction
Accumulating evidence suggests a role for IGFI in insulin resistance and cardiovascular disease. IGFI enhances glucose uptake and nitric oxide (NO) production, via similar mechanisms to insulin. Previously we have reported a mouse model with global heterozygous knockout of the IGFI receptor (IGFR/IGF1RKO) and another with heterozygous knockout of the insulin receptor (IR) and IGFI/IR (IRKOxIGF1RKO). Ex vivo assessment of vascular function was performed using murine thoracic aortic rings in an organ bath. Complementary studies were performed by disrupting IGFI/R in HUVECs using siRNA and quantifying phosphorylated eNOS (phNOS) expression.

Results
Aortic rings from ECIGF1RKO mice were hyporeactive to phenylephrine (PE) compared to those from wild-type littermates (WT; ECmax EC50 = 0.69 ± 0.03 g; n = 8, Emax WT = 0.85 ± 0.06 g; n = 7, P = 0.03). Addition of the NO synthase inhibitor L-NMMA abolished this difference (n = 4, P = 0.005). Knockout of the IGFI/IR in IRKO mice (IRKOxIGF1RKO) mice restored vascular sensitivity. Insulin led to a decrease in Emax 0.06 ± 0.04 g (n = 5) in IRKO aortic rings compared to a decrease in Emax 0.31 ± 0.11 g (n = 3) in IRKOxIGF1RKO aortic rings (P = 0.04).

IGFI/IR knockout, using siRNA in HUVECs, upregulated basal levels of phNOS compared to control by 27.3 ± 1.6% (n = 5). Insulin-stimulated phNOS expression was also increased by 28.3 ± 4.9% with siRNA IGFI/R knockout compared to insulin-stimulation alone (n = 3, P = 0.005).

Conclusion
Consistent with the favourable effects on endothelial function conferred by IGFI/IR knockout, endothelial insulin sensitivity was restored by reducing IGFI/IR numbers in a murine model of insulin resistance. This novel interaction between the IR and IGFI/IR may account for the enhanced vascular NO production seen when IGFI/IR numbers are reduced.

OC1.2 Moderate maternal undernutrition results in epigenetic changes in the fetal hypothalamic feeding centres, but not in the fetal HPA axis
Ghazala Begum1, Adam Stevens1, Mark Oliver1, Kristin Connor1, John Challis2, Frank Bloomfield3 & Anne White4
1University of Manchester, Manchester, UK; 2University of Toronto, Toronto, Canada; 3University of Auckland, Auckland, New Zealand.

Maternal undernutrition influences the development of obesity and diabetes in the adult offspring. Previous work has shown that moderate maternal undernutrition may alter the stress-response of the hypothalmic–pituitary–adrenal (HPA) axis; however, it is not clear if this is the mechanism for consequent obesity in offspring.

The aim of this study was to analyse epigenetic changes in the POMC and GR genes in the pituitary as markers of HPA axis activity and compare these to other brain regions.

For this work a sheep model of periconceptual undernutrition was used. Ewes were undernourished (UN, n = 6) for 60 days pre- and 30 days post-mating or given normal feed (N, n = 6). Brain tissues (ventral hypothalamus, hippocampus and pituitary) were dissected at post mortem (day 135). POMC and GR mRNA expression and promoter region methylation were assessed. In the stress axis there was no evidence of changes in promoter methylation and expression of the GR and POMC genes in the fetal hippocampus and anterior pituitary. In the fetal circulation there were no changes in POMC, ACTH or cortisol concentrations. In comparison, in the hypothalamic feeding centres POMC and GR gene promoter methylation were decreased in the offspring from the UN group (62 and 53% decrease respectively) and GR gene mRNA expression was increased (4.7-fold).

In summary, following maternal periconceptual undernutrition there is no evidence for epigenetic changes in stress axis genes in the fetal hippocampus and pituitary. In contrast, significant changes were observed in promoter methylation of POMC and GR genes in hypothalamic feeding centres. These observations suggest that fetal programming of the ventral hypothalamus may be involved in the development of obesity in adult offspring but similar changes were not found in the stress axis.

OC1.3 Adipose-specific knockout of androgen receptors in mice results in hyperinsulinaemia without obesity
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Background
Visceral fat is a key factor underlying type 2 diabetes. The amount and distribution of body fat is strongly influenced by sex steroids. Androgen receptors (ARs) are present in adipose tissue and are abundant in the detrimental visceral bed. Here, we sought to determine the contribution of the AR in adipose tissue to the pathophysiology of visceral obesity and type 2 diabetes.

Methods
Male fat-specific AR-knockout (IARKO) mice (12 weeks; n = 12 each group) were created by breeding floxed-AR mice with adipose-specific FABP4-Cre mice. Glucose homeostasis was assessed by intraperitoneal glucose tolerance test (IPGTT, 2 mg glucose/g body weight) following a 6 h fast. At cull, tissues were weighed and biochemical indices of insulin resistance assessed. Data are mean ± S.E.M.

Results
AR mRNA expression was significantly decreased in IARKO adipose tissue compared to floxed controls (0.29 ± 0.06 vs. 0.76 ± 0.2 Arbitrary Units, P < 0.05). (IARKO mice were lighter (27.9 ± 0.38 vs. 30.1 ± 0.28 g, P < 0.001), had decreased gonadal fat (9.9 ± 0.5 vs. 11.9 ± 0.8 g/m2 body weight, P < 0.05) and increased brown adipose tissue (5.1 ± 0.27 vs. 4.1 ± 0.28 mg/g body weight, P < 0.05) compared to floxed controls. (IARKO mice had normal fasting plasma glucose levels, glucose tolerance and NEFA suppression but showed elevated fasting (0.73 ± 0.06 vs. 0.54 ± 0.04 ng/ml, P < 0.05) and 90 min-post IP glucose bolus plasma insulin levels (0.81 ± 0.07 vs. 0.66 ± 0.06 ng/ml, P < 0.05). Liver triglycerides (11.77 ± 0.39 vs. 11.27± 0.41 mmol/ml) and plasma testosterone levels (0.79 ± 0.19 vs. 0.82 ± 0.22 ng/ml) were unchanged in IARKO mice. Conclusions
Ablation of adipose AR in mice produces hyperinsulinaemia without obesity. These findings highlight a specific role for androgen action in adipose in the pathophysiology of insulin resistance and we anticipate that therapeutic manipulation of androgen action at the level of the adipose tissue may improve insulin sensitivity.

OC1.4 The impact of saturated fatty acids and glucose on Toll-like receptor activated inflammatory pathways in human adipose tissue, in vitro
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Chronic elevation of saturated fatty acids (SFAs) and glucose (Glc) appears to activate an inflammatory response; compounded by habitual feeding. Restoration of physiological SFAs and Glc levels post-prandially may not attenuate the original insult; a concept termed ‘metabolic memory’. Therefore we investigated (1) the effect of chronic and oscillating SFAs and Glc on the inflammatory pathway in human abdominal subcutaneous (AbdSc) adipose tissue (AT) and adipocytes (Ads) and (2) whether Ads are subject to ‘metabolic memory’. AbdSc AT (age 45 ± 3.3 years; BMI 21.9 ± 2.4 kg/m2; n = 6) was obtained with ethics approval. Explants and Ads were treated with chronic low glucose (L-Glc): 5.6 mM and high glucose (H-Glc): 7.5 mM, with low (0.2 mM) and high (2 mM) doses of a palmitate:stearic tri-mix (SFA) for 48 h. Further, AbdSc AT explants and Ads were also exposed to the aforementioned treatment regimen for 12 h periods, with alternating rest periods of 12 h in L-Glc. Western blot and ELISAs were utilised to examine components of the NFkB pathway.

Chronic treatment with H-Glc and high SFAs up-regulated key proteins of the NFkB pathway in AbdSc AT explants and Ads (TLR4, NFkB, P < 0.05) whilst
OC1.5
The immune-adrenal interface: effects of endotaxin on annexin 1 and formyl peptide receptor expression, cellular morphology and steroidogenesis in the mouse adrenal cortex
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The anti-inflammatory protein, annexin 1 (ANXA1) acts via a formyl peptide receptor (FPR), possibly FPR2, in the neuroendocrine system to mediate feedback effects of glucocorticoids and thereby modulate the HPA responses to immune insults. Here, we explored further the role of ANXA1 and the FPR family in mediating the HPA responses to endotaxin (LPS), focusing on the adrenal cortex. Adult male mice treated with LPS (500 mg/kg, i.p.) showed time-dependent increases in murine ANXA1 (mANXA1), mFPRL1, mFPRL2 and mFPRL3 mRNAs in the pituitary, adrenal gland and spleen (positive control), but not in the hippocampus or hypothalamus. There were also increases in corticosterone, pro-inflammatory cytokines and at 4 h (peak upregulation) in the adrenal cortex, inflammatory cell infiltration (predominantly eosinophils) and reduced vacuolation in the steroidogenic cells were seen. In vitro, adrenal cells from LPS-treated mice were given ACTH (0.1–10 μM) but failed to release corticosterone, unlike saline-treated mice. The LDS-induced inflammatory cell infiltration was unaffected by ANXA1 or FPR2 gene deletions, but vacuolation was present, unlike their WT counterparts. In mice depleted of polymorphonuclear leucocytes (PMNs), LPS still induced increases in corticosterone and pro-inflammatory cytokines and reduced vacuolation. With inflammatory cell infiltration in the adrenal cortex absent or dramatically reduced, an upregulation of mRNA for mFPRL2 only occurred (mFPRL1 and mFPRL3 unaffected) in LPS treated mice. As before the N-terminal ANXA1 peptide (ANXA112–30, 10–30 μM) inhibited ACTH-induced corticosterone release in vitro from adrenal cells from control mice; its effects were mimicked by high (1.0–10 μM) but not low (0.1 μM) concentrations of formyl peptide (fMLP), suggesting ANXA1 effects may be mediated via FPR2. These data raise the possibility that adrenal function may be compromised in endotoxaemia both through impaired steroidogenesis, due to substrate depletion, and the cytotoxic actions of infiltrating inflammatory cells. The roles of ANXA1 and FPR2 in modulating steroidogenesis require further investigation, along with the unexplored roles of FPR1 and FPR3 in the adrenal cortex.

OC1.6
Testosterone improves glycaemic control, insulin resistance, body fat and sexual function in men with the metabolic syndrome and/or type 2 diabetes: a Multicentre European Clinical Trial: the TIMES2 Study
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Hypogonadism is a common finding in men with metabolic syndrome (MS) and/or type 2 diabetes (T2D) which adversely affects quality of life, is associated with several cardiovascular (CV) risk factors and a greater risk of mortality. Testosterone replacement therapy (TRT) in pilot studies has beneficial effects on waist circumference (WC), insulin resistance, glycaemic control, lipid and inflammatory profiles. Testosterone replacement in hypogonadal metabolic syndrome and type 2 diabetes (TIMES2) was a European multi-centre, prospective, randomized, double-blind, placebo-controlled trial of TRT (transdermal 2% testosterone gel) in 220 hypogonadal men with either MS and/or T2D.

TRT reduced homeostatic model of insulin resistance (HOMA-IR) by −0.85 (P=0.018) at 6 months and −0.84 (P=0.06) at 12 months. HOMA-IR at 12 months in T2D group decreased by −0.82 (P=0.049) and MS by −0.87 (P=0.054). In the modified per protocol groups (subjects with no change in medication affecting specific parameters in first 6 months), TRT in total study group improved a) body composition −0.28% (P=0.004) at 6 and −0.91% (P=0.026) at 12 months b) total cholesterol −0.23 mmol/l (P=0.037), LDL-cholesterol −0.2 mmol/l (P=0.019) and lipoprotein a (Lpa) −0.06 mmol/l (P=0.034) all at 6 months. No change in HDL-cholesterol or triglycerides. In T2D, TRT reduced HbA1c by −0.58% (P=0.005) at 9 and −0.5% (P=0.066) at 12 months and WC by −1.59 cm at 12 months. Sexual function assessed by the International Index of Erectile Function (IIEF) score at 6 (7.77, P=0.019) and 12 (9.36; P=0.024).

TRT improves insulin resistance, the pivotal biochemical abnormality and mediator of CV risk in MS and T2D. There were beneficial effects with reductions in body composition, in body mass and in body weight.

OC1.7
Prospective changes in glucocorticoid metabolism predict alterations in metabolic phenotype
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Glucocorticoid (GC) production rates are elevated in obese, insulin resistant individuals. We and others have demonstrated decreased hepatic cortisol regeneration through reduced 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) activity that converts inactive cortisone to cortisol. In addition, there is enhanced cortisol clearance by β-ring reductions, (notably 5α-reductase).

We have argued that these changes drive the hypothalano-pituitary–adrenal axis and represent a protective mechanism to decrease tissue specific cortisol exposure to preserve metabolic phenotype. On this background, we established the Birmingham Prospective Obesity, Diabetes and Steroid metabolism cohort (BPODS) to prospectively track changes in GC metabolism with metabolic phenotype. Individuals who are unable to decrease tissue specific cortisol exposure may be at greater metabolic risk.

One hundred and fourteen obese patients (75 women, BMI>30 kg/m2) were recruited and underwent anthropometric measurements, fasting blood tests, oral glucose tolerance test (OGTT), DXA body composition and 24 h urine collections for steroid metabolite analysis (GCMS). All investigations were repeated 12 months after enrolment. Over the initial 12-month prospective follow-up period, mean change in weight was +1.1±0.6 kg and BMI +0.42±0.22 kg/m2. 36.7% of patients lost weight and 62.3% gained weight (unchanged in one patient). Mean change in glucose tolerance (AUC across OGTT) was −0.17±0.28 mmol/l per man (41.4% deterioration, 58.6% improvement). Individuals who decreased 11β-HSD1 activity (urinary GCMS analysis) lost weight (−2.0±2.5 kg), however, in contrast, those with increased 11β-HSD1 activity gained weight (+1.7±2.6 kg, P<0.05). In addition, baseline 5α-reductase activity in men predicted deterioration in glucose tolerance over the 12-month period (R=0.46, P<0.005). Independently, increasing trunk fat mass (DXA) was associated with worsening glucose tolerance (−1.2±0.5 vs +0.13±0.33 mmol/l per min, P<0.005) and increasing fasting insulin (−3.3±2.2 vs +1.3±0.9 pmol/l, P<0.05).

We have demonstrated that 11β-HSD1 and 5α-reductase activity are able to predict individuals with increasing body weight and worsening glucose tolerance. Furthermore, decreasing 11β-HSD1 activity tacks with improvements in metabolic phenotype and endorses therapeutic inhibition as a valid treatment target.

OC1.8
Cortisol metabolism and hepatic expression of 11β-hydroxysteroid dehydrogenase type 1 in patients with non-alcoholic fatty liver disease
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Non-alcoholic fatty liver disease (NAFLD) is the hepatic manifestation of the metabolic syndrome. The role of glucocorticoids (GC) in its pathogenesis, is highlighted in patients with GC excess, Cushing’s syndrome, who develop central adiposity, insulin resistance and in 20% of cases, NAFLD. Although circulating cortisol levels are normal in patients with NAFLD, hepatic cortisol availability is controlled by enzymes that regenerate cortisol from inactive cortisone (11β-hydroxysteroid dehydrogenase type 1, 11β-HSD1) or inactivate cortisone through A-ring metabolism (5α- and 5β-reductase, 5αR and 5βR).

We characterised hepatic cortisol metabolism in 16 patients with histologically proven NAFLD (eight steatosis, eight steatohepatitis (NASH)) and 32 BMI-matched controls. We also analysed 11β-HSD1 mRNA expression and protein expression by immunohistochemistry in liver from five NASH patients and five normal controls.

In patients with steatosis 5αR activity was increased (urine 5αTHF/THF ratio: controls 0.80±0.07, steatosis 1.31±0.21, P<0.05), paralleled by a decrease in hepatic 11β-HSD1 activity (cortisol concentration curve (GCC) after oral cortisone acetate 25 mg AUC±s.e.m. controls 382±22, steatosis 304±27 μg/l per min, P<0.05, urine cortololone ratio: controls 0.43±0.02 versus steatosis 0.33±0.02, P<0.05). Furthermore, total cortisol metabolism was increased consistent with increased GC production rate (12 168±1028 vs 8 869±786 μg/l per 24 h, P<0.01).

Patients with NASH had increased cortisol generation consistent with increased hepatic 11β-HSD1 activity compared with controls and with steatosis (GCC AUC±s.e.m.: controls 382±22, steatosis 304±27, NASH 496±52 μg/l per min; NASH versus controls, P<0.05, NASH versus steatosis, P<0.01).

Immunohistochemical analysis in patients with NASH showed markedly increased 11β-HSD1 expression in peri-portal septa and within the inflammatory infiltrate. The role of 11β-HSD1 mRNA expression was also increased when compared with controls (ΔCT 9.65±0.29 vs 11.96±0.29, P<0.01).

 Patients with hepatic steatosis have increased clearance and decreased regeneration of cortisol, a possible protective mechanism to decrease local GC availability to preserve hepatic metabolic phenotype. With progression to NASH, increased 11β-HSD1 activity driven cortisol regeneration may serve to limit hepatic inflammation. These results provide a crucial insight into the pathophysiology of the NAFLD disease spectrum.

Neuroendocrine tumours/pituitary

OC2.1

A novel mechanism of effect for somatostatin analogues: the role of AIP

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Background

Recently, germline mutations in the aryl-hydrocarbon-receptor-interacting-protein (AIP) gene have been found to occur in familial and sometimes in early onset sporadic somatotroph adenomas. These tumours tend to respond less well to somatostatin analogues (SSA). It has been shown previously that AIP can upregulate the transcription factor ZAC1 in liver cells, and we were able to demonstrate this in pituitary cells. On the other hand, ZAC1 is upregulated in response to SSA. Therefore, we have hypothesised that SSA might mediate their effects by a pathway involving AIP and ZAC1.

Aim

To study the effect of SSA on AIP and ZAC1 expression in patients with sporadic acromegaly and in a somatotroph cell line.

Method

Seventeen patients with sporadic acromegaly were treated with lanreotide 30 mg/1–2 weeks, 16 weeks prior to transphenoidal surgery. They were matched (age, sex and tumour size) to 17 patients with no pretreatment prior to surgery. AIP protein expression was assessed by immunostaining. GH3 cells treated with 1 μM octreotide were assessed for AIP and ZAC1 expression by ‘real time’ PCR and immunoblotting.

Results

AIP immunostaining was significantly increased in the lanreotide group (60.3 ± 19% positive cells) versus the control group (27.9 ± 11.7%) in both sexes (P<0.001). In female patients there was a correlation between AIP staining and the reduction in IGF1 levels after lanreotide treatment (R = 0.66, P<0.05).

After treatment of GH3 cells with 1 μM octreotide, both AIP and ZAC1 mRNA expression were significantly increased at 6 h (P<0.02), while AIP protein expression was significantly increased at 9 and 12 h (P<0.01).

Conclusion

Our previous data on the lack of effect of SSA in patients with AIP mutations, together with the increased AIP protein expression in somatotrophinomas after SSA pretreatment and the in vitro data on AIP and ZAC1 upregulation, suggest that AIP is an important element in mediating the effects of SSA.

OC2.2

Wnt/b-catenin signalling is down-regulated in pituitary tumours from a multiple endocrine neoplasia type 1 (MEN1) mouse model

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The tumour suppressor menin, encoded by the multiple endocrine neoplasia type 1 (MEN1) gene, has been reported to be a component of the Wnt/b-catenin signalling pathway. To investigate the effects of menin loss on this pathway, we have determined the CDNA expression of pituitary tumours from 5 Men1-/- mice and in normal pituitaries from 5 Men1+/- littersmates by extracting total RNA and by hybridizing it to Affymetrix Mouse Genome arrays.

The pituitary tumours, which were mostly somatotrophinomas, when compared to normal pituitaries were found to have significant alterations of several Wnt/b-catenin pathway components including up-regulation of the inhibitors axin2 and sfrp1, downregulation of the target genes axin2 and calcium/calmodulin-dependent protein kinase II γ (Camk2g) (−1.9- and −3.0-fold, respectively, FDR<0.01), and down-regulation of the target genes axin2 and Camk2g (−2.4, P<0.01). To further characterize the functional effects, expression of total and active b-catenin protein was assessed by western blot and densitometry analysis, using extracts from pituitary tumours and normal pituitaries. Total b-catenin expression was similar in normal pituitaries and tumours but active b-catenin protein was reduced by >50% in the tumours, although immunohistochemistry revealed the tumours to have increased nuclear localisation of the active b-catenin, which was predominantly cytoplasmic in normal pituitaries. Thus, our results show that the absence of menin in pituitary tumours inhibits Wnt target gene expression, and thus supports a role for menin in increasing histone H3K4 trimethylation of the Axin2 promoter and thereby a transcriptional activation of Wnt/b-catenin target genes. These studies provide further insights into the role of menin in the Wnt/b-catenin signalling pathway and may allow the development of novel therapeutic targets for endocrine tumours.

OC2.3

11C-Metomidate positron emission tomography (PET) scanning for Conn’s syndrome

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Primary hyperaldosteronism usually results from an aldosterone-secreting adenoma of the adrenal cortex (Conn’s adenoma) or bilateral adrenal hyperplasia. Identification of the anatomical adrenal lesion causing hyperaldosteronism typically involves CT or MR scanning, with lateralisation of aldosterone production confirmed by adrenal vein sampling (AVS). The latter is a technically difficult and invasive procedure, but current non-invasive alternatives (e.g. radio-labelled iodochlorhydroxyquin) lack sensitivity and specificity.

11C-Metomidate, a potent inhibitor of the enzyme 11β-hydroxylase, is over-expressed in adrenocortical adenomas, has recently been used as a radiotracer in position emission tomography (PET), and shows significant uptake into both normal adrenal tissue and Conn’s adenomas. We hypothesised that 11C-metomidate uptake by normal adrenal tissue would be suppressed by both dexamethasone and fludrocortisone. In the zona fasciculata 11β-hydroxylase expression is controlled by adrenocorticotropic hormone and thereby ‘switched off’ by dexamethasone. The zona glomerulosa contains all the components of the classical mineralocorticoid response pathway, and normal aldosterone secretion is suppressed by the mineralocorticoid against fludrocortisone.

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Five patients with Conn’s adenomas (aged 58.8 ± 3.0 years) underwent three PET-CT scans at least 1 week apart: (i) without pre-treatment; (ii) pre-treatment with dexamethasone (0.5 mg qds for 3 days) alone; (iii) pre-treatment with dexamethasone and fludrocortisone (400 μg od for 3 days). Maximum standardised uptake values (SUVA\textsubscript{max}) of tracer were calculated for adenoma and normal adrenal tissue and the ratio of SUVA\textsubscript{max} in tumour to normal adrenal was calculated. Pre-treatment with dexamethasone increased the ratio of SUVA\textsubscript{max} in tumour to normal by 20.1% (P = 0.037). Combined pre-treatment with dexamethasone and fludrocortisone increased the ratio of SUVA\textsubscript{max} in tumour to normal by 16.5% (P = 0.072). In conclusion, early experience using a new non-invasive technique confirms that it is possible to visualise sub-centimetre adrenal adenomas and differentiate functioning and non-functioning nodules within the same gland.

**OC2.4**

**Geographical cluster of familial isolated pituitary adenoma kindreds with an identical AIP mutation**

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Mutations in the co-chaperone molecule AIP account for a predisposition to pituitary tumours in some families with familial isolated pituitary adenomas (FI PA). We now report on four apparently-unrelated families with the same mutation and originating from the same geographical area, suggesting a possible founder mutation.

The index patient had gigantism (19 years, 208 cm) and had a female 4th cousin, once removed (13 years, 191 cm) with a large pituitary macroadenoma. A patient born in the same village was diagnosed with acromegaly (16 years), and had a first cousin with acromegaly (20 years) and a female first cousin with a prolactinoma (30 years). A 3rd family was noted to have five affected members (three with childhood-onset acromegaly, one adult-onset acromegaly and one childhood-onset macroprolactinoma) and a 4th family with two childhood-onset cases of acromegaly were both originated from the same geographical area. All of these affected subjects have a mutation in the AIP gene: R304X. A number of families from various countries have been reported to harbour a mutation at the 304 aminocacid locus. This is a typical CpG-site where both the C and the G base pairs have been reported to be mutated, one causing a stop mutation (c.910C > T;p.R304X) while the other is an aminoacid change (c.914G > A;p.R304Q). As this locus is a mutational hotspot, we are currently using microsatellite markers to study the level of relatedness between these families. The penetrance of AIP mutations is reported to be variable, with around 30% in large kindreds. In family one we found nine heterozygote carrier subjects with two affected patients, while in family 2 two out of the nine known carriers have manifest disease, while in the other two families the penetrance seems to be lower. It remains unclear as to what other factors influence penetrance.

**OC2.5**

**Differential effects of oestrogen on hypothalamic GnRH-I and GnRH-II gene expression in female rhesus macaques**

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In mammals, the preovulatory LH surge is thought to be triggered by enhanced release of GnRH into the hypothalamic–pituitary portal blood vessels. Recently, we discovered that the medial basal hypothalamus (MBH) of rhesus macaques (Macaca mulatta) contains two distinct populations of GnRH-producing neurons. One of these populations expresses the traditional mammalian form of GnRH (i.e. GnRH-I) while the other expresses a more conserved vertebrate form of GnRH (i.e. GnRH-II). Using various molecular biology approaches (i.e. *in situ* hybridization histochemistry, RT-PCR, gene microarrays and real-time PCR), we have found that oestradiol differentially affects GnRH-I and GnRH-II gene expression in the female monkey MBH, causing a suppression of the former and a stimulation of the latter. This leads us to hypothesize that the GnRH-I producing neurons play a primary role in mediating the positive feedback action of oestrogen on the preovulatory LH surge, whereas the GnRH-I neurons play a more permissive role. To test this hypothesis, we performed an additional experiment, in which oestradiol benzoate (EB) was administered to a group of ovarietomized adult rhesus macaques. We evaluated the response by measuring serum oestradiol and LH concentrations, and examining GnRH-I and GnRH-II mRNA levels in the MBH. As expected, EB induced an LH surge ~48 h later. Importantly, this surge coincided closely with a marked increase in the expression of GnRH-II, but not GnRH-I, confirming the stimulatory influence of oestrogen on the GnRH-II neurons. Because humans also express two forms of GnRH (i.e. GnRH-I and GnRH-II), it is plausible that fertility in women is regulated by the coordinated action of two distinct GnRH neuronal populations, which are differentially affected by the sex-steroid environment. Supported by NIH Grants: HD-29186 & RR-00165, and approved by the Institutional Animal Care and Use Committee.

**OC2.6**

**Laying the foundation for neuroendocrine control of human reproduction: an investigation into the development of kisspeptin and neurokinin B networks**

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The development and maturation of kisspeptin neurons is critical for activating GnRH release needed to initiate puberty. However, little is known of the development and organization of kisspeptin neurons in the human hypothalamus and the anatomical architecture of kisspeptin neurons in rodents is distinct from that of primates.

The aim of the present study was to examine the development of the hypothalamic neuroendocrine circuitry that sets the structural basis for GnRH secretion during sexual differentiation, puberty and adulthood. We analyzed the expression of neuroendocrine genes relevant to reproduction (*KISS1, GPR54, GNRH1, TAC3, and TAC3R*) in late stages of human embryonic development by Taqman quantitative reverse-transcription PCR. We also used immunocytochemistry multiple labelling techniques to examine the distribution of GnRH, kisspeptin, and neurokinin B cell bodies and their projections in the human fetal hypothalamus.

Our data show that *GNRH1, TAC3R and TAC3* genes were expressed by 60 days gestation in males and females. All five genes were detected in hypothalamic cDNA generated from second trimester specimens. Our preliminary data using immunocytochemistry mapped the expression of large populations of neurokinin B in the dorsomedial nucleus and the ventromedial nucleus of the human hypothalamus. In addition, the infundibular/arcuate nucleus was highly immunoreactive for neurokinin B with scattered neurons projecting to the median eminence. We found that the majority of infundibular/arcuate neurokinin B neurons co-express kisspeptin in both sexes by 15 weeks gestation, a feature observed in adult sheep and rodents.

These data demonstrate that many components of the neuroendocrine reproductive network important for activation of GnRH neurons are formed early in human brain development. This is the first investigation that surveys the expression and organization of kisspeptin and neurokinin B circuits in the developing human hypothalamus.
OC2.7
The spectrum of disease in diazoxide responsive hyperinsulinaemic hypoglycaemia
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Hyperinsulinaemic hypoglycaemia (HH) has traditionally been classified according to clinical response to diazoxide with milder diazoxide responsive (DRHH) and severe unresponsive cases. Loss-of-function mutations in ATP-sensitive potassium (K_ATP) channel genes (ABCC8 and KCNJ11), are the commonest cause for HH. In severe HH, genetic analysis and pancreatic imaging assist in differentiating diffuse from focal forms of HH, as the latter may be cured by lesionectomy. However, these investigations have not been routinely undertaken in DRHH.

Aim
To assess the role of genetic testing and pancreatic imaging in DRHH.

Methods
We conducted a retrospective review of 54 HH patients treated at our centre between April 2006 and September 2009. With ethical and parental consent, genotyping was performed and [18]F-DOPA PET-CT imaging undertaken in those with a genotype suggestive of focal HH.

Results
Fifty-two patients were genotyped and 18 (35%) had a genetic abnormality (ABCC8 (n = 13), KCNJ11 (n = 2), GLUD1 (n = 1), GCK (n = 1) and one unbalanced translocation between chromosomes 9 and 13). Nine patients with no mutations (n = 5) or paternal heterozygous K_ATP mutations (n = 4) underwent [18]Fluoro [F]-DOPA PET-CT, identifying three focal lesions (two tracer-positive, one tracer-negative). Three patients underwent curative surgical lesionscopic. Histology confirmed non-malignant insulinomas in two patients, one with a paternal ABCC8 mutation and one who was subsequently found to have an MEN1 mutation. Thirteen patients (25%) had spontaneous resolution of disease, of whom three were mutation positive.

Conclusions
Approximately a third of DRHH patients had a genetic abnormality associated with their disease. Of 6% had a focal lesion amenable to surgical cure and a quarter had late resolution of disease, including mutation positive patients. These findings indicate that mutation screening should be performed in all DRHH patients and [18]F-DOPA PET-CT imaging undertaken in those with a genotype consistent with a focal lesion, as DRHH does it preclude the possibility of a surgical cure.

OC2.8
Diagnosis and localisation of insulinoma: the value of modern MRI in conjunction with calcium stimulation catheterisation
Vasanthi M Mudhuppalaniyan1, Maralyn R Bruce1, Benjamin O’Leary1, Shern L Chew1, William M Drake1, John P Monson1, Scott A Akker1, Michael Besser1, Anju Sahdev1, Andrea Rockall2, Soumil Vyas1, Matthew Matson1, Daniel Berney1, Satya Bhattacharya1 & Ashley B Grossman1
1Department of Endocrinology, Barts and the London Medical School, St Bartholomew’s Hospital, London, UK; 2Department of Radiology, Barts and the London Medical School, St Bartholomew’s Hospital, London, UK; 3Department of Histopathology, Barts and the London Medical School, St Bartholomew’s Hospital, London, UK; 4Department of Surgery, Barts and the London Medical School, St Bartholomew’s Hospital, London, UK.

Objective
To review the diagnostic features and localization accuracy of different investigations for insulinomas diagnosed 1990-2009 at a single tertiary referral centre.

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Insulin-resistant diabetes is characterised by progressive pancreatic β-cell dysfunction. Elevated pancreatic islet activity of the intracellular glucoregulon amplifying enzyme 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) was hypothesized to drive this process in genetically obese rodents. To determine the direct effects of elevated 11β-HSD1 on β-cell function in diabetes in vivo we created a transgenic model overexpressing 11β-HSD1 under the mouse insulin 1 promoter (MIP-HS1D1). As predicted, MIP-HS1D1 mice showed a gene dose-dependent impairment of insulin secretion in vivo (area under curve in control diet: KI: 160 ± 21, MIP-HSD1 tg/+: 140 ± 17, MIP-HSD1 tg/+: 89 ± 6 g·kg⁻¹·h⁻¹). However, unexpectedly, MIP-HS1D1 mice completely reversed the β-cell failure and glucose intolerance caused by chronic high fat feeding in diabetes-prone C57BlKs mice (area under curve in high fat diet: KI: 2360 ± 108, MIP-HS1D1 tg/+: 214 ± 145, MIP-HS1D1 tg/+: 213 ± 562). MIP-HS1D1 mice had increased islet number (control diet KI: 580 ± 57, MIP-HS1D1 tg/+: 1049 ± 190 P < 0.05, high fat diet KI: 384 ± 27, MIP-HS1D1 tg/+: 790 ± 45 P < 0.001) and improved islet function in vitro (insulin secretion rate area under curve in high fat diet: KI: 334 ± 47, MIP-HS1D1 tg/+: 53 ± 5 4 P < 0.05). Meanwhile as expected 11β-HSD1−/− mice have appropriate insulin secretion for their improved peripheral insulin sensitisation (area under curve high fat diet C57Bl6: 629 ± 159, 11β-HSD1−/−: 415 ± 123 P < 0.011 correlated with a lower insulin production per islet in vitro (area under curve high fat diet C57Bl6: 20 ± 2.4, 11β-HSD1−/−: 15 ± 2.7 P < 0.05).

Our study shows that elevated β-cell 11β-HSD1 critically supports compensatory insulin hypersecretion. This suggests profound implications for the imminent clinical therapeutic targeting of 11β-HSD1 in diabetes.

**OC3.3 Development of urinary steroid profiling as a high-throughput screening tool for the detection of malignancy in patients with adrenal tumours**

Angela Taylor, Michael Biel, Beverly Hughes, Han Stiekema, Petra Schneider, David Smith, Peter Nightingale, Cedric Shackleton, Paul Stewart, & Wiebe Arlt

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2 Institute for Mathematics and Computing Science, University of Groningen, The Netherlands;
3 Trust Clinical Research Facility, University Hospitals Birmingham NHS Foundation Trust, Birmingham, UK.

Adrenal tumours have an incidence of 2–3% in the general population and the work-up of newly discovered adrenals represents a major burden on the health system. Differentiating adrenocortical adenoma (ACA) from adrenocortical carcinoma (ACC) represents a continuous challenge, with unfavorable sensitivities and specificities provided by tumor size, imaging and even histology. Here, we aimed to develop a reliable screening tool for the detection of adrenal malignancy. We performed urinary steroid profiling by gas chromatography/mass spectrometry (GC/MS) in scanning and selected-ion-recording mode, quantifying 32 distinct steroids. We analysed 24-h urine samples from 102 patients with ACA (39m, 64f, 19–83 years) and 45 patients with ACC (24m, 21f, 20–80 years) recruited by the European Network for the Study of Adrenal Tumours (www.eanat.org). Diagnosis in ACC was confirmed by metastasis; patients with ACA had undergone a median follow-up of 45 months without evidence of metastasis. Total steroid excretion was independent of tumour size (ACA: 0.1–92.4 nmol; ACC: 0.14–230 nmol). Steroid output data from ACA and ACC and healthy controls (n = 88) were subjected to matrix relevance learning vector quantization, carried out over 1000 random splits into training (90%) and test (10%) data sets. This identified a subset of nine steroids that performed best in differentiating ACA from ACC. Receiver-operated characteristics analysis revealed sensitivity = specificity = 91% (AUC 0.97) employing all 32 steroids and sensitivity = 89% (AUC 0.96) when using only the nine most differentiating markers. While GCMS was invaluable in documenting the distinct ACC metabolicome, it is unsuited to use in widespread screening. We are transferring the methodology to a Waters uPLC tandem mass spectrometry platform. All nine targeted steroid markers can be separated and quantified within 5 min using positive ion mode. These results suggest that urinary steroid profiling is a highly sensitive and specific biomarker tool for differentiating benign from malignant adrenal tumours.

**OC3.4 MicroRNAs, let-7 and miR-302, have an altered expression in Men1-null embryos, consistent with abnormal embryonic embryogenesis**

Michael Bowl, Paul Newey, Amita Reed, Gerard Walls, Dilair Banan, Andrew Nesbit & Rajesh Thakker

University of Oxford, Oxford, UK.

The multiple endocrine neoplasia type 1 (MEN1) gene, which when mutated gives rise to parathyroid, pancreatic and pituitary tumours, has been shown to have a role in embryogenesis, as Men1-null mice (Men1+/−) are embryonic lethal by 12.5 days post coitum (dpc). MicroRNAs (miRNAs) are emerging as potent regulators of early mammalian embryogenesis, and we therefore undertook expression profiling of miRNAs in Men1+/− and Men1−/− embryos at 11.5 dpc. Our studies identified differential expression of two developmentally important miRNAs, the mammalian embryonic stem cell-specific miR-302 cluster and the evolutionarily conserved pro-differentiation let-7 family. Validation of these findings using quantitative reverse transcriptase-PCR (qRT-PCR) showed 3.7-fold up-regulation of miR-302a, and 2.2-fold down-regulation of let-7a, in Men1−/− embryos compared to Men1+/− (P < 0.05). We next used qRT-PCR to investigate for alterations in miR-302 and let-7 known target gene miRNAs, which are CyclinD1 and Tgfb2, and Hmgn2 and Linc28, respectively. CyclinD1 and Tgfb2 miRNAs were not significantly different in Men1+/− and Men1−/− embryos, but Hmgn2 and Lin28 miRNAs were significantly increased in Men1−/− embryos when compared to Men1+/−, by 1.5- and 2.1-fold respectively, consistent with the reduction of let-7 miRNAs. Furthermore, a strong inverse correlation between let-7a and Lin28 miRNA was observed in Men1−/− embryos, while let-7e expression was reduced by 70% directly suppresses the expression of Lin28, an oncopetal protein, in the developing Men1−/− embryo, and that this regulation is lost in Men1−/− embryos. In conclusion, our results, which show high levels of miR-302 and reduced levels of let-7, indicate that Men1−/− embryos have a miRNA profile consistent with a pro-pluripotent, undifferentiated state. Thus, our results indicate that miRNAs are a facet of the embryophobically observed in Men1-deficient embryos and that a characterization of their function will provide insight into the role of the Men1 gene during mammalian embryogenesis.

**OC3.5 A multisystem selenoprotein disorder with a thyroid signature**

Nadia Gronemans, Erik Schoenmakers, Maura Agostina, Catherine Mitchell, Laura Papp, Odelia Rajanayagam, Raja Padidela, Rainer Doffinger, Jian’an Luan, Jun Lu, Irene Campi, Hannah Burton, Francesco Muntoni, Dominic O’Donovan, Andrew Dean, Anne Warren, Pascale Guichenemy, Rebecca Fitzgerald, Mark Garnett, & Krishna Chatterjee

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6 Medical Nobel Institute for Biochemistry, Karolinska Institute, Stockholm, Sweden;
7 UCL Institute of Child Health, Dubowitz Neuromuscular Centre, London, UK;
8 Department of Histopathology, University of Cambridge, Cambridge, UK;
9 Institut de Myologie, Paris, France;
10 MRC Cancer Cell Unit, Cambridge, UK;
11 Department of Clinical Neuroscience, University of Cambridge, Cambridge, UK;
12 Department of Medicine, University of Cambridge, Cambridge, UK;
13 Department of Cancer Studies, Molecular Medicine and Genetics, University of Leicester, Leicester, UK;
14 Department of Clinical Biochemistry, University of Cambridge, Cambridge, UK;
15 Department of Medical Sciences, Fondazione Policlinico, IRCCS, Milan, Italy;
16 Current Valley Hospital, Kent, UK.

Selenocysteine insertion sequence-binding protein 2 (SECISBP2) mediates translational incorporation of selenocysteine into 25 known human selenoproteins. ESR1, encoding estrogen receptor 1 (ERα), is the most common gene mutated in thyroid cancer patients. We report a patient with severe growth retardation, mental and developmental delay, microphthalmia, a meningoencephalocele, cardiomegaly, and liver cirrhosis due to a homozygous frameshift mutation in SECISBP2, leading to a novel truncated SECISBP2 protein. SECISBP2 is highly conserved and encodes a small protein (44 amino acids) that is essential for the translation of all selenoprotein mRNAs. This frameshift mutation abolishes the SECISBP2 protein and leads to secondary selenoprotein deficiency. Thus, SECISBP2 is a gene essential for the translation of all human selenoprotein mRNAs.
normal/low free triiodothyronine (T_{3}), but normal thyrotropin (TSH) levels, indicating reduced T_{3} to T_{4} conversion:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>P1</th>
<th>P2</th>
</tr>
</thead>
<tbody>
<tr>
<td>T_{3} pmol/l (9–20)</td>
<td>41.1</td>
<td>35.0</td>
</tr>
<tr>
<td>T_{3} pmol/l (3–7.5)</td>
<td>3.8</td>
<td>2.3</td>
</tr>
<tr>
<td>TSH mIU/l (0.4–4.0)</td>
<td>0.49</td>
<td>1.10</td>
</tr>
</tbody>
</table>

However, low circulating glutathione peroxidase and selenoprotein P levels suggested a more widespread selenoprotein deficiency, and both patients are compound heterozygous for defects in SEMP1.

P1 is aozooperc, with reduced levels of tissue-enriched selenoproteins (GPx4, TGR, SELV) mediating spermatogonic arrest. He has a muscular dystrophy with clinical (fetal muscle weakness) and pathological (excess type 1 fibres, minicores, low SELN) features similar to known SEMP1 mutation myopathies. He is markedly photosensitive, which is linked to dermal deficiency of antioxidant selenoenzymes (GPx1, TrxR, SELX) with increased cellular reactive oxygen species (ROS), membrane lipid peroxidation and oxidative DNA damage. Reduction of immune cell antioxidant defence is associated with impaired T cell proliferation and shortened telomeric DNA; the latter may mediate his borderline anaemia and lymphopenia, analogous to aplastic anaemia found in human telomerase deficiency. He has increased adipose mass, but enhanced insulin sensitivity with increased ERK phosphorylation in skin fibroblasts. P2 is also deficient in tissue-specific selenoproteins (e.g. GPx4, SELN) and antioxidant selenoenzymes. He too has myopathy, increased adipose mass and insulin sensitivity. Both patients also demonstrate reduced levels of other selenoproteins (e.g. SELH, SELI, SELT, SEPN) whose functions are unknown.

Thus, we describe a multisystem disorder, with a distinctive biochemical thyroid signature, involving defective synthesis of many selenoproteins, which highlights their roles in diverse biological processes and may elucidate hitherto unknown functions of members of the selenoproteome.

**OC3.6**

**Menl gene replacement therapy using a modified adenoviral vector demonstrates reduced proliferation rates in pituitary tumours from mice deleted for a multiple endocrine neoplasia type 1 allele**

Mahsa Javid1, Gerard Walls1, Manuel Lemos1, Jeshmi Jeyabalain1, Miriam Barracongrino1, Damian Tyler1, Daniel Stuckey1, Len Seymour1 & Rajesh Thakker1

1Academic Endocrine Unit, University of Oxford, Oxford, UK; 2Department of Clinical Pharmacology, University of Oxford, Oxford, UK; 3Department of Physiology, Anatomy and Genetics, University of Oxford, Oxford, UK.

Multiple endocrine neoplasia type 1 (MEN1) is characterised by the combined occurrence of pituitary, pancreatic and parathyroid tumours. The MEN1 gene encodes a 610-amino acid tumour suppressor, menin, and MEN1-associated tumours show loss of heterozygosity. This indicates that replacement of the wild-type MEN1 gene may inhibit tumourigenesis. We have previously demonstrated that a recombinant adenoviral vector could be safely injected directly into pituitary tumours of heterozygous (MEN1+/-) mice via the transarticular route. We now report the results of a blinded randomised-controlled trial to assess the efficacy of gene replacement in Menl+/- mice.

Mice were treated with either a 20 μl transarticular intra-tumoral injection of vehicle, Ad-GFP or Ad-Menl (5×10¹⁰ viral particles of recombinant adenoviral vectors delivering green fluorescent protein (GFP) or Menl genes, respectively) or no injection. Mice received 1 mg/kg 5-bromo-2-deoxyuridine (BrdU) in drinking water for 4 weeks post-injection before a repeat MRI scan. Of 91 Menl+/- mice that underwent MRI, 22 had pituitary tumours.

Intramuscular histological assessment revealed GFP expression in only the Ad-GFP treated mice. The expression of the Menl gene product, menin, was moderate to high in Ad-Menl treated mice, but minimal in other groups. Daily proliferation rates, assessed by the proportion of nuclei with BrdU incorporation, in tumours injected with vehicle (1.33±0.10%) or Ad-GFP (1.39±0.13%) were similar but Ad-Menl treatment caused a significant reduction in tumour proliferation (0.45±0.09%, P<0.0001). Apoptotic rates, assessed using a TUNEL assay, and change in tumour volumes, assessed by MRI, remained similar in the groups. In vivo Menl gene replacement therapy for pituitary tumours was effective, demonstrated stable menin expression at 4 weeks and induced reduction of tumour proliferation.

**OC3.7**

**Effects of glucocorticoids on Wnt gene expression in syngeneic fibroblasts: potential role in inflammatory bone loss**

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1School of Clinical and Experimental Medicine, University of Birmingham, Birmingham, UK; 2School of Immunity and Infection, University of Birmingham, Birmingham, UK.

Syngeneic fibroblasts (SFs) form a substantial component of inflamed rheumatoid synovium and generate endogenous glucocorticoids (GCs) during inflammation. Recently, production of DKK-1 (a Wnt signalling inhibitor that reduces bone formation) by SFs in response to TNFα has been proposed to be the master regulator of inflammatory osteoporosis. We have identified that in addition to TNFα, GCs potently induce DKK-1 secretion. This may provide a novel mechanism whereby locally generated GCs contribute to bone loss in inflammatory disease, however DKK-1 is one component of a multitude of secreted Wnt signalling factors. Consequently, we have now assessed the effects of GCs and TNFα on a range of Wnt agonists and antagonists.

Primary SFs were isolated from synovial biopsies from three patients with RA undergoing orthopaedic surgery. Fibroblasts were treated with vehicle, dexamethasone (Dex; 100 nmol/l) or TNFα (10 ng/ml) for 24 h. Gene expression of Wnt signalling components (95 in total) was determined using TaqMan gene expression plates.

As previously reported, treatment with Dex in SFs significantly increased DKK-1 mRNA expression (3.1-fold, vehicle versus Dex; P<0.005). In addition, DKK-1 treatment with DKK-1 treatment resulted in a significant increase in expression of the Wnt antagonist FRZB (10.7-fold, vehicle versus Dex; P<0.05). We did not identify a significant increase in DKK-1 or FRZB gene expression with TNFα treatments. Treatment with Dex also resulted in a significant decrease in expression of the Wnt agonist Wnt2 (7.3-fold, vehicle versus Dex; P<0.05).

This study supports our previous finding that GCs increase expression of the Wnt antagonist DKK-1 to a greater degree than TNFα. In addition, this study has identified that several other factors involved in Wnt signalling are regulated by GCs and may contribute to the imbalance in bone metabolism observed in inflammatory disease.

**OC3.8**

**Identification and functional impact of novel mutations in the gene encoding 11β-hydroxysteroid dehydrogenase type 1 in patients with hyperandrogenism**

Alexander Lawson, Elizabeth Walker, Gareth Lavery, Iwona Bujalska, Beverly Hughes, Wiebke Arli, Jonathan Ride & Paul Stewart

University of Birmingham, Birmingham, UK.

In peripheral target tissues, levels of active glucocorticoid hormones are controlled by 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) which catalyses the reduction of cortisone to cortisol within the endoplasmic reticulum. For functional 11-ketosteroid deactivase activity (11β-HSD1), requires the NADPH-generating enzyme hexose-6-phosphate dehydrogenase (H6PDH). Loss of 11-ketosteroid deactivacy results in increased cortisol clearance and activation of the HPA axis with hyperandrogenism. To date only mutations in H6PDH have been identified as the cause of disease in such cases – a condition termed apparent cortisone reductase deficiency (ACRD).

We examined the HSD11B1 gene in two cases presenting with hyperandrogenism and premature pseudopuberty with biochemical features indicative of a milder form of ACRD in whom the H6PD gene was normal. Both cases were heterozygous for mutations (R137C or K187N) in HSD11B1. Modelling the R137C mutation indicated potential disruption of a salt bridge at the dimer interface of the 11β-HSD1 protein, whilst the K187N mutation was predicted to affect cofactor binding. Following expression of the mutants in mammalian cells, only 5% of wild-type (WT) activity was detected for R137C, and no activity for the K187N mutant. Expression in Esherichia coli also demonstrated greatly reduced (R137C), or undetectable (K187N), activity. We then developed a novel bacterial 'hybrid dimer' system to examine the effects of simultaneous expression of mutant and WT 11β-HSD1. Analysis of the resulting mutant WT heterodimer revealed that, for both R137C and K187N, mutations to one monomer of the 11β-HSD1 dimer were able to decrease the yield of soluble protein, presumably by affecting protein folding. This study has identified novel hyperandrogenous mutations in the HSD11B1 gene and has shown that mutations in one monomer of this dimeric protein can have a dominant negative effect on the WT partner. "True cortisone reductase deficiency" due to mutations in HSD11B1 is a further monogenic cause of hyperandrogenism/premature pseudopuberty.
**Bone and parathyroid**

**OC4.1**

*Multiple endocrine neoplasia type 1 (MEN-1) mutation analysis in patients with primary hyperparathyroidism under the age of 40 years*

Radu Mihaş, Gregory Sadler, Lisa Walker & Rajesh Thakker

1Department of Endocrine Surgery, John Radcliffe Hospital, Oxford, UK; 2Department of Human Genetics, Churchill Hospital, Oxford, UK; 3Academic Endocrine Unit, Nuffield Department of Medicine, University of Oxford, Oxford, UK.

**Background**

Primary hyperparathyroidism (PHPT) is commonly diagnosed after the fifth decade of life. Current guidelines suggest that young patients with apparently sporadic PHPT should be screened as potential index cases for the multiple endocrine neoplasia type 1 (MEN-1) syndrome.

**Aim**

To determine the prevalence of mutations in the MEN1 gene in young patients presenting with apparently sporadic PHPT before the age of 40 years.

**Method**

A prospective database maintained in our Unit was used to identify patients who underwent parathyroidectomy before their 40th birthday. An invitation letter was sent asking them to consider genetic screening. MEN1 mutation analysis was performed using leukocyte DNA.

**Results**

Between January 2000 and October 2009, 581 patients of median age 62 years (range 15–90 years) were operated for PHPT. Only 57 patients were under the age of 40 years (range 15–40 years, median 34 years). The proportion of men was much higher in the young group (43%) compared with those aged 40–60 years (31%) or 60–80 years (22%).

Ten young patients were known to have familial PHPT due to MEN1 (n = 7), MEN2 (n = 1) or hyperparathyroidism-jaw tumour (HPT-JT) syndrome (n = 2).

One patient had a radical excision of local recurrence of parathyroid cancer. Forty-six patients had preoperative neck imaging and underwent scan-directed minimally invasive parathyroidectomy (n = 29) or bilateral neck exploration (n = 17) and were found to have single adenomas (n = 44) or multigland disease (n = 2). All 46 patients were cured after undergoing 49 operations.

All young patients were prospectively invited for genetic tests and 17 of them agreed to proceed (Table).

<table>
<thead>
<tr>
<th>Genetic screening</th>
<th>Not screened</th>
<th>Patients 40–90 years</th>
</tr>
</thead>
<tbody>
<tr>
<td>(n = 17)</td>
<td>(n = 29)</td>
<td>(n = 516)</td>
</tr>
<tr>
<td>Sex</td>
<td>mean ± s.o.</td>
<td>mean ± s.o.</td>
</tr>
<tr>
<td>6M/11F</td>
<td>11M/16F</td>
<td>132M/384F</td>
</tr>
<tr>
<td>Age</td>
<td>28 ± 11 (32 years)</td>
<td>34 ± 5 years (35 years)</td>
</tr>
<tr>
<td>Severity of hypercalcaemia</td>
<td>2.94 ± 0.2 (2.90) mg/mmol</td>
<td>2.99 ± 0.2 (2.93) mg/mmol</td>
</tr>
<tr>
<td>Weight of adenomas removed</td>
<td>884 ± 600 (746) mg</td>
<td>1538 ± 1000 (1340) mg</td>
</tr>
<tr>
<td>Length of follow-up</td>
<td>37 ± 32 (33) months</td>
<td>25 ± 19 (22) months</td>
</tr>
</tbody>
</table>

One mutation in the MEN1 gene was identified in a 27 year old woman with double adenomas (c.1045C>T, p.Gln349X). The overall prevalence of MEN1 gene mutation in patients under the age of 40 years was 14% (8/57 patients) but only 5% in apparently sporadic tumours (1/18 patients). A further eight patients were operated for MEN1-related PHPT at and age over 40 years (range 42–70 years).

**Conclusion**

These data indicate that ~15% of patients under the age of 40 years with PHPT may have MEN1 mutations.

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**OC4.2**

*Intra-cellular availability of T3 in chondrocytes is essential for normal skeletal development and adult bone mass*

Nicholas Bernstein, Marta Archcano, Rowan Sínhoe, Yan Lu, Rebecca Hernandez, Duncan Bassett & Graham Williams

Molecular Endocrinology Group, Imperial College London, Hammersmith Hospital, London, UK.

The type 3 deiodinase enzyme (D3) inactivates T4 and prevents activation of T3 to protect the fetus from premature exposure to thyroid hormones. Rapidly falling levels of D3 activity and rising levels of T3 at birth initiate the onset of cell differentiation and organ maturation during the postnatal period. Congenital hypothyroidism causes delayed ossification with reduced bone mineral deposition and short stature. We hypothesize that increased T3 availability in bone forming chondrocytes and osteoblasts initiates the acceleration of postnatal linear growth and bone mass accrual to establish the structure of the mature skeleton. Thus, to investigate the role of T3 availability during bone development, we generated transgenic mice that over-express D3 following excision of a floxed stop cassette (pCALL2/D3) and crossed them with mice expressing cre-recombinase only in chondrocytes (COL2Cre) or osteoblasts (OCCre). Restricted D3 over-expression in either chondrocytes or osteoblasts did not affect circulating T3 or T4 levels. Consistent with high levels of endogenous D3 in utero, mice over-expressing the enzyme in chondrocytes (COL2/D3) and osteoblasts (OC/C3) displayed normal endochondral and intramembranous ossification at post-natal day P1. However, at P21 COL2/D3 mice had grossly abnormal epiphyses, absent secondary ossification centres and severe growth retardation. Short stature persisted throughout adulthood. By contrast, OC/C3 mice had no abnormality of ossification or linear growth. Quantitative Faxitron micro-radiography of adult COL2/D3 mice at 10 micron pixel resolution revealed the persistence of gross morphological abnormalities that were accompanied by reduced peak bone mass and the presence of low bone mineral content even at P66. No abnormalities were observed in OC/D3 mice. These data demonstrate that local availability of T3 in chondrocytes is essential for normal post-natal bone development, accrual of peak bone mass and establishment of the adult skeleton. By contrast, T3 action in osteoblasts does not play a major role during skeletal development.

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**OC4.3**

*Mice deleted for the transcription factor Gata3 have fewer parathyroid cells expressing Gcm2, develop hypocalcaemia and have an earlier onset of mortality when challenged with a low calcium-vitamin D diet*

Irina Grigorieva, Samanta Michalk, Katie Gaynor, M Andrew Nesbit, Elena Grigorieva, Quazol Weil, Jacqueline van der Wees, William Fraser, Tertius Hough, Nancy Manley, Frank Grosveld & Rajesh Thakker

1University of Oxford, Oxford, UK; 2The National Institute for Medical Research, London, UK; 3University of Georgia, Athens, Georgia, USA; 4Erasmus University, Rotterdam, The Netherlands; 5Royal Liverpool University Hospital, Liverpool, UK; 6Medical Research Council, Harwell, UK.

Heterozygous mutations of Gata3, a dual zinc-finger transcription factor, cause the hypoparathyroidism, deafness and renal dysplasia (HDR) syndrome. To study the role of GATA3 in parathyroid function we have investigated Gata3-/- mice for hypoparathyroidism. Gancz and Gata3-/-/- mice were challenged at weaning with a diet low in calcium (0.001%) and vitamin D (0.0 IU/g). The low calcium-vitamin D diet led to a significantly higher mortality amongst the Gata3-/- mice compared to Gata3-/-/- mice (56 vs 3%, P < 0.001). Moreover, plasma concentrations of calcium and PTH, measured before the onset of mortality, were significantly lower in Gata3-/- mice compared to Gata3-/-/- mice (2.01 ± 0.23 vs 2.16 ± 0.29 mmol/l; 73.7 ± 22.06 vs 86.44 ± 22.97 pmol/l; P < 0.001). Histological analysis revealed that the parathyroid glands of Gata3-/-/- mice failed to enlarge in size to the same extent as in Gata3-/-/- mice in response to the low calcium vitamin D diet and had a reduced Ki67 proliferation index (0.08 vs 0.16; P < 0.001). Analysis of the developing parathyroid glands in 11.5 days post coitum (dpc) mouse embryos revealed that Gata3-/-/- embryos had smaller parathyroid-thymus primordia compared to Gata3-/-/-, with fewer cells expressing the parathyroid-specific glial-cell missing 2 (Gcm2) gene, whilst Gata3-/-/- embryos had absent Gcm2 expression and gross defects of the third and fourth pharyngeal pouch development that resulted in absence of the parathyroid-thymus primordia by 12.5 dpc. These data show that Gata3 is critical for the differentiation and subsequent survival of both parathyroid and thymus progenitor cells. Thus, parathyroid agenesis occurs in Gata3-/-/- mice and haploinsufficiency in Gata3-/-/- mice is associated with a smaller parathyroid mass, Gcm2 dysregulation, and parathyroid dysfunction. These findings indicate that Gata3 and Gcm2 may form part of a transcriptional cascade that determines parathyroid gland development and parathyroid cell differentiation and proliferation.
OC4.4
Rapid screening for novel bone phenotypes in 100 consecutive lines from the Wellcome Trust Sanger Institute Gene Targeting Programme
Apostolos Gogakos1, Duncan Basset1, Anne van der Spek1, Holly Evans2, Jacqui White1, Ramiro Ramirez-Solis2, Karen Steel1, Allan Bradley1, Rajesh Thukker1, Peter Croucher2 & Graham Williams3
1Molecular Endocrinology Group, Imperial College London, Hammersmith Hospital, London, UK; 2Mellanby Centre for Bone Research, University of Sheffield, Sheffield, UK; 3Mouse Gene Targeting Programme, Wellcome Trust Sanger Institute, Hinxton, Cambridge, UK; 4Academic Endocrine Unit, Oxford Centre for Diabetes, Endocrinology and Metabolism, Churchill Hospital, University of Oxford, Oxford, UK.

The Wellcome Trust Sanger Institute Gene Targeting Programme is deleting all mouse genes and has already generated 400 knockout mice in a C57BL/6N background with a further 4000 genes targeted in ES cells. Two hundred and fifty new knockouts will undergo limited phenotyping each year. However, the programme lacks a sensitive and sufficiently detailed screen for individual physiological systems, each of which requires high throughput methodology and unique expertise. Thus, we prospectively investigated the sensitivity and reproducibility of two screening methods for identification of abnormal skeletal phenotypes in 100 consecutive knockout strains. Upper and lower limbs and caudal vertebrae from 15-week-old mutant females (n=2–6 per strain) were compared to genetic background controls (n≈94). Mutants with known skeletal defects were covertly included as positive controls. Faxitron MX20 images (10 micron resolution) of limbs and vertebrae were calibrated to three internal standards and bone lengths, cortical thickness and mineral content were determined. Trabecular bone volume (BV/TV), trabecular thickness (Tb.Th), trabecular number (Tb.N) and bone mineral density (BMD) in proximal tibia were determined by microCT (SkyScan 1172a, 4.5 micron resolution) Thus far, 55 lines have been analysed by Faxitron and 76 by microCT. Faxitron identified at least one abnormality in 23% of strains, including abnormalities of bone length (4%) and femur (15%) and vertebra (17%) mineral content. Micro-CT identified at high levels of microRNAs (miRs) some of which have been implicated in regulating IGF signalling. The production of miRs depends on processing from non-functional (pre)-miRs to mature miRs by two enzymes, Drosha and Dicer. In this study we have used Dicer siRNA to inhibit miR biogenesis in placental explants and examined the impact on IGF-stimulated proliferation in cytotrophoblast. Dicer-specific or non-targeting siRNA (200 nM) was delivered to first trimester villous tissue fragments by macrofector. Following transfection, tissue was maintained in culture for 72 h, then treated with IGF1 or 2 (10 nM) for a further 24 h before immunohistochemical (IHC) or QPCR analysis. Following exposure to siRNA, Dicer protein and miRNA expression were significantly decreased. IHC analysis of cell proliferation (Ki67) revealed enhanced levels of both basal (from 17.8±2.4 to 57.6±3.2%, P<0.05, n=5) and IGF-induced proliferation (from 33.6±5.3 to 78.0±1.8% for IGF1, and from 50.4±3.2 to 75±3.5% for IGF2, P<0.05, n=5) following Dicer knockdown. To examine the potential mechanism by which Dicer-dependent miRs regulate cytotrophoblast proliferation we examined the expression of molecules within the placental IGF-axis following Dicer knockdown. IHC analysis revealed no difference in IGF receptor (IGF1R) expression. Moreover, the expression of both Akt and ERK was enhanced in the absence of Dicer. Dicer-dependent miRs regulate expression of components in two important pathways downstream of the IGF receptor to influence placental growth. Future investigations will define specific target genes. This will uncover potential therapeutic avenues for placental undergrowth, which may ultimately lead to treatments for problem pregnancies.

OC4.6
Impaired osteoblast function in mice lacking the Tβ3-responsive calcineurin inhibitor RCAN2
Duncan Bassett1, Alan Boyd2, Peter Howell1, Xiao-Yang Sun3, Sai Xu4, Yoshiharu Murata2 & Graham Williams3
1Molecular Endocrinology Group, Imperial College, Hammersmith Hospital, London, UK; 2Centre for Oral Growth and Development, Queen Mary, University of London, London, UK; 3Eastman Dental Institute, University College London, London, UK; 4Division of Molecular and Cellular Adaptation, Research Institute of Environmental Medicine, Nagoya University, Nagoya, Japan.

Similar to thyroid hormones, the calcineurin/NFAT pathway regulates bone mass via its actions in osteoblasts and by indirect effects on osteoclast function. Calcineurin is a calcium- and calmodulin-activated phosphatase that dephosphorylates the transcription factor NFAT enabling its translocation to the nucleus. RCAN2 is an inhibitor of calcineurin that is stimulated by Tβ3 in brain, heart and skeletal muscle although its expression in bone has not been studied. Thus, RCAN2 is proposed to mediate target tissue responses to Tβ3 by inhibiting calcineurin activity. To investigate the role of RCAN2 in bone, we characterized RCAN2−/− knockout mice. In juveniles, intramembranous ossification was delayed whereas endochondral ossification and growth were unaffected. Bone mineral content was reduced in RCAN2−/− compared to wild-type mice at 4 weeks of age but no difference was evident in adults at 16 weeks. Cortical bone width, bone microarchitecture and trabecular bone mineralization density were similar in adult RCAN2−/− and wild-type mice but cortical bone mineralization was increased in RCAN2−/− mice. Examination of bone surfaces by back-scattered electron scanning electron microscopy and analysis of osteoclast parameters by histomorphometry revealed no differences between RCAN2−/− and wild-type mice. Thus, RCAN2−/− mice display delayed intramembranous ossification during skull development, reduced bone mineral content during growth but increased cortical bone mineralization in adulthood. This phenotype does not result from abnormal endochondral ossification or a defect in osteoclast function, but is consistent with an isolated impairment of osteoblast function that results in delayed intramembranous ossification and reduced bone mineral deposition during growth but increased cortical bone mineralization in adult bone. The findings are similar to the discrete osteoblast defect observed in mice lacking the type 2 deiodinase enzyme, which generates T3 in thyroid hormone responsive target cells. We hypothesise that RCAN2 mediates some of the actions of T3 in osteoblasts by inhibiting calcineurin/NFAT signaling.

OC4.5
Dicer-dependent microRNAs regulate IGF-actions in the human placenta
Karen Forbes, John Aplin & Melissa Westwood
University of Manchester, Manchester, UK.

Fetal growth restriction is associated with abnormal placental cell (cytotrophoblast) proliferation. Using an explant model of human first trimester placenta, we have demonstrated that the IGF1 and II stimulate proliferation in cytotrophoblast and are probably essential for normal placental growth, and activates signalling through both Akt and ERK, so the regulation of these pathways in placenta is important for normal pregnancy outcome. The tissue contains high levels of microRNAs (miRs) some of which have been implicated in regulating IGF signalling. The production of miRs depends on processing from non-functional (pre)-miRs to mature miRs by two enzymes, Drosha and Dicer. In this study we have used Dicer siRNA to inhibit miR biogenesis in placental explants and examined the impact on IGF-stimulated proliferation in cytotrophoblast. Dicer-specific or non-targeting siRNA (200 nM) was delivered to first trimester villous tissue fragments by macrofector. Following transfection, tissue was maintained in culture for 72 h, then treated with IGF1 or 2 (10 nM) for a further 24 h before immunohistochemical (IHC) or QPCR analysis. Following exposure to siRNA, Dicer protein and miRNA expression were significantly decreased. IHC analysis of cell proliferation (Ki67) revealed enhanced levels of both basal (from 17.8±2.4 to 57.6±3.2%, P<0.05, n=5) and IGF-induced proliferation (from 33.6±5.3 to 78.0±1.8% for IGF1, and from 50.4±3.2 to 75±3.5% for IGF2, P<0.05, n=5) following Dicer knockdown. To examine the potential mechanism by which Dicer-dependent miRs regulate cytotrophoblast proliferation we examined the expression of molecules within the placental IGF-axis following Dicer knockdown. IHC analysis revealed no difference in IGF receptor (IGF1R) expression. Moreover, the expression of both Akt and ERK was enhanced in the absence of Dicer. Dicer-dependant miRs regulate expression of components in two important pathways downstream of the IGF receptor to influence placental growth. Future investigations will define specific target genes. This will uncover potential therapeutic avenues for placental undergrowth, which may ultimately lead to treatments for problem pregnancies.

OC4.7
Persistent symptomatic improvement at more than 12 months after parathyroidectomy for primary hyperparathyroidism
Preethi Gopinath, Gregory Sadler & Rashi Mihai
Department of Endocrine Surgery, John Radcliffe Hospital, Oxford, UK.

Background
Parathyroidectomy for primary hyperparathyroidism (PHPT) is followed by a decrease in the severity of symptoms reported on the Paseka’s parathyroid symptoms score (PPSS) questionnaires and such changes correlate with improved quality of life assessed by the SF36 questionnaire (World J Surg 2008 32 807). Some argue that these benefits are short-lived and only apparent in the first months postoperatively. Aim
To-determine whether there is a persistent improvement in symptoms at more than 12 months after parathyroidectomy.

Methods
A prospective database collected clinical and operative information on consecutive patients with biochemical diagnosis of PHPT. PPSS was calculated as the sum of the 13 parameters self-assessed using a visual analogue scale.
Steroids and thyroid

OC5.1
Familial GC resistance: a novel, naturally occurring mutation which has dominant negative effects on ligand-dependent and -independent GR action
P Trebble1, L Matthews1, J Blakely1, A Wayte1, G Black1, A Wilson1 & D Ray1
1Department of Medicine, University of Manchester, Manchester, UK; 2Department of Medicine and Clinical Biochemistry, Ysbyty Gwynedd, Bangor, UK.

Glucocorticoids (GCs) are the most potent anti-inflammatory agents known and are used extensively in the treatment of inflammatory disease, however an individuals response to therapy varies greatly. Resistance to GC is often attributed to mutations within the glucocorticoid receptor (GR) through which GC elicit their effects. Here, we present a kindred with GR haploinsufficiency yet no clinical phenotype. PCR and GR sequence profiling of genomic DNA isolated from patient blood reveals a single nucleotide deletion in exon 6, producing a frameshift at residue 612 and introduction of a stop codon at residue 627. This generates 15 novel C-terminal amino acids and results in truncation and loss of a significant portion of the ligand binding domain. In this study we explore the effect of 627GR in isolation, and its effect on GRs. In vitro studies show that 627GR is neither downregulated nor phosphorylated on residue serine211 following treatment with the synthetic GC dexamethasone, two markers of ligand-dependent GR activity. 627GR failed to transactivate a simple reporter (TAT3-Luc) and antagonised the effects of GRs when co-expressed (50% reduction) suggesting dominant negative activity. 627GR alone was unable to repress NRE-Luc and did not antagonise the dexamethasone-mediated repression by GRs. Interestingly 627GR antagonised ligand-independent repression of NRE-Luc by GRs. Fluorescent microscopy using GFP-tagged 627GR revealed that 627GR failed to translocate into the nucleus in the presence of dexamethasone. Since it appears unable to bind ligand and does not translocate into the nucleus to regulate target genes, it is possible that the dominant negative effects of 627GR on GRs may be via heterodimerisation and cytoplasmic sequestration of GRs or non-genomic events initiated in the cytoplasm. The fact that these patients presented with a very mild phenotype, but had dramatically impaired GR function has implications for understanding human GC sensitivity.

Postoperative symptoms

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<td>18</td>
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<tr>
<td>Non responders</td>
<td>64</td>
<td>409 ± 326</td>
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</table>

*Possibly related to subsequent diagnoses of other medical conditions (e.g. fibromyalgia and osteoarthritis).

OC5.2
De novo cortisol synthesis by primary human keratinocytes
Rosalind Hennes1,2, Anthony Michael1, Jacky Burin1 & Michae Philpott1
1BICMS, Barts and The London, London, UK; 2William Harvey Research Institute, London, UK; 3St George’s, University of London, London, UK.

Cortisol-based therapy is still the most potent anti-inflammatory treatment available for skin conditions such as psoriasis and atopic dermatitis. Previous studies have demonstrated the presence of components of the steroidogenic pathway in keratinocytes, though surprisingly none have reported that these cells, which form up to 90% of the epidermis are able to synthesise cortisol. Here, we demonstrate that primary human keratinocytes (PHK) are capable of de novo cortisol synthesis and that keratinocyte steroid metabolism can be manipulated by calcium.

Immunofluorescence histochemistry shows that steroidogenic acute regulatory protein is predominantly expressed in the basal layer of normal epidermis. Cultured PHK isolated from redundant facelift skin (ethics approval T01/034) transcribed steroid enzymes that are obligatory for de novo cortisol synthesis. The levels of S17, CYP11A1 and CYP21 mRNA were greatest at 0.09 mM Ca2+ that select for proliferating keratinocytes but decreased with the addition of 1.2 mM Ca2+ that promotes keratinocyte differentiation. Radiometric assay showed PHK also metabolised [14C]-pregnenolone through each intermediate steroid to cortisol. The cortisol metabolite was detected at both 0.09 mM Ca2+ and 1.2 mM Ca2+ but more intermediate sterols for cortisol synthesis were identified with 0.09 mM Ca2+.

De novo steroid synthesis was confirmed by pregnenolone radioimmunoassay and cortisol ELISA. Over 24 h, the detected basal level of pregnenolone in spent keratinocyte culture media was 7.98 ± 1.85 nmol/L, which was enhanced over threefold to 29.2 ± 4.96 nmol/L (P < 0.05) with the use of the pregnenolone metabolism inhibitors trifluoroacetic (100 µM) and ketonocarboxyl (10 µM). The basal level of cortisol detected by ELISA was 1.02 ± 0.22 nmol/L in spent keratinocyte medium after 24 h in culture.
Therefore we have mapped the endogenous cortisol synthesis pathway in PHK and shown that the greatest metabolism occurs in proliferating keratinocytes. Since cortisol is such a potent anti-inflammatory compound, this pathway could be essential for maintaining a healthy epidermis.

References

OCS5.3
Differential roles of PAPSS1 and PAPSS2 in the control of androgen synthesis
IvanMcNelis, IanIdkowiak, EdsonNogueira, AlexandraWard, Vivek Dhir & WiebkeArlt
School of Clinical and Experimental Medicine, Centre for Endocrinology, Diabetes and Metabolism, University of Birmingham, Birmingham, UK.

A key component of androgen synthesis is the availability of the pro-hormone DHEA, which is either converted to active androgens or inactivated to its sulfate ester DHEAS by DHEA sulfotransferase (SULT2A1). The latter reaction requires provision of a universal sulfate donor 3’-phosphoadenosine-5’-phosphosulfate, PAPS. In humans, PAPS is generated by the PAPS synthase isoforms PAPSS1 and PAPSS2. Recently, inactivating PAPSS2 mutations have been identified in a patient with androgen excess but undetectable DHEAS3, implicating DHEA sulfation as a gatekeeper to human androgen synthesis. To examine why ubiquitously expressed PAPSS1 cannot compensate for mutant PAPSS2, we have investigated the differential impact of PAPSS1 in DHEA sulfation. Firstly, we determined the subcellular localisation of PAPSS2 by indirect immunofluorescence in NCIH292, HepG2, and HEK293 cells, derived from adrenal, liver and kidney, respectively. We found that PAPSS1 invariably showed nuclear localisation whereas PAPSS2 was predominantly cytoplasmic in HEK293 cells. By contrast, in adrenal and liver cells, capable of DHEA sulfation by cytosolic SULT2A1, PAPSS2 was almost exclusively nuclear. We generated bicistronic constructs for concurrent overexpression of SULT2A1 with PAPSS1/2 in HEK293 cells that lack SULT2A1 or PAPSS2 and only have low endogenous PAPSS1. Realtime PCR demonstrated successful overexpression of all targets (ΔCt6–8). Enzymatic activity assays measuring conversion of triitated DHEA to DHEAS demonstrated that SULT2A1 overexpression provides HEK293 cells with some ability to sulfate DHEA. However, concurrent overexpression of SULT2A1 with PAPSS yields a twofold (PAPSS1) and fivefold (PAPSS2) increase in DHEA sulfation rate. Differential siRNA knockdown of both PAPSS isoforms in the adrenal NCIH292 TR cell line demonstrated upregulation of PAPSS1 mRNA by PAPSS2 knockdown and vice versa. These findings demonstrate a tight co-regulation of PAPSS isoforms in support of sulfation and suggest that a nuclear localisation of PAPSS2 is a precondition for efficient DHEA sulfation.

Reference

OCS5.4
Induction of hypothyroidism with radioactive iodine therapy is associated with improved survival in patients with hypothyroidism
KristenBoeckert1, PatrickMaismoneau2, BarbaraTorlinska1 & JayneFranklyn1
1University of Birmingham, Birmingham, UK; 2European Institute of Oncology, Milan, Italy.

Hyperthyroidism is known to result in excess all-cause and circulatory mortality. We set out to identify which factors predict mortality in 1290 patients (1019 females and 271 males) with overt hyperthyroidism and determined if treatment with antithyroid drugs or radioactive iodine (131I) affect outcome. All individuals were aged ≥40 years (median: 57.5 years) and presented to our clinic between 1989 and 2003. Cause of death was compared with age- and period specific mortality in England and Wales. In 13,443 person-years of follow-up (median duration of follow-up 10 years), 345 subjects died versus 287 expected deaths (all cases SMR: 1.20 (95% CI: 1.08–1.34), P=0.001) and mortality from circulatory (SMR 1.25 (1.06–1.48), P=0.009) and respiratory diseases (SMR 1.33 (1.02–1.71), P=0.03) were significantly increased. On multivariate analyses, all cause mortality was higher in males (HR 1.37 (1.05–1.79), P=0.02), in smokers (HR 1.66 (1.31–2.10), P<0.001) and in those presenting with atrial fibrillation (HR 1.88 (1.44–2.45), P<0.001). The underlying cause of hyperthyroidism, classified as Graves’ disease (n=365), toxic nodular hyperthyroidism (n=286) or indeterminate aetiology (n=639), did not significantly affect outcome. 466 subjects were treated with antithyroid drugs only and 824 received 131I therapy resulting in hypothyroidism in 532. Mortality rates were similar when comparing those treated with drugs only with subjects rendered euthyroid following 131I (HR 0.95 (0.72–1.24), P=NS). Induction of hypothyroidism following radioiodine was associated with significantly reduced all-cause (HR 0.69 (0.52–0.91), P=0.01) and circulatory mortality (HR 0.65 (0.42–1.00), P=0.05), even after correcting for other vascular risk factors.

Conclusions
We have confirmed significantly increased all-cause and circulatory mortality in patients with hypothyroidism. Furthermore, these data indicate that the administration of doses of radioiodine to induce hypothyroidism is associated with significantly improved survival versus therapy with lower doses of 131I as well as treatment with antithyroid drugs only.

OCS5.5
Akt activation in a murine thyroid goitre model induced by the tumourigenic factor PBF
Martin Read1, GregLewy2, NeilSharma3, JimFong3, VickiSmith3, RobertSeed2, PerkinKwan4, WendyLeadbeater4, AdrianWarfield4, JohnWatkinson2, JayneFranklyn1, KristenBoeckert1 & ChrisMcCabe3
1University of Birmingham, Birmingham, West Midlands, UK; 2UHB NHS Foundation Trust, Birmingham, West Midlands, UK.

PTTG binding factor (PBF) is a poorly characterised transforming gene that is overexpressed in thyroid tumours and inhibits the activity of the sodium iodide symporter (NIS) in vitro. Our recent investigations demonstrated that PBF mRNA expression was 2.6-fold higher in thyroid tissue excised from patients with multinodular goitres (MNG) than in normal thyroid tissue (n=25, P<0.01). We have now generated a murine transgenic model of targeted overexpression of PBF in the thyroid to investigate its function in vivo. Thyroid glands of transgenic PBF mice were markedly enlarged with a mean weight of 6.0±1.1 mg (n=16) at 6 months of age compared to 2.95±0.5 mg (n=11, P<0.0001) in wild-type (WT) mice. By 12 months of age the mean weight of thyroids harvested from PBF mice had increased further to 16.0±2.7 mg (n=11). Histological examination showed that the percentage of large follicles was significantly greater (20% vs 7% follicles>100 μm in diameter, P<0.0001) in PBF mice compared to age-matched WT mice. Real-time RT-PCR analysis showed a significant twofold reduction in NIS mRNA levels (0.52±0.06, n=7; P<0.0001) in transgenic thyroid glands. There was no difference in thyroid function tests (serum T4 (3.1±0.9 vs 3.2±0.5 μg/dl, P=0.207), T3 (118.4±23.6 vs 140.5±27.2 ng/dl, P=0.128), and TSH (0.13±0.3 vs 0.74±0.17 μg/ml, P=0.460) between PBF and WT mice, respectively, which suggested that altered thyroid function was not responsible for inducing goitrogensis. Interestingly, there was no significant increase in mRNAs encoding growth factors known to induce thyroid cell proliferation including FGFG2, EGF, VIGFG, GF1 and TGFβ. Western blot analysis however showed significant activation of the serine/threonine kinase Akt in transgenic thyroids, a known regulator of thyroid cancer cell proliferation. These results demonstrate that PBF is overexpressed in MNG and that targeted overexpression of PBF causes significant enlargement of the thyroid gland independent of thyroid function. Further, these results implicate Akt in mediating PBF-induced thyroid cell proliferation in vivo.

OCS5.6
Is it safe for patients taking thyraxone to have a low but not suppressed serum TSH concentration?
GrahamLeese & RobertFlynn
University of Dundee, Tayside, UK.

For patients taking thyroxine replacement guidelines generally recommend aiming for a target TSH within the laboratory reference range. The evidence for this guidance is generally based on an extrapolation of data from patients with endogenous subclinical thyroid disease. We aimed to examine the safety of having a TSH which was either suppressed (≤0.03 mU/L), low (0.04–0.4 mU/L), ‘normal’ (0.4–4.0 mU/L) or raised (>4.0 mU/L) in a population-based cohort of patients all of whom were treated with thyroxine.

We used a population-based thyroid register (TEARS) linked to outcomes data from hospitalisation records, death certification data and other datasets between

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1993 and 2001. The endpoints of cardiovascular disease, dysrhythmias and fractures were assessed. Patients were categorised, using a time weighted mean of all TSH recordings. There were a total of 16,426 patients on thyroxine replacement (86% female, mean age 60 years) with a total follow-up of 74,586 years. Cardiovascular disease, dysrhythmias and fractures were increased in patients with a high TSH (adjusted hazards ratio 1.95 (1.73–2.21), 1.80 (1.33–2.44) and 1.83 (1.41–2.37) respectively), and patients with a suppressed TSH (1.37 (1.17–1.6), 1.6 (1.1–2.33) and 2.02 (1.55–2.62) respectively), when compared to patients with a TSH in the laboratory reference range. Patients with a low TSH did not have an increased risk of any of these outcomes (HR: 1.1 (0.99–1.123), 1.13 (0.88–1.47) and 1.13 (0.92–1.39) respectively.

People on long-term thyroxine with a high or suppressed TSH are at increased risk of cardiovascular disease, dysrhythmias and fractures. People with a low but not suppressed TSH did not have an increased risk of these outcomes in this study. It may be safe for patients treated with thyroxine to have a low but not suppressed serum TSH concentration.

OCS.7

X chromosome inactivation: the key to the female preponderance in Graves’ disease?

Matthew Simmonds, Paul Newby, Laura Jackson, Chantal Hargreaves, Oliver Brand, Jackie Carr-Smith, Jayne Franklin & Stephen Gough

University of Birmingham, Birmingham, West Midlands, UK.

Graves’ disease (GD) affects >2% of the population and occurs more frequently in females than males. Several hypotheses have been put forward to explain the female preponderance including increased immune responsiveness, gonadal steroids, sex chromosome susceptibility loci and, more recently, skewed X inactivation (XCI). XCI occurs in females causing one of their X chromosomes to be randomly inactivated enabling dosage compensation with males who only have one copy of the X chromosome. Although each X chromosome should be inactivated with a parent of origin ratio of 50:50, skewed XCI can occur whereby >80% of a specific copy of the female X chromosome is inactivated (or extreme skewing with <90% inactivation). Several smaller GD datasets have detected association of skewed XCI, suggesting a possible role for skewed XCI in GD onset in females. The aim of this study was to use microsatellite marker genotyping within the androgen receptor to determine levels of XCI in a large UK Caucasian GD cohort consisting of 417 female GD patients and 385 female controls. All subjects gave informed written consent and the project was approved by the local ethic committee. Skewed XCI was found to be strongly associated with GD (P=2.14×10−3, OR=2.17 (95% CI=1.43–3.30)), although no evidence of extreme skewing was detected (P=0.236). When we investigated parent of origin effects of the skewed chromosome we found that in 60% of skewed GD cases the father’s X chromosome was preferentially skewed. This suggests that products from the skewed father’s X chromosome are less likely to be seen and tolerated by the immune system of these GD subjects. Components from the skewed X chromosome when encountered later in life could not be recognized as self and an autoimmune response could occur. Further work is currently establishing to what extent XCI skewing explains the increased female preponderance in GD.

OCS.8

The effect of genetic variation in PDE8B and DIO1 on thyroid hormone levels

Peter Taylor1, Vijay Panicker1,2, Ahmed Iqbal1, Nicholas Timpson3, John Walsh4 & Colin Dayan1

1Henry Wellcome Laboratories for Integrative Neurosciences and Endocrinology, University of Bristol, Bristol, UK; 2School of Medicine and Pharmacology, University of Western Australia, Crawley, Western Australia, Australia; 3Department of Social Medicine, MRC Centre for Causal Analyses in Translational Epidemiology, University of Bristol, Bristol, UK.

Background

Single-nucleotide polymorphisms (SNPs) allow us to study effects of genetic variation on thyroid hormone levels. Phosphodiesterase 8B (PDE8B) is a protein responsible for cAMP generation, found in thyroid and brain tissue, but not normal pituitary tissue. Variation at this locus has been reported to influence TSH levels, but its effect on T3 and T4 has not been studied in the general population. Deiodinase 1 (DIO1) is known to influence T3/T4 ratio, but not TSH levels. We examined the effect of SNPs rs4704397 (PDE8B) and rs2235544 (DIO1) on serum thyroid hormone levels.

Methods

We studied 917 patients with thyroid hormone levels in the reference range. Thyroid function was measured on two occasions in 1981 and 1995 from the Brunelton Health Study (T3 not measured in 1981).

Results

TSH varied by PDE8B genotype, highest in the AA genotype (P=7.08×10−6 (1981) P=8.03×10−7 (1995) causing a TSH rise equal to 0.26 s.d. Variation in PDE8B had no effect on serum T3 or T4 concentrations. Variation in DIO1 genotype had no effect on TSH but affected T3 levels, P=0.016 (1981) and P=0.001 (1995), with greatest effect seen on T3/T4 ratio (P=5.93×10−4 (1995)).

Conclusion

We confirmed the effect of PDE8B on TSH and DIO1 on serum T3 and serum T3/T4 ratio. Surprisingly there was no effect of PDE8B on T3 and T4 levels. Rather than influencing intrinsic thyroid hormone production variation in PDE8B appears to alter an individual’s ‘set point’ for TSH. There is no effect of DIO1 variation on TSH levels implying that the alteration in T3 and T3/T4 ratio is perceived by the brain as ‘neutral’. This study highlights that common genetic variation can affect different aspects of the thyroid hormone pathway, independently from each other.
Poster Presentations
Bone

P1

Hypercalcaemia following a road traffic accident
Nyi Hwe & Koshy Jacob
Pilgrim Hospital, Boston, Lincolnshire, UK.

A 52-years man was admitted following a Road Traffic Accident. He was a known smoker but usually fit and well. During the road traffic accident he sustained fracture of all four limbs, clavicles, facial bones as well as multiple rib fractures. Following initial stabilisation in ITU he underwent multiple orthopaedic surgeries for his bony fractures over the next 2-3 months. He was then noticed to have elevated corrected calcium of 3.04 mmol/l, phosphate 1.65 mmol/l and alkaline phosphatase of 218 μl and referred to the endocrinologists.

A subsequent PTH was suppressed at 0.3 pmol/l (1.5-6.9). A chest X-ray, serum immunofluorescence, urine for Bence-Jones protein, TFF, 24-h urinary catecholamine levels and PTHeP were all normal. A 0900 h cortisol was 291 nmol/l. 25 OH Vit D was 35 mmol/l.

He was treated with i.v. pamidronate and fluids for his hypercalcaemia and advised active mobilisation early. On mobilisation his calcium levels stabilised to normal levels at 2.43 mmol/l and phosphate of 1.31 mmol/l.

Immobilisation is an unrecognized cause of hypercalcaemia and can occasionally cause quite elevated hypercalcaemia as in our case. The hypercalcaemia was adequately managed with i.v. pamidronate and fluids. Early mobilisation is the key to normalising calcium levels.

P2

Protracted hungry bone syndrome post parathyroidectomy for primary hyperparathyroidism
Subhash Rana, Vijay Bangar, Khaled Al-Zwae & Abdusalam Mousa
Calderdale Royal Hospital, Halifax, West Yorkshire, UK.

Objective
To highlight that an occasional case of parathyroidectomy may be followed by protracted symptomatic hypercalcaemia requiring calcium infusion and high doses of vitamin D.

Case
We report a 61 years male who presented with hypercalcaemia and brown tumour. He was diagnosed as a case of primary hyperparathyroidism. He had normal FBC, U&Es, LFT, TFT and negative Endomyosal antibody. Just 4 days post-parathyroidectomy he was admitted with symptomatic hypercalcaemia. He needed 3 weeks continuous Ca infusion and large doses of Alfacalcidol and oral Ca before he became symptoms free. His Mg was also low which was replaced orally. Even now he was to be kept on high doses of vitamin D and Ca to maintain his Ca.

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Discussion
The hungry bone syndrome post-parathyroidectomy is well known. It is due to avid Ca retention by bones after the source of high Parathormone causing increased mobilization of Ca from bones is removed. It usually lasts up to a week. In our case it has lasted many months still needing high doses of Ca and Alfacalcidol. The protracted hungry bone syndrome lasting up to a year has been reported following parathyroidectomy for secondary hyperparathyroidism in chronic renal failure. We did not find any case of prolonged hungry bone syndrome following parathyroidectomy for primary hyperparathyroidism. The reason for this prolonged hypercalcaemia was the late recovery of the remaining hypoplastic parathyroid as is evident from the results in the table. The low vitamin D before surgery in this case might have contributed to delayed recovery as has been suggested in a report from India.

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P3

Hypertrophic pulmonary osteoarthropathy: not all longstanding raised bony alkaline phosphatase is Paget's disease
Ahmed El-Laboudi & Emma Ward
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Objective
Highlight hypertrophic pulmonary osteoarthropathy (HPOA) and partial HPOA as a cause for raised bony alkaline phosphatase (ALP).

Case
We report the case of a 59-year-old lady who was referred to the endocrine clinic with 2 years history of bilateral leg pain and raised bony ALP. She denied any other symptoms. She had no past medical history. She was not on any regular medications and she has never smoked. Apart from mild tenderness over both legs, examination was unremarkable with no evidence of clubbing.

Investigations
A bone scan showed low grade linear increased tracer activity along the outer border of both tibias. X-rays showed bilateral, symmetrical, periosteal reaction along the distal tibia raising the possibility of HPOA. A CXR showed a large mass at the left base. CT thorax showed that this is a 10×7 cm bilobed soft tissue mass pleurally based in the left lower chest which was felt to be a pleural fibroma. A PET scan showed low grade uptake in the mass and there was no evidence of disease in any other site.

Management
The patient underwent left thoracotomy with resection of the mass. Histology confirmed a diagnosis of benign solitary fibrous tumor of the pleura (SFTP).

Post-operatively, her leg pain resolved with normalization of alkaline phosphatase level within 2 months of surgery.

Conclusion
Hypertrophic pulmonary osteoarthropathy, especially periositis without clubbing (Partial HPOA), may go unrecognized. A raised bony alkaline phosphatase can be the only early abnormality that leads to the diagnosis of the cause of secondary HPOA. Including HPOA (or partial HPOA) as in our case) in the differential diagnosis list when assessing a patient with raised bony alkaline phosphatase is very important especially as timely intervention can be crucial for effective management of secondary causes.

P4

Vitamin D sufficiency is rare in patients attending the Endocrine Antenatal Clinic even in the Caucasian population
Ammar Tarik, Deepan Bathia & Emma Ward
Endocrine Department, St James University Hospital, Leeds, West Yorkshire, UK.

Vitamin D is not only essential for maternal health but insufficient levels during pregnancy and breast feeding can lead to infantile rickets or osteomalacia in their offspring. NICE recommends that vitamin D supplements should only be offered to pregnant women with dark skin, those who usually cover their skin, vegans and women between the ages of 19-24. Although it is widely known that these women are at high risk of vitamin D deficiency, the incidence of vitamin D insufficiency amongst Caucasian women has been much less widely studied.

We wished to investigate the incidence of vitamin D deficiency in pregnant women attending an Endocrine Antenatal Clinic. Vitamin D status in 29 pregnant women was ascertained between April and June 2009. Half of the patients were Caucasians while the rest were of Indian subcontinent or Middle Eastern origin. In the 14 non Caucasians, vitamin D levels were deficient (<20 nmol/l) in 57% (8 patients), depleted (20-60 nmol/l) in 36% (5 patients) and sufficient (>60 nmol/l) in only 1 patient (7%).

In the 15 Caucasian women, vitamin D levels were depleted in 12 women (80%) and sufficient only in 3 (20%).

Discussion
We conclude that vitamin D depletion is a significant and common problem in pregnant women attending the Endocrine antenatal clinic. An unexpected finding was the high number of Caucasian women with vitamin D insufficiency. We measured vitamin D levels during the Spring and Summer months and it is likely that hypovitaminosis D would be even more prevalent during the winter. Further work is underway to establish whether this is a finding peculiar to pregnant patients with endocrine abnormalities or if it is a more widespread problem amongst the general pregnant population in our city.
Investigations of skeletal dysplasias which are often inherited have yielded important insights in the molecular mechanisms of bone development, osteoporosis and osteoarthritis. However, these studies have been hampered by the lack of available patients and affected families. To overcome this limitation, we have investigated mice treated with the chemical mutagen N-ethyl-N-nitrosourea (ENU) for hereditary musculoskeletal disorders. Mice were kept in accordance with national welfare guidelines and project license restrictions. At 12 weeks of age, all mice underwent phenotypic assessments that included dysmorphology, radiography and serum biochemistry. These processes identified a mutant mouse with a waddling gait designated Slip. Slip mice had bilateral deformation of the calcaneum, extended tuberosities, and abnormal metaphyses in the knee joints, metatarsals and metacarpals. Micro-computed tomography scanning of Slip mice revealed a shortening of bone lengths, decreased total bone volume, cortical bone volume, trabecular bone volume and trabecular number. The tribute of the Slip mice also had an increased structure model index suggesting a more rod-like scaffold indicative of low bone density. These features had similarities to those for Jansen’s metaphyseal chondrodisplasia which is due to activating mutations of the parathyroid hormone receptor (PTHR1). However, only male mice were affected with the Slip phenotype and inheritance testing demonstrated an X-linked inheritance. Furthermore, genetic mapping studies using DNA from 14 affected mice localized the Slip locus to a 151 Mb region on the X chromosome indicating that the phenotype could not be due to a mutation in the PTHR1 which is located on mouse chromosome 9. Thus, our studies have established a mouse model for an X-linked metaphyseal chondrodisplasia that may potentially identify a component in the PTHR1 signalling pathway encoded by a gene on the X chromosome.
change over several months. Clinical observations in patients with COPD have noticed that those patients have muscular pain accompanied with loss in the bone mineral density which can be assured by Bone Densitometry and some laboratory tests. Aim of the study To study the use of Bone Densitometry in the early diagnosis of metabolic bone disorders – especially osteoporosis – in patients with COPD before the onset of clinical symptoms. Methods The study evaluated bone mineral density (BMD) in 50 patients with Chronic Obstructive Pulmonary Disease by using Bone Densitometry. All patients have been selected randomly from those who were admitted in the Division of Chest Medicine and diagnosed with COPD. Results Thirty-seven males and 13 females have been recruited in this study. Patients were classified according to the WHO criteria of Osteoporosis to 4 classes: i) Normal ii) Osteopenia iii) Osteoporosis iv) Severe Osteoporosis The classification depends on the T-score of BMD for both lumbar and femur bones. The BMD of those patients was matched with the BMD of healthy control from the same age, gender and race. Of 50 patients, 4 patients only had normal BMD (4% of all patients), 25 patients (50%) had osteopenia, 23 patients had osteoporosis (46%). None of the patients had severe osteoporosis which should be associated with fractures. Conclusion Patients with COPD are at high risk of developing osteoporosis and having clinical symptoms related to that. This study suggests performing BMD test as effective diagnostic method for osteoporosis in patients with COPD, which will help in taking the correct decision for them before increasing the severity of osteoporosis and affecting the quality of life.

P10
Recurrence of spontaneously resolving hypercalcaemia, an unusual case
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A 67-year male presented to his GP with polydypsia, polyuria and bone pains. Routine blood tests showed hypercalcaemia (corrected calcium 2.77 mmol/l). Patient was otherwise well. He had a history of ethanol abuse, hypertension and gout. Medications: Allopurinol, ramipril simvastatin and co-dydramol. Repeat blood tests after 4 weeks show a rising calcium of 3.24 mmol/l with intact PTH 33.6 pmol/l (<6.4). He was referred to our endocrinology department after initial treatment on MAU with i.v. fluids.

Relevant investigations FBC and ESR, U&E, LFT, Vit D levels, CXR were all normal. Serum phosphate low normal (0.75 mmol/l). An ultrasound of the neck followed by a CT neck/chest/mediastinum and Sestamibi scan was normal. Venous sampling was undertaken but this did not localize an adenoma. He was posted for parathyroid exploration and pre operative assessment bloods, done 5 months after initial diagnosis, showed calcium has returned to normal (2.37, PTH reverting to normal 7.7). Patient remained well and was discharged by surgeons. Three months later repeat blood tests (patient remains asymptomatic and well) show calcium is rising again to 2.66 with an elevated PTH 16.5.

Discussion
Although rare, spontaneous resolution of hyperparathyroidism has been occasionally described. The postulation is there is a spontaneous infarction of the parathyroid adenoma resulting in normocalcaemia. However these cases do not recur. In our case there is a recurrence of the hyperparathyroidism with no obvious adenoma during the initial investigations. We are now repeating the venous sampling and arranging further imaging. He may need exploration and parathyroidectomy.

P11
Assessment of vitamin D status in patients with primary hyperparathyroidism
Mary Jane Brassill, Muhammed Adreess, John O’Mullane & Antionette Tuthill
Cork University Hospital, Cork, Ireland.

Vitamin D insufficiency is common in the Irish population. Patients with primary hyperparathyroidism (PHT) and co-existing vitamin D insufficiency have higher PTH levels, increased bone turnover and increased risk of postoperative hypocalcaemia. The 3rd International Workshop on PHTP recommended measurement of serum 25-OH Vitamin D in all patients with PHTP, and treatment where required to maintain 25-OH Vitamin D > 50 nmol/l. Our study aimed to assess vitamin D status in patients with PHTP referred to our tertiary referral centre. Using the biochemistry database, 58 patients with PHTP were identified over a 1 year period (October 2007 to September 2008). Fifty-two (90%) were female with a mean age of 62 years. Forty-three were seen by Endocrinology services with 15 referred directly to surgical outpatients by General Practitioners and other specialties. Twenty-two of those seen by Endocrinology (51%) had their vitamin D status assessed. Six were vitamin D deficient (25-OH Vitamin D < 50 nmol/l) with the remaining 16 vitamin D insufficient (25-OH Vitamin D < 50 nmol/l). Equal numbers were deficient/insufficient in spring/summer and autumn/winter, so no significant seasonal variation was noted. None of the 15 patients referred directly to surgeons had their vitamin D status assessed. Those with vitamin D deficiency had higher mean parathyroid hormone (PTH) and alkaline phosphatase levels than those with vitamin D insufficiency.

In conclusion, over half of the patients with PHTP in our cohort did not have vitamin D status assessed as a routine check. Some of the 22 patients whose vitamin D status was assessed were vitamin D sufficient indicating high levels of vitamin D insufficiency in our PHTP cohort.

P12
Secondary preventative alendronate use in the prevention of fragility fractures in women above 75 years: the implementation of NICE guidance
Ramalingam Srinivasan, Mazhar Zaidi & Adam Devany
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Background
NICE has issued the guidelines regarding the use of alendronate in the secondary prevention of osteoporotic fragility fracture in women above 75 years in whom DXA scan is clinically inappropriate or not feasible and considered to have osteoporosis by the responsible clinician.

Objectives
We conducted an audit on patients at the James Paget University Hospital to examine whether patients are receiving the appropriate interventions as stated in NICE guidance.

Methods
We reviewed the lumbar and thoracic spine X-ray reports of 83 (57 females, 26 males) patients aged seventy-five-years and over admitted between August 2008 and August 2009. Twenty-eight (20 females, 8 males) patients’ reports had one or more of the following words – wedge/compression, fracture, or loss of height suggesting osteoporotic fragility fracture. We had access to look into 17 (13 females, 4 males) patients notes

Results
Only 38% (513) of the females received the appropriate medications (calcium, vitamin D and alendronate) as recommended in NICE guidance. None of the men (04) received the full secondary prevention. Four patients received either calcium and vitamin D or alendronate.

Discussion
A woman with a vertebral fracture has an increased relative risk (RR) of 4.4 for a further vertebral fracture, 2.3 for a hip fracture, and 1.4 for a wrist fracture and it is estimated that 180 000 osteoporosis related symptomatic fractures occur annually in England and Wales, resulting in significant morbidity, mortality and financial burden, of which up to 19% are preventable (www.nice.org.uk/TA161). This audit highlights that a significant proportion of patients at high risk of fragility fractures are not receiving appropriate preventative medication despite recent NICE guidance of the potential benefits. It is imperative that awareness of this guidance is improved so that patients can benefit from the risk reduction offered by bisphosphonates such as alendronate.

P13
Is it time to replace the 24 hour urine calcium: creatinine clearance ratio in the investigation of PTH-dependent hypercalcaemia?
Jaimini Cegla, Sharan Saroya, Barbara McGowan, Mandy Donaldson & Tricia Tan
Imperial College, London, UK.

Background
Primary hyperparathyroidism (PHTP) and familial hypercalcaemic hypocalciuria (FHH) can both present with hypercalcaemia, but their management and
P14

Mutational analysis of the PHEX gene in three patients with X-linked hypophosphatemic rickets: discovery of a novel point mutation

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Introduction
X-linked hypophosphatemic rickets is the most common form of familial hypophosphatemic rickets. It is caused by a defect in renal phosphate transport leading to phosphate wasting and hypophosphatemia. Furthermore, 1,25-dihydroxyvitamin D concentrations are inappropriately normal in regard to hypophosphatemia. Clinical manifestation of the disease are skeletal deformities, short stature, osteomalacia, dental abscesses, bone pain, and loss of hearing.

PHEX is located on Xp22.1 and inactivating mutations of PHEX have been identified as the gene defective in X-linked hypophosphatemic rickets.

Clinical characteristics
We report three cases of hypophosphatemic rickets. Two patients are female (55 and 24 years old), one is male (49 years old). Age at diagnosis ranged from early childhood to the age of 35 years. One patient underwent hip replacement at the age of 41 due to secondary arthritis caused by coxa vara. Two patients have mild calcification of kidney tissue. One patient suffers from loss of hearing since the age of 17. All three patients have been treated with phosphate supplements, two patients receive 1,25-dihydroxycholecalciferol (for treatment of secondary hyperparathyroidism). Under this regimen blood levels of calcium, phosphate and parathyroid hormone are in normal range. In all patients at least one family member is affected by rickets, as well.

Genetical analysis
Genetic mutational analysis of the PHEX gene was performed in all patients. In both female patients known mutations were found: 683delITC (exon 6, codon 220) and c.1952G>C (exon 19, codon 651, R651P). However, in one patient and an unknown nonsense mutation was found in exon 7, codon 245 (c.735T>G, Tyr245Ter, Y245X).

Conclusions
We report a novel nonsense mutation of PHEX that has not been identified so far. It underlines that genetic testing of patients and family members is needed to guarantee early diagnosis and treatment.

P15

A study on accuracy of Sestamibi and ultrasound scanning in identifying a solitary parathyroid adenoma in patients with primary hyperparathyroidism

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Background
The success of Minimally Invasive Parathyroidectomy (MIP) is closely linked to accurate preoperative localisation of a solitary parathyroid adenoma. Sestamibi/SPECT scan (SS) and ultrasonography (US) are the preferred imaging modalities although their accuracy varies considerably between different centres.

Aim
To assess the efficacy of SS and US in identifying a solitary parathyroid adenoma in patients with primary hyperparathyroidism (PHPT) at our centre.

Methods
A retrospective study based on demographic, biochemical and surgical details of patients with PHPT who underwent SS and US during a 5-year period. Imaging results were analysed on patients who were considered to have a confirmed solitary parathyroid adenoma as indicated by single adenoma on bilateral neck exploration or biochemical cure after MIP.

Results
Sixty-three patients had SS, 61 had US and 33 had surgery. Sixty-four percent were females, median age 68 years, median serum calcium 2.86 mmol/l and median serum PTH 15.26 pmol/l. SS identified a single adenoma in 35 (56%) patients, multil gland disease in 7 (11%) and was normal in 21 (44%) patients. Corresponding figures for US were 35 (57%), 3 (5%) and 23 (38%). Positive and negative predictive value, sensitivity and specificity for SS in identifying a confirmed solitary adenoma were 80, 67, 86 and 55% and for US 73, 44, 76 and 40% respectively. Concordance between the two imaging studies was obtained in 37 (60%) patients although concordant imaging resulted in a minor increase in PPV to 83%.

Conclusion
Sestamibi/SPECT and ultrasonography were individually highly effective in identifying a parathyroid adenoma at our centre which could allow the use of MIP in a higher proportion of patients with PHPT. Discordance between the two forms of imaging did not add significantly to the chances of detecting a solitary adenoma.

P16

A novel GATA3 mutation, Tyr345Cys, in hypoparathyroidism, deafness and renal dysplasia (HDR) syndrome results in abolished DNA binding

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GATA3 mutations cause the congenital autosomal dominant hypoparathyroidism, Deafness and Renal dysplasia (HDR) syndrome. GATA3 belongs to a family of dual zinc-finger transcription factors that recognise the consensus (A/T)GGATAAA(G) motif and are involved in vertebrate embryonic development. We investigated a HDR proband for GATA3 abnormalities. Venous blood was obtained after informed consent, as approved by the local ethical committee, and leukocyte DNA extracted. GATA3 specific primers were used for PCR amplification and the DNA sequence of both strands determined. This revealed a novel missense mutation, Tyr345Cys, in the basic region C-terminal to zinc finger 2 (ZnF2) of GATA3. Sub-cellular localisation studies revealed that this mutation did not interfere with correct localisation of the mutant GATA3 protein to the nucleus. However, luciferase reporter assays using a construct containing a functional GATA3 binding site in the promoter region, revealed that the Tyr345Cys GATA3 mutant was unable to transactivate expression of the reporter gene, and electrophoretic mobility shift assays confirmed that this was due to loss of DNA binding. The effect of the Tyr345Cys is in contrast to a previously characterised Leu348Arg mutation which is located in close proximity but did not cause loss of DNA binding or abolish transactivation activity. Three-dimensional modelling revealed that the Tyr345 residue lies in close contact with the major groove of the DNA and the mutation results in a polar neutral, tyrosine, being substituted for a non-polar neutral, cysteine. This subtle change in charge may affect the protein-DNA interactions, with the change in electrostatic interactions affecting the specificity and strength of binding. These results elucidate further the molecular mechanisms of the altered function of this zinc finger transcription factor and its role in causing this developmental anomaly.

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P17
Adenine A2b receptors induce osteoblastogenesis whereas A1 receptors induce adipogenesis
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Trans-differentiation of osteoblasts to adipocytes may in part be responsible for diseases such as osteoporosis and arthritis. The processes involved are however complex and largely unknown. Our previous data showed that the adenine A2b receptor (A2bR) is important for human osteoprogenitor cell function. In this study, we investigated the expression of adenine receptors in an osteoblast cell line (7F2) and during its differentiation into adipocytes (7F2A).

Oil Red O and Nile Red coupled with FACS analysis showed that 45% of cells in the 7F2A cultures contained lipid after 7 days of induced adipogenesis; such cells were undetectable in 7F2 cultures. Adipogenesis was accompanied with the loss of adenine or NECA (universal adenine receptor agonist) induced cAMP responses, at 100 μM, the stimulations were respectively 1900±400 and 3900±300 for 7F2 cells (P<0.001) and 500±200 and 300±60 fmol/ml for 7F2A cells (P<0.001). Induced adipogenesis was also associated with increased A1 receptor (A1R) and decreased A2a receptor (A2aR) and A2bR expression. In 7F2A cells, A1R mRNA increased up to 1500 fold (P<0.001) and that for A2aR and A2bR decreased by up to 76% (P<0.001) when compared with expression in 7F2 cells. On adipogenesis, A1R and A2aR proteins paralleled changes in mRNA and the A2bR protein showed increased phosphorylation.

Stable transfection of human A1R into 7F2 cells induced adipogenesis (20-30%, P<0.001) as determined by Oil Red O staining and Nile Red labelling and increased expression of the adipogenic markers, lipoprotein lipase and glycerol 3-phosphate dehydrogenase. A2bR transfection decreased adipogenesis (20-30%, P<0.001) and increased expression of the osteoblast markers, osteocalcin and alkaline phosphatase with no change in the expression of adipogenic markers.

These data indicate that specific adenine receptors are important for osteoblast and adipocyte lineage specific differentiation and targeting such pathways may be therapeutically beneficial in those diseases where osteoblastogenesis or adipogenesis is imbalanced.

P18
Management of primary hyperparathyroidism: are we following the guidelines?
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In 2008, we presented data showing that combining sonography and scintigraphy investigations resulted in the correct identification of a parathyroid adenoma in 97% (28/29) patients undergoing parathyroid surgery for primary hyperparathyroidism (PHPT). Further to this audit we compared the treatment of PHPT against the standards set by NICE in 2008. All patients who had Setamul /+ ultrasound scan over 18 month (July 2006 until December 2007) for biochemically confirmed PHPT were audited. Of a total of 92 patients who had either one or both scans, 42% (39/92) had surgical removal of the adenoma. The remaining 58% (53/92) were not operated on.

We further assessed the ‘not operated’ subgroup to assess for end-organ damage secondary to PHPT. Twenty-three percent (12/53) had osteoporosis, 43% (23/53) had no DEXA scan and the remaining 34% (26/53) had either normal bone density or osteopenia. Also, 43% (23/53) had no imaging to detect renal calculi, 51% (27/53) had normal imaging and 6% (3/53) had renal calculi on imaging. In addition, patients in the ‘not operated’ subgroup did not have surgery for several reasons.

<table>
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We also noted that 3 patients had parathyroid imaging which was requested by a general physician. Our audit highlights several recommendations:

i) Jointly agreeing a single clinical pathway for the investigation and treatment of PHPT to reduce unnecessary investigations and referrals if patient not fit or unwilling for surgery

ii) Set up ‘Joint’ parathyroid clinic with endocrine surgeon

iii) Parathyroid imaging to be requested by a specialist endocrinologist only.

iv) Parathyroid surgery to be done only by a single experienced surgeon.

P19
Hereditary renal calcification locus, Rclac1, is associated with altered expression of cell survival genes
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Renal stone disease is a common disorder for which the underlying causes remain largely unknown. We have investigated a hereditary renal calcification mouse model, Rclac1, that is not associated with hypercalciuria for underlying mechanisms. Kidney RNA from 30 to 33 week-old Rclac1 and control BALB/c and C57BL/6 female mice were extracted via the bead column method, and gene expression was determined using the Affymetrix 430 2.0 arrays. Following Robust Multichip Average normalization, pair-wise comparisons of expression data was performed using Student’s t-test. Due to the small number of genes that were significantly different between Rclac1 and both wild-type strains at a 5% false discovery rate level, a less stringent selection criteria of transcripts that were >1.2-fold up- or down-regulated in Rclac1 mice relative to both wild-type strains, at P values ≤ 0.01, were selected for further investigation. Amongst the 451 protobesets, 56 genes met these criteria, 33 genes were up-regulated and 23 genes were down-regulated in Rclac1 kidneys compared with both wild-type strains. Analyses revealed that the vitamin D3-24-hydroxylase (Cyp24a1) transcript was up-regulated by 1.9- to 3.1-fold, and mRNA levels of calcium-binding protein calbindin-D28k (Calb1) and vitamin D-binding protein (Gg) were down-regulated by 1.4- to 1.5-fold in Rclac1 kidneys, whereas other genes involved in systemic vitamin D-mediated calcium regulation were not affected. However, cell survival genes were down-regulated (idil: −1.92- to −3.26-fold, il1: −1.77- to −2.63-fold) in Rclac1 kidneys when compared to those from control mice. These results were validated by quantitative real-time PCR, and suggest vitamin D-mediated cell survival is suppressed. Furthermore, TUNEL staining of kidney sections from Rclac1 mice (n=7), when compared to control mice (n=4), revealed that calcified lesions in Rclac1 kidneys are associated with apoptotic cells. Thus, our results show that Rclac1 calcification in the renal papillae is associated with suppressed vitamin D-mediated cell survival.

P20
Is Vitamin D deficiency a common cause of elevated PTH post-parathyroidectomy?
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Serum parathyroid hormone (PTH) and other parameters are measured post-parathyroidectomy to assess cure. A persistently raised serum PTH may indicate an unsuccessful procedure. However, vitamin D deficiency is increasingly recognised in the general population, and may be a cause of raised PTH post-surgery (secondary hyperparathyroisim). Persistently elevated post-operative PTH may continue to stimulate bone turnover and secondary hyperparathyroidism. Our aim was to determine whether an elevated PTH is associated with hypovitaminosis D following parathyroidectomy.

The hospital database (eCAMS) was accessed and cross-referenced with our parathyroid database for the last 12 months. The following data was recorded: pre- and post-operative PTH, pre- and post-operative serum calcium, and 25-hydroxy vitamin D for all patients undergoing parathyroid surgery. Of the 35 patients having parathyroidectomy within the last 12 months (with post-operative normalcalcaemia), 20 (57%) of patients had an elevated PTH despite surgery. 17 (85%) of these patients had vitamin D levels assessed. All had vitamin D levels of <30 μg/l (possible vitamin D deficiency) with 11 (65%) of patients had levels <20 μg/l (overt vitamin D deficiency). This compared to only 1 patient

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(14% of cohort) with a normal PTH and a vitamin D measured at <20 pg/mL post-parathyroidectomy. In assessing cure post-parathyroidectomy, in patients without hypercalcemia, vitamin D deficiency is a common accomplishment of elevated PTH levels and is not an indicator of failed intervention. Assessment of D is not undertaken routinely in patients with primary hyperparathyroidism either pre or post surgical intervention. Measurement of vitamin D status may be helpful in patients undergoing parathyroid surgery in order to not only reduce the risks of post-operative hypercalcemia and ‘hungry bone syndrome’, but also to aid with the definition of ‘cure’ post surgery.

P21
Transient receptor potential cation channel, subfamily Vanilloid, member 5 (TRPV5) mutation (Ser682Pro) results in loss of apical membrane expression in the distal convoluted tubule, thereby resulting in hypercalciuria
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Transient receptor potential cation channel, subfamily Vanilloid, member 5 (TRPV5) is a member of the TRP superfamily. TRPV5, which functions as a tetramer, is localized to apical membranes of distal convoluted tubules (DCT) and connecting tubules (CNT) of the kidney, and is involved in vitamin D-regulated calcium reabsorption. Mice with a targeted deletion of TRPV5 (TRPV5-/-) develop severe hypercalciuria, compensatory hyperabsorption of dietary calcium and osteopenia. We recently identified an N-ethyl-N-nitrosourea-derived mouse model with autosomal dominant hypercalciuria, Hcals1, due to a TRPV5 missense mutation, Ser682Pro. To investigate the molecular/functional consequences of this mutation, we assessed wild-type and mutant TRPV5 function in vitro by electrophysiology and examined kidneys from wild-type (TRPV5+/+) and heterozygous (TRPV5+/Ser682Pro) mutant mice by immunofluorescence and western blot analyses. Mice were kept in accordance with UK Home Office welfare guidelines and project licence restrictions. Whole-cell patch-clamp analysis of wild-type and mutant TRPV5 channels in transiently transfected human embryonic kidney (HEK) 293 cells showed calcium currents similar to those seen in primary cultures: at ~80 mV, wild-type: ~915.0 ± 82.26 pA/Pf, n = 7, mutant: ~1000.0 ± 87.33 pA/Pf, n = 10. However, immunofluorescence studies using antibodies to TRPV5 and markers to the DCT (anti-sodium/chloride cotransporter) and CNT (anti-aquaporin 2), revealed a dramatic loss/reduction of TRPV5 expression from apical membranes of the DCT in the kidneys of the mutant mice. Furthermore, semi-quantitative immunofluorescence and western blot analyses revealed a ~50-90% reduction in expression of the intracellular calcium binding protein, calbindin-D28K, in TRPV5+/Ser682Pro kidney. Similar downregulation of calbindin-D28K abundance has also been observed in TRPV5 and Klotho deficient mice, both serve as models of impaired TRPV5 transport. Thus, these findings show that Ser682Pro TRPV5 mutation identified in the Hcals1 mouse model is associated with a loss of apical membrane expression in the DCT, and this likely contributes to the defect in renal calcium reabsorption in the Hcals1 mutant mice.

P22
AMP-activated protein kinase (AMPK) regulates in vitro bone formation and bone mass in vivo
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Adenosine 5'-monophosphate-activated protein kinase (AMPK), a regulator of energy homeostasis, has a central role in mediating the appetite-modulating and metabolic effects of many hormones and neuromodulators. We previously demonstrated similar neuromodulatory activation of AMPK in bone-forming osteoblasts. In this study, we tested whether stimulation of AMPK activity in osteoblasts plays a role in their function and whether deletion of the catalytic AMPKα1 subunit, the most abundant isofrom in bone, affects bone mass in vivo. Primary osteoblasts were obtained from rat calvaria by trypsin/collagenase digestion and cultured for 14–17 days in the presence of two activators of AMPK, AICAR (a cell-permeable AMP analogue) and metformin. Formation of ‘trabecular-shaped’ bone nodules was evaluated following alizarin red staining. AMPKα1−/−mice were generated by Benoit Violet (INSERM U567, Paris). Tibia were harvested from 4-month old male wild-type (WT) and AMPKα1−/−KO mice (n=10/group) and bone characteristics determined using micro-CT. We demonstrated that both AICAR and metformin dose-dependently increase trabecular bone nodule formation. Quantitative analysis of alizarin red-stained cultures confirmed the potent dose-dependent stimulatory effects of AMPK activators on the number and size of bone nodules formed by osteoblasts. AMPKα1−/− deficient mice showed a bone loss in both cortical and trabecular bone compartments of the tibia. The knockout mice showed a 30-50% decrease in trabecular bone volume (~32%), trabecular number (~31.1%) and trabecular thickness (~12.2%) compared to WT mice. As expected, trabecular separation was significantly increased in the tibia. These data are in accordance with mice lacking AMPKα1. Bone area (~28%) and cortical thickness (~17%) were significantly reduced in mice lacking AMPKα1 versus WT. Our data are consistent with AMPK playing an important role in osteoblast function. AMPK activators stimulate in vitro bone formation and ablation of AMPKα1 signaling decreases bone mass. Further studies will determine the role of AMPK in skeletal physiology.

P23
A mouse model of early-onset renal failure, tertiary hyperparathyroidism and renal osteodystrophy
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Abnormalities of calcium homeostasis such as secondary or tertiary hyperparathyroidism, and renal osteodystrophy often occur in patients with kidney failure. However, investigations of the underlying molecular mechanisms have been hampered by the lack of available tissues and the lack of suitable animal models. We therefore sought to overcome this limitation by investigating mice treated with the chemical mutagen N-ethyl-N-nitrosourea and identified a mouse model with renal failure and features consistent with normal nationwide guidelines and project license restrictions. Renf mice were found to be smaller than their littermates at weaning, failed to thrive and did not live beyond the age of 8 weeks. Renf mice had kidneys that were smaller and irregularly shaped. Histological analysis using haematoxylin and eosin stained sections revealed focal enlargements of tubular epithelial cells, interstitial damage, and concretions in the lumen of tubules which also stained positive for periodic-acid Schiff. TUNEL analysis of renf kidney sections showed increased apoptosis that predominantly affected the tubules. These features are consistent with tubulointerstitial nephritis. Analysis of plasma samples from renf mice revealed elevated concentrations of urea, creatinine, phosphate, calcium and alkaline phosphatase consistent with tertiary hyperparathyroidism and renal osteodystrophy. Inheritance testing demonstrated that renf was transmitted as an autosomal recessive disorder and genetic studies using DNA from 10 affected mice and 91 single nucleotide polymorphisms mapped the renf locus to a 9.3 Mb region on chromosome 17. Thus, we have established a novel mouse model for autosomal recessive tubulointerstitial nephritis and early-onset renal failure that results in tertiary hyperparathyroidism and renal osteodystrophy. This mouse model will help to increase our understanding of the molecular mechanisms associated with end-stage renal disease.

P24
The association among bone mineral density (BMD), 24-h mean sex hormone binding globulin (SHBG) concentration and its circadian rhythm
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Introduction
The role of hormones and their underlying mechanisms in the development of osteoporosis are complex and not completely understood. Serum SHBG
P25
Extra-pituitary expression of the prolactin gene in the bone and cartilage of an hPRL-Luc transgenic rat model
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Prolactin (PRL) is a peptide hormone produced by the anterior pituitary gland and is commonly known for its lactogenic and mammaryotropic effects. In humans and primates, prolactin is also expressed in extra-pituitary sites where it is associated with numerous biological functions, but its spectrum of expression varies in different species. Hyperprolactinemia-induced osteoporosis is believed to be mediated by hypogonadism, but the expression of the prolactin receptor (PRLR) on osteoblasts suggests a direct effect of prolactin on bone remodelling. Very little is known about the expression of prolactin in chondrocytes and osteoblasts, and the possible autocrine/paracrine mode of action of PRL in these tissues. In order to study extra-pituitary human prolactin gene expression, we have generated a transgenic rat that expresses luciferase under the control of extensive regulatory regions of the human prolactin gene in the genomic context of a 166 kb BAC construct. The rat has already proven to be ideal for in vivo imaging of the pituitary gland and of immune-related organs after intra-peritoneal injection of LPS (Semprini et al. 2009).

In vivo optical imaging of hPRL-Luc rats revealed luciferase signal in the ears, tail and paws, suggesting that the prolactin gene might be normally expressed in bone and cartilage. This was confirmed on human cartilage by rPCR and immunohistochemistry, showing that the transgenic rat model faithfully reports sites of human prolactin expression, and that this extra-pituitary transgene expression was not merely an aberrant integration site effect. Immunohistochemistry of rat knee joints revealed luciferase protein and endogenous rat prolactin in cartilage from the growth plate and in osteoblasts of young rats. Prolactin, but not luciferase staining was also detected in rat osteocytes. These observations indicate that prolactin is produced in bone and cartilage and might act locally in an autocrine/paracrine manner to regulate bone physiology.

P26
Prevalence of various morbidity in North Indian adult obese patients
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Aim
To study distribution and magnitude of morbidities (cardiovascular, renal, endocrine, CNS, hypertension, dyslipidemia) in North Indian adult obese patients.

Methods
We assessed 100 obese patients based on BMI criteria for Asian adults (BMI > 27) attending endocrine and metabolic clinic and subjected to quantification of various morbidities in the form cardiovascular, renal, endocrine, CNS, hypertension, dyslipidemia.

Results
We studied 100 obese patients and 30 controls and the prevalence of hypertension, coronary artery disease, dyslipidemia, diabetes, cholelithiasis, fatty liver, polycystic ovarian disease were 35, 11, 58, 8, 11, 13.6% respectively for patient groups in contrast prevalence of dyslipidemia, fatty liver were 10 and 7% respectively for control group.

Conclusion
Our study is unique in the sense that no study till date available from SEAR countries, showing prevalence of composite complications in obese population. This study actually quantify obesity related comorbidities in North Indian adult and which was found to be significant in comparison to normal weight patient.

Recommendation
Any obese patients with BMI > 27 kg/m² body surface area should be screened for various morbidities.

P27
Intervention to improve the management of diabetes in an academic primary care practice
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Diabetes mellitus is a serious health problem that requires continuous medical care to reduce the risk of its long-term complications. Although several guidelines have been developed to improve diabetes care, the compliance with diabetes clinical practice recommendations remains inadequate in some primary care clinics. The objective of this study was to assess the effectiveness of an intervention targeted at primary care physicians to improve the screening for diabetes related long-term complications. Thirty three primary care physicians were randomly assigned to work either with helper (intervention group), or without helper (control group). The study was carried out in the period between 1/8/2008 and 31/7/2009. The helper reviewed patients’ charts and entered information into a specially designed computer program. A checklist was generated by the program with the clinical and laboratory tests that need to be done for the patient at his/her current visit according to the recommended clinical practice guidelines. The checklist was then handled to the physician upon seeing the patient. At the end of the study, the achievement of the standards of care in screening for diabetes complications was assessed in both groups (total of 299 patients). Both groups had met the clinical practice recommendations in measuring blood pressure, and checking HbA1c and lipids. However, the control group failed to achieve the standards of care, when compared to the intervention group, in performing annual eye examination (23.9 vs 93.8%), annual complete foot - examination (23.1 vs 86%), and annual screening for nephropathy (35.8 vs 70.9) (P <0.001 in all). We concluded that the intervention used did significantly improve the standards of care for patients with diabetes in primary care clinics.

P28
Are adrenal incidentalomas routinely referred to endocrinology services? An audit of referral pattern and appropriate investigation
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Adrenal incidentaloma is a clinically silent adrenal mass detected on imaging performed for unrelated reasons. The concern is whether the incidentaloma is malignant or hypersecreting. The most comprehensive existing guidelines on investigation and management come from NIH Consensus Development Programme. We aimed to determine how many are identified by radiology over 12 months; how many of these are referred to endocrinology; how many are appropriately investigated using NIH guidelines.

We searched Clinical Radiology Information System reports between 01/11/2007 and 01/11/2008 for the terms adrenal adenoma, nodules, incidentaloma, mass and tumour. Exclusions: patients with current malignancy/still under surveillance; patients having suspected adrenal pathology; cysts; haemorrhage; angiomyolipoma; tuberculosis, deceased. Of those with adrenal incidentaloma, we established if referral had taken place to ourselves and searched the results server for evidence of investigation by clinicians other than us. We found 394 reports for 306 patients. Exclusions: ‘no adrenal masses’ (88), deceased patients (8) patients with active malignancy/under 5 year surveillance (102), specific adrenal imaging (12), incidentalomas found prior to 01/11/2007 (15), angiomyolipoma (2); haemorrhage (1), cysts (2), mycobacterial disease (2), extra-adrenal disease (2). In total, 69 patients with adrenal incidentaloma. Fifty were <4 cm; 4 were 4–6 cm; 1 was > 6 cm and 14 had no size reported. One patient was referred to us; 2 patients were referred for endocrine conditions other than adrenal lesions. Three patients had assessment for catecholamine excess; 1 had aldosterone/resin levels. Fourteen patients had follow-up imaging with no significant growth.

We conclude that patients with adrenal incidentaloma are not routinely referred to endocrinology services and undergoing adrenal hypersecretion is likely to be missed in a significant minority of cases. We are currently addressing this issue locally but acknowledge this will have some resource implications.

P30

Unusual presentation of a phaeochromocytoma

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Background

Phaeochromocytoma are rare catecholamine secreting tumours and may present with hypertension, orthostatic hypotension, tachycardia and even cardiogenic shock. These tumours can easily be missed particularly in patients presenting with other disorders. We present a case of a patient with severe community acquired pneumonia who despite overwhelming sepsis was severely hypertensive.

The case

JP was a 33-year-old man who was previously fit and well presented with fever, myalgia and shortness of breath. A chest X-ray demonstrated a right lower lobe pneumonia. He was treated with i.v. co-amoxiclav and clarithromycin and blood cultures grew Streptococci and Staphylococci.

However, despite antibiotic treatment his CRP was 418 and his temperature remained persistently elevated four days after admission. Paradoxically he remained hypertensive with systolic blood pressures of over 200 mmHg despite a marked systemic inflammatory response. Due to poor response to antibiotics, a CT scan of chest and abdomen was performed. This demonstrated a large right sided pleural effusion with consolidation of the lung above. A CT guided chest drain was placed to drain the effusion which turned out to be an empyema. Furthermore, an incidental right sided 4.2 cm adrenal mass was noted.

In view of the raised blood pressure, a phaeochromocytoma was suspected and investigations carried out after the acute phase showed a raised 24 h urinary metanephrine levels of 19 and 12 μmol/L (0–7). Free urine cortisol excretion marginally elevated at 644 mmol/24 h. He was commenced on Phenoxybenzamine 10 mg tds.

Three months after discharge for the pneumonia, he had an MBiSC scan and elective laparoscopic adrenalectomy. Urine normetanephrine excretion and BP normalized following the surgery.

Discussion

Severe sepsis may unmask underlying phaeochromocytoma and should be suspected in patients with severe hypertension during profound sepsis.

P31

Severe abdominal pain as presentation of Addison’s disease in undiagnosed case of autoimmune polyglandular disease

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A 58-year-old previously fit and well man presented with sudden onset severe epigastric pain associated with frequent episodes of vomiting. The pain was constant and relieved only with morphine. He was febrile otherwise systemic examination was normal. Serum sodium 132 mmol/l and serum potassium 4.4 mmol/l. The rest of blood tests including; full blood count, liver functions, glucose, amylase, corrected calcium, D-dimer, troponin, ura and creatinine were all normal. ECG, chest X-ray and abdominal X-ray were unremarkable. On further review, it was noted that he was extensively tanned and pigmented with few patches of vitiligo that he never noted before. He had a significant postural drop of his blood pressure. Furthermore he revealed that he was feeling sleepy and tired most of the time.

His 0900h cortisol was 59 mmol/l and ACTH – 406 ng/l. Short synacthen test showed a flat response. Further investigations showed; Free T4 5.5 μmol/l, TSH 49.22 μU/l, Testosterone 5.4 μmol/l, LH 3.8 IU/l and FSH 3.8 IU/l. These investigations confirmed that he had primary adrenal failure, primary hyperthyroidism and hypogonadism. Therefore, he was diagnosed as having autoimmune polyendocrine syndrome type 2 and was commenced on replacement treatment. Two years follow up he remains in full time job and both his sons developed hypothyroidism. Lessons to be learned from this case are:

i) The diagnosis of Addison’s disease is often delayed due to non-specific nature of its presentations and this case report highlights the importance of high index of suspicion in relevant settings to make a diagnosis.

ii) Severe abdominal pain which could present as an apparent acute abdomen is a well recognised feature of Addisonian crisis.

In Polyendocrine syndromes it is important to identify and treat adrenal failure prior to treating hypothyroidism with Thyroxine as this could lead into Addisonian crisis.

P32

A case of polyglandular autoimmune syndrome type II and sarcoidosis

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I would like to present a case of autoimmune poly glandular syndrome who first presented as profound hypothyroidism and secondary amenorrhea 8 years after her first baby. TSH was > 100 and LH and FSH 44.3 and 27 at diagnosis with oestradiol of 18. As patient remained symptomatic after being on thyroxine replacement she went on to undergo a synacthen test which showed a much blunted response (baseline cortisol 264, 30 min 273 and 60 min 264). While being investigated as an inpatient, she was found to have a cyst on her forehead which was subsequently excised and the histology report was consistent with Non
P33
The importance of HLA haplotype analysis in the polyglandular autoimmune syndromes
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A 45-year-old woman was diagnosed with hypothyroidism at 26, Addison’s disease at 35 and primary ovarian insufficiency at 42. She is positive for adrenal and thyroid microsomal antibodies but negative for ovarian antibodies. She has a strong family history of autoimmunity. Interestingly, her mother has pernicious anaemia and hypothyroidism and her sister has hypothyroidism. A diagnosis of autoimmune polyglandular syndrome (APS) type 2 has been supported by the HLA haplotype B8 DR3, and DR4.

The APS syndromes types 1 and 2 have dissimilar characteristics and there is emerging evidence to suggest that the genetics and pathogeneses of these two entities are different. The genotype for a particular syndrome does not always correlate with the phenotype hence clinical presentations are variable. The syndromes are characterised by multiple endocrine organ dysfunction associated with circulating organ specific antibodies. A family history of autoimmunity is characteristic and the syndromes show specific patterns of disease associations. There are 2 main types. Autoimmune polyendocrinopathy–candidiasis–ectodermal dystrophy (APECED) syndrome is a rare autosomal recessive disorder with an onset primarily in childhood or early adulthood. APECED has been linked to mutations in the autoimmune regulator gene on chromosome 21q22.3 and may produce different phenotypes. Adrenal insufficiency and hypoparathyroidism are the main endocrinopathies. These may coexist with non-endocrine disorders such as chronic active hepatitis, pernicious anaemia and malabsorption. APS type 2 is far more common than the type 1 syndrome. About 50% of cases are familial in origin and the mode of transmission is variable. There is a striking female preponderance and the onset occurs mainly in the middle aged. Adrenal sufficiency is the main manifestation and autoimmune thyroid disease and type 1 diabetes mellitus are common associations. The HLA DR3 and DR4 haplotype is significantly associated with Type 2 APS and helps with genetic screening and counselling.

P34
Marked hypercalcaemia in a case of primary hyperparathyroidism
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We report the case of a 77-year-old lady who presented to hospital with confusion, agitation and polyuria. She had type 2 diabetes mellitus but was otherwise well with no history of weight loss, nephrolithiasis or fractures. There was no relevant family history. There were no abnormal findings on examination apart from dehydration and no focal neurological signs or lymphadenopathy. Investigations revealed a serum corrected calcium level of 4.71 mmol/l (normal 2.1–2.6). Her parathyroid hormone level was also markedly elevated at 113 pmol/l (normal 1.5–7.6). Vitamin D level was 29 nmol/l. A previous calcium level 3 years ago had been normal. Myeloma screen was negative. She was treated with i.v. fluid rehydration and 90 mg i.v. pamidronate. Her serum calcium levels corrected over the next 10 days with improvement in her symptoms. Ultrasound scan of the neck revealed an incidental 2×1.5 cm mass in the lower pole of the right thyroid gland. Nuclear medicine parathyroid (sestamibi) scan was consistent with a left sided parathyroid adenoma. On the basis of these investigations a left upper parathyroidectomy was performed. Histology revealed 0.96 g nodular parathyroid tissue consistent with an adenoma. The patient was well and asymptomatic three months after the procedure with normal calcium levels.

Corrected calcium levels of >3 are unusual in primary hyperparathyroidism and levels >4 are rare. The extremely high calcium and parathyroid hormone levels accounted for this lady’s florid symptoms and were initially suggestive of parathyroid carcinoma. Findings following parathyroidectomy confirmed an adenoma.

P35
‘Mighty oaks from little acorns grow’: a review of the development of Endocrine Nursing in the UK
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Historically, many endocrine specialist nurses (ESN) worked in ‘isolation’, with little peer support. ‘Learning on the job’, there were few external training opportunities and their roles developed according to local expectations and needs. In 1997, a small group of ESN met to establish a support network for themselves and their colleagues. Within a year they had successfully developed links with the Society for Endocrinology (S for E), industry and patient support groups (PSGs). A committee was formed with good geographical representation across the UK and began by organising dedicated nursing symposia at the annual S for E meetings. Since 1998, they have organised an annual training course, now a conference, attracting ~75 delegates from as far as field as Australia, Sweden, Singapore, Turkey and The Netherlands. A quarterly newsletter (current distribution ~274 nurses) is produced. ESN can now work towards obtaining a Certificate by successfully complete four compulsory elements for the eight credits required. These include attending three ESN conferences and one S for E meeting, successfully submitting an abstract and either, an essay ‘My contribution to good endocrine nursing practice’, or a portfolio of evidence to reflect their area of expertise and show their development as an ESN.

The last decade has seen an increase in the development of ESN posts. New posts have been created, part-time jobs have become full-time and roles have expanded as ESN have established nurse led clinics and taken on their own caseloads. ESN are now involved in supporting patients with a far greater number of endocrine conditions and their expertise is sought for many and varied roles in addition to their clinical posts, e.g. trustees of PSGs and experts on pharmaceutical nurse advisory boards. Working closely with their medical colleagues and well supported by them they are going from strength to strength.

P36
Altered time-effect profile of insulin glargine in overdose: a case report
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Introduction
Glargine is a recombinant human insulin analogue, used in the treatment of diabetes mellitus. Although widely used, information regarding the altered time-effect profile of glargine taken in overdose is scarce. We describe a case which highlights this phenomenon.

Case report
An 82-year-old female was found by her neighbour, 18 h after attempting suicide by injecting herself with 600 units of glargine at multiple sites. Her capillary blood glucose (CBG) was 1.5 mmol/l at the scene. On treatment with oral glucagon and i.m. glucagon it rose to 3.9 mmol/l.

She was transferred to A&E 19 h post-overdose, her CBG was 6.7 mmol/l and her serum potassium was 2.5 mmol/l. She became hypoglycaemic 3 h later with a CBG of 1 mmol/l necessitating treatment with 50 ml 50% glucose.

She was admitted to a Medical ward and was commenced on an i.v. infusion of 5% dextrose at 100 ml/h. Her CBG was monitored every hour and her electrolytes every 4 h, with rate of infusion and potassium concentration being adjusted accordingly.

Her last episode of hypoglycaemia (CBG 1.9 mmol/l) occurred 63 h post-overdose which was attributed to interruption of infusion by extravasation of her i.v. cannula. Her dextrose/potassium infusion was stopped safely after 102 h. She required a total of 141 of i.v. 5% dextrose and 540 mmol of potassium over 4 days. Although she continued to eat and drink, she remained euglycaemic for 9 days without any anti-diabetic treatment. She was recommenced on glargine 9 days post-overdose, and was transferred for inpatient Psychiatric care.

Conclusion
This case highlights that insulin glargine taken in overdose, has a much longer duration of action than is reported on product literature. Cases should be closely monitored for hypokalaemia and hypoglycaemia and treated with intravenous infusion, of which early interruption should be avoided.
Polymyalgia Rheumatica (PMR) is a condition of widespread inflammation which mainly affects large muscles manifesting as muscle pain, stiffness and weakness, usually associated with elevated circulating inflammatory markers. Typically PMR responds briskly to the introduction of glucocorticoids but hasty withdrawal can precipitate re-activation of the condition, a message illustrated by our case report in a patient identified with Cushing’s syndrome.

A 52-year-old woman was referred with a year history of lethargy, proximal muscle pain and weakness, poor wound healing, subocital BP control and recent weight gain of 10 kg. She appeared clinically Cushingoid and had marked proximal muscle weakness. Cushing’s syndrome was confirmed by biochemical investigation (cortisol 367 nmol/l after high dose dexamethasone suppression test). MRI scanning revealed a left 3.2 cm adrenal adenoma. She received metyrapone prior to left adrenalectomy. There was marked resolution of her symptoms and her blood pressure was more readily controlled. She also lost 7 kg in 2 months.

However, over the forthcoming weeks she experienced reduced energy levels and lassitude associated with progressive neck/shoulder pain. She reported proximal muscle stiffness/weakness and had difficulty combing her hair and getting out of her chair. Recurrence of her Cushing’s syndrome and adrenal insufficiency were both excluded by biochemical testing. A clinical diagnosis of PMR (confirmed by a rheumatologist) was supported by an elevated CRP of 42 mg/l. Prednisolone 20 mg/day was commenced and consistent with PMR, she noticed dramatic improvement within 48 h.

We postulate that the onset of PMR predates the diagnosis of Cushing’s syndrome, but the patients clinical manifestations of the disease were suppressed by endogenous glucocorticoid over-production. Overt clinical presentation with symptoms suggestive of PMR became evident only after surgical resection of the adenoma associated with rapid decline in supraphysiological cortisol levels. The gradual withdrawal of steroids is mandatory in the successful treatment of PMR.

P38

A case of microprolactinoma in a young man presenting as obesity despite regular heavy exercise

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Introduction

Hyperprolactinaemia may be associated with weight gain and obesity, suggesting that prolactin may modulate body composition and weight. Treatment that normalises prolactin levels using dopamine agonists frequently leads to weight loss and provides further evidence for the association.

Case

A 28-year-old South Asian male with a body mass index of 27.2 kg/m² was referred to a weight management clinic with a five year history of weight gain despite regularly attending the gym for 2 h 4–5 days per week. He had frequently been seen by his GP who had consistently given lifestyle and dietary advice despite his regular attendance at the gym with no improvement. He also complained of short-term memory loss, feeling tired and lethargic for several years. He was not diabetic and blood pressure and cholesterol were normal. Examination findings were unremarkable. Prolactin level was 6251 mU/l (86–324) testosteron was 8.8 nmol/l (8.6–33.0) and his TSH was 1.5 mU/l (0.3–4.6).

An MRI scan showed a 9 × 8 mm bulge arising from the pituitary gland consistent with a microprolactinoma. He was commenced on carbergoline 250 µg twice weekly and his prolactin level 3 months later had improved considerably to 443 mU/l and testosterone had shown some improvement at 9.8 nmol/l. His weight went from 85 to 86.2 kg but measurement of bioimpedance showed that his fat mass had dropped from 29.1 to 26.8%, and fat mass went up from 60 to 63 kg, suggesting a loss of fat and gain in muscle mass.

Discussion

Although it is widely accepted that dietary intake and lifestyle factors are the major contributors to weight gain and subsequent obesity, weight gain is associated with a number of endocrine disorders including hyperprolactinaemia, and should be considered as a differential diagnosis for endocrine obesity when changing lifestyle factors has not affected weight.
P41
Extensive hirsutism, a valuable clue to a sinister pathology
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Background
Hyperandrogenemia is one of the most commonly encountered endocrine disorders in reproductive-aged women. By far the most common causes of androgen excess are functional which account for more than 90% of cases. Androgen and ovarian tumours producing excess androgen are however very rare and only account for about 0.2% of cases.

Case history
A 62-year-old lady admitted to hospital with pneumonia was found to have an ill defined, firm, non-tender right upper quadrant mass, extensive hirsutism involving the face, lower abdomen and extremities, male pattern baldness, facial plethora and proximal myopathy. Initial investigation with ultrasound of the abdomen confirmed the presence of a 21 cm diffuse mass infiltrating the right kidney. A clinical diagnosis of androgen and cortisol secreting adrenocortical cancer was made and further investigation of full adrenal hormonal profile and CT scan imaging was arranged.

Her hormonal profile confirmed a high testosterone level of 52 nmol/l (normal <2.4 nmol/l) and androstendione of 35 nmol/l (normal 1-11.5 nmol/l) but normal DHEAS level. She also had raised 24 h urinary free cortisol level of 47 mol/24 h (normal <280 mol/24 h) and failed to suppress morning serum cortisol level following 1 mg. overnight dexamethasone suppression test (9000 h cortisol of 1369 nmol/l, normal <50 nmol/l). Her plasma methamphetamine levels were within normal range. A CT scan of the abdomen confirmed the presence of a 24 x 15 x 14 cm heterogeneous suprarenal mass infiltrating the kidney along with evidence of liver and pulmonary metastasis and tumour thrombus in the inferior vena. An ultrasound guided biopsy of the mass confirmed the diagnosis of adrenocortical carcinoma.

Conclusion
Although hirsutism is commonly related to functional (non-tumour) causes, the presence of rapidly progressive and extensive hirsutism should alert physicians to undertake basic hormonal and imaging investigations to facilitate early diagnosis of malignant adrenal and ovarian secreting tumours.

P42
Aetiology of hyperprolactinaemia
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Elevated serum prolactin (PRL) is common, with many potential causes. We performed a retrospective audit of patients with hyperprolactinaemia from 2006 to 2008, aiming to identify the clinical indications for prolactin testing, the aetiology identified and the treatment instigated. Medical notes for 83 patients (55 female, mean age 41 years (range 17-95)) with PRL >700 mU/l were retrospectively reviewed. Indications for testing included: seizure or suspected seizure (35), headache (14), routine psychiatric admission (13), oligomenorrhoea (11), galactorrhoea (7) and erectile dysfunction (3). Aetiology of elevated PRL was: presumed seizure-related (30), medication-related (16), pituitary tumour (15), macroprolactininaemia (8), pregnancy (2), and unknown (12). Medications associated with hyperprolactinaemia included: neuroleptics (12), selective serotonin re-uptake inhibitors (SSRIs) (3), and dopamine antagonists (1). Mean PRL for the different medications was 1596, 1361 and 1052 mU/l respectively (P=0.49, ANOVA). Four patients on neuroleptics/SSRIs had medication adjustments based on the PRL result. Of 15 patients with pituitary tumours, 4 were macroprolactinomas, 4 microprolactinomas and 7 other pituitary tumours, with mean PRL of 9066, 2876 and 1343 mU/l respectively (P=0.01, ANOVA). Mean PRL in 30 patients with seizure-related hyperprolactinaemia was 1328 mU/l. Repeat blood testing to confirm normalization of prolactin was performed in one patient. In patients with macroprolactinoma, mean PRL was 1008 mU/l, with mean post-fractionation PRL 302 mU/l. In conclusion, seizure was the most common cause of an elevated PRL, but follow-up of these patients to rule out co-incidental pituitary disease was poor. A large number of tests were performed, many of which did not alter management.

P43
Testosterone replacement in the setting of Finasteride therapy: a therapeutic dilemma
Christian Hariman1, Laks Varadhan1,2, Adrian Walker3 & George Varughese3
1University Hospital of North Staffordshire, Stoke on Trent, Staffordshire/West Midlands, UK; 2Keele University Medical School, Stoke on Trent, Staffordshire/West Midlands, UK.

Introduction
Benign prostatic hypertrophy is common amongst elderly men, and usually treated with the testosterone blocker, finasteride. However, the treatment of such a condition alongside hypo-pituitarism and low testosterone levels presents with a therapeutic dilemma.

Case report
An 88-year-old gentleman who was admitted following recurrent episodes of confusion and falls was found to have a serum sodium level of 114 mmol/l, serum osmolality of 258 mosmol/l and urine osmolality of 418 mosmol/l. Investigations for underlying neoplastic causes were all negative. Subsequent cranial imaging shows a pituitary macroadenoma occupying the sella and the sphenoid sinus. He has reported no visual disturbances, and visual field tests were satisfactory. Further detailed investigations have shown hypogonadotropic hypogonadism with undetectable testosterone with suppressed LH and FSH levels. Following an inadequate response with a short synacthen test, he was prescribed hydrocortisone and three monthly testosterone injections, of which he is tolerating well. He was diagnosed with hypo-pituitarism from a non-functioning pituitary macroadenoma which warranted replacement with hydrocortisone and testosterone. A few months following this treatment, he was admitted by the urologists for haematuria due to benign prostatic hypertrophy after prostatic carcinoma was ruled out. He was then prescribed finasteride as a testosterone blocker for symptomatic control of his urinary tract symptoms. As the patient was on testosterone replacement, treatment with finasteride was thought to be counter-intuitive, as it would block the effects of the testosterone replacement. Conclusion
Finasteride blocks the actions of 5α-dihydrotestosterone that mainly has actions on the prostate and hair loss. Therefore, testosterone replacement can be safely prescribed alongside finasteride in patients with low testosterone (predominantly for general well being and bone strength) and benign prostatic hypertrophy. We discuss the implications of testosterone replacement therapy in such situations.

P44
Addison’s disease unmasking an occult systemic cause for cerebral ischaemic event
Christian Hariman1, Laks Varadhan1,2, Indira Natarajan1, Adrian Walker3 & George Varughese3
1University Hospital of North Staffordshire, Stoke on Trent, Staffordshire/West Midlands, UK; 2Keele University Medical School, Stoke on Trent, Staffordshire/West Midlands, UK.

Introduction
Cerebral ischaemic events in young patients due to patent foramen ovale are rare. A more plausible diagnosis had clouded the initial management and diagnosis of such a case in a young male patient.

Case report
A 19-year-old gentleman was admitted with vomiting and profound hypotensive state of 99 mmHg. He was diagnosed to have Addison’s disease (with positive adrenal antibodies) following a flat short synacthen test. He warranted ITU admission and subsequently developed new neurological features of facial drooping, speech deficit and subtle weakness in his right upper and lower limb following rapid sodium correction over a few days. His sodium was corrected to 131 mmol/l within 72 h of admission, and MRI brain scans did not demonstrate any evidence of central or extra pontine myelinolysis. However subsequent imaging showed features consistent with ischaemic changes. The patient’s Addison’s disease is now well controlled with oral hydrocortisone and fludrocortisone replacement, and a subsequent repeat cranial MRI showed high intensity at the left temporal lobe and bilateral pre-central sulcus that was in keeping with ischaemic events. His thrombophilia screen was negative, and he underwent a bubble contrast Echocardiogram that showed evidence of a patent foramen ovale on valsalva manoeuvre. He is now listed for closure of his patent foramen ovale under the care of the cardiologists.

Conclusion
The likelihood of the diagnosis was clouded by another more plausible diagnosis, the initial clinical presentation, and the rarity of cerebral ischaemic events in young patients. Although clinical suspiscions were initially biased towards extra pontine myelinolysis, such rare conditions can occur and although well recognised, such situations are less commonly perceived in clinical practice.
**P45**

**Bariatric surgery in renal transplant recipients**

Salma S Kamaleldin1, Marco Buiter2, Ahmed Ahmed1 & Carel W Le Roux3

1Imperial College, London, UK; 2King’s College London Medical School, London, UK.

Objectives

The long-term outcome of bariatric surgery on renal function in the morbidly obese renal transplant recipient is still unknown. We describe three cases of morbidly obese renal transplant recipients who have subsequently undergone bariatric surgery.

Patient 1: A 44-year-old man (BMI 42.2 kg/m²) had received a renal transplant for chronic renal disease secondary to childhood reflux nephropathy. Laparoscopic Roux-en-Y gastric bypass resulted in 25.1 kg weight loss over 3 months. Long-standing proteinuria, glucosuria and urine blood trace resolved by 4 months and serum creatinine and cholesterol improved. His blood pressure medications were reduced post-bypass.

Patient 2: Patient 2 is a 51-year-old man (BMI 43.3 kg/m²) with a pancreas-kidney transplant for end stage renal failure (ESRF) secondary to diabetes. He required insulin and metformin following pancreatic graft failure. He lost 8 kg in 4 months following sleeve gastrectomy. Insulin requirement was nil for 7 days post-sleeve gastrectomy but returned to pre-operative levels. Elevated blood urea, bicarbonate and potassium levels normalised. Creatinine remained normal, although urine protein creatinine ratio was raised and there was a need to readjust immunosuppressant medication.

Patient 3: Patient 3 is a 56-year-old man (BMI 39 kg/m²) who had received a kidney transplant for ESRF secondary to diabetes. Weight loss was 15 kg, 4 months following sleeve gastrectomy. Insulin requirement was reduced from 260 to 40 units post-sleeve gastrectomy, although HbA1c was consistently high. Ibsuprasan was commenced for hypertension. Serum creatinine and eGFR remained normal.

Conclusion

Bariatric surgery can be safe and successful in reducing weight and obesity related co-morbidities in the morbidly obese renal transplant recipient. Bariatric surgery in this cohort of patients can stabilise the renal transplant and improve some markers of renal function.

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**P46**

**Intermittent galactorrhoea due to vacant episodes**

Sampath Satish Kumar & Chimnadori Rajeswaran

Dewsbury District Hospital, Dewsbury, UK.

Introduction

Epilepsy and anti-epileptic medications can be associated with various endocrine disorders namely, polycystic ovary syndrome, premature ovarian failure, hypothyroidism, amenorrhoea, functional hyperprolactinaemia and rarely galactorrhoea. Here, we present a lady who developed intermittent episodes of galactorrhoea suspected to be due to primary generalized seizures.

Case report

A 50-year-old lady was referred for investigation of elevated prolactin and galactorrhoea for two years. Detailed evaluation of medical history revealed history of epilepsy, but was not on anti epileptics as she was seizure free. She continued to be seizure free but had intermittent vacant episodes lasting for a few seconds. She was not on any medication except mirena coil. General examination was unremarkable and visual field was normal. Initial serum prolactin was 1027 IU/L. Repeat blood test showed a serum prolactin of 884 IU/L, TSH, FSH 14.4 IU/L and LH 3.8 IU/L. Magnetic resonance imaging did not reveal any pituitary lesion.

In view of the suspicion of galactorrhoea secondary to seizure activity associated with vacant episodes, an EEG (Electroencephalogram) was requested. EEG revealed continuous generalized seizure activity suggesting a diagnosis of primary generalized epilepsy. She was referred to the neurologist for the primary treatment of epilepsy. The plan is to start her on carbamazepine if galactorrhoea does not resolve with anti-epileptics.

Discussion

Elevation of serum prolactin level is seen following generalized tonic-clonic, complex partial, some times in simple partial seizures and moderately high serum prolactin levels seen in interictal epileptiform discharges. We suspect our patient has been having interictal epileptiform discharges and probably generalised seizures activity intermittently during the vacant episodes. This would explain moderately high serum prolactin levels causing intermittent galactorrhoea. Endocrine dysfunction has a complex mechanism in people with epilepsy and needs judicious investigation before any therapeutic intervention.

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**P47**

**Assessment of risk factors given in GP referral letters for DEXA imaging**

Ghulam Mustafa Shahi1, Mortimer B O’Connor2, Jeetandra Rathi3, Fredo Lainais4, Ursula Bond4, Joan Swan4, Matthew Murphy5 & Mark J Phelan3

1Endocrine Unit, South Infirmary Victoria Hospital, Cork, Ireland; 2Rheumatology Department, South Infirmary Victoria Hospital, Cork, Ireland.

Introduction

Osteoporosis poses a significant public health issue, causing significant morbidity and mortality. It leads to an increased fracture risk through a reduction in the bone mineral density (BMD), disruption of bone microarchitecture, and alteration of the amount and variety of non-collagenous proteins in bone. Treatment aims are to prevent fractures and maintain the quality of life of the aging adult. The advent of the WHO assessment tool ‘Fracture Risk Assessment Tool’ (FRAX®) has been revolutionary in GP assessment of patients regarding need for treatment, need for further evaluation by DEXA imaging and those not requiring any treatment. This study examines GP requests for DEXA imaging and if they contain sufficient details to justify imaging.

Methods

Two hundred randomly chosen GP request letters were analysed using the FRAX® tool. All letters were from April 2007 to July 2008. Resulting data was analysed using the statistical package SPSS

Results

Of the 200 letters, 4% (n=8) were male and 96% (n=192) were female, with a mean age of 64.3 years. One GP service provided a proforma referral letter with the remaining letters being individually composed. Table 1 shows the percentage of letters containing each of the FRAX® criteria. Of importance only 1 request (not a proforma letter) contained all the details allowing for FRAX® assessment (P<0.005) despite the use of a proforma by one GP service.

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Percentage (n value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>100% (n=200)</td>
</tr>
<tr>
<td>Sex</td>
<td>100% (n=200)</td>
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<tr>
<td>Weight</td>
<td>&lt;1% (n=1)</td>
</tr>
<tr>
<td>Height</td>
<td>&lt;1% (n=1)</td>
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<tr>
<td>Previous fracture</td>
<td>7% (n=14)</td>
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<tr>
<td>Parental hip fracture</td>
<td>5% (n=10)</td>
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<tr>
<td>Current smoker</td>
<td>6% (n=12)</td>
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<tr>
<td>Glucocorticoids</td>
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<tr>
<td>Rheumatoid arthritis</td>
<td>3% (n=6)</td>
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<tr>
<td>Secondary osteoporosis</td>
<td>9% (n=18)</td>
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<tr>
<td>Alcohol 3 or more units/day</td>
<td>2% (n=4)</td>
</tr>
</tbody>
</table>

Conclusions

The majority of GP referral letters for DEXA imaging do not contain adequate data to make recommendations using the FRAX® tool. Incorporating this data is likely to improve requesting systems for DEXA scanning to GPs.

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**P48**

**Forensic endocrinology: a case report of factitious hypoglycaemia**

Yee Seun Cheah1, Atim Elok1, Andrew Gough1, Pauline Kane1, Simon Aylwin1, Klaus-Martin Schulte1 & Jackie Gilbert1

1King’s College Hospital NHS Foundation Trust, London, UK; 2Medway Maritime Hospital NHS Foundation Trust, Gillingham, UK.

A 51-year-old female presented with a 9 month history of symptoms suggestive of hypoglycaemia and associated episodes of unconsciousness, which were prevented by frequent food intake but with subsequent weight gain. She reported a history of lactose-induced anaphylaxis and the use of lactose-free subcutaneous prednisolone since sustaining a traumatic cardiac injury in 1987.

Two supervised prolonged fasts demonstrated hypoglycaemia with elevated insulin levels. C-peptide was detectable but below the reference range (Table).

**Table 1** FRAX® details contained in GP DEXA imaging request letters.

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Percentage (n value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
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</tr>
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<td>100% (n=200)</td>
</tr>
<tr>
<td>Weight</td>
<td>&lt;1% (n=1)</td>
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<tr>
<td>Height</td>
<td>&lt;1% (n=1)</td>
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<tr>
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<td>5% (n=10)</td>
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<tr>
<td>Current smoker</td>
<td>6% (n=12)</td>
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<tr>
<td>Glucocorticoids</td>
<td>8% (n=16)</td>
</tr>
<tr>
<td>Rheumatoid arthritis</td>
<td>3% (n=6)</td>
</tr>
<tr>
<td>Secondary osteoporosis</td>
<td>9% (n=18)</td>
</tr>
<tr>
<td>Alcohol 3 or more units/day</td>
<td>2% (n=4)</td>
</tr>
</tbody>
</table>

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The cortisol level was 759 nmol/l. The 24 h urine collection did not detect sulphonylurea. CT and MRI scans showed normal pancreatic appearances. An octreotide scan was also normal. Selective arterial catheterisation with calcium gluconate stimulation showed a flat insulin response in the hepatic, gastroduodenal; distal, mid and proximal splenic and superior mesenteric arteries (glucose 2.4–4.6 mmol/l, insulin 48.2–57.1 mIU/l). Therefore no abnormal pancreatic region was localised.

Further discussions with the laboratory confirmed that the ratio of insulin to C-peptide was not consistent with an insulinoma. It subsequently transpired that the ‘subcutaneous prednisolone’ was not obtained from any healthcare practitioner. To determine whether this was a source of exogenous insulin we offered an alternative oral, lactose-free preparation of prednisolone which she declined. It is understood that she is currently under investigation for criminal activities. It was postulated that she presented with factitious hypoglycaemia in order to avoid a custodial sentence and is undergoing psychiatric evaluation.

Determining the cause of hypoglycaemia amongst the many possible differential diagnoses can be difficult, and a systematic approach is necessary. This may have a significant medico-legal bearing, which our case illustrates. Psychiatric evaluation is recommended for the management of factitious hypoglycaemia.

<table>
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<tr>
<th>Date</th>
<th>Glucose (mmol/l)</th>
<th>C-peptide (266–1332 pmol/l)</th>
<th>Insulin (4.4–26.0 mU/l)</th>
<th>Pro-insulin (0–7 pmol/l)</th>
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<tbody>
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<td>26.01.09</td>
<td>2.4</td>
<td>142</td>
<td>50.7</td>
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</tr>
</tbody>
</table>

P49

Severe hypoglycaemia leading to death in a patient with type 2 diabetes: insulinoma, sulphonylurea overdose or some other cause?

Lina Pui Lin Chong, Nihad Jalali & Darryl Meeking
Queen Alexandra Hospital, Portsmouth, UK.

We present a 52-year-old lady with type 2 diabetes mellitus, referred by her GP with poor glycaemic control. Her past medical history included haemochromatosis, undifferentiated connective tissue disorder, depression, hypothyroidism, pernicious anaemia and alcohol abuse. Relevant medications were metformin 1 g bd, novorapid 28 units tds, levarterenol 30 mg od and prednisolone 5 mg od. Her HbA1c was 8.1%, renal function and baseline pituitary function was normal. Following an admission with unexplained hypoglycaemia, her diabetic medications were stopped. An abdominal ultrasound reported a fatty liver, enlarged spleen and limited view of the pancreas. On follow-up, she had an episode of witnessed hypoglycaemia. Serum glucose, C-peptide, insulin and pro-insulin were sent. She denied exogenous administration of insulin/ oral hypoglycaemic agents (OHAs). A month later she had symptomatic hypoglycaemia and metformin was commenced. Three weeks following that, she was found unconscious with a GCS of 3, profoundly hypoglycaemic (venous glucose 0.8 mmol/l) and hypertensive (BP 160/100). CT brain showed basal ganglia changes in keeping with haemochromatosis. Renal function, liver enzymes, electrolytes and paracetamol/salicylate levels were normal. TTFs suggested probable noncompliance with thyroxine. Full blood count revealed macrocytosis. There was no evidence of insulin/OHAs overdose from collateral history. She did not make any neurological recovery and subsequently died 2 weeks after admission. Initial results from witnessed hypoglycaemia:

Glucose 2.6 mmol/l
C-peptide 381 pmol/l (0–480)
Insulin 91.6 mU/l (0–10)

Disproportionately high insulin to C-peptide suggests probable exogenous insulin use given that additional history revealed possible Munchausen’s syndrome with deliberate overdose of azathioprine resulting in neutropenic sepsis. However, pro-insulin measured 52 pmol/l (<10) which is significantly elevated. This raises the possibility of an insulinoma or sulphonylurea overdose. Postmortem identified a pale area in the pancreas and a small well-circumscribed lung lesion. Histology is pending.

P50

Hypocalcaemia post total thyroidectomy: a clinical experience

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University Hospital Lewisham, London, UK.

Hypocalcaemia following thyroidectomy may be temporary or permanent, usually occurring within 14–72 h. The British Association of Endocrine Surgeons 2009 audit reported long-term hypocalcaemia rates of 7%. Total thyroidectomy for Graves’s disease carries the greatest risk (reported in 6–30% of cases) and currently there is no national consensus on management of this complication.

We present three healthy young women who underwent total thyroidectomy for Graves disease. Medical management failed following poor compliance and attendance to endocrinology. Radioactive iodine was declined or deemed impractical.

Postoperatively all women were hypocalcemic and required oral calcium. In addition they received i.v. high dose calcium plus alfalcacidol as they become symptomatic with falling serum levels (lowest immediate eCa 1.7 mmol/l). Calcium replacement was not given preoperatively. The longest immediate inpatient stay was 14 days however all women required subsequent prolonged admissions after presenting acutely with symptoms in the 1st 8 weeks (lowest eCa 1.6 mmol/l).

They had daily endocrine review and one lady commenced recombinant PTH for 8 months.

PTH measured at 4 weeks was undetectable and in one case remains undetectable at 12 weeks. Histology did not identify parathyroid tissue and there was no documentation of damaged parathyroid tissue in theatre notes.

This series highlights the significant morbidity associated with hypocalcaemia. In our patients, compliance was already an issue and they all continue on multiple daily tablets. We continue to see them regularly with close serum monitoring to prevent repeated admission.

This has highlighted in our own centre the need for a local management protocol. At present intraoperative PTH measurement is not available however data is varied to support its use as a positive predictor. There is a move for some centres to perform total thyroidectomy for benign disease as day case and we would advise caution following our clinical experience.
P52
A case of fatal acute severe multi-factorial hypoponatraemia
Agnieszka Falinska, Dina Saleh, Alex Comminos & Khalid Ahmed
West Middlesex University Hospital, London, UK.

Hypoponatraemia is the commonest electrolyte abnormality observed in clinical practice. It is a potential cause of substantial morbidity and mortality. Drug history, fluid volume status in addition to serum and urine biochemistry is essential for optimal management. We report a case of a 50-year-old female with known psychosis admitted to the Mental Health Unit and treated with Citalopram, Mirtazapine, Risperidone, Clonazepam and Procycolidine. Admission plasma sodium was 125 mmol/l. During admission she was noted to be drinking large amounts of fluids. Eight days later she had a respiratory arrest. Respiratory rate was 6 breath per minute and GCS 3/5. She was intubated, infused 1.51 0.9% saline i.v. and a CT brain scan performed. The latter demonstrated extensive cerebral oedema. Sodium on arterial blood was 99 mmol/l.

On admission to ITU plasma sodium was 103 mmol/l, urine osmolality 76 mosmol/kg, plasma osmolality 223 mosmol/kg and urine sodium 13 mmol/l. Renal, thyroid and adrenal function were normal.

After 5 h on fluid restriction in ITU, her sodium rose from 103 to 115 mmol/l. She was started on 5% dextrose 500 ml/h. Retrospectively, fluid balance charts showed urine output of 0.1 h and an 11 l deficit over 17 h. Her plasma sodium was 135 mmol/l at that stage.

Throughout ITU admission her GCS remained 3/5 and pupils were fixed and dilated. Brainstem death was confirmed 48 h post admission to ITU.

The most likely cause of hypoponatraemia in this case is a combination of psychogenic polydipsia with anti-psychotic-induced SIADH. Cerebral oedema is the likely cause of the respiratory arrest. Pontine demyelination due to rapid correction of plasma sodium is a likely cause of brainstem death.

This case highlights the challenges in management of acute severe multi-factorial hypoponatraemia. It also emphasizes importance of regular sodium and fluid balance evaluation on patients receiving multiple agent therapy for mental health disorders.

P53
An unusual presentation of polyendocrinopathy
Maya Vena, Haris Marath & Francesco Swords
Norfolk and Norwich University Hospital, Norwich, UK.

A 52-year-old gentleman was referred for the evaluation of hypercalcaemia. He gave a four week history of feeling generally unwell with dizziness, abdominal discomfort, weight loss, nausea and vomiting. He was an ex-heavy smoker and was on Lithium for about 20 years for bipolar disorder. He had had a recent admission to hospital with sepsis, secondary to chest infection, with a brief ITU stay and was discharged only 3 weeks prior to the onset of current illness.

On examination he was dehydrated, tremulous and deeply tanned with increased pigmentation of surgical scar on his knee. There was no palpable goitre, lymphadenopathy or evidence of buccal pigmentation. His vitals were stable and the rest of the examination was unremarkable.

Initial investigations revealed Na 123 mmol/l, K 6.3 mmol/l, Ur 14.9 mmol/l, Cr 172 mmol/l, Cor.Ca 3.35 mmol/l, Phos 1.81 mmol/l, Li 1.1 mmol/l (0.4-0.8). FBC, LFTs and CRP were normal.

Further investigations showed a PTH 0.9 pmol/l (1.6-6.9), TSH 0.03 mIU/l (0.35-3.5), FT<sub>3</sub> 33 pmol/l (8-21), FT<sub>4</sub> 8.9 pmol/l (3.8-6.0), no thyroid auto antibodies detected. Short synchtest test showed a flat response with pre-test ACTH >1000 pmol/l. In view of weight loss, hypercalcaemia and adrenal insufficiency, malignancy with adrenal metastasis was suspected. However upper GI endoscopy, CT scan of chest, abdomen and pelvis failed to show any evidence of a primary malignancy. Initial CT abdomen showed bilateral bulky adrenals which, on serial scans have shown progressive shrinkage, suggestive of adenral infarction rather than metastasis.

A final diagnosis of lithium induced thyrotoxicosis and sepsis induced bilateral adrenal infarction with adrenal insufficiency was made. Hypercalcaemia was probably secondary to either adrenal insufficiency or thyrotoxicosis or both as it resolved with steroid supplementation and carbamazepine treatment. After liaising with psychiatrists, Lithium has been gradually reduced and stopped.

Endocriological diagnosis of hypercalcaemia can be challenging as illustrated by this case.

P54
Acute fatty liver due to poor diabetic control
Agnesieka Swiezicka & Manjumah Malige
Royal Bolton Hospital, Bolton, UK.

A 25-year-old lady with poorly controlled type 1 diabetes presented acutely with vomiting, peripheral oedema and abdominal pain.

She was diagnosed with type 1 diabetes at the age of 12 and her glycaemic control has always been suboptimal predominantly due to poor compliance with the treatment. She had had numerous admissions with diabetic ketoacidosis in the past.

On examination a tender hepatomegaly was noted. The investigations revealed Ast of 1675 µ/l, ALP 242 µ/l, LDB 2171 µ/l, total cholesterol 8.8 mmol/l and triglycerides 10.9 mmol/l. Her HbA1C at the time was 11%. Both the ultrasound and the CT of the abdomen showed enlarged liver with diffuse granularity. All vessels were patent and there were no signs of cholestasis or ascites. Viral hepatitis and autoimmune screen was negative. Haemochromatosis, Wilson’s disease and α-1 antipyrin deficiency were ruled out. Liver biopsy revealed abnormal glycogen accumulation suggesting a possibility of a late presentation of glycogen storage disease which, however, did not fit into the clinical picture. The RBC phospholipase-kine activity was within normal limits.

The case was discussed on the multidisciplinary team meeting involving diabetologists and histopathologist at the tertiary centre. The general consensus was that this was an acute fatty liver due to poor glycaemic control.

The patient was started on continuous subcutaneous insulin infusion therapy resulting in a dramatic improvement in her diabetes control. Eighteen months after the diagnosis of acute fatty liver was made, her HbA1c was 8.5%. total cholesterol 6.1 mmol/l, triglycerides 2.0 mmol/l. The follow-up CT of the abdomen demonstrated resolution of hepatomegaly with no fatty changes observed.

P55
A case of pseudo-carcinoid
Craig Stiles, Manika Sunamattileke & Will Drake
St Bartholomews and the Royal London NHS Trust, London, UK.

A 31-year-old Caucasian male with chronic fatigue syndrome and additional symptoms of abdominal pain and diarrhoea, was referred by his neuro-gastroenterologist for investigation of possible carcinoid tumour after finding a raised 24 h urinary 5HIAA level – 220 µmol/24 h (normal range <50 µmol/24 h).

Full blood count, urea and electrolytes, liver function tests, plasma and urinary catecholamines were all within normal range.

A CT of the chest, abdomen and pelvis had detected no tumour.

A detailed history revealed that the patient had become disillusioned with conventional medical therapy for his Chronic Fatigue and had seen a nutritionist in the alternative healthcare sector.

He had been ‘prescribed’ a large number of nutritional supplements.

One of these supplements was ‘5HTP’. This is 5-hydroxytryptophan, a precursor of serotonin. A rise in urinary 5HIAA to a median of 204 µmol/24 h with 5HTP ingestion in healthy subjects had previously been observed<sup>3</sup>

The 5HTP tablets were stopped and two weeks later, repeat 24 h urinary 5HIAA analyses returned within normal limits (13 and 12 µmol/24 h).

This, coupled with an unremarkable CT scan effectively excluded a carcinoid tumour.

It has also been shown<sup>4</sup> that certain foods elevate urinary 5HIAA. A 1985 paper<sup>5</sup> demonstrated that foods like walnuts, pecans, plantain and pineapple are especially high in serotonin and their consumption can cause a raised urinary 5HIAA. 1 Black walnut can cause a rise of 5 µmol/24 h and 1 banana by 10 µmol/24 h<sup>2</sup>.

This case highlights the importance of alternative medicines and diet as confounding factors in the biochemical investigation of Carcinoid.

References
P56
Investigating for Cushing's syndrome in a patient with increased BMI on rifampicin
Mansoor Ali, Julie Andrew, Wycliffe Mbagaya & Steve Orme

A 39-year-old rugby league player was admitted with back pain to an Endocrinology/General Medicine ward. He was diagnosed with osteomyelitis of the spine and was treated with antibiotic. During his prolonged in-patient stay (6 weeks i.v. antibiotic treatment), the presence of purple striae on his lower abdomen was noted. Although he did not have other classical features of a Cushing's syndrome including proximal myopathy, he had an increased BMI (38 kg/m²) and hypertension. He underwent investigations to exclude Cushing's syndrome. An oral low dose dexamethasone suppression test, 24 h urinary cortisol and 09:00 h plasma ACTH level were performed. His 48 h cortisol was 463 nmol/l with 24 h urinary cortisol of 130 nmol/day (nr 10–147). His Plasma ACTH was 19 ng/l (nr <47). It was then realised that his results could have been affected by him being on oral rifampicin for the treatment of osteomyelitis and an elevated BMI was also considered to be a possible cause of a false positive result.

We then performed an i.v. dexamethasone suppression test. A baseline cortisol was 613 nmol/l. Following an i.v. dexamethasone infusion of 5 μg/kg per h given for 5 h, samples were collected at 7 and 9 h, both showed cortisol levels <50 nmol/l.

The i.v. dexamethasone suppression test is usually indicated in cases where an oral dexamethasone suppression test is considered to be false positive and this has been evaluated in patients with simple obesity, PCOS, and Cushing’s disease and has been shown to be diagnostically accurate. This case demonstrates the diagnostic utility of the i.v. dexamethasone suppression test in a patient taking an enzyme inducing drug who also has a raised BMI.

Reference

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P57
Granulomatous infiltration of the pituitary gland
Jackie Gilbert, Victor Oguntolo, Dulka Manawada & Jon Salisbury
Kings College Hospital, London, UK.

Pituitary sarcoidosis is a rare form of neurosarcoid, commonly associated with multisystemic sarcoidosis. The most common intracranial site of sarcoidosis leading to endocrine involvement is the hypothalamus and the pituitary gland. The prevalence of hypothalamic-pituitary involvement in multisystemic sarcoidosis is reported to be around 3%. Granulomatous infiltration of the hypothalamus and the pituitary gland always results in endocrine disorder, GH deficiency and diabetes insipidus in about 40% of cases.

We report a case of pituitary sarcoid without the multi-organ involvement typical observed in systemic sarcoidosis.

Case report
A 24-year-old male presented with 2 months history of headache, fatigue, loss of libido, polyuria and polydipsia. Clinical examination revealed multiple enlarged lymph nodes within the neck. Visual field assessment showed a bitemporal hemianopia. A Computed Tomogram scan revealed mediastinal, hilar and retroperitoneal lymph nodes.

Endocrine tests confirmed diabetes insipidus and panhypopituitarism with undetectable gonadotrophins, low testosterone, cortisol, free thyroxine, and insulin-like growth factor 1.

A pituitary MRI demonstrated an enhancing intrasellar and suprasellar mass in contact with the optic chiasm. There was a loss of the pituitary bright spot. A lymph node biopsy was performed which revealed well formed non-caseating epithelioid granulomas with numerous giant cells. Ziehl-Nielsen staining for mycobacteria and PAS staining for fungi were all negative. A diagnosis of sarcoidosis was made based on clinical, biochemical and histology results. He was commenced on high dose corticosteroids, anterior and posterior pituitary hormone replacement with levothyroxine, testosterone, hydrocortisone and desmopressin.

Clinical course
Two months after the initial presentation, and the initiation of steroid, the patient described significant improvement in his symptoms. A repeat visual field examination was normal. A follow up pituitary MRI revealed a marked reduction in the bulk of the pituitary mass.

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P58
Audit on the safety of the insulin stress tests in a tertiary endocrine referral centre
Jen Min Ng, Myint Aye, Leanne Ward, Deepa Narayanan, Stephen L Aitkin & Eric S Kilpatrick
1Michael White Research Centre, Hull, UK; 2Department of Clinical Biochemistry, Hull, UK.

Background
The insulin stress test (IST) is accepted as the gold standard investigation of the hypothalamic-pituitary-adrenal axis (HPA). The test has been shown to be reliable though potentially unpleasant for the patient and has been associated with complications such as loss of consciousness, seizures, arrhythmias, angina, myocardial infarction and death. To this effect close medical supervision is always required when an IST is performed.

Methods
We have audited the number of adverse events for all of the IST’s performed in our tertiary referral centre over a 2 year period. All patients who underwent an IST from October 2007 to October 2009 were included in the analysis. Data was obtained from the central laboratory and from review of patient and nursing notes.

Results
There were a total of 90 patients who had an IST done over the study period. There were 8 patients (9%) who met 1 of the following criteria: (mean age 42.7 (range 15 to 52) A history of ischaemic heart disease, previous seizures and an abnormal ECG were considered absolute contraindications to performing an IST. There were 2 (2.2%) adverse events over the 2 years. One patient developed chest pain and required admission to hospital. The ECG performed prior and after the test was normal. The troponin test was normal and the patient was discharged the day after a diagnosis of non cardiac chest pain. One other lost consciousness and required third party help for correction of her hypoglycaemia.

Conclusion
Our audit re-highlights the importance of experienced medical supervision when conducting an IST due the potential adverse risks associated with the procedure that can be considered safe in the correct hands.

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P59
The use of insulin stress test as an assessment of tiredness
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1Michael White Diabetic Centre, Hull, UK; 2Department of Clinical Biochemistry, Hull, UK.

Background
The insulin stress test (IST) is accepted as the gold standard investigation of the hypothalamic-pituitary-adrenal axis (HPA). The short synacthen test (SST) has been advocated as an alternative to the IST. Fatigue can be a common presenting symptom of patients suffering from abnormalities in the HPA axis.

Methods
We audited the results of ISTs following failed SSTs for the assessment of patients who present with symptoms of fatigue. We included all patients from July 2007 to July 2009 who failed a short synacthen test (defined as a cortisol of <540 nmol/l after 30 min) who subsequently had an IST. Data was obtained through the central laboratory database serving the Trust and review of patient notes and clinical letters.

Results
There were a total of 16 patients who were included in this analysis. All patients presented with fatigue with no abnormal physical signs and had normal biochemistry and full blood count. Of the 16 patients (14 F, 2 M mean age 44.7 years), 12 (75%) patients passed the IST (peak cortisol > 500 nmol/l) and four (25%) failed to achieve this value. None of the 30 min cortisol results from the SST were below 400 nmol/l. Of the four patients who failed the IST, three patients had autoimmune disease (two with hypo/hyperthyroidism, one with pernicious anaemia). Of the 12 patients who passed the IST, only 1 patient had a previously established autoimmune disease.

Conclusion
For patients with fatigue and an established autoimmune disease the IST is most likely to show an abnormality of the HPA axis following a suboptimal SST.
P60
A case of hypomagnesemia
Pradeep Kurar & Godwin Simon
Royal Blackburn Hospital, Blackburn, UK.

Low potassium and magnesium are common findings found in patients seen on acute medical take which is often not followed up.

A 23-year-old previously well Asian lady, presented with history of fatigue, weight loss and palpitations. She has been always thirsty and polyuric at night. There was history of tetany in childhood. There was a family history of an aunt who had Barter’s syndrome and her parents were first cousins.

She had low potassium (2.7–3.3 mmol/l) and low magnesium (0.08 mmol/l). Her symptoms returned with fall in magnesium levels to 0.21–0.3 mmol/l and was put back on fortnightly magnesium infusions for the last more than 3 years. Currently she is on 16 tablets of oral magnesium, 20 mg BD of Amiloride and fortnightly magnesium infusions.

Currently pre infusion magnesium levels are 0.24–0.56 mmol/l and post infusion levels are 1.5 mmol/l and patient continues to be asymptomatic. This case illustrates the need for follow up investigations for these electrolyte abnormalities.

P61
Hyperandrogenism with an abdominal mass but an ovarian source
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A 55-year-old female presented to her General Practitioner with hirsutism, male pattern baldness, weight gain and a deepening of voice. Her past medical history included well controlled type 2 diabetes on glimepiride, and hypertension for menorrhagia.

She had an elevated serum testosterone after solvent extraction (18 mmol/l) and free androgen index (17.6). The patient was referred for further investigation.

17-Hydroxyprogesterone and dehydroepiandrosterone-sulphate were within reference limits. Androstenedione was slightly elevated. Urinary cortisol was not elevated and suppressed following dexamethasone, however testosterone remained elevated.

Abdominal CT scan revealed a lesion above the right adrenal bed which was suspicious of malignancy. The scan also suggested a bulky left ovary, however trans-vaginal ultrasound was unremarkable. Catecholamines and CA-125 were normal.

She underwent laparoscopic resection of her right-sided abdominal mass, histopathology of which was reported as an insulina. Post-operatively she required insulin to control her diabetes, possibly following the reduction in insulin secretion.

Her testosterone fell immediately to 1.2 nmol/l, however later climbed again to 12.8 nmol/l. She received a trial dose of a gonadotrophin-releasing hormone analogue and the serum testosterone concentration fell to 0.6 nmol/l. This indicated an ovarian source for the excess androgens. She then underwent bilateral salpingo-oophorectomy.

Pathology revealed marked stromal hyperplasia in both ovaries which was likely to be the cause of her hyperandrogenism.

P62
Diabetes insipidus secondary to caseating granulomatous disease
Taimour Alam, Steven Thomas & Vijay Bangar
Calderdale Royal Hospital, Halifax, UK.

We present a case of a 25-year-old male with no significant past medical history presenting with extensive bilateral cervical lymphadenopathy, thirst and polyuria.

He denied night sweats, weight loss or a change in appetite. There was no reported previous contact with tuberculosis and he had not travelled to high risk areas. A water deprivation test was consistent with a diagnosis of diabetes insipidus.

Computerised Tomography scanning of the chest demonstrated extensive lymphadenopathy throughout the neck, chest and abdomen in addition to patchy change in the right lung apex. Cervical lymph node biopsy showed caseating granuloma. Ziehl-Neelsen stain was negative but unfortunately samples were not sent for culture for Mycobacterium tuberculosis at the time of biopsy. Assessment of anterior pituitary function was normal as was pituitary imaging using magnetic resonance imaging.

The patient went on to have a bronchoscopy which was unremarkable. Upper lobe bronchial biopsy samples have shown no evidence of granulomatous change with no acid-fast bacilli seen on auramine staining. Results from lavage samples as well as Quantiferon-TB GOLD are awaited. Original lymph node samples have been sent for PCR for tuberculosis. The patient is likely to require further lymph node biopsy in order to obtain samples for isolated acid-fast bacilli.

Whilst it is clear that this patient has cranial diabetes insipidus, we are still challenged as to what the primary cause is. Cranial diabetes insipidus due to sarcoidosis is rare; that due to tuberculosis without TB meningitis is extremely rare. Caseating granuloma is classical but not exclusive for tuberculosis. Treating the patient with both steroids and tuberculous drugs could prove to be extremely problematic should he go on to develop complications of treatment such as hepatitis. This presents us with a challenge as how best to proceed.

P63
Case of post menopausal steroid cell ovarian tumor
Anupam Brahma, Maya Vema, T Duncan & G Ceccarelli
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Androgen secreting ovarian tumours account for 0.1% of ovarian tumours. They can appear at any age (mean, 42 years) and can be slow growing.

We report a case of androgen secreting ovarian tumour diagnosed at a delayed stage. It is unusual as it evaded diagnosis because of various factors, which we believe should be learning lessons.

A 56-year-old lady with medical history of sub-arachnoid haemorrhage (SAH), presented with features of gradual onset abdominal discomfort. GP was concerned of malignancy.

On examination, she was found to be strikingly virilised. Her endocrine assessment showed testosterone above 52 nmol/l (0.2–2.9), androstenedione >35 nmol/l (4–10.2) with DHEAS at 0.9 nmol/l (0.4–4.7) with <0.1 nmol/l of FSH/LH. She had raised ovarian tumour marker CA 125 >1183 (0–35 KU) raising the possibility of ovarian malignancy. CT abdomen/pelvis confirmed a right ovarian mass with normal appearing adrenals. She subsequently underwent bilateral salpingo-oophorectomy and hysterectomy. Histopathology confirmed the mass as Steroid cell tumour.

She was admitted to secondary care centre 30 years ago for sub-arachnoid haemorrhage. She had recorded features of Cushing’s and hirsutism during that admission. Her assessment then had shown normal testosterone, FSH/LH, prolactin, thyroid functions with negative screening for Cushing’s. She had several generalised seizures during the period of hospital stay for SAH managed with long-term phenytoin. She had started using a wig since 10 years because of male pattern balding and we could not find any medical consultation for that.

Steroid cell tumours are slow growing and we believe this might be a possible reason for the delay in diagnosis. Also the fact she had assessment for her hirsutism 30 years ago when she was acutely unwell. This emphasises the importance of repeating hormonal assessment when clinical suspicion is high and the unreliability of hormonal investigations, during an acute illness.

P64
A man with short stature and absent testis
Prashant Singh, Pat Pickett, Adwin Rookey, Prabal Moulik & Andrew MacLeod
The Hummingbird Diabetes Centre, Royal Shrewsbury Hospital, Shrewbury, UK.

A 63-year-old phenotypic male presented with absent testes and enlarged adrenal glands. He had attended the urology clinic for persistent microscopic haematuria. He was found to have an empty scrotum and hypospadias. The patient recalled having genital surgery in childhood but could not recall any endocrine assessment. He was taller than his peers in early childhood but stopped growing after age 13 years. He described himself as ‘potent’. He was unmarried and did not have any children.
He was 1.49 m tall and had an empty scrotum sac, small penis and hypogonadism. Endocrinological evaluation is summarized in the Table. Thirty minutes after an i.v. injection of 250 µg synacthen his cortisol went from 359 to 375 nmol/l. Urinary androgen profile was consistent with a diagnosis of congenital adrenal hyperplasia secondary to 21 hydroxylase deficiency. Ultrasound scan and magnetic resonance study failed to identify testis in abdomen or inguinal canal but both adrenal glands were enlarged. Congenital adrenal hyperplasia is a recognized cause of short stature and genital abnormalities, however, this man presented unusually late in life. His adrenal glands seem to be his only source of androgens. Despite an inadequate cortisol response to synacthen, he was able to withstand infections and surgery without any reported problems. His current needs are glucocorticoid replacement and androgen replacement as his adrenal glands are the sole source of his testosterone.

Table 1 Patient’s endocrine profile.

<table>
<thead>
<tr>
<th>Hormone</th>
<th>Levels</th>
<th>Reference range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Testosterone</td>
<td>22.2 nmol/l</td>
<td>9.9–27.8</td>
</tr>
<tr>
<td>FSH</td>
<td>136</td>
<td>1.0–11.0</td>
</tr>
<tr>
<td>LH</td>
<td>41.6</td>
<td>1.0–8.0</td>
</tr>
<tr>
<td>ACTH</td>
<td>&lt;46 ng/ml</td>
<td></td>
</tr>
<tr>
<td>Aldosterone</td>
<td>532 pmol/l</td>
<td>28–445</td>
</tr>
<tr>
<td>Plasma cortisol activity</td>
<td>16.30 nmol/l</td>
<td>0.39–2.03</td>
</tr>
<tr>
<td>17 Hydroxyprogesterone</td>
<td>&gt;300 nmol/l</td>
<td>0.9–4.1</td>
</tr>
<tr>
<td>DHEAS sulphate</td>
<td>17.4 µmol/l</td>
<td>1.1–7.9</td>
</tr>
<tr>
<td>D4-Androstenedione</td>
<td>&gt;34.9</td>
<td></td>
</tr>
</tbody>
</table>

P65

A rare case of isolated Cushing’s disease from an ACTH and GH staining pituitary adenoma

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A 31-year-old female presented with menstrual irregularities, weight gain and hirsutism. She was recently diagnosed with type 2 diabetes requiring Metformin therapy.

Investigations

Serum cortisol levels failed to suppress on overnight dexamethasone suppression test (ODST) and low dose dexamethasone suppression tests (LDDST) (ODST: unsuppressed cortisol at 343 nmol/l, LDDST 48 h post dexamethasone cortisol of 397 nmol/l). The serum cortisol showed <50% suppression from basal value on high dose dexamethasone suppression test (HDDST) (69.45 nmol/l). ACTH levels were ranging between 66 (0900 h) and 47 (midnight). Her 24 h urinary cortisol levels were elevated with values between 850 and 1000 nmol/24 h (normal range <250 nmol/24 h). Rest of the baseline pituitary hormone profile was normal.

MRI pituitary showed a 4 mm micro adenoma in the left lateral wing of the gland. In view of concordant clinical, biochemical and radiological results, diagnosis of Cushing’s disease was made.

Treatment

She was initially commenced on Metyrapone 250 mg tds. The pituitary adenoma was extirpated by transphenoidal surgery. Histology confirmed normal adenohypophysis and neurohypophysis with extensive immunoreactivity to GH and ACTH.

Follow up

Postoperative glucagon stimulation test confirmed inadequate ACTH reserve with peak cortisol of 176 nmol/l. GH reserve was adequate with no evidence of acromegaly.

Post operative MRI pituitary was normal.

Her diabetes mellitus reversed to impaired glucose tolerance test.

Two years post surgery, confirms remission with no biochemical or radiological evidence of recurrence.

Her recent glucagon stimulation test is now suggestive of inadequate GH reserve (peak GH 1.24 µg/l).

Conclusion

Our case illustrates a rare presentation of Cushing’s disease due to a dual staining pituitary adenoma. At the same time, it also raises the question of safety of treating such a case with GH replacement therapy.

P66

What a difference a dose makes! Unexpected test results lead to improved communication

Katherine Powell, Sondra Gorick & Mike Sampson

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We would like to present a case which, through unexpected results, caused us to examine the communication, both written and verbal, that is provided to patients prior to undergoing dynamic tests in our unit, and which subsequently led to a change in our practice.

A 48-year-old lady was referred from the GP with a raised testosterone of 5.0 nmol/l (reference range 0.2–2.9 nmol/l), irregular periods and late onset hirsutism. A low dose dexamethasone test (LDDT) was requested to exclude the possibility of an androgen secreting tumour.

Alarmingly, day 1 pre-dexamethasone 0900 h. cortisol was reported as 16.0 nmol/l (reference range 140–700 nmol/l). Telephone contact was immediately made with the patient, at which point she denied taking any steroids or over the counter medication which could affect the result.

An urgent short synacthen test (SST) was arranged for the next day, together with emergency oral hydrocortisone cover. GP surgery agreed to fax through a list of the patient’s medications, which arrived just prior to commencement of the SST. This included triamcinolone i.m. injection administered four days prior to day 1 LDDT. Even on direct questioning, the patient still denied receiving any steroid injection, until her husband reminded her that her tennis elbow had recently been treated with an injection. ‘Was that a steroid?’

This is an important reminder of the need to clarify terminology and to make no assumptions with regard to a patient’s understanding. On the strength of this, we have amended, and hopefully improved, the information sheet that is sent out to patients, not only for this test, but also for any dynamic test where cortisol is being measured. We are also more mindful of the way in which we phrase our verbal questions. Only by asking the right questions do we get the right answers!

P67

Hypercalcaemia due to PTH-related peptide secretion by small cell carcinoma of the ovary

Katherine Simpson, George Tharakan, Andy Coady, Malcolm Padwick & Michael Clements

Watford General Hospital, West Hertfordshire NHS Trust, Watford, UK.

A 45-year-old woman presented with a 6-week history of constipation and generalised abdominal pains. Blood biochemistry revealed a corrected serum calcium of 4.99 mmol/l, phosphate 0.82 mmol/l, and intact parathyroid hormone (i-PTH) of 0.2 pmol/l (normal range 1.6–6.1 pmol/l). Serum parathyroid hormone related peptide (PTH-P) was 5.3 pmol/l (normal range <1.8 pmol/l). Computed tomography demonstrated an 11.0 x 11.6 cm ovarian tumour with cystic and solid areas and high vascularity. The patient was treated with i.v. saline and disodium pamidronate. Over the course of 3 days the serum calcium fell to 2.42 mmol/l. The patient underwent laparotomy and the tumour was excised intact. Histopathology was consistent with a small cell undifferentiated carcinoma of the ovary. Exploration at the time of the operation revealed no evidence of metastases and no invasion of the tumour capsule or local lymph nodes. The patient is now well, normocalcaemic and has no evidence of recurrence 8 months postoperatively. Small cell carcinoma of the ovary is rare and can also cause inappropriate ADH secretion whilst small cell carcinoma of the vagina has been associated with ectopic Cushing’s syndrome. The prognosis of hypercalcaemia of malignancy is extremely poor. This case illustrates an unusual cause of malignant hypercalcaemia in which the outcome of treatment has so far been unexpectedly favourable.

P68

Low sodium and neurological findings: consider the diagnosis

Mary Jane Brassill & Antoinette Tuthill

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A 61-year-old gentleman, with a background of chronic schizophrenia, presented with hyponatraemia in 2002 and was diagnosed with Addison’s disease. He had two previous admissions with hyponatraemia in 2002 and 2006. During his first admission his hyponatraemia was attributed to volume depletion secondary to vomiting, and he responded to fluid rehydration. On the second occasion, SIADH (syndrome of inappropriate antidiuretic hormone) was diagnosed.
secondary to antipsychotic medication. The patient was noted to be wheelchair-bound on both occasions but there was no formal neurological diagnosis made. He was referred to Endocrinology out-patients in December 2008 for further management of his Addison’s disease. On examination he was noted to have a spastic paraparesis. There was no family history of adrenal insufficiency or neurological dysfunction.

Anti-adrenal antibodies were negative and very long chain fatty acid (VLCP) levels were requested. VLCPs showed increased C24 and C26 and C24/C22 and C26/C22 ratios consistent with a diagnosis of X-linked adrenoleukodystrophy; this was confirmed by subsequent genetic analysis with a missense mutation, c.761C>T in exon 1 of the ABCD1 gene. Family members have now been referred for genetic counselling and testing. This case highlights the importance of proper evaluation of hypotarnaemia, as well as testing for adrenoleukodystrophy in males with adrenal insufficiency without neurological findings.

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P69
Androgen-producing tumour in a transposed ovary: a diagnostic difficulty
Wasala M H S Chandrasekara, Saravanan Balaguruswamy, Sid McNulty & Niall Furlong
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Case report
A 52-year-old woman presented with an 11-month history of increasing hirsutism and deepening voice. She had a past history of total abdominal hysterectomy (ovaries preserved) with adjuvant chemotherapy and radiotherapy for cervical carcinoma. She was on no medication. Examination revealed evidence of virilisation with no other abnormality.

Investigations
Total testosterone = 10.7 nmol/l (<2.9).
FSH = 31.7 IU/l (37-125).
Androstenedione = 6.8 nmol/l (3.5-14).
LH = 35.5 IU/l (10.5-42).
DHEAS = 3.9 nmol/l (0.9-11.6).
E2 = 229 pmol/l.
Renal and liver function normal.
17α-OHPC = 4.7 nmol/l.

Overnight dexamethasone suppression test normal.
CT abdomen and pelvis showed normal adrenals with no ovarian mass but a ‘soft tissue mass’ (2.0×2.5 cm) was identified in the region of the proximal ascending colon. Barium enema showed a persistent induration in the bowel wall in mid ascending colon but colonoscopy was normal. An Octreotide stress CT scan revealed tracer uptake in the region of ascending/transverse colon with more marked uptake in right iliac fossa. Review of her gynaecology surgical notes revealed preservation of the right ovary with transposition to the right paracolic gutter (removing it from the field of subsequent radiotherapy). Further review of the imaging suggested a tumour within the transposed ovary.

Treatment
At laparotomy, the right ovary (4.0×2.0×1.5 cm) was identified in the right paracolic gutter and removed. Histology revealed a steroid cell tumour without capsular invasion (mixture of Leydig-like (predominant) and adrenal cortical like cells with mild atypia). Serum testosterone normalised post-operatively (1.4 nmol/l) and symptoms improved.

Discussion
Rapid onset of virilisation in a post-menopausal woman is usually due to androgen secreting tumour of adrenal or ovarian origin. In our patient, initial investigations were suggestive of a testosterone producing ovarian tumour but transposition of ovarian tissue during previous surgery created diagnostic difficulty. Endocrinologists should be aware of this aspect of gynaecological practice.

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P70
Insulin autoimmune syndrome: a rare case of hypoglycaemia
Suma Sugunanand & Mohamed Malik
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Background
Insulin autoimmune syndrome (IAS) is characterised by spontaneous hypoglycaemia in the absence of exogenous insulin administration and high serum levels of immunoreactive insulin along with high titres of insulin antibodies. Although it is the third leading cause of hypoglycaemia in Japan, it is very rare in the western world. We present the first reported case of IAS in the UK.

Case report
A 37-year-old lady from south east Asian background presented to Accident and Emergency with hypoglycaemia. The blood glucose was 2.9 mmol/l. There was no history of diabetes nor was she on any drugs causing hypoglycaemia. She was recently diagnosed with Graves disease and was started on Carbimazole. Hypoglycaemic work-up revealed 12 h fasting glucose of 2.5 mmol/l along with insulin – 17.750 pmol/l and C- Peptide-8520 pmol/l. Abdominal CT and MRI scans revealed normal pancreas. Serum insulin antibodies (IgG) was positive and Sulphonylurea area was negative. Genetic typing for HLA DRB1*0406 was negative.

The diagnosis of Carbimazole induced IAS was made. Discontinuation of carbimazole resulted in complete resolution of hypoglycaemia with normalisation of fasting glucose, insulin and C-peptide levels. She was later treated with radioiodine with good outcome and advised regarding offending drugs.

Discussion
IAS is one of the rare causes of hypoglycaemia. It occurs in the setting of autoimmune disease and has a strong HLA association. Many drugs containing sulphonylurea compounds can cause this syndrome in genetically susceptible individuals. The drugs implicated are Methimazole, Glutathione, Alpha mercaptopropionyl glycin, Alpha-lipoic-acid, Tolbutamidine, Imipenem and Hydralazine. Stopping these drugs can reverse hypoglycaemia.

Conclusion
We should consider IAS in the differential diagnosis of spontaneous hypoglycaemia especially in the setting of an autoimmune disease and/or receiving treatment with the implicated drugs. Thus the recognition of IAS can prevent unnecessary investigations and surgery in vain search of an insulinoma.

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P71
Improving patient awareness of antithyroid medications
Lina Pui Lin Chong & James Lawrence
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Background and aims
Antithyroid medications are first line treatment for thyrotoxicosis in the UK. The risk of agranulocytosis is ~1:2000 cases. Between 1963 and 2003, 809 adverse reactions related to antithyroid medications were reported. Of these 179 were due to Methimazole. Forty-two fatal events were recorded and half of these were related to agranulocytosis.

Patient awareness is vital in preventing drug associated morbidity and mortality. We conducted an audit to assess patient awareness of side effects relating to antithyroid medications.

Methods
An audit cycle was completed over a 2 year period. Patients taking antithyroid medications were given a questionnaire on knowledge of drug side effects. Data was collected over a 3-month period. Recommendations included handing out drug information sheet to patients and face-to-face communication at every clinic opportunity, and re-auditing.

Results
Fifty-four patients completed the questionnaire at the initial audit and 42 patients when repeated. At baseline, 42 patients (85%) were aware of some side effects, 30 patients (55%) were aware of agranulocytosis and 29 patients (53%) stated appropriate action in response to drug side effects. These figures were 86, 71 and 69% respectively after re-auditing. The initial audit found 30% patients remembered being given drug side effect information in clinic, which was more than doubled (69%) in the re-audit.

Conclusion
This audit highlights the importance of a proactive approach from clinicians, supplemented with written information, in raising patient awareness to potentially fatal and avoidable drug adverse events. Targeting other forms of communication such as education groups, media coverage and the internet may help maximise patient understanding.

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P72
A case of hypogonadotrophic hypogonadism due to pituitary stalk tuberculoma
Amalia Iliopoulou, Joachim Stolte & Dinesh Nagi
Pinderfields Hospital, Wakefield, West Yorkshire, UK.

A 25-year-old Asian man presented with a 6 month history of erectile dysfunction, loss of libido and reduced exercise tolerance. His past medical
P73

Recurrent pituitary apoplexy
Bini Krishnan, Voon Loh & Gul Bano
St George’s Hospital, Tooting, London, UK.

Pituitary apoplexy is uncommon and the incidence is quoted to be between 5 and 16% in pre-existing pituitary adenomas. Recurrent pituitary apoplexy is rarer; we report 2 case.
A 22-year-old female underwent transphenoidal resection of an expanding intrasellar lesion in 1987. She was commenced on hydrocortisone for cortisol deficiency and on subsequent follow-up, on thyroxine. She required repeated surgery in 2003 and 2005 after she presented with headaches. Imaging on both occasions confirmed haemorrhage into the pituitary lesion. She underwent stereotactic radiotherapy in 2006 and continues to be under follow-up.
A 45-year-old lady presented with sudden onset severe headache in 1998 and subsequent imaging confirmed pituitary apoplexy. She underwent transphenoidal hypophysectomy in 2004 following a similar presentation with headaches and pituitary haemorrhage on MRI. Following dynamic testing, she was commenced on thyroxine, hydrocortisone and growth hormone replacement for hypopituitarism. Postoperative MRI showed a small amount of residual pituitary tissue. She presented with a further episode of headache in 2006 and an MRI confirmed a large lesion filled with blood in the pituitary fossa suggestive of a bleed into the residual pituitary tumour. As her visual fields were normal, she was managed conservatively. She required a further transphenoidal surgery in 2007 due to enlargement of pituitary mass with visual field defect, followed by radiotherapy. She is under continued follow-up at the endocrine clinic.
The cause of the rare phenomenon of recurrent pituitary apoplexy is not fully understood. We hypothesise that this may be related to good vascular supply in younger patients, which makes them prone to recurrent pituitary bleeds.

P74

A case of connective tissue disease complicated by multiple metabolic disorders
Milan Pya1,2, Abd Tahraii1,2, Philip Dyer1, Jayadev Shakher1 & Alan Jones1
1Birmingham Heartlands Hospital, Birmingham, UK; 2University of Birmingham, Birmingham, UK.

A 24-year-old Pakistani woman presented one day after returning from a 6-week holiday in Pakistan with a 3-day history of generalised weakness, difficulty in walking and left flank pain. She was known to have mixed connective tissue disease (MCTD). Clinically she was pyrexial and had generalised muscular weakness (power 3/5), and hypotonia. Biochemically, she was found to have hypokalaemia (1.8 mmol/l), raised serum ura (8.1 mmol/l), and creatinine (160 mmol/l), high ESR (106 mmol/l), hypocalcaemia (1.96 mmol/l) and normal serum sodium. She had a metabolic acidosis (pH 7.14, HCO3− 11.2 mmol/l and a base deficit −16.8) with a normal lactate and (PCO2) and a normal anion gap of 15.5 mmol/l (12-20 mmol/l). Urine pH was 7.1. A diagnosis of distal renal tubular acidosis (RTA) was made in the view of hypokalaemic acidosis and an inability to acidify the urine. Additionally she was polyuric. The urine osmolality was 135 mOsm/kg and plasma osmolality 296 mOsm/kg. After DDAVP, urine osmolality was 185 and plasma osmolality 305. A diagnosis of nephrogenic diabetes insipidus possibly secondary to the potassium and calcium abnormalities was made. Further questioning revealed a history of intermittent diarrhoea, and further investigations showed vitamin D deficiency (3.8 μg/l, normal >20 μg/l) and a duodenal biopsy showed histological evidence of coeliac disease. The patient improved with antibiotics and electrolyte/ fluid replacement, initially in a high dependency unit (HDU). The patient was started on gluten-free diet, KCO3, Sando-K and ergocalciferol.
MCTD can be complicated by distal RTA, in turn resulting in the development of hypokalaemia and nephrogenic diabetes insipidus. The autoimmune disease was also associated with coeliac disease which may have contributed to vitamin D deficiency and hypocalcaemia. This case highlights that multiple metabolic complications can develop in the context of autoimmune disease. Such complications can be life-threatening and precipitate acute hospital admission.

P75

The dangers of drinking liquorice tea
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Salisbury District Hospital, Salisbury, UK.

A 59-year-old lady with headaches and malaise was found to be hypertensive with a blood pressure (BP) 210/105. An MRI brain revealed a haemorrhagic lesion in the right temporal lobe. There were no other signs of end organ damage. Her medications included atenolol 25 mg OD, ramipril 10 mg OD and bendroflumethiazide 2.5 mg OD. It transpired that she has been drinking 5 cups of liquorice tea per day for over the last 18 months. Following a vomiting illness she stopped liquorice tea consumption. Her BP normalised and antihypertensive medications were weaned off.
Biochemically, her potassium (K+) was 5.2 mmol/l, sodium (Na+) 139 mmol/l and creatinine 113 μmol/l. Retrospectively, she had low/nominal K+ (3.6-4.1 mmol/l) and Na+ at the upper limit of normal (142-145 mmol/l). Renin and aldosterone were not measured. However, given her history and biochemistry, the cause of hypertension is likely secondary to liquorice ingestion. Liquorice consumption is an unusual but well-reported cause of secondary hypertension. Excessive consumption of liquorice can cause hypertension and hypokalaemia, which may lead to cardiac arrhythmias and myopathy. The pathogenesis involves the action of glycyrrhetinic acid (GA), the active metabolite of liquorice, on the enzyme 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2). GA inhibits the action of 11β-HSD2 resulting in a mineralocorticoid excess state causing hypertension hypokalaemia, alkalosis, sodium and water retention, and suppression of renin-aldosterone system. The lowest observed dose resulting in adverse effects is 100 mg of GA daily. Hence, using a safety factor of 10, a daily intake of 10 mg GA per person is regarded as an acceptable safe dose. This means no more than 10-30 mg liquorice, i.e. no more than half a cup of liquorice tea per day. This case demonstrates a rare cause of hypertension and highlights the importance of taking a detailed history to avoid unnecessary investigations and treatment.

P76

A case of functional parathyroid adenoma
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A 60-year-old woman presented with 2 months of aching limbs and 2 weeks of nausea and a left sided neck mass. She was clinically euthyroid with a 5 cm non-tender left thyroid nodule. There was no cervical lymphadenopathy and the remainder of the examination was unremarkable. There was no family history of any endocrine disorder.
Investigations showed serum TSH concentration 5.94 μU/l and serum free T4 concentration 12 μmol/l. Thyroid peroxidase antibody titres were not raised.
P77

A rare cause of a common problem

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A 24-year-old Asian lady with no past medical history was admitted under the surgical team with right sided abdominal pain, nausea and vomiting, postural dizziness and weight loss. She was given threptomiphram for a possible urinary tract infection along with paracetamol, tramadol and diclofenac. Blood tests on admission demonstrated normal renal, liver and thyroid function, a normal C-reactive protein and a low sodium of 131 mmol/l. An abdominal ultrasound was unremarkable. Over the following few days her abdominal pain deteriorated thought to be a combination of constipation and menstruation. Four days into the admission she was found collapsed in the toilet with little recollection, having been incontinent of urine. She then suffered a witnessed generalised seizure which self terminated. Her sodium levels had fallen to 112 mmol/l, with urine osmolality of 700 mmol/kg and plasma osmolality of 240 mmol/kg. A CT head scan was normal.

She became increasingly confused and sexually disinhibited. She was biochemically dehydrated, hypotensive, tachycardic, afebrile, with no focal neurology and passing ‘port wine’ like urine. She was treated with i.v. normal saline. A synacthen test demonstrated good cortisol reserve with a 30 min sample of 1730 nmol/l.

Serum and urinary porphyrin levels were elevated, consistent with an acute porphyria attack. She improved following withdrawal of tramorphin and diclofenac, a high calorie diet, and infusion of 10% dextrose. Her sister was admitted the following week with a very similar history and course of illness and similar elevated blood and urinary porphyrins.

The episode of acute intermittent porphyria was thought to be precipitated by a combination of menstruation and a viral illness prior to admission. It was exacerbated by certain medications during the inpatient stay causing significant hypotenaemia through inappropriate anti-diuretic hormone (ADH) secretion.

P78

Chronic lymphocytic infundibulitis with visual field defects, partial hypopituitarism and diabetes insipidus

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A 68-year-old female was found to have a supra sellar mass on CT scanning for investigation of long-standing tremor. She had experienced thirst, polydipsia, polyuria, nocturia and malaise for 1 year. Primary hypothyroidism had been diagnosed 5 years earlier. A left temporal visual field defect found 3 years earlier had been attributed to a structural anomaly of the optic nerve head. MR scanning confirmed the presence of a mass lesion of the infundibulum with displacement of the optic chiasm.

Investigations

Visual fields revealed progression of the left visual field defect plus a right field defect. Anti-thyroid peroxidase antibodies > 500 IU/ml, anti-nuclear antibodies 1:1280, T3 21 pmol/l, T4 6.2 pmol/l, TSH < 0.02 mU/l (taking thyroxine 75 µg od), cortisol (0900 h) 448 nmol/l, FSH 0.5 mU/l, LH 0.1 mU/l, prolactin 4579 µIU/ml, IGF1 4.5 µg/ml, plasma osmolality 317 mOsm/kg and urine osmolality 226 mOsm/kg. Short Synacthen test: cortisol 0 min (0900) 244, 30 min 651 and 60 min 767 nmol/l. Metoclopramide test: prolactin 0 min 4126, 30 min 5465 and 60 min 4951 µIU/ml. CSF: protein 1.3 µl/l, white blood cells 130/mm³ (5% lymphocytes), ACE normal, AIP and HCG not measurable.

Impression

History, physical signs and investigations suggestive of chronic lymphocytic infundibulitis of at least 3 years duration. Treatment: methylprednisolone 250 mg i.v. daily for 3 days resulted in improvement in visual fields and shrinkage of the infundibular mass. Oral prednisolone 30 mg daily decreasing to 15 mg daily over 2 months resulted in further improvement in visual fields, pituitary function and diabetes insipidus with normal appearance of the infundibulum. We describe a new variant chronic form of lymphocytic infundibulitis with a good therapeutic response to glucocorticoid treatment.

P79

Mislaided adrenal carcinoma with recurrence of Cushings syndrome complicated by spontaneous hypoglycaemia

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A 34-year-old female presented with an 18 months history of secondary amenorrhoea, hirsutism, weight gain and low mood. Examination confirmed a typical stigmata of Cushings syndrome with skin thinning, purpura, acne, facial plethora, central adiposity and hypertension. Investigations revealed FT4 14.7 nmol/l, FT3 4.1 pmol/l, TSH 0.71 mU/l, prolactin 377 µmU/ml, FSH 3.5 µl, LH 2.2 µl, oestradiol 140 pmol/l, testosterone 5.1 nmol/l, undestoredinone 23.2 nmol/l, DHEAS 8.8 µmol/l, SHBG 24 nmol/l and urinary free cortisol 1244 nmol/l/24h. Pre low dose dexamethasone cortisol 672 nmol/l and ACTH<5.0 ng/l. Post low dose dexamethasone cortisol 600 nmol/l and post high dose dexamethasone cortisol 600 nmol/l CT scan demonstrated a 4.5 cm diameter right adrenal mass and no other abnormalities. Laparoscopic right adrenalectomy was performed at a tertiary centre and benign histology reported. At review 2 months post operatively Cushingoid features had improved and mean cortisol level on a day profile was 383 nmol/l. Four months post operatively Cushingoid features had worsened to presentation severity with a 0900 h cortisol 1054 nmol/l and ACTH<5.0 ng/l. CT scan demonstrated a 12.5 cm diameter mass invading the right lobe of the liver and pulmonary metastases. Biopsy of the liver lesion and review of original histology both confirmed adrenocortical carcinoma. Despite treatment with chemoembolisation, metyrapone, flucuconazole and mitotane she died 12 months post initial presentation. The last month of life was complicated by spontaneous hypoglycaemia which responded well to i.v. octreotide. Investigations confirmed the aetiology of the hypoglycaemia with IGF2 grossly elevated at 96.2 mmol/l and IGF2/IGF1 ratio > 30.

This case demonstrates the diagnostic pitfalls in histological diagnosis of adrenal carcinoma and the very rare complication of hypoglycaemia due to tumour secretion of IGF2.

P80

Successful treatment of severe premenstrual dysphoria by a GnRH analogue and hormone add-back

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A 41-year-old lady presented with a long history of very severe premenstrual dysphoria, commencing 2 weeks prior menstruation. Her symptoms included
behavioural problems and in particular aggression, impaired memory, palpitation, bloating and diarrhoea. She also experience cyclical left head and left eye pain without visual symptoms. She was para 3 + 0 with a past medical history of polycystic ovary syndrome, eventually treated by ovariectomy. During pregnancy her symptoms resolved, but subsequently they did not improve during three cycles of subcutaneous gonadotropin implant.

Previous unsuccessful treatments included tricyclic antidepressants, serotonin uptake inhibitors, vitamin B complex, Gamolenic acid and various "natural" remedies. On Dianette and other combined oral contraceptive pills, both with and without a pill free week, she felt worse. Treatment with continuous oestrogestrogen following insertion of a Mirena intrauterine device was unsuccessful as she was unable to tolerate the device.

Family history included primary hyperparathyroidism, primary thyroid failure, type 1 diabetes, stroke and depression. Investigations showed a normal short synarin test, bone profile, renal function, thyroid function, prolactin, CRF, full blood count and CT brain.

As her symptoms had become increasingly intolerable, she was treated with Nafarelin 200 µg intranasally bd, followed 2 months later by the addition of continuous combined hormone replacement therapy, using oestradiol hemihydrate 1 mg and hydrogestosterone 5 mg. She did not menstruate for the first 2 months and felt tired and lachrymatory. However, following the addition of HRT her symptoms completely resolved.

After 8 months she experienced some menstrual bleeding and recurrence of her dysphoric symptoms. Nafarelin was therefore increased to 400 mg bd and her add back changed to Tibolone 2.5 mg daily. This was followed by a complete and persistent resolution of symptoms.

P81
Hypertension in familial adenomatous polyposis (FAP): don't be coun't!
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A 36-year-old man, presenting to his GP with severe intermittent headaches, was found to have a blood pressure of 210/110 mmHg. He had been diagnosed with familial adenomatous polyposis (FAP) in early childhood, and had had a total colectomy with ileo-rectal anastomosis at the age of 17. Routine surveillance CT imaging the following month revealed an incidental finding of bilateral adrenal masses (right 4×2×2 cm, left 2.7×2.9 cm).

On assessment in our unit, the patient was found to still be hypertensive at 150/100 mmHg on lisinopril and amlodipine. Serum potassium concentration was normal at 3.8 mmol/l (ref. range 3.5–5.3). Plasma aldosterone was 590 pmol/l, with a plasma renin activity (PRA) of 0.2 pmol/m1 per h, giving an aldosterone:PRA ratio of 2950. Saline infusion testing revealed a lack of suppression of aldosterone (plasma levels >280 pmol/l).

Selective adrenal vein sampling (AVS) was undertaken, and indicated dominant secretion of aldosterone from the left adrenal gland, but without complete suppression of secretion from the right. Although large, it was felt that radiologically there were no other suspicious features in the right adrenal mass. With the additional feature of dominant left-sided aldosterone secretion in this case, it was decided that the patient would benefit from laparoscopic left adrenalectomy, and he is currently awaiting surgery.

Adrenal gland enlargement is commoner in patients with FAP than the general population, over and above the expected ascertainment bias because these patients undergo frequent abdominal imaging. The majority of adrenal tumours associated with FAP are non-functioning and require no treatment unless they enlarge. However, primary hyperaldosteronism has been reported previously in patients with FAP. Recent research had identified a potential pathophysiological link between adenomatous polyposis coli (APC) gene mutations (which cause FAP), and hyperaldosteronism. This may have implications for the long-term follow-up of patients with FAP.

P82
Audit of hypothyroid management in pregnancy
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Introduction
Hypothyroidism in pregnancy may be associated with neurodevelopmental delay of the unborn baby. Timely management of abnormal thyroid function tests (TFTs) in pregnancy is important. In our centre, some patients attend an Endocrinology-led dedicated antenatal thyroid clinic, whilst others attend an Obstetrician-led general antenatal clinic.

Objectives
The management of hypothyroid pregnant patients attending our centre was audited against the American Endocrine Society clinical practice guidelines which recommend targeted case finding at the first ante-natal clinic visit and thyroxine adjustment to maintain TSH ≤2.5 µU/ml.

Methods
Pregnant patients with hypothyroidism attending both clinics between 2007 and 2008 were identified from the biochemistry database. Data was recorded from case-notes including whether targeted case finding was done and gestation at 1st general antenatal clinic visit, 1st TSH measurement and referral to thyroid antenatal clinic. TSH values were recorded together with action following abnormal results.

Results
Fifty-three patients were identified. Targeted case finding was done only in 11 patients (20.7%). Fifty-two patients were seen in the general antenatal clinic in the 1st trimester (98%). Thirty patients (56.6%) were referred to the dedicated antenatal thyroid clinic (11 in the 1st trimester, 10 in the 2nd trimester and 9 in the 3rd trimester).

Conclusions
Management of hypothyroid patients attending the general antenatal clinic alone was suboptimal suggesting endocrinology involvement in these patients’ care is required. The results of this audit have implications both for education and service provision.

P81.1
Pituitary apoplexy in Cushing’s disease
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A 40-year-old female presented with lethargy, poor mobility and blurred vision for 2 weeks and 6 months of amenorrhoea. On examination she was markedly Cushingoid with proximal myopathy and normal visual fields. She was hypertensive, with peripheral oedema and leg ulcers. Bloods revealed leucocytosis, hyperglycaemia, and ketonuria. She was commenced on insulin. CXR was clear.

Endocrine review diagnosed Cushing’s syndrome. Cortisol did not suppress overnight with 1 mg dexamethasone but poor compliance limited a 24 h urine collection. Prolactin, T3FT, IGF1 were normal. MRI showed deviated pituitary stalk but no adenoma. CT chest and abdomen showed mild consolidation in the right lung but no masses or adrenal abnormalities.

Two weeks later, she collapsed with marked hypoxia and hypotension. CXR revealed a white-out of left lung. Despite ventilation and inotropes she died later that day. Post-mortem revealed a 9 mm basilar pituitary adenoma containing both fresh and old haemorrhage. ACTH levels (returning subsequently) were elevated. A diagnosis of fatal pituitary apoplexy was made. During resuscitation on ITU the patient had not been given glucocorticoids. The Coroner declared accidental death as the patient had been prescribed prophylactic clexane.

Though the reported incidence of pituitary apoplexy is around 10% in pituitary tumors, it rarely occurs in Cushing’s disease. It is usually seen in macroadenomas and there are only very few reported cases in literature of apoplexy in ACTH secreting microadenomas. Classical symptoms are headache, visual problems and meningism. In some cases patients may be asymptomatic like our case. Precipitating factors include pregnancy, pituitary stimulation tests, large tumour growth etc.

Apoplexy should be suspected in collapsed patients even with suspected, but unproven pituitary disease and steroid treatment initiated empirically in such emergencies. Also apoplexy, though rare, may be a higher risk in patients treated with even low dose anticoagulants.
Severe hypomagnesaemia associated with proton pump inhibitor therapy
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Hypomagnesaemia usually occurs secondary to decreased intestinal absorption or excessive renal excretion. There have been a few reports recently suggesting an association between PPIs and hypomagnesaemia. A 65-year-old lady was referred to the endocrine clinic for investigation of hypomagnesaemia found on routine testing. Serum magnesium was 0.27 mmol/l (normal 0.7–1.0), with corrected serum calcium of 2.0 mmol/l (2.12–2.63), and normal PTH and Vitamin D levels. Renal function was normal. She had no vomiting or diarrhoea, had a healthy diet and was not on diuretics. She had a history of peptic ulcer disease for which she was taking omeprazole. She had been commenced on calcichew D3 forte and magnesium glyceroxophosphate 24 mmol/day (6 tablets) by her GP. Despite this, repeat investigations in clinic revealed that serum magnesium was only 0.45 mmol/l (0.7–1.15). Serum potassium was 3.7 mmol/l (3.5–5.5), corrected calcium 2.3 mmol/l, PTH 40 pg/ml (15–65) and serum bicarbonate 25 mmol/l. Twenty-four hours urinary calcium and magnesium were low (0.2 mmol/l (2.5–7.5) and 0.5 mmol/l (3–5) respectively) excluding the possibility of a renal tubular defect causing the hypomagnesaemia.

Six months later serum magnesium was only 0.34 mmol/l despite magnesium supplements. Omeprazole was considered as a possible precipitating cause and was discontinued. Eight days later her magnesium level had risen to 0.66 mmol/l and after 6 weeks to normal (0.82 mmol/l). Unfortunately dyspeptic symptoms warranted recommencement of PPI therapy. Serum magnesium dropped to 0.55 mmol/l within 6 weeks and to 0.39 mmol/l within 16 weeks. Omeprazole was substituted by ranitidine and serum magnesium normalised.

Our patient had renal magnesium retention suggesting a failure of intestinal magnesium absorption. Her hypomagnesaemia resolved rapidly on cessation of PPI therapy. PPIs may reduce active magnesium absorption in the small intestine by affecting function of the transient receptor protein channel TRPM6. PPIs are widely used and clinicians need to be aware of potential metabolic sequelae.

Recurrent pregnancy-related hypokalaemia associated with Gitelman’s syndrome
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Gitelman’s syndrome (GS) is a rare autosomal recessive renal tubular disorder caused by defects of thiazide sensitive Na-CI co-transporter due to mutations of the SLC12A3 gene. We report a case of a 23-year-old woman, who presented with excessive tiredness in her second pregnancy. She had a past history of hypokalaemia in a previous pregnancy (attributed to ‘vomiting’), which required multiple admissions for i.v. potassium replacement despite oral potassium supplementation. Her serum potassium returned to normal following delivery.

In her second pregnancy she reported no history of vomiting, diarrhoea, laxative or diuretic use, or excessive intake of liquorice ingestion. She was normotensive with no Cushingoid features. Investigations revealed a severe hypokalemia metabolic alkalosis with serum potassium 2.5 mmol/l (3.5–5.5) and bicarbonate 42 mmol/l (22–30). Further investigations revealed: serum magnesium 0.60 mmol/l (0.7–1.0), corrected calcium 2.55 mmol/l (2.15–2.60), and phosphate 1.08 mmol/l (0.80–1.50). Urinary potassium excretion was 89 mmol/24 h (40–120). Urinary calcium excretion was low at 2.12 mmol/24 h (2.5–7.5) and cortisol excretion was normal at 64 mmol/24 h (50–350). Ambulant serum renin (38.3 ng/ml per h [1.5–7.5]) and aldosterone (2939 pmol/l [140–850]) were significantly elevated. A urine screen for diuretics was negative. She was treated with magnesium and potassium supplements but despite this required frequent hospital admissions for i.v. potassium replacement. Both pregnancies resulted in uneventful obstetric and neonatal outcomes.

Subsequent maternal DNA analysis detected one mutation of the SLC12A3 gene. This case illustrates that i) GS may be unmasked during pregnancy and ii) in GS increased tubular loss of potassium may result in severe pregnancy-related hypokalaemia. GS should be considered as a cause of failure related hypokalaemia where on-going renal losses are apparent and no other cause is evident. Pre-pregnancy counselling should be offered to women with GS who should be monitored closely.

An unusual cause of hyperparathyroidism
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A 28-year-old man presented to his GP in Turkey complaining of fatigue. As part of his work up, serum PTH was assessed and was found to be markedly elevated at 29.9 pmol/l (normal range 1.6–6.8), with normal calcium, phosphate, alkaline phosphatase, renal function, 1,25- and 25-hydroxy vitamin D3 levels. A DEXA scan was normal and a Sestamibi scan did not reveal any parathyroid adenoma. Prior to any further investigations or treatment, he moved to the UK. He represented to a local UK GP explaining his ‘hyperparathyroidism’ and previous investigations in Turkey. Repeat measurement of PTH was however normal at 1.4 pmol/l (normal range 0.5–4.4). He was reassured but insisted on further investigations, which showed a high PTH of 35.4 pmol/l. He was then referred on to our unit.

The patient’s history and examination were normal and an erroneous result was suspected. Our laboratory had recently replaced its analytical equipment in between the UK based PTH measurements (Siemens Immulite 2500 replaced by Roche Cobas 6000) and we investigated the possibility that analytical interference could explain the discrepancy between the two assays. Analysis after PEG precipitation or incubation in a heterophile antibody blocking tube yielded normal PTH levels, indicating the presence of heterophile or anti-animal antibodies. An explanation of the sequence of events was made to the patient and he was reassured. He was also made aware that antibody interference could potentially affect other blood tests.

Antibody interference can cause falsely high or low results. It should be considered when results do not fit the clinical picture, and particularly when discrepant results are obtained using different analytical methods. Although this is well described in the context of thyroid function tests, to our knowledge this is the first case described in the UK of antibody interference in a PTH assay.

Cushing’s syndrome: a rare cause of hypertension in pregnancy
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A 34-year-old primigravida, 11 weeks into pregnancy, presented with hypertension (blood pressure 170/110 mmHg) proteinuria and hypokalaemia. She gave a 6-week history of tiredness, weight gain, and easy bruising. On examination, there was marked facial puffiness, non pitting oedema, thin skin with multiple bruises, marked proximal myopathy and no pigmented striae. Pregnancy associated Cushing’s was suspected.

Investigations
Hypokalaemia (K+ 2.9 mmol/l) and alkalosis, otherwise normal renal function.
Urinalysis confirmed proteinuria. Midnight cortisol 755 nmol/l, corresponding ACTH undetectable. USG showed a 12 week normal viable foetus. Urinary cortisol was 9552 and 11 344 nmol/l on successful dexamethasone suppression tests showed no suppression of cortisol levels. Adrenal androgen profile was unremarkable apart from a mildly raised androstenedione level. Random aldosterone level and random plasma renin activity were mildly suppressed. Serum and urinary metanephrines were normal.
A non contrast MRI showed a 4 cm left sided adrenal mass.
Blood pressure was controlled with methyl dopa, and potassium corrected orally.
She underwent laparoscopic adrenal surgery at 12 weeks gestation. Histology was in keeping with an adrenocortical tumour. Immediately post operatively, hydrocortisone replacement therapy was instituted. Within 24 h, off all treatment, her blood pressure and hypokalaemia had normalised. Proteinuria, as well as clinical features of cortisol excess disappeared within 6 weeks. To date, the pregnancy is progressing normally. Full re-assessment of HPA axis, withdrawal of hydrocortisone and repeat imaging have all been deferred until after delivery.

However, ACTH is now detectable and pre dose cortisol is suggestive of early recovery of the contralateral gland.

The diagnosis of pregnancy associated Cushing’s syndrome is difficult to make, due to significant overlap of physical and biochemical findings. Less than 150 cases have been reported to date. Untreated this condition carries extremely high maternal and fetal morbidity and mortality, hence prompt recognition and treatment are crucial.
P87
An unusual case of vitamin D deficiency and Wernicke-Korsakoff syndrome
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A 30-year-old Eritrean female presented with a 1 month history of nausea and vomiting, with 15 kg weight loss but no bowel disturbance. Past medical history included an open cholecystectomy. There was no family history of mineral or bone disorders. She denied any prescribed/over-counter medication and abstained from alcohol. Examination revealed marked cachexia (BMI 16.1 kg/m²). ECG revealed ST and T wave inversion. Investigations indicated profound mineral deficiency (Na 135 mmol/L, K 2.6 mmol/L, phosphate 0.35 mmol/L, corrected calcium 2.09 mmol/L, magnesium 0.66 mmol/L), severe vitamin D deficiency (<4.0 μg/L) with secondary hyperparathyroidism (PTH 112 ng/L, 10-70 ng/L). Liver, renal, thyroid function and vitamin A levels were normal. The presence of a metabolic acidosis (pH 7.28, bicarbonate 19 mmol/L) with a urinary pH of 6.0, suggested a diagnosis of Type 2 renal tubular acidosis due to profound vitamin D deficiency.

Anti-tissue transglutaminase, faecal elastase and calprotectin levels were normal as was a porphyria screen. Liver ultrasound, abdominal CT, oesophagogastroduodenoscopy and video capsule enteroscopy were all normal. Therefore no malabsorptive cause of vitamin D deficiency was found.

She initially declined all forms of vitamin and mineral replacement. She subsequently developed acute confusion, visual disturbance with bilateral nystagmus and ataxia. Acute Wernicke-Korsakoff syndrome was suspected. Symptoms resolved after treatment with i.v. thiamine, i.m. ergocalciferol, and calcium supplementation. Brain MRI demonstrated abnormal bilateral T2 hyperintensity of the mamillary bodies, periaqueductal gray matter, medial thalami and inferior cerebral peduncle in keeping with Wernicke’s encephalopathy. Cerebrospinal fluid analysis did not reveal dietary restriction in the context of anxiety from refusal of asylum status. This unusual case of profound vitamin D and B1 deficiency illustrates the devastating effects of dietary restriction and malnutrition.

P88
Phaeochromocytoma/paragangliomata patients in a joint endocrine genetic clinic setting
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A 19-year-old student was referred with a history of increasing anxiety attacks, palpitations and breathlessness. Blood pressure and urinary catecholamine levels were elevated. MRI imaging confirmed a para-aortic phaeochromocytoma and MIBG scan highlighted bony metastases. The patient underwent surgery for the primary lesion and targeted MIBG therapy for metastases.

Age of onset, malignant disease and metastases increase the likelihood of a genetic cause and family history revealed a paternal uncle who died from renal cell cancer aged 22. The patient underwent genetic counselling and testing and a heterozygous SDHB splice site mutation was found (c.423 + 1G > A).

DNA testing of relatives to date has identified another 7 mutation carriers (age 7-74 years) in the family with metastatic and paragangliomata disease. SDHB mutation carriers are more likely to develop malignant disease including renal cell and thyroid cell carcinomas.

Paragangliomatas and phaeochromocytomas are rare complex tumours requiring tertiary care and a multidisciplinary approach, and genetic classification of these tumours is key to follow-up screening and treatment protocols. 25-30% may be familial associated with one of six genes inherited in an autosomal dominant pattern (SDHB, C or D, VHL, RET or rarely NF1).

With the establishment of our joint endocrine genetic clinic, and open access to an endocrine genetic clinical nurse specialist, patients and families receive optimum streamlined care and management.

Costly and time consuming genetic testing is prioritised according to clinical risk factors such as age at onset, sex, tumour site and number and accurate family history.

15/26 phaeochromocytoma/ paragangliomata patients tested in our clinic between 2007 and 2009 were sporadic in origin. 9/26 were familial tumours and prompted 21 pre-symptomatic cascade relative tests. Ten (47.6%) of these were found to have a mutation.

Accurate and supportive counselling is essential prior to genetic screening. Cascade screening/pre-symptomatic testing for relatives of gene positive patients provides early diagnosis and best practise preventative medicine in a specialist clinic setting.

P89
Severe primary hyperparathyroidism (PHPT) and osteomalacia in pregnancy complicated by post parathyroidectomy hypocalcemia related transient congestive cardiac failure (CCF): a case report
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Introduction
PHPT is reported to be rare in pregnancy, with 150 cases described in the literature. Its incidence is 8/100 000 in childbearing women/year. PHPT may lead to a loss of up to 50% of pregnancies through miscarriage or stillbirth, dehydration, hyperemesis, nephrolithiasis, pre-eclampsia, fractures and pancreatitis in mothers; and death, hypocalcaemia and tetany in neonates. Intratertine growth retardation, low birth weight and pre-term labour were described. Rate of maternal and neonatal complications reaches 80%.

Case presentation
Twenty-one-year-old female was referred to clinic, complaining of generalised myalgia/arthralgia for 1 year. Diagnoses of PHPT and osteomalacia (CCA 2.66 mmol/L, PTH 142 pmol/L, ALP1700 IU/L, vitamin D13 mmol/L, skeletal survey) was made. USS of neck and Tc99m sestamibi scan confirmed presence of single large parathyroid adenoma. Parathyroid surgery was planned.

She DNA’d to any further appointments until 14 weeks pregnant, when she was seen in joint endocrine and antenatal clinic and underwent minimally invasive parathyroid surgery at 18 ±3 weeks, and benign nature of adenoma was histologically confirmed.

Post-operatively, she developed symptomatic hypocalcaemia and required daily i.v. infusions of 100 ml 10% of calcium gluconate for 5 days and pre-operatively commenced, oral treatment. On day 6, post-operatively; breathlessness and orthopnoea developed. Clinical examination revealed bilateral lung crepitations with lower limbs and bilateral pedal oedema. Chest X-ray indicated left ventricular failure and cardiomegaly. Troponin T was negative. ECG normal. CCA was 1.87 mmol/L. She started on diuretics and calcium replacement and swiftly recovered. Next day ECHO showed normal LVVRF and LVEF 70%: CCF secondary to hypocalcaemia-induced transient cardiomyopathy diagnosed.

Patient monitored in antenatal clinic until healthy baby delivered at 38 weeks.

Conclusion
We show a multidisciplinary approach to management of PHPT in pregnancy and safety of the parathyroidectomy in 2nd trimester. Post-operative cardiomyopathy and CCF, exacerbated by ‘hungry bones’ and vitamin D deficiency related hypocalcaemia, is the first report of such complication in pregnancy.
P91

Failure of renin determined by immunnoassay to suppress in a case of adrenocortical carcinoma secreting excess mineralocorticoid
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A 48-year-old woman presented to her GP with polyuria and proximal muscle weakness. She was found to have newly developed hypertension at 180/96 and her serum potassium was 2.7 mmol/l. Lisinopril 40 mg od and Slow-K 1 tablet bd were started and she was referred for further investigation. On clinical review blood pressure was 188/110 supine and 190/110 standing. Serum sodium was 147 mmol/l, potassium 3.7 mmol/l and total bicarbonate 29 mmol/l. Her Lisinopril was replaced with Doxazosin and her Slow-K dose was increased to 2 tablets tds. Six weeks later, serum potassium was 4.0 mmol/l, urinary sodium excretion was 100 mmol/24 h and potassium excretion was 110 mmol/24 h. After overnight recumbency, plasma renin determined by immunnoassay was 36.4 µU (supine 2–30), 35.7 µU (ambulant 3–40), aldosterone was 104 ng/l (10–160 ng/l) and 11-deoxy cortisol was 71.3 µmol/l (<20.9 µmol/l). Serum cortisol suppressed to 34 nmol/l in a 1 mg overnight dexamethasone test. Urine catecholamine excretion was normal. Abdominal CT revealed a 6.6 x 7.8 cm left adrenal mass with appearances suggestive of an adenocortical carcinoma. This was surgically excised by radical adrenalectomy together with her left kidney and spleen. In contrast to the result of renin immunnoassay measurement made earlier, plasma renin activity measured preoperatively was <1.1 pmol/l per hr (2.8–4.3 pmol/l per hr).

Postoperatively her blood pressure normalised rapidly (125/72), her serum potassium rose to 5.0 mmol/l, and she maintained an eGFR of 54 ml/min consistent with unilateral nephrectomy. She remains normotensive and normokalaemic whilst taking adjuvant Mitotane prophylactically. There is an increasing trend for clinical laboratories to report plasma renin by immunnoassay rather than by activity. In this case plasma renin level measured by immunnoassay failed to suppress despite clear evidence of excess mineralocorticoid secretion by an adrenocortical carcinoma. The possibility that renin determinations by immunnoassay may occasionally generate false negative results in patients being screened for Conn’s syndrome should be examined.

P92

A case of spontaneously resolving occult ectopic ACTH-dependent Cushing’s syndrome
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A 70-year-old woman was referred to the endocrine clinic with recently diagnosed diabetes mellitus and poorly controlled systemic hypertension. At presentation she had clinical features of Cushing’s syndrome including central obesity, Cushingoïd facies and marked proximal myopathy. BP was 220/190. Initial biochemistry revealed plasma sodium 140 mmol/l, potassium 3.6 mmol/l and creatinine 82 mmol/l. Overnight 1 mg dexamethasone suppression test revealed cortisol 1148 mmol/l and 24-h urinary free cortisol was markedly increased at 2334 mmol/24 h. ACTH was also elevated at 169 ng/l (NR 0–50 ng/l). A diagnosis of ACTH-dependent Cushing’s syndrome was made. The patient was further investigated by means of low-dose dexamethasone suppression test (48-h cortisol 956 nmol/l). Pituitary MRI and whole body CT did not identify a tumour as source of excessive ACTH production. A corticotroph-releasing hormone stimulation test with petrolatum sinus sampling failed to identify a pituitary source of ACTH. We proceeded to an octreotide scan which revealed increased uptake in the right iliac fossa, however abdominal CT did not identify any tumour and she never developed gastrointestinal symptoms.

As a primary source of excess ACTH was not identifiable the patient was commenced on metyrapone 500 mg three times daily. This suppressed urinary free cortisol into the normal range (238 mmol/24 h). She had repeated octreotide scans at one and two years that remained unchanged however repeated CT scans did not show a source of ACTH. For the subsequent 8 years she continued to stay well. The clinical symptoms of Cushing’s syndrome resolved on metyrapone and urinary free cortisol remained normal.

Ten years from diagnosis she discontinued metyrapone of her own accord. Repeated urinary free cortisol measurements off metyrapone were found to be normal and no clinical features of Cushing’s syndrome have returned. There are no previous reports of spontaneously resolving ectopic ACTH-dependent Cushing’s syndrome and this should be considered in the evaluation of patients with ectopic ACTH-dependent Cushing’s.

P93

Reasons for treatment changes in hypogonadal men undergoing testosterone replacement
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Aims
Treatment regimes for low testosterone are well established but reasons for treatment change and patient preferences are less well known. We looked at the type of testosterone replacement, subsequent modification of replacement and reason for that change.

Methods
Data were collected from the electronic case notes of 50 patients on testosterone replacement for at least 6 months attending endocrine clinics in a University Teaching Hospital.

Results
Median age was 52 years (range: 19–88). Forty-two percent (n = 21) had primary gonadal failure, 42% secondary gonadal failure, 16% late onset hypogonadism. Transdermal preparations were used in 52% of cases for first line therapy of which Andropatch accounted for 77%, gel preparations 23%. Oral preparations were used in 4% and implants in 2%. Im. preparations were prescribed in 34% of cases, Testosterone enantate 6%, testosterone undecanoate 41% and testosterone propionate 53%. Three cases (6%) were unspecified. Two thirds of patients changed their treatment and a third of those changed medications at least twice. Recent clinic letters show transdermal preparations now account for 38% of treatments; Andropatch 5% and gel preparations 95%. Oral and implant preparations are no longer in use and unspecified treatments account for only 2% of cases. Im. preparations have increased to 60% of the total; Testosterone undecanoate 90% and testosterone propionate 10%. Patient inconvenience, ‘messy’ or ‘difficult to apply’, caused 21% of medication changes. Thirty-three percent of patients found their original treatment choice resulted in disappointing outcomes or no change in symptoms. Low testosterone was cited as a cause in 13% of cases. Six percent were changed due to raised haemacrit and 6% for unspecified side effects. Twenty-one percent were not recorded.

Conclusion
Our audit has identified a tendency to start patients on transdermal preparations but a high proportion will eventually require i.m. injections, and in particular testosterone undecanoate, for satisfactory control.
Evidence is emerging that patient-responsive clinical services may deliver improved outcomes.

Aims
To assess the perceptions of obese young people about weight and weight-management services.

Method
Anonymised, postal questionnaire survey of 116 obese young people (9–20 years), who had attended a clinic and/or participated in obesity research at our centre. Data was analysed using SPSS.

Results
Forty-four questionnaires were returned (38%). Respondent percentages are reported. 98, 75 and 75%, respectively, ‘agreed’ or ‘strongly agreed’ that they were motivated to lose weight, change eating and exercise habits. 91, 68 and 75%, respectively, were ‘likely’ or ‘very likely’ to go walking, attend a gym or participate in exercise with similar young people. 51 and 47%, respectively, reported ‘always’ feeling anxious or sad about their weight. 53 and 32%, respectively, ‘agreed’ or ‘strongly agreed’ that their weight affected their sports activities and social life. Perceived causes for obesity were eating habits (22%), lack of exercise (20%), family history (14%) and stress (11%). 71 and 82%, respectively, ‘agreed’ or ‘strongly agreed’ that their obesity affects their current health and will affect their adult health.

Motivation to lose weight was correlated with impact on friendships and social activities (r = 0.4, 0.3, respectively, P < 0.05) and with impact on sports activities, sadness and anxiety about weight (r = 0.5, 0.4, 0.6, respectively, P < 0.01). There was no correlation between motivation and perceived impact upon health.

Young people would like to receive information from a personal trainer (59%), dietician (54%) doctor (43%), group discussion (40%). The most popular information delivery formats were leaflets (42%) and website (40%).

Conclusions
An adolescent weight-management service incorporating personal mentors, peer support and website may harness motivation more successfully than the conventional medical model.

P95
Addison’s disease: a new indication for continuous s.c. insulin infusion (CSI)?
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Insulin induced hypoglycaemia is a life threatening complication in T1DM. Sub-optimal counter regulatory response, with hypocortisolaemia, threatens the recovery from insulin induced hypoglycaemia. We present a 28-year-old man with polyglandular autoimmune syndrome type 2 and life threatening hypoglycaemia, whose severe hypoglycaemic events were improved following introduction of CSI.

The patient was diagnosed with Addison’s disease in 2000 and T1DM in 2004. Basal bolus insulin treatment was associated with variable glycemia control, HbA1c 7.5–10.0%. He experienced recurrent unpredictable episodes of hypoglycaemia requiring third party intervention. Three episodes lead to hospitalisation, one where he was unconscious at home for 24 h. A lack of endogenous cortisol response may have prevented recovery from the hypoglycaemic event.

Intensive optimisation of hydrocortisone dose and basal insulin, including the use of continual glucose monitoring (GCM), proved difficult to cope with early morning hypoglycaemia and daytime episodes were not reduced despite the use of a carbohydrate counting approach.

CSI is indicated in patients with T1DM in those unable to maintain satisfactory glucose levels ‘without disabling hypoglycaemia’. Agreed goals for CSI were HbA1c < 7.5%, reduction in fear and rates of severe hypoglycaemia. CSI was initiated with a reduced basal rate for 4 h before waking/hydrocortisone. Post initiation GCM also demonstrate glucose peaks following hydrocortisone which can be addressed by an increase in basal rate at these times. He has met and maintained his goals since initiation of CSI. HbA1c has fallen from 8.6 to 7.3% with no hypoglycaemic events in the last 3 months. Suboptimal endogenous cortisol response is a threat to recovery from insulin induced hypoglycaemia. The ability to vary basal insulin rates is an effective way of adjusting to the variations in cortisol levels associated with oral hydrocortisone replacement that can be associated with profound and prolonged hypoglycaemic episodes.

P96
Primary antiphospholipid syndrome presenting as accelerated hypertension and adrenal haemorrhage associated with elevated urinary catecholamines
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A 51-year-old man with no significant past medical history presented with left flank pain, accelerated hypertension, progressive deterioration in renal function and left sided pleuritic chest pain. CPK raised pulmonary oedema and left adrenal haemorrhage. Urinary catecholamines were marginally elevated (24 h Urinary Metadrenalines 7.58 μmol/24 h) raising the possibility of an underlying phaeochromocytoma. Short synacthen test showed a sub-optimal response (0 mm cortisol 199 nmol/l, 30 min 433 nmol/l).

Routine coagulation screen showed a prolonged APTT (APTT 81.5, PT 4.0), prompting antiphospholipid syndrome (APS) screen. Anticardiolipin antibodies and lupus anticoagulant were present (IgG Cardiolipin Abs 21 GPLU/ml, IgM Cardiolipin Abs 45 MPLU/ml) consistent with an underlying diagnosis of APS. Autoimmune screen was negative consistent with primary APS. Diagnosis was confirmed on repeat testing 12 weeks later.

Blood pressure was well controlled with appropriate combination therapy. Significant acute kidney injury developed with peak creatinine 517 μmol/l. Urological analysis showed blood and protein. A further vesical scan was negative and renal biopsy performed. This showed features of microvascular ischaemia alone. Adrenal haemorrhage has reduced in size on serial imaging. Twenty-four hour catecholamine levels have fallen back into the normal range. Antihypertensive treatment has been withdrawn and renal function has improved significantly (creat 165 μmol/l). Therapeutic anticoagulation had been deferred due adrenal haemorrhage, but has now been instituted following serological confirmation of APS.

Conclusions
1. Adrenal haemorrhage more commonly presents with hypertension and hypoadrenalism rather than hypertensive crisis.
2. Adrenal haemorrhage should always raise the possibility of underlying APS in absence of sepsis, trauma and coagulopathy.
3. Rise in urinary catecholamines may be associated adrenal haemorrhage. Elevation is typically marginal and transient.

P97
The relationship between electrolyte disturbance and treatment with proton pump inhibitors
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Up-to-date, there have been 7 case reports of hypomagnesaemia associated with hypocalcaemia and hypokalaemia in patients on long-term treatment with proton-pump inhibitors (PPIs). The suggestion is that these reports present a tip of an iceberg in clinical practice. Inspired by a case of severe symptomatic electrolyte loss, which was corrected by discontinuation of PPIs, we performed an audit of a potential relationship between hypomagnesaemia and PPIs in our hospital.

From October 2008 until October 2009, our biochemistry department reported 34 cases of low magnesium (Mg) in the outpatient department and on the medical admission unit (26 female, age 65±14). Therapeutic information was available in 26/34 and included PPIs in 22/28. Other potential contributors to electrolyte disturbance could be speculated in 11/30 for whom the full medical history was available. Mean serum Mg was 0.5±0.1 mmol/l. Following treatment, which included PPI withdrawal in 1/22 and Mg infusion in 15/16 patients, it rose to 0.7±0.4 mmol/l, remaining subnormal in 10/18 patients in whom Mg was repeated. Serum calcium (Ca) was measured in 32/34 patients and was 2.08±0.24 mmol/l (presented as corrected Ca), with readings below reference range in 13/32. It increased to 2.24±0.13 mmol/l in 32/34 patients in whom the measurement was repeated, remaining low in 5/29 despite Ca and vitamin D supplements. Hypomagnesaemia was associated with hypokalaemia in 1/32 patients (mean 3.8±0.7 mmol/l). Three out of 18 patients were symptomatic with tetany (2) and seizures (1).

There is a little awareness of the relationship between PPI treatment and electrolyte disturbances in clinical practice. Despite hypomagnesaemia being a fairly common finding, PPI therapy rarely figures in the differential diagnosis. Without recognition of possible causality between the two, it is difficult to achieve electrolyte normalisation purely with replacement therapy. Greater recognition of this phenomenon is required and H2 antagonist substitution should be considered.
P98
Challenges in management of aggressive GH secreting tumors in adolescents
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Despite rarer in children than in adults, GH-secreting pituitary adenomas are often more aggressive, being challenging cases during childhood. Somatic changes (tall stature and acromegalic features), optic chiasma syndrome or metabolic and cardiovascular impact of GH excess trigger diagnosis and requires aggressive management. Treatment options are surgery, radiotherapy and medical therapy using somatostatin analogues, dopamine agonists and recently, GH receptor antagonists – Pegvisomant, all with pro and cons.

A girl aged 12 was admitted for cavernous sinus syndrome due to an aggressive pituitary adenoma. She was submitted twice to pituitary surgery at 3 months interval (Jan and Apr 2007), with partial tumor removal, immediately followed by y knife radiosurgery. Despite signs were not suggestive for GH secretion, immunohistochemistry proved intense GH secretion. GH and IGF-1 values certified autonomous GH secretion. She underwent a third surgery in Erlangen, Germany (Sep 2008), with a forth re-intervention in the same center in Jan 2008, with efficient debulking of suprasellar mass, but remnants in the cavernous sinuses. In the same time, since Oct 2007 she started SMSA Lanreotide, initially at 60, then at 90 mg/month, with poor response ( nadir GH=66 ng/ml), to which Cabergoline 3 mg/week was added since May 2009. In Sep 2008, she underwent fractionated additional stereotaxic radiotherapy in Erlangen, in the area of cavernous sinuses extension.

At the last admission (June 2009) the patient aged 16 years-old was 180 cm height, 66.5 kg and had only discreet facial acromegalic features. Pubertal development was Tanner stage B2P4. GH hypersecretion was detected with very high levels of serum GH without suppression during OGGT ( nadir serum GH 61.4 ng/ml) and increased serum IGF-1 (970 ng/ml, normal range for age 163–584 ng/ml). The thyroid hormones and cortisol tests was normal but she had hypogonadotropic hypogonadism. Pituitary MRI scan showed an intrasellar tumor mass with invasion of cavernous sinuses and retrostellar extension. Ophthalmologic examination revealed normal bilateral visual field but paresis of right common oculomotor nerve, while heath evaluation showed signs of acromegalic cardiomyopathy. She was treated with Cabergoline 3 mg/week and estradiol 1 mg/day for induction of puberty. At this time, due to the aggressive tumor and the lack of control of tumor secretion using extensive surgery, radiotherapy and high doses of somatostatin analogues, GH receptor antagonists therapy was started. The treatment with Pegvisomant 10 mg/day, well tolerated, without side effects. However, pro and cons concerning radiotherapy and extensive surgery, timing and availability of use of expensive radiopharmaceuticals in which fight against tumor must be balanced against a life-long preservation of pituitary function and visual pathway in a young patient. The use of Pegvisomant has shown great promise in adults with acromegaly, but experience in pediatric patients is lacking. In our patient a close follow-up is necessary.

P99
Vitamin status after gastric bypass and lifestyle intervention: a comparative prospective study
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Context
Bariatric surgery can lead to vitamin deficiencies.

Objective
To assess changes in blood vitamin concentrations after gastric bypass surgery.

Design
Twenty-nine patients underwent gastric bypass and 24 unmatched controls underwent lifestyle intervention in a prospective, non-randomized trial. Patients in the surgical group received multivitamin, iron, calcium, vitamin D, and vitamin B12 supplements. No supplements were prescribed to the lifestyle group. Median BMI (kg/m2) decreased from 46 to 32 after surgery and from 40 to 39 after lifestyle intervention.

Results
Out of 53 included patients, 50 completed the 1-year follow-up (94%). Compared with lifestyle patients, the surgical group had increased vitamin B-6, folic acid, vitamin B-12, and lipid-adjusted vitamin E (P < 0.020 for each concentration); but decreased vitamin A concentrations (P < 0.01) during follow-up. No significant difference between the groups was found for vitamin B-1, vitamin C, and 25-hydroxyvitamin D. Most surgical patients reported taking their supplements.

Conclusions
Gastric bypass patients adhering to a set of dietary supplements had mostly stable or increased vitamin concentrations, as compared both with their baseline values and with the changes in a non-surgical control group.

Ethics
The Regional Ethics Committee approved the study protocol. The trial was registered in ClinicalTrials.gov.

P100
An unusual case of hypercalcaemia in an HIV positive man
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A 60-year-old HIV positive man was referred from the gerontiurgical physicians for investigation of hypercalcaemia (calcium 3.20 mmol/L). He described non-specific symptoms of tiredness and polyuria. Past medical history included well-controlled HIV with an undetectable viral load, secondary syphilis and a history of renal stones. Medications included Didanosine, Emtricitabine, Darunavir and Ritonavir. Initial investigations demonstrated a suppressed parathyroid hormone (PTH) of 0.7 pmol/l, ESR 115 mm/h. Other investigations were normal including urinary calcium and 25-hydroxy Vitamin D (77.3 pg/ml). He had impaired renal function with a serum creatinine of 177 mmol/l and an eGFR of 35 units. Serum ACE was elevated at 110 IU/I (normal 8-65 IU/I). CT of the abdomen and chest demonstrated the presence of renal stones but no other abnormalities. In view of the suppressed PTH with no other cause apparent, referrals were sent to the endocrine department as well as the renal physicians in view of the impaired renal function. A renal biopsy was consistent with an interstitial nephritis, however in view of the presence of hypercalcaemia and elevated serum ACE, a diagnosis of sarcoidosis was suggested. On reviewing the radiology and renal histology in our unit, there was no good evidence of sarcoidosis. The patient admitted to prior injection of silicone into the genital area, which had been complicated by exudation of silicon into the dermis necessitating surgical debridement. Further histological examination of the debried tissue confirmed the presence of silicone granulomata. He was treated with prednisolone with rapid improvement in his hypercalcaemia. He is now steroid free and there are no plans for further debridement.

Hypercalcaemia associated with granuloma has been well-described – usually due to either sarcoidosis or tuberculosis. Silicone granuloma is a rare cause of hypercalcaemia and is usually associated with medical implantation of silicone such as in breast augmentation surgery. Silicone granulomata need to be considered in the differential diagnosis of unusual hypercalcaemia.
Further results are tabulated below:

Table 1

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<th>Pre-treatment</th>
<th>Post-treatment</th>
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<tr>
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<td>IGF1 (ref range 6–36 nmol/l)</td>
<td>IGF2 (ref range 6–36 nmol/l)</td>
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<tr>
<td>Case 1</td>
<td>5.5</td>
<td>10.2</td>
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<tr>
<td>Case 2</td>
<td>3.4</td>
<td>97.6</td>
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Treatment with dexamethasone (2 mg BD and 8 mg BD respectively) and recombinant GH (35 μg/kg daily) was initiated in both cases. Octreotide scans did not demonstrate increased uptake in either tumour. Despite re-embolisation of the leiomoma, case 1 remains GH and steroid dependent. Combined therapy with glucocorticoids and recombinant GH in these two cases has effectively controlled NICHTH.

P102

DIPNECH: precursor to pulmonary neuroendocrine tumors
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Diffuse idiopathic pulmonary neuroendocrine cell hyperplasia (DIPNECH) is regarded as a precursor to the development of carcinoid tumourlets. It is confined to the neuroendocrine cell of the airway mucosa without penetration through the basement membrane and appears in a diffuse pattern, typically in association with obliterative bronchiolar fibrosis. DIPNECH belongs to the preinvasive lesions defined by the 1999 World Health Organization classification, along with atypical adenomatous hyperplasia and squamous dysplasia – carcinomas in situ. A 58-year-old lady, with a background of Dukes A Carcinoma Colon resected 14 years previously, was referred with symptomatic primary parathyroidism and was subsequently found to have a two gland parathyroid hyperplasia on open neck exploration. She underwent subsequent imaging and biochemical tests to exclude Multiple Endocrine Neoplasia Type 1. These revealed a raised Somatostatin level on fasting gut hormone profile and a 4.3 cm lesion in the right middle lobe of lung along with multiple lung nodularities on thoracoabdominal CT scanning. A CT guided biopsy of the 4.3 cm lesion revealed the presence of neuroendocrine cells suggestive of carcinoid with concordance of the large lesion on Octreotide and MIBG scanning. No evidence of metastasis was revealed on PET–CT.

She subsequently underwent a middle and lower lobectomy and histology confirmed that the lesion to be a central thoracic carcinoid. The rest of the lung showed diffuse multiple foci of neuroendocrine tumourlets and multiple small carcinoids (DIPNECH) throughout with evidence of pulmonary thrombotic veno-occlusive phenomenon. It was felt that the central carcinoid was an independent finding with the surrounding DIPNECH being secondary to veno-occlusive disease.

A review of the literature suggests that DIPNECH is associated with peripheral sub-clinical carcinoid formation but does not lead to development of central bronchial carcinoids. DIPNECH neuroendocrine cells usually do not lead to clinically significant endocrinopathies and are in general an incidental finding. This case therefore highlights two seemingly unrelated rare pathologies.

P103

Ghrelin levels in patients with post hepatitis “C” liver cirrhosis and hepatocellular carcinoma (HCC)
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Background
Anoexia is a problem of paramount importance in patients with advanced liver cirrhosis contributing to malnutrition. In turn, malnutrition is a risk factor for the development of life-threatening complications and increased mortality. Ghrelin hormone; an important orexigenic agent has been postulated to integrate anabolic changes in the body.

Aim
To assess ghrelin level in Egyptian patients with liver cirrhosis and hepatocellular carcinoma.

Method
Ghrelin levels were determined in 25 patients with post-hepatic (HCV) liver cirrhosis, 25 patients with hepatocellular carcinoma and compared to 25 healthy controls. Ghrelin levels were correlated with the clinical and biochemical parameters.

Results
Ghrelin levels were significantly reduced in patients with post-hepatic liver cirrhosis (52.4±14 pg/ml) unlike patients with hepatocellular carcinoma who showed markedly elevated levels (453±36 pg/ml). BMI was significantly low in HCC than liver cirrhosis group (22.4±2.1 vs 17.6±0.9). Ghrelin levels were significantly higher in Child C patients in both groups. There was an inverse correlation with between serum ghrelin and BMI.

Conclusion
Ghrelin levels were significantly reduced in patients with post-hepatic liver cirrhosis in comparison to control. There was no significant difference between the liver cirrhosis and the control group in BMI. However, the low levels in liver cirrhosis group could be attributed to portal hypertension and portosystemic shunting present in the cirrhotic patients. Patients with hepatocellular carcinoma showed higher levels in serum ghrelin than the control group, this could be attributed to the markedly lowered BMI in the HCC. Ghrelin levels were significantly higher in Child C patients in both groups. There was an inverse correlation with BMI.

P104

A case of Verner–Morrison syndrome with solitary VIPoma and an incidental serous mucinous adenoma
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Introduction
Neuroendocrine tumours (NET) are very rare and often present a diagnostic challenge. They are often misdiagnosed due to non-specific symptoms. We report a rare case of NET presenting to a non endocrine unit.

Case
A 72-year-old Caucasian female was referred for refractory diarrhoea with hypokalaemia. In addition, there was history of significant weight loss over few months. She had past medical history of diabetes, hypertension and gastroesophageal reflux disease.

Given her symptoms, she was referred to the gastroenterology department. Her baseline bloods were normal albeit a low potassium. Colonoscopy revealed a benign rectal ulcer. Computed tomography of the abdomen showed a pancreatic tumour measuring 2.6x2.7x2.4 cm. This was further confirmed on magnetic resonance imaging.

After ruling out primary pancreatic malignancy, she underwent further investigations for a suspected NET.

Baseline fasting gut hormone profile identified elevated plasma vasoaduct intestinal polypeptide (VIP) (99, reference range 0–30 pmol/l) and chromogranin A levels. Her 24 h urinary 5-HIAA levels were normal.

The tumor confirmed somatostatin receptor positivity on radiolabelled Octreotide scanning with no evidence of metastasis. Given her symptoms with raised VIP levels in presence of a pancreatic tumour, a diagnosis of Verner–Morrison syndrome was made.

She was commenced on subcutaneous somatostatin analogue therapy for symptomatic relief. Later, she underwent left radical pancreatectomy with splenectomy. A separate incidental serous mucinous adenoma was identified on histology.

She remains asymptomatic off treatment, 6 months post surgery.

Conclusion
Verner-Morrison syndrome is the combination of watery diarrhoea, hypokalaemia and acidosis due to secretion of VIP from a neuroendocrine pancreatic tumour. VIPomas account for <10% of islet cell tumours. We report a rare case of VIPoma and also highlight the need to remain cognisant of NETs as possible differential diagnosis for persistent diarrhoea.
P105
Risk of metabolic syndrome among Egyptian patients with schizophrenia
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Background
The high prevalence of metabolic syndrome (MS) in schizophrenia has been increasingly recognized in the mental health literature in recent years. The MS is a cluster of the most dangerous known cardiac risk factors (diabetes and prediabetes, abdominal obesity, high cholesterol level, and high blood pressure BP; according to the International Diabetes Federation Criteria, 2007). The existence of an increased mortality risk among people with mental illness due to physical health factors has been demonstrated by several authors. Poor diet, lack of exercise, negative symptoms, stress, smoking, and abnormalities in the hypothalamic-pituitary-adrenal axis have all been proposed as precursors to the link.

Objectives
This study aimed to determine the prevalence of predictors of metabolic syndrome in a sample of Egyptian patients with schizophrenia attending Ain Shams University Psychiatry Hospital.

Methods
Sixty-three patients attending Ain Shams University Psychiatry Hospital were screened for the following: age, gender, waist circumference, blood pressure, high-density lipoprotein level, low-density lipoprotein level, blood sugar levels, total cholesterol level, triglycerides level, weight, body mass index, insulin resistance level, length of time on antipsychotics, antipsychotics dose, smoking status, family history of diabetes and cardiovascular disease, and personal history of polycystic ovarian syndrome.

Results
Sixty-three patients were screened for metabolic syndrome using the International Diabetes Federation’s (2007) definition. Twenty-four (38.9%) patients met the criteria for the syndrome. Increased waist circumference, higher body mass index, raised triglycerides level, increased blood sugar level, older age, which emerged as significant predictors of metabolic syndrome in the sample. Thirty-nine (61.91%) patients did not meet the full criteria for the syndrome; however, Thirty-one out of these 39 patients (49.2%) had 1–2 criterion of the syndrome.

Conclusion
This study adds further support for the systematic screening for metabolic syndrome in patients receiving antipsychotic drugs. The need for intervention programs that screen for and address the modifiable risk factors of metabolic syndrome is recommended.

P107
Audit of the management of metformin treated diabetic patients undergoing i.v. contrast procedures
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Background
Contrast procedures are frequently performed in diabetic patients and are the third most common cause of hospital-acquired renal failure 1. Diabetic patients are at risk of Metformin associated lactic acidosis in the presence of renal failure 2.

Standards
RCR guidelines 3 recommend:
- Recent serum creatinine should be available for patients with renal disease or diabetes.
- Metformin should be withheld 48-72 hours prior to and post procedure if creatinine is raised or > 100ml contrast or intra-arterial route is used.
- If creatinine is raised, renal function should be assessed before re-starting Metformin.

Indicators and targets
- Percentage of high risk patients with recent pre-procedure creatinine value.
- Percentage of patients in whom Metformin was withheld appropriately.
- Post-procedure renal monitoring in patients with renal failure.

Method
Data was collected retrospectively from the case-notes and electronic records of diabetic patients undergoing inpatient lower limb angiograms in 2007. A total of 19 episodes were audited.

Results
Only 37% of patients had a recent creatinine value available (1 week prior to procedure). Metformin was withheld in 77% of the cases but only half were in accordance with RCR guidelines. Neither of the two patients with renal failure had timely post-procedure creatinine values. Glycaemic management off Metformin was found variable and suboptimal.

P106
Hypocalcaemia following thyroid surgery: impact on length of stay
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Introduction
Hypocalcaemia secondary to post-operative hypoparathyroidism is a recognised complication of thyroid surgery with reported rates of up to 75%. Little guidance exists on the appropriate management of post-operative hypocalcaemia and individual clinicians may develop their own preferred management strategies based on past experience. However, delay in restoring normocalcaemia may increase length of hospital stay and patient morbidity.

Aims
1) To identify the local incidence of hypocalcaemia following completion and total thyroidectomy; ii) to determine the post-operative management of hypocalcaemia and its impact on length of stay at our centre; iii) to develop a ward guideline for hypocalcaemia management.

Methods
A retrospective review of patients undergoing total or completion thyroid surgery between 1st April 2007 and 31st March 2009. Patients undergoing total thyroidectomy were significantly more likely to develop hypocalcaemia (P<0.05). The mean length of stay (LOS) was 4.2 days. The development of hypocalcaemia was significantly associated with a prolonged LOS (P=0.007). Hypocalcaemia was the primary reason for delayed discharge in 11 patients (37.9%). Mean LOS for patients who developed hypocalcaemia was 7.0 days, compared with 2.0 days for those who did not. This resulted in an additional 55 days of hospital stay during the study period. The management of hypocalcaemia was found to be variable both for oral and intravenous therapy. At 6 months only 1 patient (5.6%) was requiring calcium supplementation.

Conclusions
Our rates of hypocalcaemia are in line with the published literature with a low incidence of permanent hypocalcaemia. Hypocalcaemia increases LOS and its management in our institution appears variable. We present a management algorithm for post-operative hypocalcaemia with the aims of maintaining uniformity of management, reducing LOS and reducing patient morbidity.

P108
Hypoparathyroidism treated with Teriparatide and I.m. vitamin D
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Introduction
Hypoparathyroidism is normally treated with calcium salts and activated vitamin D. Here we report a case where synthetic 1-34 PTH (teriparatide) and i.m. vitamin D was successful in treating a case of primary hypoparathyroidism resistant to conventional treatment.

Case report
A 16-year girl presented to the emergency department with vacant episodes and mild confusion. Her childhood was eventful until the age of 12 when she developed recurrent seizures. She was known to have a prolonged QTc interval on
her ECG and was under the care of the cardiologists. Her family history was unremarkable.

On admission, her corrected serum calcium was 1.59 mmol/l, phosphate 2.49 mmol/l and magnesium 0.78 mmol/l. The serum calcium had never been checked previously. Her PTH was 0.5 pmol/l, thyroid function tests, Vitamin B12 and a 090h cortisol level were within normal limits. Her 24 h urine calcium excretion was 1.55 mmol/24 h (2.5-7.5) and her 25-OH Vitamin D was 24 nmol/l (43-144). The rest of the routine blood tests were unremarkable.

Primary hyperparathyroidism was diagnosed and i.v. calcium was used to correct the serum calcium in the acute setting. Initial treatment with 1-α calcirol was started and later calcitriol along with oral calcium supplements were unsuccessful. Magnesium supplements were also given.

Due to the failure of conventional treatment and ongoing symptoms, i.m. vitamin D2 150 000 units once a week was added. There was no response and treatment with teriparatide 20 μg s.c. on subjects. The patient initially stabilised but relapsed later and teriparatide was increased to 20 μg bd. Her serum calcium normalized with teriparatide and i.m. vitamin D2. Her symptoms of neuromuscular irritability subsided and the QTC interval improved.

Discussion
Medical literature on the use of 1-34 PTH in hypoparathyroidism is scarce. This case highlights the use of 1-34 PTH and i.m. vitamin D2 in the treatment of refractory hypoparathyroidism.

P109
Primary medical therapy of acromegaly
Daniel Flanagan, Gina Twine, Lou Pobereskin & William Adams Derriford Hospital, Plymouth, Devon, UK,

The current paradigm for management of acromegaly includes surgery as primary treatment regardless of whether or not this is likely to be curative. There is increasing evidence that somatostatin analogue medical therapy may produce shrinkage of growth hormone producing pituitary tumours. This study tests the hypothesis that primary medical therapy may produce clinically significant tumour shrinkage prior to surgery.

Eleven treatment naive subjects with acromegaly were recruited. Mean GH 51 mU/l (range 6-96), mean IGF1 99 nmol/l (range 54-161). Maximum tumour diameter at presentation 12-38 mm. All patients were treated with lanreotide autogel (90 mg sc) every 28 days for 12 months treatment. Mean GH fell to 14 mU/l (2.5-37.8). Mean IGF1 fell to 71 nmol/l (17-150). Tumour shrinkage was seen in 9 subjects with a mean reduction of 31.1% (17.1-57.6%). Although the fall in IGF1 appeared to plateau by 6 months we continued to see tumour shrinkage to the end of the study. None of the pre-study measures predicted the degree of tumour shrinkage. We did not see significant shrinkage beyond 6 months unless we had already seen tumour shrinkage at the 6 month scan. Two individuals withdrew because of lack of response to therapy and underwent surgery before trial completion.

In conclusion primary somatostatin analogue therapy may be an effective but unpredictable primary treatment of acromegaly. A 6-month scan may be used as an assessment for whether to continue treatment.

P110
Severe insulin resistance in hypothyroidism: a case report
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Background
Hypothyroidism is reported to be associated with mild insulin resistance though severe insulin resistance is rare. We report the case of a man presented with reversible severe insulin resistance in hypothyroidism.

Case report
A 23-year-old obese man with autism was presented with poor responsiveness (GCS 7/15). He had general malaise for 2 days prior to admission. On admission, his test results showed random glucose ~ 60.8 mmol/l, potassium ~ 8.9 mmol/l, arterial blood pH ~ 6.78, bicarbonate ~ 5.0 mmol/l and 3+ urine ketones. He was managed in ITU as diabetic ketoacidosis and referred to endocrine team on 5th day due to higher insulin requirement (984 units/day).

Investigations
Free T4 ~ 4.6 pmol/l (10.0-20.0), Free T3 ~ 1.7 pmol/l (3.5-6.5), TSH ~ 32.69 mIU/l (0.20-6.0), FSH ~ 0.4 IU/l (1.0-11.0), LH ~ 0.1 IU/l (1.0-11.0), Testosterone ~ 0.4 nmol/l (8.0 ~ 34.0), Prolactin ~ 1710 mIU/l (0-400).

C-peptide ~ 1348 pmol/l (190-990), GAD antibodies 15.4 nmol/l, random cortisol ~ 199nmol/l (120-620). Subsequent short synchroph test and dexamethasone suppression test were normal.

Management
Patient was commenced on levothyroxine. Once hypothyroidism was corrected, his insulin requirement progressively reduced and insulin was stopped on 39th day.

Follow up
Recent HbA1c without any diabetic treatment for 4 months was 4.8%. Serum gonadotrophins, testosterone and prolactin levels normalised. His fasting insulin and C-peptide levels are 175 and 1580 pmol/l respectively.

Discussion
Our patient presented with diabetic ketoadidosis and had severe insulin resistance due to hypothyroidism compounded by obesity and acute illness. His diabetes mellitus fully resolved in subsequent follow up. Hyperprolactinemia was second to primary hypothyroidism. Gonadotrophin abnormality (sick eugonadal syndrome) can be explained with acute illness.

P111
An unusual association of primary amenorrhoea and sleep dysfunction
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A 17-year-old female was referred to the adult endocrine clinic with a history of primary amenorrhoea. She was short in stature with a height below the 4th centile for her age. Her BMI was 24 kg/m² with a weight below the 25th centile and there was delay in bone age ~ 2 years.

She had an interesting past medical history of late-onset central nocturnal hyperventilation from the age of 8 years when she presented with agnosic episodes and weight gain. This was classified as central hyperventilation syndrome following sleep studies and was subsequently managed with nocturnal BIPAP. At this time, she was also noted to be short in stature with a delayed bone age and had a elevated prolactin of 2099 mU/l.

A series of dynamic combined pituitary function tests were subsequently undertaken, indicating significantly subdued GH responses to insulin-stimulated hypoglycaemia (peak 9.1 IU/l), with cortisol, TSH, FSH and LH indicating normal responses to normal stimulation.

The endocrine anomalies of hyperprolactinaemia and GH deficiency along with central hyperventilation raises the possibility of a rare clinical syndrome known as late onset central hyperventilation with hypothalamic dysfunction, (LO-CSH/D).

This rare syndrome presents with obesity, sleep disturbance associated with hypothalamic/endocrine dysfunction. The genetic basis of this condition has not so far been delineated. In view of the association of hypothalamic-pituitary dysfunction in LOHS patients, baseline pituitary function testing should be considered as part of their routine clinical monitoring of this condition, with further assessments and treatments appropriate.

Further studies are clearly needed to explore the relationship between hypothalamic function and respiratory physiology as highlighted in this presentation. This patient received cabergoline therapy that successfully reduced her hyperprolactinaemia, leading to menopause. GH replacement is also being offered to this patient.

P112
Somatostatin analogues as an alternative treatment for type 1 gastric endocrine tumour: case report
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Introduction
Gastric endocrine tumours (GET) are rare. Type 1 tumours are non-functioning, almost exclusively benign lesions, usually presenting as multiple polyps, usually <1 cm in diameter, which arise from gastric enterochromaffin-like cells in response to chronically elevated gastrin, secondary to (auto-immune) atrophic fundic gastritis. These tumors were traditionally treated with total gastrectomy, like adenocarcinomas. Currently, surveillance or endoscopic treatment is recommended, as surgery is reserved for cases of malignant development or recurrence. More recently, some reports showed encouraging results of medical therapy with somatostatin analogues (SA).

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Case report
A 58-year-old female, without relevant medical or family medical history, presented with dyspepsia. Upper gastrointestinal endoscopy (UGE) was performed, revealing multiple polyps <1 cm. Histology showed well-differen-
tiated endocrine tumour (WDET) with extension to muscularis mucosa, atrophic gastritis and Knörr7=2–20%. Gastrin levels were increased 9-fold (828 pg/ml; nc<90). Admitted to our hospital, UGE was repeated, revealing WDET with Knörr7<2%. Chromogranin A (CgA) was elevated (342 ng/ml; nc<134); anti-parietal cell antibodies were positive and anti-intrinsic factor were negative. After considering all therapeutic options, it was decided to start therapy with SA (20 mg, every 28 days). Six months later, UGE showed reduction of polyps number and diameter and biopsy samples revealed absence of GET, gastrin decreased to 233 pg/ml. After 12 months, UGE revealed disappearance of the tumors, CgA levels normalized and gastrin decreased to 124 pg/ml.

Discussion
We describe a case of a multiple type 1 GET <10 mm of diameter, with a Knörr7=2–20%. Medical therapy with SA resulted in regression of these tumors, normalization of CgA levels and almost normalization of gastrin, supporting the described antiproliferative effect of this treatment. SA may be a good alternative therapeutic approach for type 1 GET, although the duration of the therapy is still to be determined, based on cost-benefit studies.

P1113
Is a morning serum cortisol a useful screening test to rule out hypoachromalasia?
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Introduction
A short-synacthen test (SST) is routinely used to diagnose hypoachromalasia, however, some authors suggest using morning serum cortisol as an initial screening test to rule out hypoachromalasia. We assessed the utility of a morning cortisol as a screening test when compared with the outcome of SST.

Methods
We retrospectively analysed SSTs carried out in our endocrine outpatient clinic in the last 3 years (649 results). Of these, 113 were identified as morning tests. We evaluated baseline morning cortisol values against stimulated values, using a cut-off ≥550 nmol/l to indicate a normal test. For step-wise values of morning cortisol, we calculated the sensitivity and specificity of its use as a screening test. Due to the clinical consequence of missing a diagnosis of hypoachromalasia it was felt that a screening cortisol value would only be useful if it excluded all positive tests.

Results
Of 113 morning SSTs (109 patients, 49 males, median age 48 years), 13 confirmed hypoachromalasia. Analysing these, a morning baseline cortisol ≥450 nmol/l ruled out all patients with hypoachromalasia and a morning cortisol level <100 nmol/l was diagnostic for hypochromalasia. Twenty-eight patients had a baseline morning serum cortisol ≥450 nmol/l, 83 patients between 100 and 450 nmol/l, and two patients ≤100 nmol/l. Using a morning cortisol cut-off ≥450 nmol/l as a screening test had a sensitivity of 100.0% and a specificity of 28.0%. The positive and negative predictive values were 15.3 and 100.0% respectively. By using a morning cortisol ≥450 nmol/l as a screening test, the number of short-synacthen tests we perform would be cut down by one quarter.

Conclusion
A morning cortisol result ≥450 nmol/l rules out hypochromalasia with sensitivity of 100.0%. As the majority of morning cortisol tests would need to be re-evaluated with a SST, leading to potential delays in diagnosis of hypochromalasia, a morning cortisol is of limited value as a screening test.

P1114
The endocrine consequences of stem cell transplantation
Amy Kennedy, Jane Nunnick, Fiona Clark, Charles Craddock & Andrew Toogood
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Over the last 10 years there have been significant advances in stem cell trans-
plantation (SCT) in adults for haematological malignancies leading to improved survival. Conditioning regimes prior to transplantation often utilise high dose chemotherapy and/or total body irradiation (TBI). Significant endocrinopathies have been reported amongst children undergoing SCT. We report the effect of SCT during adult life on endocrine function.

Basal hormone levels were obtained from 155 patients (64% male), age 20 to 67 years at a median of 36 (range 8–216) months post-transplant.

There is a high prevalence of endocrine dysfunction within this cohort. FSH concentration were elevated in all except five women of whom one was aged <45 years. In men the FSH was elevated in 83%. Testosterone deficiency was evident in 7.5% of men. Thyroid dysfunction was common; 12.9% of patients had an elevated TSH. A morning cortisol of <300 nmol/l was found in 23.2%. More patients treated with TBI had an IGF1 SDS of <−2 (15.4 vs 4.5%). Six of the eight patients who had received TBI with cranial boost had IGF1 SDS of <−2.

Dyslipidaemia occurs commonly, 30% had total cholesterol over 6 mmol/l. With the exception of low IGF1, the prevalence of these endocrine disturbances does not seem to be dependent on whether the patient had received total body or chemotherapy alone for their transplant conditioning.

These data suggest that adults undergoing SCT are at a high risk of endocrine dysfunction. More detailed studies are required to determine the severity of growth hormone deficiency and ACTH deficiency in this cohort.

P1115
Endocrinopathy and low bone mineral density in thalassaemia
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Thalassaemia patients are transfusion dependent and at risk of iron overload with end organ damage. Iron deposition is reduced by chelation treatment. We present the data on endocrinopathy and bone density in the adult patients under our care with thalassaemia. In addition, we will discuss therapeutic approaches to these problems including the use of newer chelation agents and possible reversal of endocrine organ damage. We will also review the correlation in our patients between the degree of endocrinopathy and their chelation status/ferritin and cardiac T+ weighted MRI.

In our cohort of 19 predominantly South-East Asian patients, 15 have beta thalassaemia major (7 male, 8 female), 2 females have thalassaemia intermedia and 2 females have beta thalassaemia/HbE. The mean age of the males is 35 years (22–41) and females 32.9 years (19–50).

All of the patients with thalassaemia major and thalassaemia/HbE have hypogonadism. GH deficiency, hypothyroidism, hypoparathyroidism and adrenal insufficiency are present in varying degrees within this group. Eighty-five percent of patients are vitamin D deficient.

The majority of these young adults have failed to achieve peak bone mass. In the males, mean DEXA T score at the lumbar spine is −3.53 (−2 to −5.4) and femoral neck is −2.37 (−1.8 to −3). In the females, mean DEXA T score at the lumbar spine is −2.46 (0.4 to −4.2) and at the femoral neck is −1.67 (−0.1 to −3.2).

Advances in treatment mean that these patients are surviving into adulthood and will need long term endocrine care. There are few studies in adult compared to paediatric patients with thalassaemia and the data presented provides some insight into the management challenges.

P1116
Unemployment and return to work after the diagnosis of a chronic endocrine condition
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Introduction and aim
Long-term unemployment leads to poorer health and increased inequalities. The Welfare Reform Bill, published on 14 January 2009, supports the progression back to work for incapacity benefit claimants. Due to the chronic nature of endocrine conditions, affected patients may be at an increased risk of long-term unemployment. We do not currently hold data describing the unemployment or return to work rate for this group. Our aim is to describe this data.

Patient population, sampling and methods:
Adult patients of working age (≥65 years), registered on the institution’s patient database with the following conditions were included: Addison’s disease, Cushing’s syndrome/disease, Cerebro-ophthalmic syndrome and Kleinleiter’s syndrome. Patients excluded: current inpatients, patients with terminal disease and patients
for whom no contact details were available. Our final sample included 174 patients. All patients were contacted by telephone, after working hours and at least 3 attempts to contact patients were made. One hundred and thirty (74.7%) responded, 2 (1.2%) declined participation and 42 (24.1%) were not contactable.

Results

<table>
<thead>
<tr>
<th>Employed at time of diagnosis</th>
<th>Unemployment related to disease</th>
<th>Period of unemployment</th>
<th>Benefits claimed</th>
<th>Return to work or started working</th>
<th>Currently employed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>130</td>
<td>83</td>
<td>79</td>
<td>2 weeks–34 years</td>
<td>55</td>
</tr>
<tr>
<td>Male</td>
<td>49</td>
<td>55</td>
<td>49</td>
<td></td>
<td>52</td>
</tr>
<tr>
<td>Female</td>
<td>21</td>
<td>21</td>
<td>21</td>
<td></td>
<td>21</td>
</tr>
<tr>
<td>Age</td>
<td>40.8</td>
<td>Mean</td>
<td>61.5</td>
<td>m/s0.1</td>
<td>24.3</td>
</tr>
<tr>
<td>%</td>
<td>63.8</td>
<td>60.8</td>
<td>–</td>
<td>40</td>
<td>59.2</td>
</tr>
</tbody>
</table>

Seventy-seven patients (59.2%) were satisfied with their current working status and ability to work. Nine of the 53 (40.8%) unemployed patients said that they would like to work but felt unsupported. A graded return to work was experienced as being useful.

Conclusion

Although our study is relatively small and does not include all endocrine conditions, it shows a high rate of unemployment: 40.8% vs 27.5% for the UK population (September 2009). By supporting this group of patients, an improved return to work rate may be achieved.

P117

The use of the urine calcium/creatinine clearance ratio in patients with hypercalcaemia

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Background

Hypercalcaemia is defined as an elevated serum calcium level above 2.6 mmol/l and occurs in 1 in 1000 of the population. It commonly results from primary hyperparathyroidism (PHP) which is usually treated with parathyroidectomy. A less common cause of hypercalcaemia is familial hypocalciuric hypercalcaemia (FHH) which is caused by mutations inactivating the calcium-sensing receptor; however FHH is relatively benign and requires no treatment. It is clearly important to distinguish between hypercalcaemia caused by PHP which requires parathyroidectomy, and hypercalcaemia caused by FHH for which no surgery is required. The urine calcium/creatinine clearance ratio (UCCR) is widely used to distinguish between FHH and PHP in patients with hypercalcaemia.

Aim

To determine how reliable the UCCR is in patients with hypercalcaemia to distinguish between FHH and PHP.

Methods

One hundred and three hypercalcaemic patients with a diagnosis of PHP were identified from patients attending our endocrine clinic. A UCCR > 0.01 suggested the diagnosis of PHP, and a UCCR < 0.01 suggested the diagnosis of FHH. The diagnosis of PHP was further supported by measurement of detectable serum PTH, together with normalisation of serum calcium post-surgery or positive parathyroid histology.

Results

Fifteen percent of patients with hypercalcaemia who were ultimately diagnosed with PHP, had a UCCR < 0.01, which falsely suggested the diagnosis of FHH. In patients with hypercalcaemia, a UCCR > 0.01 had 84% sensitivity and 85% specificity with respect to the diagnosis of PHP.

Conclusion

This study suggests that the UCCR may fail to reliably differentiate between hypercalcaemia due to PHP and FHH in up to 15% of patients, which could lead to inappropriate management.

P118

Salivary cortisol is a reliable marker to monitor hydrocortisone replacement

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Context

Hydrocortisone day curves (HDCV) are used to monitor hydrocortisone replacement by obtaining serum samples to measure cortisol levels throughout the day. Salivary measurement of free cortisol offers a non-invasive alternative to serum measurements.

Objective

To validate salivary cortisol measurement as an alternative method to assess the adequacy of hydrocortisone replacement in patients with hypoadrenalism.

Setting, patients, and design

Dual-centre cross-sectional study of 23 patients, taking hydrocortisone for hypoadrenalism and admitted for a HDCV to day-case clinical investigation centres (09/H0711/7). Simultaneous blood and saliva samples were obtained to determine total and free cortisol, respectively.

Outcome measures

Serum free cortisol was estimated using measurements of total serum cortisol, corticosteroid-binding globulin, albumin and previously validated mass-action formulae. Salivary cortisol levels were also measured in 24 healthy volunteers to determine a control profile.

Results

Serum total and salivary free cortisol correlated well (Spearman r=0.850, P<0.0001). Salivary free cortisol correlated well with estimated serum free cortisol calculated by the Coolens formula (r=0.8764, P<0.0001) and the Dorin formula (r=0.9635, P<0.0001). Salivary cortisol measurements were significantly higher in patients than controls in the samples taken after doses of hydrocortisone but not significantly different in the samples taken before doses of hydrocortisone.

Conclusion

Salivary cortisol measurements offer a more cost-effective, non-invasive and well tolerated alternative to serum cortisol during HDCV.

P119

Screening for glucose intolerance in young women with polycystic ovary syndrome: what is the optimum strategy?

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Introduction and aims

Women with polycystic ovary syndrome (PCOS) are at significantly increased risk of developing impaired glucose tolerance (IGT; prevalence 9–35%) and type 2 diabetes (T2DM; prevalence 2–10%), hence screening for these complications is recommended. The American Androgen Excess Society (AAES) recommends a biannual oral glucose tolerance test (OGTT) in all women with PCOS but this is costly and inconvenient. Alternative strategies which minimise the need for OGTT have thus been proposed, based on anthropometric variables alone (clinical decision tree modelling, DTM), anthropometry, clinical history, age and fasting plasma glucose (FPG) (Royal College of Obstetricians and Gynaecologists, RCOG), or FPG and a risk assessment questionnaire (All Wales Guidelines; AWG). We sought to determine the prevalence of glucose intolerance in our patients with PCOS and compared the performance of these 3 strategies with OGTT in identifying subjects with IGT/T2DM.

Methods

R&D and ethical approval was granted for this study. Clinical details and anthropometric measurements were collected in 28 consecutive clinic patients.

Table 1 Performance of each screening strategy.

<table>
<thead>
<tr>
<th>RCOG</th>
<th>AWG</th>
<th>DTM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reduction in OGTT (n)</td>
<td>6</td>
<td>10</td>
</tr>
<tr>
<td>Missed IGT/T2DM (n)</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>66.6%</td>
<td>55.6%</td>
</tr>
<tr>
<td>Specificity</td>
<td>15.8%</td>
<td>31.6%</td>
</tr>
</tbody>
</table>
with PCOS (16–45 years). Patients with previously documented IGT/T2DM and those taking metformin were excluded. Subjects underwent a 75 g OGTT after an overnight fast.

Results
Nine patients (32%) were identified with abnormal glucose tolerance on OGTT; 5 with IGT and 4 with T2DM. Of these 8 had FPG values of <5.6 mmol/l.

Conclusions
These preliminary results confirm a high prevalence of IGT/T2DM in our local population of women with PCOS and highlight the limitations of FPG as a screening test for glucose intolerance in this population. The AAES guidance represents the only strategy which reliably identifies all patients with glucose intolerance but DTM may offer a reasonable alternative.

P120
The role of adrenal vein sampling (AVS) in the diagnosis and management of primary hyperaldosteronism: an audit of 10 years experience at a tertiary referral centre
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Hyperaldosteronism is a significant cause of secondary hypertension, but there are often delays in obtaining the diagnosis. The recent BES publication of Guidelines for the investigation and management of this condition represents a move to standardise the work-up of these patients.

We present an audit of cases, dating back to 2000, with confirmed biochemical hyperaldosteronism (on the basis of plasma aldosterone/renin activity ratio or lack of aldosterone suppression on salt loading testing). All patients went on to have bilateral adrenal vein sampling (AVS) and CT/MRI adrenal imaging.

Data was collected for 45 patients. The average age of the cohort was 47.6 years and 51% were female. Imaging identified potential causative adrenal lesions in 73% of patients, with AVS results indicating significant lateralisation in 78% of patients. There was concordance between imaging and AVS results in 68.8% of cases, which agrees well with previously published data. Interesting and informative examples will be highlighted from the cohort. On the basis of investigation results, patients were selected for either surgical or medical management of their hyperaldosteronism. Age and gender were equally distributed between both groups. Although blood pressure reduction and potassium levels were comparable, patients who underwent surgery were able to reduce their number of antihypertensives, from a mean of 2.7 to 1.2. In comparison, after optimisation of antihypertensive therapy in the Endocrine clinic, there was no reduction in the number taken by the medically-managed group. Long-term follow up data regarding cardiovascular morbidity (up to 9 years) will also be presented.

Our results reinforce the potential dangers of basing treatment decisions on imaging alone in primary hyperaldosteronism. Management in a tertiary referral centre, alongside expertise in AVS procedure, is important. In view of reductions in medication burden, surgical management is the best option in cases where unilateral secretion has been confirmed.

Cytokines and growth factors
P121
Peripheral blood mononuclear cells from active rheumatoid arthritis patients show a defective induction of adrenomedullin
Laura Green, Andrew Berry, Rachelle Domn & David Ray
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Rheumatoid arthritis (RA) is a common chronic systemic inflammatory disease. Several new therapies exist for RA but long-term patient management remains problematic.

We have discovered that hypoxia, a characteristic of inflamed tissues, potently inhibits glucocorticoid (Gc) sensitivity. This effect is mediated, in part by HIF-1α, whose activity is induced in hypoxia. HIF-1α gene expression is also induced in primary peripheral blood mononuclear cells (PBMCs) by inflammatory stimuli, even under normoxic conditions. HIF-1α has two paralogs, HIF-2α and HIF-3α and several splice variants which impact on HIF function.

To determine if differences in the hypoxia signalling cascade are present in RA PBMCs were isolated from 9 healthy volunteers and 8 patients with active disease.

We find co-expression of multiple HIF1α splice variants in PBMCs and a dramatic increase in two of six HIF2α splice variants in cells activated by inflammatory stimuli in all subjects. HIF2α but not HIF3α alpha is also expressed in PBMCs, but is differentially regulated, suggesting a different control mechanism, and so a possible function in inflammation.

HIF1α target genes, including VEGF, Hexokinase 2, Interleukin 6 and Adrenomedullin (ADM), also showed increased expression in response to inflammatory stimuli. Specifically, ADM, a mainly anti-inflammatory mediator, showed significantly lower transcriptional activation in response to inflammatory stimuli in cells isolated from the RA patients compared with the healthy subjects (P<0.001).

In summary, we show activation of the hypoxia sensing cascade in PBMCs in response to inflammatory stimuli, and a dramatically deficient induction of the HIF target, and locally acting anti-inflammatory mediator ADM in patients with active RA. This inadequate induction of ADM may contribute to the progression of the chronic inflammatory response characteristic of RA.

P122
Impact of adiposity on dynamic changes of serum adipokines after OGTT
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1Manchester University, Manchester, UK; 2Central Manchester Foundation NHS Trust, Manchester, UK; 3Liverpool University, Liverpool, UK.

Introduction
Data are conflicting on whether and how serum adipokines alter following acute dietary intake. Here, we used the specific challenge of standard 75 g glucose tolerance tests (GTG) to assess timed changes in TNF-α, IL-6, leptin and adiponectin in healthy younger women and how these would be affected by adiposity.

Methods
Consenting participants in the Manchester Mothers’ Heart and Vascular health study had serum adipokines measured at fasting, 30 and 120 min of a GTT. Standardised anthropometry, blood pressure and blood biochemistry were measured and results analysed by body mass index (BMI) tertiles and insulin resistance (‘HOMA-IR’) status as insulin sensitive (IS) or more resistant (IR) groups.

Results
In 115 women, there was no change during GTT in serum TNF-α and IL-6. Serum leptin declined significantly by 10–18% (P<0.05) at 30 min in all groups and fell further for women in the highest BMI tertile by 120 min. In the 1st thinnest tertile and IS groups, serum adiponectin increased slightly at 120 min (±4–4.5 mg/l, P<0.05). On multiple regression analysis, BMI displaced IR status as a strong independent predictor of change in serum leptin from 0 to 30 min and adiponectin from 0 to 120 min.

Conclusions
In younger age groups serum leptin concentrations fell and adiponectin rose in slimmer women, so that adiposity was the major determinant of changes in both adipokines, but TNF-α and IL-6 did not alter following glucose challenge.

P123
Interaction of endothelin, aldosterone and bone morphogenetic proteins on mitotic actions of pulmonary arterial smooth muscle cells isolated from pulmonary arterial hypertension
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Primary arterial hypertension (PAH) is a life-threatening disease that has prevalence of 1 to 2 occurrences per one million individuals. This disease is characterized by excessive proliferation of vascular endothelium and smooth muscle cells, causing thickening the walls of pulmonary arteries. Recent studies have uncovered a link between familial and idiopathic PAH to BMPR-II mutations. The pathology of PAH is characterized by the remodeling of pulmonary arteries due to pulmonary artery smooth muscle cell (PASMC) hyperproliferation. However, the detailed mechanism has yet to be elucidated.

Here we investigated the functional link of BMP and vasoactive factors (including endothelin (ET), angiotensin II and aldosterone) in the mitotic actions of

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PASMCs of PAH lungs. ET1 and aldosterone stimulated PASMC proliferation of idiopathic PAH more efficaciously than that of secondary PAH, whereas angiotensin II and ET3 failed to activate mitosis in either cell type of PASMC. The effects of ET1 and aldosterone were blocked by an ET type-A/B receptor (ETα/BR) antagonist bosentan and a selective MR blocker eplerenone, respectively. BMP-2 and -7 but not BMP-4 and -6 significantly increased mitosis in both cell types of PASMCs. Notably, ET1- and aldosterone-induced mitosis as well as MAPK phosphorylation were increased in the presence of BMP-2 and -7 in PASMC isolated from idiopathic PAH, although the additive effects were not observed in PASMC isolated from secondary PAH. Inhibition of ERK suppressed basal, ETα- and aldosterone-induced PASMC mitosis more potently than that of SARK/INK inhibition. Given that BMP-2 and -7 upregulated ETA/BR and MR expression and that BMP-2 decreased 11βHSD2 levels in PASMC isolated from idiopathic PAH, BMP/Smad signaling may play a key role in amplifying the ETA/BR- and/or MR-ERK signaling in PASMCs of PAH lung. The link between BMP and ETβ1 and/or MR system may be involved in the progress of PASMC mitosis leading to clinical PAH.

P124
PTTG promotes mitogenic mechanisms in thyroid cells through autocrine pathways of interaction with TGFβ, EGF and IGF1

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The human pituitary transforming gene (hPTTG) is overexpressed in thyroid cancers; it induces genetic instability and propagates growth through the induction of growth factors. We set out to investigate the autocrine and paracrine pathways of interaction between hPTTG and epidermal growth factor (EGF), transforming growth factor-β (TGFβ) and insulin-like growth factor 1 (IGF1) in vitro and in vivo. Synchronised K1 papillary thyroid carcinoma cells were treated with TGFβ (5 nM), EGF (5 nM), or IGF1 (10 ng/ml) and hPTTG protein expression was determined by western blotting at 24, 36 and 48 h. Treatment with TGFβ resulted in a ~3-fold upregulation of hPTTG at all 3 time points and dose-response experiments confirmed significant PTTG induction with 5 and 50 nM TGFβ. IGF treatment induced a ~4-fold increased expression of hPTTG at 36 and 48 h. Treatment with IGF1 resulted in a 2-fold upregulation of hPTTG at 36 and 48 h. To investigate if hPTTG in turn results in increased expression of these growth factors, we determined TGFβ, EGF and IGF1 mRNA expression through TaqMan RT-PCR following transient transfection of primary human thyrocytes with hPTTG. EGF (1.7-fold, n = 6, P = 0.006) and IGF1 mRNA (1.6-fold, n = 6, P = 0.03) were significantly upregulated by hPTTG, whereas TGFβ mRNA was not significantly induced (1.6-fold, n = 6, P = NS). To investigate these findings in vivo, we subsequently evaluated mRNA expression of these mitogenic factors in our recently generated transgenic mouse model of targeted hPTTG overexpression in the thyroid gland. Upregulation of mEGF (2.7-fold, n = 3, P = 0.012) and mIGF1 (3.0-fold, P = 0.02) was confirmed when comparing 6-week old PTTG+/+ mice to age-matched WT.

Conclusion
These results indicate that PTTG is involved in autocrine signalling mechanisms with growth factors such as TGFβ, EGF and IGF1 in the thyroid. We propose that aberrant control of these pathways may enhance tumour development and that further elucidation of these pathways may provide novel therapeutic targets for the prevention of thyroid tumour progression.

P126
Differential effects of eicosapentaenoic acid (EPA) and palmitate on tumour necrosis factor (TNF-α) and insulin-like growth factor (IGF1) action in murine skeletal muscle cells

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Introduction
Sarcopenic obesity prevails with ageing, encompassing excess weight gains and muscle mass or strength losses. Free fatty acids and proinflammatory cytokines are elevated in obese adults. Palmitate decreases the anabolic effects of IGF signalling, while EPA elicits anti-inflammatory activities.

We aimed to examine the effects of palmitate and EPA on TNFα/IGF1 interactions in established models of skeletal muscle cell loss.

Methods and results
Palmitate (750 μM) pretreatment increased myoblast death by >50% versus controls (P < 0.05) and reduced myoblast differentiation by 84% versus IGF1 (97 ± 7 vs 600 ± 29 CK Units/mg; P < 0.05). By contrast, TNF-α (10 ng/ml) induced myoblast apoptosis was reduced by co-incubation with EPA (50 μM, 26 ± 2 vs 14 ± 3%; P < 0.05). Although basal and IGF-induced myoblast differentiation were significantly (P < 0.05) increased by EPA (572 ± 27 vs 782 ± 32 basal and 600 ± 29 vs 800 ± 24 Units/mg IGF1), it could not rescue TNF-α induced (1.25 and 10 ng/ml) inhibition of differentiation. IGBP5, IGBP3, MyoD and myogenin mRNA expression were significantly (P < 0.05) down-regulated by palmitate versus EPA pre-administration (97 ± 18, 68 ± 11, 70 ± 16, 84 ± 27%, respectively) but were not significantly different between control and EPA. Expression of Id3, an inducer of proliferation and hence inhibitor of differentiation, was increased 6-fold (P < 0.05) by palmitate at 72-h compared with control. Critically, co-administration of palmitate and EPA significantly dampened the negative effects of palmitate, increasing myoblast survival and differentiation. Although still down-regulated palmitate/EPA co-treatment increased IGBP5 and myogenin levels by 89 ± 26 and 54 ± 15% respectively relative to palmitate alone but IGBP5, MyoD and Id3 levels remained unchanged.

Conclusion
Palmitate-induced myoblast apoptosis and inhibition of differentiation is associated with elevated Id3 and reduced IGBP5, IGBP3, MyoD and myogenin expression. EPA elicits protective action against the apoptotic effects not only of TNF-α but also of palmitate. These findings encourage investigations of the potential therapeutic effects of EPA in inflammatory conditions, such as sarcopenic obesity.

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P127

Atrophy or hypertrophy: differential responses of C2 and C4C12 mouse skeletal myotubes in the absence or presence of tumour necrosis factor-α (TNF-α)

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Inflamed ageing is associated with reduced muscle mass and increased susceptibility to TNF-induced muscle protein degradation. We therefore aimed to elucidate mechanisms supporting reduced hypertrophy and increased atrophy of skeletal myotubes. C2 myotubes display diminished differentiation and increased susceptibility to TNF-α-induced apoptosis versus daughter C4C12 cells, providing us with relevant models. C2 and C4C12 cells were cultured for 72 h ± TNF-α (20 ng/ml), to assess differentiation (contractile kinase), proliferation (protein), death (trypan blue) and anabolic/catabolic parameters (RT-PCR). Under basal conditions, larger myotubes were evident in C4C12 versus C2 cells. Significantly higher CK activity (320 ± 2.6 ± 7.7 ± 2.5, P < 0.05, 31 ± 1.1 ± 6.8 ± 1.9, P < 0.05) and myogenin mRNA (241.8 ± 40 vs. 368.0 ± 19.3, P < 0.05, 441.0 ± 100.5 vs. 201.1 ± 86, P < 0.05) were detected at 48 and 72 h, respectively. Fold increases in IGFBP mRNA (243.1 ± 3.1 vs. 105.7 ± 21.9, P < 0.05), together with reduced proliferation and significantly lower protein expression (1.21 ± 0.28 vs. 1.79 ± 0.29 mg/ml, P < 0.05) was evident at 72 h. Significant fold reductions in IGF2BP mRNA occurred in the C4C12 cells (15.04 ± 2.1, 8.27 ± 2.47, 4.21 ± 0.29, P < 0.05) versus increase in the C2 cells (1.61 ± 0.89, 11.98 ± 2.26, 18.39 ± 3.56, P < 0.05) at 48 and 72 h, respectively. TNF-α induced apoptosis in the C2 cells (2.67 ± 1.54, 34.42 ± 5.39, 29.71 ± 7.9% (0, 48, 72 h), P < 0.05), was without effect in the C4C12 at 48 h but caused a small significant increase at 72 h (9.88 ± 4.02% (TNF-α) versus 0.17 ± 0.749% (DMSO, 72 h)). TNF-α and TNFRI mRNA were unchanged, however, larger reductions in IGFBP (8.2 and 7.5 vs 4.5 and 4.1-fold (48, 72 h)) and IGFBP5 (2.7 versus no reduction (48 h) P < 0.05) mRNA were observed in C2 versus C4C12 cells in the presence of TNF-α. This model provides insight into altered hypertrophic/atrophy and myogenic regulators of basal and TNF-induced adaptations of skeletal muscle and may provide insight into therapeutic initiatives for ageing and wasting disorders.

P129

Diabetes and metabolism

Profile of elderly patients with diabetes admitted into Obafemi Awolowo University Teaching Hospital

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Introduction
The average life expectancy and the prevalence of diabetes are increasing. Among the elderly population, type 2 diabetes is a growing problem, with a large proportion of newly diagnosed patients being aged 65 years or older. Treatment of elderly diabetic patients presents unique challenges. Impaired cognitive and physical functioning among some elderly patients can make adjusting to a diabetes care routine more difficult and thus, contribute to the complexity of managing diabetes.

Aims
To determine in an elderly diabetic population, the frequency of related disorders, the morbidity, and outcomes compared with other age group.

Method
The medical records of all diabetics admitted between 2003 and 2007 were studied retrospectively. A comparison was made between two age groups: elderly patients (65 years) with type 2 diabetes. The presence of associated cardiometabolic and outcome parameters were entered into a database and analysed statistically.

Results
The prevalence of diabetes among the admitted medical cases was 10% (398), and elderly patients represented 27% (66) of these. Diabetic foot disease (30.3%) and hyperglycemia emergencies (27.2%) were the main reasons for admission in the elderly group. Similarly, each of these accounted for 29.8% of the diagnosis in the order group. 80.3% of the elderly patients were hypertensive, while 66.9% of the patients were hypertensive in the younger group. The mortality rate was 28.8 and 30.9% in the elderly and younger patients respectively. Diabetic foot disease (36.8%) accounted for the major cause of death in the elderly group, while hyperglycemia emergencies (27.3%) accounted for the major cause in the young patients. The average time spent on admission was 25 days (s.o. 22) in the elderly and 23 days (s.o. 29) in the younger patients.

Conclusion
Treating geriatrics with diabetes requires the care giver to take a multidisciplinary role. The goals should always be the reduction of diabetes-related complications and improving the quality of life in this vulnerable group.

P128

Blood pressure, renal cytokines and biochemical parameters improve in morbidity obese patients after bariatric surgery

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Introduction
Obesity related glomerulopathy (ORG) is an emerging epidemic alongside increasing incidences of obesity. ORG pathophysiology has not been explored despite poor prognosis in untreated patients. MCP-1, MIF, CCL-18 and CCL-15 are novel cytokines that may be pathologically involved in obesity-induced renal injury and explored in this study.

Methods
Blood pressure, urine and blood samples were collected from 34 morbidly obese patients before and 4 weeks after bariatric surgery. Biochemical parameters including serum creatinine, albumin, cholesterol, estimated glomerular filtration rate (eGFR) and C-reactive protein (CRP) alongside urine albumin and creatinine were recorded. Urinary and serum chemokines MCP-1, MIF, CCL-18 and CCL-15 were detected using ELISA.

Results
Thirty-four patients were analysed postoperatively with an average weight loss of 9.3 kg. Systolic blood pressure decreased from 142.9 to 128.1 mmHg (P < 0.001), diastolic blood pressure decreased from 87.1 to 79.2 mmHg (P < 0.001) and mean arterial blood pressure decreased from 105.7 to 95.5 mmHg postoperatively (P < 0.001). Urinary albumin decreased from 45.3 to 21.9 mg/l (P < 0.001), urinary creatinine decreased from 25.9 to 17.2 mmol/l postoperatively (P < 0.01) whilst eGFR increased from 68.1 to 86.1 ml/min per 1.73 m2. Serum creatinine decreased from 73.6 to 68.1 umol/l postoperatively (P < 0.05). Serum CRP values decreased from 25.2 to 8.0 mg/l postoperatively (P < 0.001). Urinary MCP-1 decreased from 203.1 to 90.4 ng/mmol Cr postoperatively (P < 0.001). Urinary MIF decreased from 26.1 to 16.7 ng/mmol Cr postoperatively (P < 0.001). Urinary CCL-18 levels decreased from 98.6 to a postoperative value of 22.0 ng/mmol Cr (P < 0.05), urinary CCL-15 changes were not statistically significant. Serum CCL-18 decreased from 576.2 to 122.6 ng/mmol Cr postoperatively (P < 0.001). There were no significant changes in serum MIF, MCP-1 or CCL-15.

Conclusion
This study demonstrates the early benefits of surgically induced weight loss upon blood pressure and markers of renal function. Reduced urinary cytokines suggest bariatric surgery attenuates inflammatory status which may have an aetiological role in ORG.
discharge he was shown how to use a glucometer, advised to eat normally and abstain from alcohol. At outpatient review he reported no further hypoglycaemia and outstanding blood tests showed a plasma insulin of 720 pmol/l, C-peptide of <94 pmol/l and negative sulphonylurea screen, consistent with exogenous insulin administration.

Conclusion
Exogenous insulin administration should be considered in all patients who present with unexplained hypoglycaemia, as they may not admit to this. Insulin may be misused by athletes for its anabolic effects, and by patients who crave the euphoria associated with rapid lowering of their blood glucose levels, or have suicidal intent. Our patient’s motives are unclear.

P131
Gender and the metabolic syndrome in type 2 DM
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Background
The prevalence of diabetes mellitus (DM) is on the increase and with this scenario, a possible increase in burden of DM which may be largely attributed to cardiovascular complications is expected. The objective of this report is to determine the prevalence and compare gender characteristics in subjects with type 2 DM.

Methods
Subjects with type 2 DM were recruited from an urban hospital for the study and clinical data obtained. The anthropometric indices and blood pressure measurements were documented. Laboratory parameters analysed for included total cholesterol, high density and low density cholesterol, triglyceride and glycosylated haemoglobin.

Results
Nine hundred and sixty-three patients with type 2 DM aged between 35 and 85 years were recruited for the study. The main outcome measures included the prevalence of the metabolic syndrome and the gender differences of its components. The prevalence of the metabolic syndrome (Mets) was 86%. The prevalence of the Mets was similar for men (83%) and women (86%) respectively and increased with age in both sexes. The prevalence of Mets increased from 11% among participants aged 20-29 years to 89% in participants aged 70-79. In our patients with DM, the commonest occurring and least detected Mets defining parameters are central obesity and elevated triglyceride levels respectively. The components of the Mets that differed significantly in both sexes was HDL-C. The combinations of the components of the Mets were comparable in both genders and 5.8% of the subjects with the Mets had all components of the Mets.

Conclusion
The prevalence of the Mets in type 2 DM is high in both genders and increases with age thus posing a potential high cardiovascular risk in this group of patients.

P132
Positive correlation between serum omentin and thrombomodulin-1 in gestational diabetes despite lack of correlation with insulin resistance indices
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Background
Gestational diabetes (GDM) is characterized by insulin resistance and a pro-inflammatory state, both factors possible related to adipokine expression. In our study we have endeavoured to assess serum level of insulin-sensitising omentin and pro-inflammatory thrombomodulin-1 (TSP-1) in GDM.

Subjects and methods
The study included 20 women with GDM, diagnosed according to the WHO criteria, age 29.7 ± 5.6 (mean ± s.d.), BMI 28.1 ± 4.7 kg/m2 and 23 controls matched for age (28.3 ± 3.9 years), and BMI (28.3 ± 4.8 kg/m2). Omentin and TSP-1 were measured by ELISA assays. Insulin resistance was assessed by HOMA and Insulin Resistance Index (IRI) and takes into account glucose and insulin levels during oral glucose tolerance test (OGTT).

Results
There were no differences in fasting glucose levels between women with GDM and controls (76.9 ± 2.8 vs 78.8 ± 6.5 mg/dl (conversion: 1 mmol/l = 18 mg/dl). As expected, women with GDM had significantly higher glucose (162.9 ± 16.6 vs 117.0 ± 15.7 mg/dl, P < 0.01, and insulin levels 93.48 ± 59.11 vs 59.91 ± 32.47 μU/ml, P = 0.016, at 120 min of OGTT). There were no significant differences in omentin and TSP-1 levels between subjects with GDM and controls (48.0 ± 12.0 vs 50.2 ± 7.9 ng/ml and 2150 ± 1661 vs 1569 ± 1160 ng/ml, P = 0.64 and P = 0.29, for omentin and TSP-1 in GDM and control subjects, respectively).

There was no significant correlation between either omentin or TSP-1 with HOMA or IRI, however, there was a significant positive correlation between thrombomodulin-1 and omentin (r = 0.49, P = 0.010).

Conclusions
Concentrations of novel adipokines i.e. omentin and thrombomodulin-1 seem to be inter-related in pregnancy, however, in contrast to the data from non-pregnant individuals, there are no differences in serum levels between women with normal glucose tolerance and those with glucose intolerance, characteristic for gestational diabetes. These observations suggest that regulation of concentrations of these adipokines in pregnancy is mediated though different mechanisms than in non-pregnant subjects.

P133
Pancreatic polypeptide: a novel substrate for the endopeptidase nephrilysin
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Pancreatic polypeptide (PP) is a 36 amino acid peptide, secreted from the endocrine pancreas. Previous work has shown that peripheral administration of PP inhibits food intake in rodents and humans. However, PP has a short circulating half-life that limits its use as an anti-obesity agent. Determining the mechanisms involved in the physiological breakdown of PP will allow the rational design of long-acting analogues with greater clinical utility in the treatment of obesity. PP is a member of the PP fold family of peptides which includes Neuropeptide Y (NPY) and Peptide YY (PYY). The endopeptidase nephrilysin (NEP) is known to break down PYY. We investigated whether NEP breaks down PP in vitro.

In vitro, we examined the effect of co-administration of the NEP inhibitor phosphoramidon with PP on food intake and plasma levels of PP in mice. Our results demonstrate that NEP breaks down PP in vitro. Incubating 2 mmol PP with 200 μg NEP for 90 min at 37 C resulted in breakdown of 33% ± 5.09 (n = 3) of PP, as analysed by HPLC. MALDI-TOF MS analysis suggests NEP cleaves PP at amino acid positions 20 and 30 in vitro. Co-administration of the NEP inhibitor, phosphoramidon, significantly increased the plasma levels of PP at 45 min post-injection in mice compared to PP administration alone (n = 3, P = 0.01). Co-administration of PP with phosphoramidon, demonstrated a non-significant trend of an increased anorectic effect following s.c. injection of 150 nmol/kg of PP to fasted male mice at 2 h post-injection (n = 10).

PP may play a role in the physiological breakdown of PP. PP analogues resistant to breakdown by NEP may prove useful drug targets for obesity.

P134
Bariatric surgery in renal transplant recipients
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Objectives
The long-term outcome of bariatric surgery on renal function in the morbidly obese renal transplant recipient is still unknown. We describe three cases of morbidly obese renal transplant recipients who have subsequently undergone bariatric surgery.

Patient 1
A 44-year-old man (BMI 42.2 kg/m2) had received a renal transplant for chronic renal disease secondary to childhood reflux nephropathy. Laparoscopic Roux-en-Y gastric bypass resulted in 25.1 kg weight loss over 3 months. Long-standing proteinuria, glucosuria and urine blood trace resolved by 4 months and serum creatinine and cholesterol improved. His blood pressure medications were reduced post-bypass.
Patient 2
Patient 2 is a 51-year-old man (BMI 43.3 kg/m²) with a pancreas-kidney transplant for end stage renal failure (ESRF) secondary to diabetes. He required insulin and metformin following pancreatic graft failure. He lost 8 kg in 4 months following sleeve gastrectomy. Insulin requirement was nil for 7 days post-sleeve gastrectomy but returned to pre-operative levels. Elevated blood urea, bicarbonate and potassium levels normalised. Creatinine remained normal, although uric acid to creatinine ratio was raised and there was a need to readjust immunosuppressant medication.

Patient 3
Patient 3 is a 56-year-old man (BMI 39 kg/m²) who had received a kidney transplant for ESRF secondary to diabetes. Weight loss was 15 kg, 4 months following sleeve gastrectomy. Insulin requirement was reduced from 260 to 40 units post-sleeve gastrectomy, although HbA1c was consistently high. Ibesarian was commenced for hypertension. Serum creatinine and eGFR remained normal.

Conclusion
Bariatric surgery can be safe and successful in reducing weight and obesity related co-morbidities in the morbidly obese renal transplant recipient. Bariatric surgery in this cohort of patients can stabilise the renal transplant and improve some markers of renal function.

P135
Testosterone replacement therapy has no effect on ultrasound assessed carotid artery stiffness and intima-media thickness in men with insulin treated type 2 diabetes
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Context
Testosterone replacement therapy (TRT) has shown benefit on insulin resistance, glycaemic control and cardiovascular risk markers in hypogonadal men with diabetes. Arterial stiffness and intima-media thickness (IMT) are vessel wall properties associated with future cardiovascular risk which allow the progress of atherosclerosis to be assessed non-invasively. Low testosterone has been associated with the presence and progression of carotid atherosclerosis as assessed by IMT.

Methods
Double-blind placebo controlled parallel group study of 24 hypogonadal men with insulin treated type 2 diabetes examining the effect of TRT on ultrasound assessed markers of atherosclerosis. Participants were randomised to 1 mg testosterone (n = 11) or placebo (n = 13) every 2 weeks for 6 months. Carotid artery stiffness (stiffness index beta) and IMT were assessed by ultrason at baseline, 3 and 6 months. Other assessments included conventional cardiovascular risk markers and hypogonadal symptoms (AMS score).

Results
The testosterone and placebo groups did not significantly differ at baseline, 3 or 6 months in terms of arterial stiffness, IMT, HbA1c, anthropometrics, blood pressure, lipids, PSA or AMS score. Testosterone was associated with an increase in haematocrit (+ 0.054 vs – 0.006; P = 0.003) but no patient developed a raised haematocrit. One patient in the placebo group withdrew from the trial after a fall ulceration worsened. There were no significant adverse events in the testosterone group.

Conclusion
TRT given to men with insulin treated type 2 diabetes had no effect on ultrasound assessed atherosclerosis and we failed to confirm the beneficial effects of testosterone on weight, glycaemic control and lipid parameters seen in other trials. The trial was underpowered to draw strong conclusions from these negative results. Additional reasons that the study failed to demonstrate the effects of testosterone may have been an advanced state of atherosclerosis and insulin resistance at baseline and the short duration of the study.

P136
A novel role for neuropeptide Y in the regulation of energy homeostasis
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The hypothalamus regulates multiple homeostatic systems, and is essential for the regulation of appetite and energy balance. Neuropeptide Y, a 36 amino acid peptide and member of the PP-fold family, is a key hypothalamic neuropeptide involved in the regulation of energy balance, potentially stimulating food intake following central administration. NPY is expressed in the arcuate nucleus (ARC) and NPYergic neurons project to multiple hypothalamic nuclei and extra-hypothalamic sites. NPY is also expressed in the hypothalamic dorsomedial nucleus (DMN) and there is data to suggest that NPY is differentially regulated in the DMN compared to the ARC. In diet-induced obesity and a number of genetic models of obesity NPY mRNA levels are reduced in the ARC but increased in the DMN. In order to study the role of NPY in the DMN in the regulation of energy balance, the gene transfer vector recombiant adeno-associated virus (rAAV) expressing NPY was administered with stereotactic microinjection to over-express NPY in the DMN of male Wistar rats. Body weight and food intake were monitored for 94 days.

Over-expression of NPY within the DMN resulted in a reduction in food intake and body weight gain compared to controls injected with rAAV encoding enhanced green fluorescent protein (EGFP). This effect was accentuated in animals fed on a high fat diet. Plasma leptin was reduced in NPY injected rats compared to controls, reflecting a decrease in adiposity. Measurement of circulating thyroid hormone levels, brown adipose tissue (BAT) weight and BAT uncoupling protein-1 mRNA expression indicated that the reduction in body weight gain was not due to an increase in energy expenditure.

These results suggest that NPY may have a different role in the DMN compared to the ARC adding further complexity to the neuronal circulatory involved in the regulation of food intake.

P137
Salicylate induces insulin sensitivity by alleviating endoplasmic reticulum (ER) stress in cultured primary human adipocytes
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Background
Adipose tissue (AT) may have a significant role in obesity associated inflammation but, the mechanisms underlying the pathogenesis of obesity induced inflammation remains unclear. Recent murine studies indicate that ER stress is critical to the initiation and integration of inflammation and insulin signalling pathways. The ER stress occurs primarily due to accumulation of unfolded proteins and results in activation of the unfolded protein response to restore ER homeostasis. This up-regulates PERK, IRE1α, ATF6 and other chaperones. Therefore, our aims were to determine the existence and causes of ER stress in human adipocytes along with therapeutic interventions.

Methods
Human abdominal subcutaneous (AbSc AT) was obtained from Caucasian non-diabetic population (BMI: 27.9 ± 3.7 kg/m², age: 36–49 years; n = 4; female subjects) that underwent elective liposuction. Preadipocytes were isolated from stromal fraction, grown and fully differentiated into adipocytes (n = 5). Adipocytes were then treated with LPS (100 ng/ml), tunicamycin (750 ng/ml), high glucose (25 mM, HG) and in combination with 20 mM salicylate, salicylate alone and without (controls). To characterise markers of ER stress total protein and RNA was extracted from adipose tissue and cultured adipocytes. Western blots and qRT-PCR were performed to examine expression levels.

Results
ER stress proteins Calnexin1, BiP1, Erp1, PDI, IRE1α and phospho-PERK were significantly up-regulated in obese AbSc AT compared to lean (n = 4; P < 0.05 to P < 0.001). phospho-eIF2α (n = 5; P < 0.005) and calnexin (n = 5; P < 0.02) were both significantly induced by LPS, tunicamycin and HG in differentiated human adipocytes and were significantly reduced when treated with salicylate. Similarly, both phospho-Akt (S473) (n = 5; P < 0.002) was activated and GLUT4 up-regulated, when ER stress was down-regulated by salicylate.

Conclusion
Our findings highlight, up-regulation of ER stress in human obese AbSc AT compared to lean subjects. From this study we also conclude that salicylates improve insulin sensitivity and alleviate ER stress induced by LPS and high glucose in cultured primary human adipocytes.

P138
Herbal remedies as a cause of recurrent hypoglycaemia
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Objective
Factitious hypoglycaemia presents diagnostic difficulties. We report a case presenting with hypoglycaemia, due to use of herbal remedies for enhancement of sexual performance.
P139
The effects of chloroquine on blood glucose and plasma insulin concentrations in male Sprague–Dawley rats
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Chloroquine, the commonly used in the treatment of malaria has been reported to elicit a hyperglycaemic effect due to unknown mechanism. In particular, literature to date has revealed that the effects of the drug on insulin concentrations remain obscure. Our investigation therefore focuses on establishing a possible mechanism of CHQ-induced hyperglycaemia with regards to its effects on plasma insulin concentrations. The purpose of this study was to investigate if the effects of chloroquine on blood glucose and insulin concentrations in male Sprague-Dawley rats. Oral glucose tolerance (OGT) responses were conducted in separate groups of rats given drug load (0.86 g/kg, p.o.) after 18 h fast, followed by various CHQ doses (30, 60, 120 mg/kg, p.o.). Rats treated with deionized water (3 ml/kg, p.o.) served as control animals. Blood glucose was monitored at 15 min intervals for the first hour, and hourly thereafter for 3 h. Plasma insulin concentrations were measured in separate groups of rats treated with chloroquine (60 mg/kg, p.o.) after 0 h (control) and 4 h. Plasma obtained was analysed for chloroquine and insulin concentrations. Blood glucose concentrations in untreated rats significantly increased from a fasting level of 3.66 ± 0.14–6.50 ± 0.20 mmol/l 15 min after a glucose challenge. The mean blood glucose concentration declined by the end of the experiment to reach levels that did not significantly differ from the initial concentration. The blood glucose lowering effects of all doses of CHQ were pronounced in the first hour following the glucose load. All doses of CHQ showed blood glucose lowering effects which did not exhibit dose-dependency. Chloroquine additionally increased plasma insulin concentrations. Since CHQ reduced blood glucose concentration with concomitant increase in plasma insulin concentration, we suggest that the reported hypoglycaemic effect of the anti-malarial is mediated via increased pancreatic insulin secretion.

P140
The impact of thiazolidinediones on microalbuminuria in adults with type 2 diabetes mellitus: a systematic review
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1School of Medicine, Health Policy and Practice, University of East Anglia, Norwich, UK; 2Endocrine Signalling Group, Veterinary Basic Sciences, Royal Veterinary College, University of London, London, UK; 3Eliza Beatrix Diabetes Centre, Norfolk and Norwich University Hospital NHS Trust, Norwich, UK.

Background
Microalbuminuria is an early marker of diabetic nephropathy in type 2 diabetes mellitus (T2DM) which predicts the development of later stages of nephropathy. Thiazolidinediones are a class of oral hypoglycaemic drugs used in diabetes glycemic management that act through PPARy receptors. Studies have suggested they significantly reduce urinary albumin excretion; however, most trials were small. We therefore performed a systematic review to clarify the effectiveness of thiazolidinediones on microalbuminuria in adults with T2DM.

Methods
Eleven electronic databases were searched from inception to March 2007 and references from included trials were checked. Randomised, controlled trials were included if the patients had T2DM, were being treated with rosiglitazone or pioglitazone in comparison to other hypoglycaemic medication and had their urinary albumin excretion measured as either urinary albumin excretion rate (UAER) or urinary albumin creatinine ratio (UACR) at the study endpoint. Secondary outcomes were HbA1c, blood pressure, serum lipids and adverse events.

Results
Thirteen trials reporting on 3473 subjects were included in the systematic review and five studies were suitable for meta-analysis. Thiazolidinediones significantly reduced UAER at the study endpoint when compared to control, (pooled weighted mean difference (WMD) = −69.52 μg/min; 95% CI: −78.61 to −60.44; P < 0.00001). Twelve months therapy (WMD = −103.66 μg/min; 95% CI = −119.70 to −87.63 (P < 0.00001)) was more effective than 3–6 months therapy (WMD = −56.01 μg/min; 95% CI = −65.24 to −46.79 (P < 0.00001)). Qualitative analysis showed that in the majority of studies (75%), thiazolidinediones exerted a significant positive effect by reducing UAER.

Conclusions
This systematic review shows that thiazolidinediones sufficiently reduce urinary albumin excretion in patients with T2DM and suggests that they might be useful in preventing the progression of microalbuminuria to the later stages of diabetic nephropathy. The adverse side effect profile of the glitazones has been more emphasized in recent years and this potential benefit may be overlooked.
P142
Healthcare professionals frequently fail to recognize severe obesity
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Severe obesity continues to dramatically increase in prevalence and is associated with significant mortality and morbidity. The American Society of Metabolic and Bariatric Surgery recommends that bariatric surgery only be performed on those with severe obesity. Gastric bypass surgery decreases mortality by 40% at 7.5 years. Using photographs of volunteers from a hospital based weight management clinic, we performed a survey of endocrinologists, general practitioners (GPs), dietitians, physiotherapists and final year medical students. Members from each group were asked to estimate the body mass index (BMI) of the subjects in the photographs. We hypothesised that healthcare professionals underestimate the BMI of severely obese people.

Estimated BMI (kg/m²)

<table>
<thead>
<tr>
<th>Actual BMI (kg/m²)</th>
<th>Endocrinologists (n=19)</th>
<th>GPs (n=103)</th>
<th>Dietitians (n=26)</th>
<th>Physiotherapists (n=25)</th>
<th>Medical students (n=33)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>32</td>
<td>31.8 (2.3) 29.0 (2.3) 31.1 (3.4) 28.8 (5.6)</td>
<td>33.1 (2.7) 30.8 (2.3) 31.2 (2.7) 29.4 (4.0)</td>
<td>28.2 (2.4)</td>
<td>&lt;0.0005</td>
<td></td>
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<tr>
<td>40</td>
<td>43.2 (2.8) 37.8 (2.6) 41.8 (5.2) 39.2 (4.9) 34.9 (3.1)</td>
<td>42.7 (2.5) 38.1 (4.3) 42.0 (5.4) 41.0 (5.3) 35.1 (4.0)</td>
<td>&lt;0.0005</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>50</td>
<td>50.2 (2.7) 42.0 (4.9) 48.0 (6.5) 44.4 (4.7) 39.0 (3.4)</td>
<td>&lt;0.0005</td>
<td></td>
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</table>

Data are expressed as mean (± s.d.). P values were calculated using Kruskall–Wallis analyses.

We found that 25–72% of healthcare professionals fail to recognise severe obesity (BMI>40 kg/m²) in a patient with a body mass index of 51 kg/m². In order to facilitate appropriate therapy, all patient encounters with a healthcare professional must include formal assessment of weight and height.

P143
Starvation ketoadiposis in pregnancy
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John Radcliffe Hospital, Oxford, UK.

Introduction
Starvation ketoadiposis outside pregnancy is a rare phenomenon and is unlikely to cause a severe acidosis. Pregnancy is an insulin resistant state due to placental production of hormones including glucagon and human placental lactogen. Insulin resistance increases with advancing gestation and this confers a susceptibility to ketosis, particularly in the third trimester. Starvation ketoadiposis in pregnancy has been reported and is usually precipitated by a period of severe vomiting. Ketoadiposis has been associated with intrauterine death. Case report
A 22-year-old woman in her third pregnancy presented at 32 weeks gestation with a 24 h history of severe vomiting. She had been treated for an asthma exacerbation with prednisolone and erythromycin the day prior to presentation. She was unwell, hypertensive (145/70 mmHg) with a sinus tachycardia and Kussmaul breathing. Urinalysis showed ++ + ketones, + protein and pH 5. Fingerprick glucose was 4 mmol/l and ketones were 4.0 mmol/l. Arterial blood gas showed pH 7.27, PaCO₂ 1.1 kPa, base excess −23, bicarbonate 8.6 mmol/l and lactate 0.6 mmol/l. The anion gap was 20. Serum ethanol, salicylates and paracetamol levels were undetectable. She was fluid resuscitated but her biochemical parameters did not improve. She was intubated and underwent emergency caesarean section. A healthy boy was delivered and her acidosis resolved over the subsequent 8 h. Discussion
We believe this case is explained by starvation ketoadiposis. There was no evidence of diabetes mellitus or other causes of a metabolic acidosis. In view of the hypertension, proteinuria and raised urate the differential diagnosis was an atypical presentation of pre-eclampsia. This case illustrates the metabolic stress imposed by the feto-placental unit. It also highlights the importance of regular meals in pregnancy and sufficient calorific intake in patients who are vomiting, as well as the need for cautious prescribing of medications, which may worsen the insulin resistant state.

P144
Protective effects of annexin A1 in experimental endotoxaemia are mediated by an FPR-dependent mechanism
Ellen L. Hughes, Julia C Buckingham & Felicity N E Gavins
Imperial College, London, UK.

Sepsis is a major clinical problem, caused by a hyperactive immune response following infection. Worldwide prevalence is estimated at 1.8 m/year and mortality at around 40%.1 Protective effects of the endogenous anti-inflammatory protein annexin A1 have previously been shown in many models, including sepsis.2 We therefore chose to investigate the role of the annexin A1 peptide mimetic, Ac2–26, in murine experimental endotoxaemia, and to ascertain whether this is mediated by the formyl peptide receptor (FPR) family. We have previously profiled differences in leukocyte-endothelium interactions following LPS injection over time3 and found that optimal responses for further study occurred 2 h after treatment. Male C57BL/6 mice were treated with LPS (10 µg/mouse, i.p.) and 20 min later with peptide Ac2–26 (100 µg/mouse), the pan-FPR antagonist Boc2 (10 µg/mouse), or Ac2–26 and Boc2 together; controls received the relevant vehicles. The mice were then anaesthetised, the mesentry was exteriorised and leukocyte-endothelium interactions in post-capillary venules were quantified by intravital microscopy 2 h after the injection of LPS. All animals treated with vehicle or drug showed a reduction in rolling leukocyte flux compared to LPS alone, indicating a small effect of second injection. No differences were seen between groups in leukocyte rolling interactions. However, animals given LPS + Ac2–26 showed significantly fewer adherent leukocytes than those given LPS alone, LPS + Boc2 or LPS + Ac2–26 + Boc2. For the first time we demonstrate that Boc2 blocks the effects of Ac2–26 in this model, and therefore suggest that Ac2–26 is acting by an FPR-dependent mechanism to afford protection in experimental endotoxaemia. Further work is now required to establish whether a single-family member may be responsible.

References

P145
Toll-like receptor 2 (TLR2) as a biomarker for cardiovascular diseases in type 2 diabetes mellitus patients
Gihan Mohamed Kamal, Rania Sayed Abd ElBaki, Rehab Saleh ElIhagragi, Rasha Youssif Shaheen & Dina Mohamed Samir
 Ain Shams University, Cairo, Egypt.

Content
Type 2 diabetes mellitus (T2DM) may be associated with disorders in innate immune system. Toll-like receptor 2 (TLR2) is an important mediator of the innate immunity with subsequent proinflammatory reactions that thought to be involved in the cardiovascular complications in T2DM. Objective
We evaluate the role of TLR2 in T2DM patients with cardiovascular complications Patients and methods
The study was conducted on 60 patients with T2DM recruited from Ain Shams University Diabetes clinic from November/2007 to May/2009 compared to 25 controls. Patients were further subdivided to Group-Ia:30 patients without cardiovascular complication and Group-Ib:30 patients known to have cardiovascular complications. Peripheral blood samples were taken for measurement of TLR2 expression by flowcytometry and hs-CRP by PCR. In addition to full clinical, anthropometric measures, Fasting and 2hrPP serum glucose level, HbA1c and lipid profile evaluation. Results
TLR2 expression and hs-CRP were significantly higher among T2DM patients compared to controls (P=0.00) and in Group Ib compared to Group Ia (P=0.00). Further, Group Ib had significantly higher diastolic blood pressure (P=0.01) and HbA1c, (P=0.01) with a lower HDL-c (P=0.02). Moreover, positive correlation detected between TLR2 expression and hs-CRP as well as correlated to systolic blood pressure, HbA1c, and BMI among diabetic patients. Conclusion
Over expression of TLR2 and hs-CRP in T2DM with cardiovascular complications especially hypertensive and poorly controlled patients could reflect the inflammatory process. Measurement of TLR2 and hs-CRP could be a new marker for cardiovascular risk in T2DM. Future target therapy for TLRs can be a promising for T2DM patients.

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P146
Regulation of the melanocortin receptor accessory protein (MRAP) in Y1 adrenal cells
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Melanocortin receptor accessory protein (MRAP) is a single transmembrane domain protein, which has been identified as essential for the cell surface trafficking and signalling of the ACTH receptor (melanocortin 2 receptor (MC2R)). MRAP is essential for the function of MC2R, but regulation of its expression remains unclear. This study aims to identify factors regulating MRAP gene expression and whether these factors would affect the signalling of MC2R.

The mouse adrenal cell line (Y1) was stimulated with a range of hormones/stimulants for 4 h and MRAP mRNA expression was quantified using real time RT-PCR.

There was a four-fold increase in MRAP expression following stimulation with adrenocorticotropic hormone (ACTH) (10^{-8} M) and forskolin (10^{-5} M), whilst dexamethasone (10^{-6} M) induced a two-fold increase. Time course studies showed that the peak expression of MRAP was after 2 h stimulation with ACTH. Forskolin caused a gradual increase in MRAP expression, reaching its peak around 6 h, while dexamethasone caused maximal effect after 4 h. MC2 receptor function studies showed that treatment of cells with ACTH resulted in a lower cAMP response, which can be said that ACTH desensitises this receptor. In comparison the other agents do not effect the sensitisation of MC2R.

This study has shown that MRAP expression can be up-regulated by ACTH, forskolin and to a lesser extent dexamethasone. Activation of the MC2R by ACTH leads to cAMP signaling and positive regulation of its expression. This contrasts with the regulation of the MC2R, which in previous studies has been found to be modest. Thus it seems likely that regulation of MRAP gene expression is one mechanism by which the ACTH receptor complex is controlled. Further studies will address the protein expression of MRAP and its effect on signalling of MC2R in response to ACTH.

P147
Annexin 1 affords cerebroprotection in sepsis
Felicity Gavins, Ellen Hughes, Honeysha Patel & Julia Buckingham
Imperial College, London, UK.

Sepsis continues to be a leading clinical problem, with ~1.8 million people worldwide affected. This continual increase in sepsis and related deaths is in part due to age, increased frequency of invasive procedures and widespread bacterial antibiotic resistance, with mortality often related to underlying disorders that often accompany sepsis. Sepsis affects the brain, and the impairment of brain function is often associated with severe infectious disease. The endogenous anti-inflammatory protein Annexin 1 affords its protection via the FPR family. Here we assessed whether Annexin 1 could protect against the cerebral inflammation associated with experimental endotoxaemia.

Adult male mice (C57BL/6) treated for 2 h with LPS (10 µg/mouse, i.p.) exhibited a decrease in rolling and an increase in adherent leukocytes in the cerebral microcirculation, as demonstrated using intravitreal microscopy. Following the administration of Annexin 1 N-terminus-derived peptide Ac2–26 (100 µg/mouse, i.v.), a significant decrease in the number of adherent leukocytes was observed. This effect was abrogated following the co-administration of the FPR antagonist Boc2, suggesting that the protective effect of Annexin 1 peptide Ac2–26 was mediated by a member(s) of FPR. Serum samples were taken and inflammatory cytokines were measured, e.g. tumor necrosis alpha (TNFα) and IL-1β. LPS caused an increase in these inflammatory cytokines, which were suppressed following peptide Ac2–26 administration.

For the first time, these results demonstrate that the Annexin 1 peptide Ac2–26 affords cerebroprotection in experimental endotoxaemia and furthermore, these effects appear to mediated via an FPR-family mechanism.

References

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P148
The lipoprotein lipase HindIII polymorphism and susceptibility to hypertension
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Faculty of Medicine, Ain Shams University, Cairo, Egypt; 4 Fayoum University, Cairo, Egypt.

Background
Lipoprotein lipase (LPL) enzyme plays a central role in lipid metabolism. The primary function of LPL enzyme is the hydrolysis of the core triglycerides of circulating chylomicrons and very low-density lipoprotein (VLDL). It releases monoglycerides and free fatty acids, which are taken up by skeletal muscle or adipose tissue.

Aim
The present work aimed to study the association of the common variant of LPL HindIII (H^3) and hypertension.

Method
HindIII (H^3) variant allele of LPL were determined by polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) assay in 150 hypertensive patients and 150 normotensive subjects as a control group. Serum lipoproteins were also observed in both groups.

Results
Allele frequencies were H^3 = 0.783 and H = 0.267 for LPL HindIII in the hypertension group compared to H^3 = 0.683 and H = 0.317 in the control group. Individuals with homoygous (H^3/H^3) genotype were at high risk of developing hypertension compared to the (H/H) genotype (OR = 2.13, 95% confidence interval CI = 0.957-4.8). Serum triglyceride (TG) level were also higher in the individual with the (H^3/H^3) genotype compared to (H/H) genotype, while HDL showed negative correlation with the presence of (H^3+).

Conclusion
It can be concluded that the LPL HindIII (H^3) variant allele of LPL may influence the blood lipid metabolism and increase risk for hypertension.

P149
Intravitreal bevacizumab injection for proliferative diabetic retinopathy
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HMMI Meknes, Meknes, Morocco.

Introduction
To report the efficacy and safety of intravitreal bevacizumab (avastin) as an adjunctive treatment for proliferative diabetic retinopathy (PDR).

Objectives and methods
Thirteen patients (13 eyes) with PDR underwent an intravitreal injection of 1.25 mg of bevacizumab. All patients underwent a complete ophthalmic examination, anterior segment or fundus photographs at baseline and before bevacizumab injection.

Results
Eleven patients (84%) showed complete regression of anterior segment retinal, and/or optic disc neovascularisation between 1 and 3 weeks after injection. Treatment was well tolerated and no complications were observed.

Discussion
Short-term results suggest that intravitreally administered bevacizumab (avastin) for PDR is effective and safe.

Conclusion
Further studies are required to better assess the efficacy and safety of intravitreal bevacizumab as adjunctive treatment of PDR.

P150
Diabetic ketoacidosis: a 5-year retrospective analysis
Tromna O’Shea, Marie Louise Healy, John Nolan & Siobhan McQuaid
St James Hospital, James Street, Dublin 8, Ireland.

Introduction and methods
Despite advances in diabetes treatment, diabetic ketoacidosis (DKA) remains a significant cause of morbidity. We performed a 5-year retrospective analysis of DKA episodes (2004–2008). Results were compared with an earlier study (1997–2001).
Results
Ninety-four episodes of DKA (defined as hyperglycaemia, ketosis and acidosis) were identified through Hospital Inpatient Enquiry (HIE). Patients with Type 1 diabetes mellitus (DM) accounted for 89% of episodes, Type 2 DM (6%) and pancreatic diabetes (4%). Mean patient age was 31.4 ± 14.8 years with duration of diabetes 14.8 ± 13.0 years. 57.4% of episodes occurred in females.

Table 1 Biochemical characteristics.

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<tbody>
<tr>
<td>PH</td>
<td>7.18 ± 0.09</td>
<td></td>
</tr>
<tr>
<td>Bicarbonate (mmol/l)</td>
<td>9.22 ± 4.8</td>
<td></td>
</tr>
<tr>
<td>Glucose on admission (mmol/l)</td>
<td>32.8 ± 12.8</td>
<td></td>
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<tr>
<td>HbA1c (%)</td>
<td>11.1 ± 2.3</td>
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</table>

Data are presented as mean (s.d.) unless otherwise stated.

Mean length of hospital stay was 6.7 ± 8.9 days, with 12.8% requiring admission to HDU/ITU (mean length of stay 4.4 ± 3.6 days). Severe DKA (serum bicarbonate < 10 mmol/l) occurred in 37.2%. One patient died during admission. Precipitants for DKA were poor compliance with insulin therapy (54.5%), infection (20.2%), first presentation with DM (11.7%), unknown in 8.5%, continuous subcutaneous insulin infusion pump failure in 3.2% and alcohol-related in 3.1%. Repeat offenders accounted for 36.2% of episodes, with addiction or social factors contributing significantly to these recurrent episodes.

There has been a 15% reduction in cases of DKA since the previous study. However, DKA in patients with Type 2 DM and pancreatic diabetes has increased (0.9% vs 10.6%, respectively). There was a 33% reduction in HDU/ITU admissions.

Conclusions
DKA remains a common cause of hospital admissions and omission of insulin continues to be the main precipitant. For repeat offenders, social and psychological support is required in the management of this vulnerable group.

P151
Effect of ramonabant and metformin on oxidative stress in obese patients with polycystic ovary syndrome (PCOS)
Myint M Aye1, John Shepherd2, Liwei Cho1, J M Ng1, Anne Marie Coady2, Eric S Kilpatrick3, Stephen L Akpin1 & Thorzbahat Sathyapalan1
1University of Hull, Hull, UK; 2Hull Royal Infirmary, Hull, UK.

Background
Insulin resistance and obesity are characteristic features of PCOS. Oxidative stress leads to the formation of lipid peroxidation products in the skeletal muscles and has the potential to interfere with insulin signaling and thereby contribute to insulin resistance. Malondialdehyde (MDA) is a lipid peroxidation end product and is widely used as a marker of oxidative stress. Ramonabant has been shown to reduce weight and insulin resistance in obese PCOS patients. Pavlović et al. (2000) found that ramonabant reduced MDA level in patients with type 2 diabetes mellitus with average body mass index (BMI) 31 kg/m². This study was conducted to determine the effect of ramonabant and metformin on oxidative stress in obese patients with PCOS.

Subjects and methods
A randomized open labelled parallel study of metformin and ramonabant for 12 weeks in 20 patients with PCOS with a mean BMI 37 kg/m² was undertaken. MDA was measured before and after treatment with either metformin or ramonabant.

Results
MDA decreased (0.81 ± 0.14 vs 0.72 ± 0.89 μmol/l, P = 0.03, 95% CI 0.01-0.16) after treatment with ramonabant, but not following metformin treatment (0.77 ± 0.16 vs 0.89 ± 0.23 μmol/l, P = 0.17 CI -0.30-0.07). The baseline MDA were comparable (0.81 ± 0.13 vs 0.77 ± 0.16 μmol/l, P = 0.55) in the ramonabant and metformin groups. However, ramonabant group showed statistically significant reduction of MDA (9.6 ± 12.7 vs -17.9 ± 27%, P = 0.01, CI 7.5-47.6) compared to metformin group.

Conclusion
Ramonabant reduced lipid peroxidation likely through weight reduction, and improved insulin resistance, that was not seen with metformin. The negative result for metformin may reflect the reported lack of its efficacy in patients with a higher body mass index over 31.

P152
At high dietary fat levels, dietary fish-oil may enhance metabolic efficiency but may favour a reduction in the deposition of adipose tissue
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Background
Obesity is the excessive accumulation of adipose tissue to the extent that health may be impaired. Determination of the mechanisms governing adipose deposition and body weight regulation are now a scientific priority. High dietary fat intake is generally considered to be a factor in obesity’s multi-factorial aetiology and the metabolic syndrome. Yet not all dietary fats appear to be obese genic to the same degree.

Objective
To study the role of saturated (butter–milk) and polyunsaturated (fish–oil) dietary fats (DF) in the development of obesity.

Methods
Male Wistar rats were randomised (n = 10 per group) to receive one of four experimental diets which contained either moderate amounts of DF (23% of dietary energy) as either butter–milk (23.4%) or fish-oil (23.4%), or high levels of DF (42% of dietary energy) as butter–milk (42.5%) or fish-oil (42.5%). Rats were given ad-libitum access to their respective diets for 94 days. Total body adiposity was measured in car cases by magnetic resonance spectroscopy (MRS).

Results
At termination, there were no significant differences in the energy intake or body weight gain between the 23.4F and 23.4B groups. Whilst body weights were not significantly different between the 42.5F and 42.5B groups, there was a significant reduction in the cumulative energy intake in the 42.5F group (P < 0.01). Despite similar body weights, preliminary MRS analysis showed a trend towards reduced total adiposity in the rats fed the 42.5F diet compared to the 42.5B group.

Discussion
At high dietary fat levels, rats fed a fish-oil enriched diet appear to exhibit an enhanced metabolic efficiency, they consume less energy but have equal body weight gain to those animals consuming an isoenergetic saturated-fat diet. However, MRS analysis suggests any enhanced efficiency does not cause an increase in total adiposity, rather a leaner and therefore a more metabolically favourable phenotype may prevail.
P154
Indolentity of ectopic transcription using a pseudo splice site: lessons from HNF-1β mutation causing familial juvenile hyperuricaemic nephropathy
Brian Perri, Anita Reed, Jennifer Reilly, Neil Turner & Rajesh Thakker
University of Oxford, OXDEM, Oxford, UK. *Centre for Inflammation, Queen’s Institute for Medical Research, Edinburgh, UK.

Ectopic (or ‘illegitimate’) transcripts, which have been widely used to study disease-causing mutations when samples from the appropriate tissue cannot be obtained, are generally faithful representations of the normal tissue-specific counterparts. Here, we report the occurrence of ectopic transcripts of the hepatocyte nuclear factor-1 beta (HNF-1β) gene, mutations of which may result in maturity onset diabetes of the young type 5 (MODY5), the renal cysts and diabetes (Rcad) syndrome, and familial juvenile hyperuricaemic nephropathy (FJHN). We identified a donor splice site (IVS2a +1g→a) HNF-1β mutation in a proband with FJHN, and whilst investigating the functional consequences in Epstein-Barr virus transformed lymphoblastoids, detected the occurrence of ectopic HNF-1β transcripts that are not representative of the normal tissue-specific counterparts. Thus, two such novel splice variants resulting from use of a pseudo splice donor site within exon 2, were identified in the FJHN patient and 5 normals. These were not detected in RNA obtained from normal liver, kidney, thymus or spleen, thereby indicating that their occurrence in the lymphoblastoids is consistent with an indolent in ectopic transcription. Awareness of such indolentness of ectopic transcription, which has been previously reported for dystrophin and COLA4AS only, is important to ensure the correct interpretation of the functional consequences of DNA sequence variants identified in patients with metabolic and endocrine disorders.

P155
Patients with Turner syndrome have a high incidence of metabolic abnormalities
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Objectives
A retrospective review of adult patients with Turner syndrome (TS) attending a joint Turner syndrome clinic.

Methods
Sixty-two adult patients with TS clinic were identified. Data was collected from case notes and electronic records.

Results
Diagnosis was prenatal in 20%, 1–10 years in 29%, 11–20 years in 47% and after 21 years in one patient. Common presenting complaints were short stature (51%) and primary amenorrhoea (27%). The overall median height was 1.48 m (range 1.30–1.62 m). Twenty-one percent were above the 95th Turner height centile, 39% between the 75 and 95th centile, 34% between the 25–75th, and 6% were under the 25th. GH increased height range by 10 cm. Four TS mosaic patients achieved spontaneous puberty, one having had three spontaneous pregnancies. All premature patients were taking oestrogen replacement–sequential combined HRT (43%), OC (30%), continuous combined HRT (17%), topical oestrogen (6%), ethynylestradiol alone (4%). Mean uterine volume was 47.24 cm³ ± 16.17.

Nineteen percent had structural cardiovascular anomalies: 12% coarctation of the aorta, 7% bicuspid aortic valve and 2% dilated aortic root. Twenty-four percent had structural renal tract abnormalities: horseshoe kidney was the commonest (9.6%). BMD was available in 65%: 51% osteopenic, 18% osteoporotic. Forty-one percent were hypertensive and 80% have hypercholesterolaemia. Twenty-nine percent patients had a BMI >30. Eleven percent had type 2 diabetes, 4% had type 1 diabetes and 6% have glucose intolerance. Twenty-two percent of patients had hypothyroidism and 2% Graves’ disease. Coeliac antibodies were present in 6.5% of patients. Thirty-two percent had abnormal LFT, 15% had hepatic steatosis. Patients on continuous combined HRT had higher LFT than those on other oestrogen preparations (P<0.05).

Discussion
These data describe the multisystem nature of TS, and in particular highlight the obesity, hypercholesterolaemia and hypertension in these patients. However this is a small cohort. In order to further improve the care of our patients, multicentre long-term studies are required.

P156
Impact of extra cellular matrix changes on adiogenesis
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1School of Medicine, Centre for Endocrine and Diabetes Sciences, Cardiff University, Heath Park, Cardiff, UK; 2School of Medicine, Institute of Nephrology, Cardiff University, Heath Park, Cardiff, UK.

Obesity (excess adipose tissue generated by hypertrophy and hyperplasia) predisposes to poor health. Adiogenesis (hyperplasia) involves the differen-
tiation of adherent preadipocytes into non-adherent adipocytes and is accompanied by alterations to the extracellular matrix of which hyaluronan (HA) is a component. Used in vitro to promote lineage specific differentiation, little is known of HA’s role in adiogenesis. We hypothesise that HA modifications must occur during adiogenesis and have undertaken studies to investigate.

Experiments were performed in complete (CM) and differentiation medium (DM), containing a PPARγ agonist. Human preadipocytes were obtained (with ethical approval and informed consent) from sub-cutaneous adipose tissue and treated with or without 0.1 mM 4-methylumbelliferone (4 MU, inhibitor of HA synthesis) for 20 days. HA in medium (IILISA) and HA synthesise (HAS) transcripts (QPCR) were measured. Adiogenesis was assessed by oil red O (ORO) staining, counting adiogenic foci and QPCR measurement of differentiation markers (LPL). Protein expression of phospho-Akt and PPARγ were analyzed by western-blot.

Human primary preadipocytes (n=6) cultured for 20 days in DM displayed morphological and other signs of adiogenesis; this was accompanied by significant decreases in transcripts for HAS1, HAS2, and HAS3 and HA secreted into the culture medium compared with CM. Addition of 4 MU to DM, enhanced adiogenesis and produced fold increases of 4.1 ± 0.63 (foci), 2.6 ± 0.21 (LPL transcripts) and 1.52 ± 0.18 (ORO). Surprisingly, although 4 MU treatments decreased HA production in preadipocytes in CM, this did not occur in DM. However, levels of pAKT and PPARγ protein were increased 20% (P=0.04) and 40% PPARγ (P=0.01) respectively when comparing DM with/without 4 MU. Our study demonstrates that HAS transcripts and HA are decreased during adiogenesis and that the process is enhanced by an HA inhibitor. However the mechanism underlying the observations may involve signaling within the cell (e.g. pAKT) rather than through a surface receptor, further investigations are required.

P157
The hyperphagic effect of ghrelin is inhibited by diets high in fat in mice
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Background and aims
Ghrelin is the only known peripheral hormone, which increases food intake. It is released from the stomach and is thought to function as a meal initiator and signal of energy deficit. We used bacterial artificial chromosome transgenesis to generate a mouse model with increased ghrelin expression and production in stomach and brain. These mice exhibited increased circulating bioactive ghrelin and as expected were hyperphagic and glucose intolerant. We hypothesised that exposure to a high fat diet would exacerbate this phenotype.

Methods
We investigated the effect of high fat feeding on energy and glucose homeostasis in these mice. Subsequently, we determined dietary preference, expression of hypothalamic neuropeptides known to control food intake and using FPLC the circulating forms of ghrelin present under both feeding paradigms. Finally we measured food intake during continuous administration of ghrelin in wild type mice fed either regular chow (RC) or high fat diet (HFD).

Results
Firstly, we found our mice were resistant to diet induced obesity due to a significant reduction in food intake. This was not caused by alterations to food preference, hypothalamic signalling of neuropeptides known to control food intake or an alteration in the form of circulating acylated ghrelin. Secondly, we found long-term administration of ghrelin to wild type mice failed to increase ingestion of a HFD but as expected increased food intake on RC.

Conclusion
This is the first report that diets high in fat inhibit the hyperphagic effect of ghrelin and suggests that dietary environment is critically important in determining the function of ghrelin. This may be of importance when developing anti-obesity drugs targeting the ghrelin system.
P158

Nmu mice with an activating calcium sensing receptor mutation, Leu723Gln, have a metabolic acidosis and impaired urinary acidification

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The calcium sensing receptor (CaSR) is a G protein coupled receptor that is expressed in type A intercalated cells of the distal convoluted tubule and cortical collecting ducts, where it is involved in renal proton excretion. We have therefore investigated the Nmu mouse, which has an activating CaSR mutation (Leu723Gln) that leads to hypocalcaemia, hyperparathyroidism, renal acidification, a urinary concentrating defect, and cataracts, for disturbances in acid-base homeostasis. Mice were kept in iStatTran® with UK Home Office welfare guidelines and project license restrictions and housed in metabolic cages with ad libitum feeding for 4 days to allow for aclimation. The day 4 urine samples were obtained for measurement of pH, and venous and whole blood samples were collected and analysed immediately using an i-stat blood gas analyser (Abbott diagnostics). Homozygous affected (Nmu/Nmu) mice (n = 17) had a significantly more alkaline urine when compared to wild-type litter mates (n = 21) (p = 0.01) that was associated with significantly lower venous whole blood bicarbonate and base excess levels (blood bicarbonate = 17.3 ± 0.52 vs 21 ± 0.53 mmol/l, P < 0.0001; base excess = -9.29 ± 0.69 vs -4.53 ± 0.61, P < 0.0001). These results demonstrate that Nmu/Nmu mice have a metabolic acidosis that is related to a deficit of urinary acidification, and indicates that the CaSR plays a role in the regulation of systemic acid-base homeostasis. In addition, the presence of alkaline urine may explain why patients with activating CaSR mutations are susceptible to renal calcification and stone formation.

P159

Brain natriuretic peptide shows no response to acute hypobaria hypoxia in humans

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Background

Effective acclimatization to high altitude involves a natriuresis and diuresis facilitated by a suppressed aldosterone. Acute mountain sickness (AMS) is common but poorly understood. A cardiac hormone, brain natriuretic peptide (BNP), is released primarily in response to cardiomyocyte stretch but animal models suggest BNP secretion is also stimulated by acute hypoxia. An increase in BNP causes a diuresis, natriuresis and a reduction in aldosterone. We hypothesized that acute hypobaria hypoxia would elevate BNP.

Method

Seven healthy subjects (28 ± 2.4 years; 70.3 ± 14.8 kg; 172 ± 8 cm) gave informed consent. Blood samples were taken for assay of BNP at rest. Subjects then entered a hypobaric chamber where barometric pressure was reduced at a rate equivalent to 4000 ft. The chamber was aspirated to a chamber equivalent to 7700 ft. After 25 min at this altitude a standardised 1 min exercise test was performed and blood samples taken pre- and post-exercise (with simultaneous recordings of oxygen saturation and heart rate). Subjects breathed ambient air throughout. BNP was analysed using Biosite Triage BNP test kits at sea level after satisfactory quality control checks and had been passed.

Results

Mean (± s.e.m.) SaO2 was 98.1 ± 0.69; 68.1 ± 2.89 and 62.2 ± 5.3 at sea level; pre-exercise and post-exercise at 17 500 ft. Mean HR (± s.d.) was 65.4 ± 9.2; 81.1 ± 7.1 and 134 ± 13.5 at sea level; 17 500 pre-exercise and 17 500 post-exercise. BNP remained <5 pg/ml in 6 of 7 subjects and showed no significant rise in the seventh subject (BNP 18.1 pg/ml pre-exercise, 18.9 post-exercise).

Conclusion

Acute hypobaria hypoxia with exercise induced significant hypoxaemia and tachycardia in all subjects. No significant rise in BNP occurred. BNP may not therefore be implicated in the natriuresis and diuresis of high altitude adaptation. Alternatively, it may be that the relatively acute (< 40 min) hypoxic exposure is an insufficient stimulus to BNP secretion. Further experimentation under prolonged hypoxic conditions is underway.

P160

Total pancreatitis and pancreatic islet autotransplantation in patient with intractable pain caused by chronic pancreatitis

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Total pancreatitis for chronic pancreatic pain is often referred because it leaves the patient with unstable insulin-dependent diabetes. NICE (2008) has recently approved pancreatotomy with simultaneous islet autotransplantation and we here describe our first case. A 49-year-old female with a 6-year history of intractable, opioid requiring abdominal pain had become depressed to the point of suicide attempts. Imaging showed pancreatic atrophy and duct irregularity (subsequent histology confirmed exocrine acinar atrophy and fibrosis). Glucose levels were normal (HbA1C = 5.2%) and glucagon stimulation showed adequate β-cell function (C-peptide 1046 nmol/l basally, 2572 at 6 min). A total pancreatotomy was performed with simultaneous splenectomy. Following pancreatic digestion with Svera collagenase and neutral protease, 433 000 islet equivalents were infused into the portal system via the umbilical vein. Postoperatively, she was given oxygen, tinzaparin and insulin. She was discharged 2 weeks post-transplant with greatly reduced pain. However, 4 days later, she developed right upper quadrant pain and vomiting. Platelets had risen to 1000×10^9l and a CT scan demonstrated partial portal vein thrombosis. She was anticoagulated with heparin, followed by warfarin for 6 months. C-peptide levels rose transiently within 1 h post-transplant, probably due to release from dying β-cell, fell to undetectable levels by day 3 but a glucagon stimulation test at 4 months demonstrated basal and stimulated C-peptide levels of 357 and 540 nmol/l respectively indicating satisfactory β-cell engraftment. Her glycaemic control was stabilised on small amounts of insulin. A real time subcutaneous glucose sensor showed stable glucoses levels as seen in Type 2 diabetes and insulin is being gradually replaced by exenatide. Our patient underwent successful simultaneous pancreatotomy and islet autotransplantation resulting in satisfactory pain relief and stable glycaemia. This procedure should be considered earlier in the course of painful chronic pancreatitis. However, if the spleen has to be removed, portal vein thrombosis due to the consequent thrombocytopenia is a risk.

P161

Weight and glycaemic outcomes following bariatric surgery in people with type 2 diabetes

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Bariatric surgery in people with morbid obesity results in effective long-term weight loss, and resolution or reduction in the severity of several co-morbid conditions. The majority of obese people with type 2 diabetes who undergo bariatric surgery, achieve remission of their diabetes or a significant reduction of their glucose lowering therapy in the long-term. We conducted a retrospective observational study to assess changes in body weight and glycaemic control in obese type 2 diabetic patients in the first year after bariatric surgery to assess early efficacy.

Sixty-four obese type 2 diabetic patients (39 women) with a mean age of 49.5 years who had undergone bariatric surgery were identified from electronic patient records. Data recorded included preoperative and postoperative body weight and glucose control at 3, 6 and 12 months, and changes to diabetes treatment. The mean preoperative body weight was 143 kg and mean body mass index of 49.4 kg/m². The mean duration of diabetes was 9.8 years and 53% of patients were receiving oral hypoglycaemic agents whilst 26% were on insulin with or without oral agents. The mean preoperative glycosylated haemoglobin (HbA1c) was 7.99%. The mean postoperative weight loss at 3, 6 and 12 months was 28.1 kg (19.7%), 37.0 kg (25.9%) and 47.9 kg (33.5%) from baseline respectively, with a corresponding fall in mean HbA1c to 6.97, 6.55 and 6.15% respectively. Of those patients who were on glucose lowering treatment preoperatively, 67% achieved complete withdrawal of treatment whilst 32.5% had their medication dosage reduced. This study confirms significant weight loss comparable to the results of similar studies reported in the literature. It also demonstrates early remission or improvement in type 2 diabetes following bariatric surgery. Bariatric surgery should be regarded as an effective therapeutic intervention in the management of obese people with type 2 diabetes.
**P162**

Effect of Ramadan fasting on glycaemic control in type 2 diabetic patients on oral hypoglycaemic agents

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Ramadan is a month for Muslims during which they abstain from eating, drinking and smoking from dawn to sunset. The recommendations regarding the safety of fasting for type 2 diabetic patients have been based on expert opinion rather than evidence-based medicine and sometimes controversial. In this study, we looked at the effect of Ramadan fasting on glycaemic control of type 2 diabetic patients on oral hypoglycaemic agents.

Eighty-eight diabetic patients (45 males and 43 females, mean age 51 ± 10 years) on oral hypoglycaemic agents with a stable glycaemic control 3 months prior to the Ramadan were recruited. Local research ethics committee approval obtained. The subjects should have been fasting for at least 10 days (out of 29 days) during month of Ramadan. Fasting blood samples were taken at the beginning, at the end and 1 month after Ramadan. Anthropometric values, fasting blood sugar (FBS), insulin, lipids profile and HbA1c were measured at these time-points.

There was a significant deterioration in FBS and HbA1c after Ramadan (P < 0.005, paired t-test). There was also a significant improvement in HDL level after Ramadan (P < 0.005, paired t-test). Interestingly, the HbA1c showed a reduction 1 month after Ramadan (HbA1c 8.4 ± 2% 1 month after Ramadan; P < 0.005, paired t-test).

This study shows fasting during Ramadan deteriorates the glycaemic control in type 2 diabetic patients. The glycaemic control was improved 1 month after Ramadan. Also there was a significant improvement in HDL value. However, measuring other short-term markers of the glycaemic control such as fructosamine and a study with a bigger sample size, controlling other habitual changes and a control group is needed.

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<th>FBS (mg/dl)</th>
<th>HDMA-IR</th>
<th>Cholesterol (mg/dl)</th>
<th>TID (mg/dl)</th>
<th>HDL (mg/dl)</th>
<th>BMI (kg/m²)</th>
<th>LDL (mg/dl)</th>
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<td>8.2 ± 0.2</td>
<td>158 ± 64</td>
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<td>197 ± 2</td>
<td>198 ± 5</td>
<td>43.7 ± 8</td>
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<td></td>
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<tr>
<td>After</td>
<td>9.4 ± 0.2</td>
<td>171 ± 64</td>
<td>3.8 ± 2.9</td>
<td>234 ± 4</td>
<td>168 ± 3</td>
<td>47.8 ± 8</td>
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<td>&lt; 102</td>
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**P163**

An exploration of the predictive factors for the early pleiotropic responses to exenatide therapy in patients with type 2 diabetes mellitus

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Aims

Our aim was to explore predictive factors for the pleiotropic responses to exenatide during the first 12 weeks of therapy, and associations between these responses.

Methods

This was an observational study based on patients (n = 83) with type 2 diabetes mellitus (T2DM) in whom exenatide had been added to existing oral hypoglycaemic therapy. Exenatide was administered subcutaneously (5 mcg bd for the first month, 10 mcg bd thereafter). Data collected (at baseline and following up to 12 weeks of exenatide therapy) included weight, waist circumference (WC), HbA1c, fasting lipid profile and blood pressure (BP). Analyses included paired-sample t-tests and Pearson correlations. Data are shown as mean (± s.e.), as appropriate (or s.e.) at baseline and following exenatide therapy respectively.

Results

Duration of exenatide therapy (n = 83) was 1–12 weeks (mean 7 weeks (± 3.5)). Following early exenatide therapy, there were significant reductions in weight (112.3 kg (20.4) versus 108.7 kg (19.3), P = 5.1 × 10^-5), WC (125.7 cm (16.2) versus 121.6 cm (13.2), P = 3.9 × 10^-5), HbA1c (9.5% (1.4) versus 8.9% (1.8), P = 0.03) and systolic BP (139 mmHg (13) versus 133 mmHg (16), P = 0.03). There were no significant changes in fasting lipid profile or diastolic BP.

In separate multivariate analyses using total weight loss and reduction in HbA1c as dependent variables, initial weight (P = 4.6 × 10^-5) and initial HbA1c (P = 3.3 × 10^-5) were significant and independent variables respectively. Baseline weight correlated significantly with weight loss (r² = 0.30, P = 4.8 × 10^-5), and baseline HbA1c with reduction in HbA1c (r² = 0.47, P = 1.2 × 10^-5). Weight loss and reduction in HbA1c during exenatide therapy did not correlate with each other.

Conclusions

Baseline weight and HbA1c independently associate with early reduction in weight and HbA1c respectively, during treatment with exenatide therapy. Obese T2D patients with poor glycaemic control are most likely to derive early benefit from exenatide treatment, although weight reduction does not necessarily correlate with improved glycaemic control suggesting variable independent effects of exenatide on appetite and β-cell function between patients.

**P164**

Expression of store-operated Orai channels in normal and diabetic kidney

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Cytosolic calcium signalling controls a vast number of cellular functions, such as contraction, secretion, cell growth and cell division. The increase of [Ca²⁺]evoked by G-protein coupled receptor activation has two closely related components, i.e. the rapid phase of inositol 1,4,5-trisphosphate-mediated Ca²⁺ release from ER stores, and followed by Ca²⁺ entry through store-operated channels (SOCs) in plasma membrane. Orai and STIM proteins are recently identified store-operated channel molecules, but their expression and function in human kidney are unknown. Here, we aimed to determine the expression and distribution of Orai in normal and diabetic human kidney. Methods

Human kidney samples were obtained from Hull and East Yorkshire NHS Trust with approval of the local ethics committee from patients undergoing a nephrectomy, or from patients with diabetes undergoing a kidney biopsy. The mRNA expression was detected by RT-PCR or real-time RT-PCR. The protein expression and localisation were detected by western blotting and immunostaining. Results

The mRNA and protein expression of Orai1, Orai2 and Orai3 was detected in normal human kidney samples by RT-PCR and western blotting, respectively. Orai proteins were highly expressed in proximal tubules in the kidney, but very weakly expressed in the glomerulus. The expression of Orai isoforms in diabetic kidney was less abundant than that in normal kidney. In primary cultured human kidney tubular cells, Orai 1 was localised in the plasma membrane detected by fluorescence staining, while Orai 2 and 3 were intracellularly located. Conclusions

We have demonstrated that the store-operated Orai channels existed in human kidney, and highly expressed in the proximal tubules. The expression level could be changed in some kidney diseases, such as diabetes. The finding provides the first evidence of Orai proteins in normal human kidney and diabetic nephropathy.

**P165**

Metabolic phenotype in brothers’ of women with polycystic ovarian syndrome

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Women with polycystic ovary syndrome (PCOS) have twice the risk for metabolic syndrome (MetS) compared to women from the general population. Mothers and sisters of affected women also have an increased prevalence of MetS. We undertook this study to evaluate whether the abnormalities are also present in brothers’ of affected women.

Design and setting

A total of 59 brothers (age ≥ 18 and ≤ 40 years) of women with PCOS and 59 healthy men (of same age) as controls without any first degree female relatives with PCOS visiting endocrine clinic were included in the study. They underwent anthropometric measurements (BMI, waist circumference (WC), waist hip ratio (WHR)), 75 gm oral glucose tolerance test (OGTT), fasting insulin and fasting lipids measurements.
Results
The prevalence of MetS was increased in brothers (29 vs 8%; P = 0.001) compared to the controls. Brothers had higher BMI (28 ± 1.9 vs 23 ± 1.1 kg/m²), WC (95 ± 11.2 vs 81 ± 9.9 cm) and WHR than controls. Fasting insulin levels (29 ± 2.6 vs 15 ± 1.6 μU/ml), LDL cholesterol (125 ± 12 vs 109 ± 9 mg/dl) and triglyceride levels (138 ± 13 vs 119 ± 10 mg/dl) were also higher compared to controls and HDL levels (42 ± 5 vs 49 ± 3 mg/dl) were lower in the study group. OGTT also revealed the diagnosis of diabetes in four and IGT in 14 brothers' of women with PCOS.

Conclusion
Brothers' of women with PCOS had increased prevalence rates of MetS and obesity as compared to control population. This implies that brothers' of women with PCOS should also be evaluated to detect previously undiagnosed metabolic abnormalities.

P166
Nuf mice with an activating calcium-sensing receptor mutation, Leu723Gln, have impaired glucose tolerance and reduced insulin secretion
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The calcium-sensing receptor (CaSR) is a G protein coupled receptor that is expressed widely, including pancreatic beta cells where it has been shown to modulate insulin secretion in vivo. However, the role of the CaSR in regulating in vivo insulin secretion and glucose homeostasis remains unknown. We have therefore investigated the Nuf mouse, which has an activating CaSR mutation (Leu723Gln), to determine whether abnormal CaSR function in vivo affects glucose tolerance and plasma insulin concentrations. Mice were kept in accordance with UK Home Office welfare guidelines and project license restrictions. A glucose tolerance test was performed by fasting mice overnight and administering an intraperitoneal 2 g/kg glucose bolus. Blood samples were obtained at 0 and 120 min. Plasma glucose concentrations were measured using an analog glucose analyser and fasting plasma insulin concentrations determined using a multiplex mouse endocrine immunoassay. The glucose tolerance tests revealed that male and female homozygous Nuf mice (Nuf/Nuf) had significantly raised plasma glucose concentrations at 120 min when compared to wild-type litter-mates (male glucose = 25.4 ± 1.7 vs 20.5 ± 2.1 mmol/l, P < 0.05; female glucose = 13.0 ± 0.6 vs 10.6 ± 0.3 mmol/l, P < 0.01). In addition, male and female Nuf/Nuf mice had significantly reduced fasting plasma insulin concentrations, when compared to wild-type litter-mates (male insulin = 65.4 ± 9.1 vs 200.9 ± 49.5 pmol/l, P < 0.01; female insulin = 63.6 ± 9.5 mmol/l vs 100.4 ± 17.0 pmol/l, P < 0.05). Thus, our finding demonstrate that CaSR activation impairs insulin secretion and glucose tolerance and highlights that the CaSR is an in vivo determinant of glucose homeostasis.

P168
Glucocorticoid and insulin regulation of lipid metabolism in human adipocytes
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Patients with glucocorticoid excess develop a phenotype characterized by central obesity, however, the impact of glucocorticoids upon the processes that regulate lipid accumulation has not been fully explored. We hypothesize that intracellular generation of cortisol from cortisone by 11b-hydroxysteroid dehydrogenase type 1 (11bHSD1) is an important regulator of lipid metabolism.

In adipocytes, acetyl-CoA carboxylase 1 and 2 (ACC1/2) convert acetyl-CoA to malonyl-CoA. ACC1 predominates and is the rate-limiting step in lipogenesis. ACC2 is localized to the mitochondrial membrane, and the malonyl-CoA generated inhibits β-oxidation. Using a human subcutaneous cell line, Chub-67, we characterized the expression of ACC1/2 and the regulation of lipogenesis (11b-hydroxyo rt steroid dehydrogenase type 1 (11bHSD)) in human adipocytes. The expression of ACC1/2 is regulated by sterol and malonyl-CoA.

P167
Liraglutide prevents endothelial damage triggered by glucose oscillations
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CSRI, Warwick University, Coventry, UK.

Background
Since it has been shown that markers of oxidative stress persist in endothelial cells that have been exposed for a prolonged time to chronic constant high glucose levels, long after glucose normalization, a phenomenon defined as ‘metabolic memory’, evaluation of chronic exposure to oscillating high glucose, its impact on the induction of cellular stress markers and the onset of a ‘metabolic memory’ might be of pivotal interest. In doing so we also tested the efficacy of Liraglutide in reducing the induction of cellular stress factors, and in preventing the onset of the metabolic memory.

Methods
HUVECs were incubated in 5 or in 30 mmol/l glucose or for 24 h in 5 followed by 24 h in 30 mmol/l glucose for 3 weeks or subjected to 1 week of constant 5 mmol/l glucose after being exposed for 2 weeks to continuous 30 mmol/l high glucose or oscillating high glucose. All the conditions were examined in the presence or absence of Liraglutide 100 nM. Protein analysis was conducted thought western blotting.

Results
Exposure to chronic high or oscillating glucose significantly upregulated PTEN, ATM, H2AX and PKCζ (P < 0.05), and remained significantly elevated after glucose normalization compared to control (P < 0.05). Adding Liraglutide to the media, the stress markers upregulation was not significant compared to control with or without Liraglutide, but significantly increased Akt phosphorylation in all the conditions taken in exam (P < 0.05).

Conclusions
Exposure to chronic oscillating glucose confirmed to be more deleterious condition compared to constant high glucose and able to induce a metabolic memory effect after glucose normalization. Adding Liraglutide to the culture media not only prevented the onset of this effect but also reduced expression of oxidative stress markers both in constant high and oscillating glucose.

P169
MOPDII and Alström syndrome: two centrosomopathies featuring severe insulin resistance and impaired adipogenesis
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2MRC Human Genetics Unit, Western General Hospital, Edinburgh, UK.

Genetic defects in PCNT, encoding the centrosomal protein pericentrin, cause a rare syndrome of primordial dwarfism, skeletal dysplasia and facial dysmorphism, known as Majewski Osteodysplastic Primordial Dwarfism Type II (MOPDII). We now report that 11 out of 15 patients with PCNT defects had clinical and/or biochemical evidence of severe insulin resistance (IR), many also with severe lipodystrophy; the remaining four were under 4 years-old. The metabolic profile of MOPDII therefore resembles an extreme, early-onset (but not congenital) form of the prevalent metabolic syndrome. Such an exaggerated
metabolic phenotype is also seen in Alström syndrome (US), caused by genetic defects in ALMS1, which encodes another large centrosomal protein. These findings suggest that the centrosome may play a general role in maintaining metabolic homeostasis in humans. The centrosomal localisation of both pericentrin and ALMS1 implicates them circumstantially in mitotic and/or interphase microtubule organization. However, specific roles in canonical insulin action have not been sought. We have therefore tested two hypotheses regarding the basis of severe IR in MOPFDI and/or AS: first, that it is associated with cell autonomous defects in insulin action; and second, that it is explained by a relative failure of adipose tissue differentiation, and thus ‘fat failure’. Stable knockdown of either pericentrin or ALMS1 in murine 3T3-L1 preadipocytes impaired induction of adipocyte gene expression and lipid accumulation. Furthermore, pericentrin knockdown was associated with a slower rate of cell proliferation. Proximal insulin signalling events were unaffected in the presence of either knockdown, and although insulin-stimulated deoxyglucose uptake was decreased, this was commensurate with the adipogenic defect. Our findings suggest that partial failure of adipocyte differentiation and thus adipose tissue expandability may contribute to the severe metabolic syndrome of MOPFDI and AS, but provide no evidence for a role of either pericentrin or ALMS1 in cell autonomous insulin action.

### P170

A novel syndrome of IGFI and insulin supersensitivity
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1Metabolic Research Laboratories, Institute of Metabolic Science, University of Cambridge, Cambridge, UK; 2Whiston Hospital, Prescot, Merseyside, UK.

GH-secreting pituitary adenomas are by far the commonest cause of acromegalic soft tissue overgrowth. However the differential diagnosis includes pseudo-acromegaly in rare patients with severe insulin resistance, with or without lipodystrophy, and some congenital overgrowth syndromes such as Sotos’ syndrome, due to mutations in the NSD1 gene. We now report the case of two siblings, born to non consanguinous, clinically unaffected Europid parents, with childhood overgrowth and progressive adult soft tissue overgrowth closely resembling acromegaly, but without any evidence of GH hyperscretion or a pituitary adenoma. Serum IGFI levels were also repeatedly normal. Moreover, far from exhibiting severe insulin resistance, both siblings showed strikingly low insulin excursions during an oral glucose tolerance test: the male sibling, despite a body mass index of 37 kg/m², had fasting and peak insulin levels of 10 and 85 pmol/l respectively, while his sister, with a BMI of 34 kg/m², had fasting and peak insulin levels of 17 and 118 pmol/l, well below the third centile for sex, age and BMI matched control subjects. Serum adiponectin levels were also abnormal, with both siblings having elevated adiponectin relative to matched controls. NSD1 screening was normal in both siblings. In summary, both siblings present with a previously unreported syndrome of biochemically-determined insulin supersensitivity, and clinical features consistent with lifelong excess IGFI action. We hypothesized that this disorder is caused by genetic loss of function of a negative regulator of both insulin and IGFI  action. Genetic screening to date has ruled out coding defects in the genes encoding the IGFI receptor, protein tyrosine phosphatase 1B, and the adaptor proteins GRB10 and GRB14. Elucidating the cause of this syndrome is likely to give valuable insights into the function in vivo of a negative regulator of insulin action, and thereby to efforts to target it pharmacologically.

### P171

**Biomarkers of the hypothalamic–pituitary–adrenal (HPA) axis for analysis of in vivo 11β-hydroxysteroid dehydrogenase 1 inhibition**
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1University of Manchester, Manchester, UK; 2CVCV, AstraZeneca, Alderley Park, UK.

Inhibition of 11β-hydroxysteroid dehydrogenase 1 (11β-HSD1) as a means of reducing tissue corticosteroids is showing marked potential as a treatment for type 2 diabetes. However, reduction in tissue corticosteroids may lead to upregulation of the HPA axis. Therefore this study investigates the effect of 11β-HSD1 inhibition on HPA axis biomarkers and examines whether time of dosing impacts on the biomarkers. C57Bl6/Jax mice were fed 60% kcal fat diet for 16 weeks. Mice were given an 11β-HSD1 inhibitor (or vehicle) or neither at the previously determined corticosterone peak or nadir or both. Samples were taken at peak or nadir after 8 days. Pro-opiomelanocortin (POMC) peptide levels were measured as a biomarker of chronic effects but not acute stressors. No changes were seen at peak or nadir after any treatment. There was a trend towards increased adrenocorticotropic hormone (ACTH) concentrations. The corticosterone diurnal rhythm was maintained in naive but not vehicle treated mice. When the inhibitor was administered at nadir and samples taken 2 h later corticosterone was elevated.

### P172

Aldosterone production by the mouse adrenal gland is compromised by a high fat diet and 11β-hydroxysteroid dehydrogenase type 1 deficiency
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Deficiency of the intracellular glucocorticoid (GC) reactivating enzyme 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) invokes compensatory activation of the HPA axis. Nevertheless, 11β-HSD1−−/− mice resist dietary-induced obesity indicating that target tissues are protected from GC actions. Since endocrine adaptation to diet is mediated by altered HPA activity, we investigated whether the adrenal response to high fat (HF) diet is compromised in 11β-HSD1−−/− mice. Control and null male mice (n=5) were fed normal or HF diet whilst being infused sc with bromoexoxy-aniline (BrdU) to mark cell proliferation. After 2 weeks, tissues and blood were collected. HF diet and 11β-HSD1−−/− genotype increased adrenal mass by 10 and 20% (P<0.01). Mid-sections of adrenal glands were stained immunocytochemically for BrdU. Cell size and percentages of BrdU+ve nuclei were measured in the aldosterone-synthesizing zona glomerulosa and the GC-synthesizing zona fasciculata and reticularis. HF diet increased cell size by 45% in null but not control mice (P<0.01). BrdU labelling was highest in the glomerulosa and declined by 90% in the reticularis; labelling in the medulla was also low. HF diet and 11β-HSD1−−/− genotype increased percentages of labelled cells by 2.5 fold in the glomerulosa and fasciculata (P<0.001). Plasma renin and steroid concentrations were measured by radioimmunoassay. Neither diet nor genotype affected corticosterone. HF diet raised renin by 50% and reduced aldosterone by 40% (P<0.05); the aldosterone renin ratio was reduced from 1.11±0.15 to 0.33±0.08. We conclude that resistance to obesity in 11β-HSD1−−/− mice is not due to insufficient adrenal corticosterone production. Both HF diet and 11β-HSD1 inactivation caused adrenal hypertrophy. Hyperplasia of glomerulosa cells is probably due to HPA activation and as well as increased renin. Glomerulosa and renin changes were associated with decreased rather than increased plasma aldosterone indicating a novel mechanism of aldosterone clearance that is secondarily influenced by glucocorticoid metabolism.
P173
Transdermal delivery of insulin using amimated pectin hydrogel patches
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In type 1 diabetes mellitus, tight glycaemic control is required to attenuate chronic complications and often requires daily injectable insulin therapy, which can result in non-compliance because of patient discomfort. As a result of this, some studies are concerned with optimal routes of insulin administration. In our laboratory, we have developed amimated pectin (polygalacturonic acid) hydrogel beads for oral insulin administration. The aim of present was to investigate whether insulin can be transported through the skin using pectin hydrogel insulin patches. Four groups of diabetic male Wistar rats (n=10) fasted overnight were used. Basal blood glucose concentrations were taken and the patches (containing 6, 15, 30 and 60 µg of insulin in each group, respectively) applied before the administration of an oral glucose tolerance test (OGTT) (0.86 g of glucose/kg body weight). Blood glucose was measured using the tail-prick method at 30 min, 1, 2, 3 and 4 h after glucose administration. After 4 h, rats were sacrificed and blood collected for plasma insulin assay using the rat ultra-sensitive insulin ELISA. The patches were dissolved in Sorenson’s buffer and the insulin released measured by ELISA. At each time point during the OGTT, the blood glucose concentration was significantly lower in the high insulin dose group than the low insulin dose groups, indicating that insulin was transported across the skin and that a dose-dependent effect on blood glucose occurred. Similarly, plasma insulin concentrations after 4 h were 18.7±4.9, 26.9±3.9, 35.7±2.6 and 112.7±9.1 pmol/l for the 6, 15, 30 and 60 µg insulin patch group, respectively. The dissolution studies revealed an insulin yield from the patches of greater than 70%. Results of this study suggest that transdermal insulin delivery using pectin hydrogel patches occurs in a rat model of type 1 diabetes mellitus.

P174
Low testosterone level is associated with significant increase in mortality in patients with type 2 diabetes
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Background
Men in type 2 diabetes there is high prevalence of low levels of testosterone. There is accumulating evidence that low testosterone levels are associated with greater morbidity and mortality. There is no published data regarding mortality among men with type 2 diabetes in relation to testosterone levels.

Aim
We report preliminary findings from our 4 to 7 years follow up study to examine the effect of baseline testosterone on all cause mortality in men with type 2 diabetes.

Methods
A total of 587 patients with type 2 diabetes who had testosterone levels done between 2002 and 2004 were followed up for a mean period of 5.8 years (SD 1.3). Patients who died in the first 6 months of screening were excluded. Patients were analysed in three groups: i) with normal TT levels (above 12 nmol/l) ii) with borderline TT (between 8 to 12 nmol/l) and iii) low TT levels (below 8 nmol/l).

Results
Of the 587 patients 277 (47%) had normal TT levels, 179 (30%) borderline low TT levels and 131 (22%) had low TT levels. Mean age was 60 (SD 11) and they were significantly matched for other groups. 72 (12.2%) deaths occurred during the follow up period. The mortality was highest in low TT group compared to that with normal TT (27 out of 131 (21%) versus 26 out of 277 (9.4%) in the Kaplan-Meier model, survival was significantly decreased from 49 months (SE 3.8) in normal TT group to 37 months (SE 2) in patients with low TT (P=0.041). In the Cox regression model of the age adjusted survival was significantly lower (Hazard ratio=1.4, P=0.005) in patients with low testosterone in comparison with those with a normal TT levels.

Conclusions
This data shows that in men with type 2 diabetes low levels of testosterone is associated with significant increase in mortality.

P175
The effects of plant derived oleic acid bioactive compound on glycolygenic enzymes in streptozotocin-induced diabetic rats
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We have reported that the anti-hyperglycaemic effects of Syzygium cordatum leaf triterpene mixtures (oleanolic acid (OA) and ursolic acid) in streptozotocin (STZ)-induced diabetic rats are mediated in part via increased hepatic glycolytic synthesis. To further elucidate the mechanism(s) of the hyperglycaemic effects of the triterpene, this study investigated the effects of plant derived OA on glycolygenic enzyme activities in STZ-induced diabetic rats. Pure OA was obtained from Syzygium aromaticum flower bud ethyl acetate solubles following recrystallization with ethanol and its structure confirmed by spectroscopic analysis using 1D and 2D, 1H and 13C Nuclear Magnetic Resonance (NMR) techniques. Hepatic and gastrocnemius muscle glycogen concentrations and activities of hexokinase and glucokinase enzymes were measured in separate groups of non-diabetic and STZ-induced diabetic rats after 5 weeks of twice-daily treatment with OA (60 mg/kg, p.o). Rats treated with deionised water (3 ml/kg, p.o), or standard hyperglycaemic drugs (insulin, 200 µg/kg, s.c.; metformin, 500 mg/kg, p.o.) acted as untreated and treated positive controls, respectively. Hexokinase and glucokinase activities were measured spectrophotometrically in reactions where the oxidation of glucose-6-phosphate formed was coupled to NADP+ reduction catalyzed by glucose-6-phosphate dehydrogenase. OA increased hepatic and muscle glycogen concentrations of both non-diabetic and STZ-induced diabetic rats (P<0.05) as did standard drugs, metformin and insulin. OA and insulin significantly increased muscle hexokinase activity in the non diabetic animals. There was no significant effect on muscle hexokinase activity with all treatments in the STZ-induced diabetic animals, however; metformin showed a significant decrease in hexokinase activity compared to control animals (P<0.05). On the contrary, OA and insulin significantly increased hepatic hexokinase and glucokinase activities in STZ-induced diabetic rats. These findings suggest that the plant-derived OA increases glycogen synthesis, partly by increasing the activities of the enzymes hexokinase and glucokinase in the muscle and liver, respectively thereby reducing blood glucose levels.

P176
Testosterone replacement may be beneficial in hypogonadal men with cardiovascular disease
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Hypogonadism is more prevalent in populations with type 2 diabetes (T2D) and/or cardiovascular disease (CVD) and is associated with increase in all-cause and CVD mortality. Testosterone replacement therapy (TRT) improves visceral adiposity, insulin resistance, glycaemia, lipids inflammatory cytokines and cardiac ischaemia. The long-term safety of TRT in men with CVD and/or T2D is not known and the British National Formulary advises use with caution these groups. This is a retrospective analysis of 182 patients of hypogonadal patients with diabetes and/or CVD on TRT. Mean age 58.3±0.83 years, weight 103±2.24 kg, total testosterone 7.34±0.29 nmol/l. Prior to TRT 53% had T2D, 51.9% CVD (40% had CABIg), 36.6% hypertension. 18.58% had a previous CV event defined as either myocardial infarction, cerebrovascular accident or TIA, amputation due to peripheral vascular disease (PVD). Of 73.2% had erectile dysfunction. Mean follow-up 32 months – total 574.5 patient years of TTR. There were six deaths (3.3%) one attributable to CVD. Sixteen CV events occurred in 13 patients (7.1%). After 8-13 months of TTR total cholesterol (TC) decreased (4.67±0.14-4.15±0.11 mmol/l, P=0.002, n=65). No significant change in HDL cholesterol (1.04±0.03-1.02±0.04 mmol/l, P=0.45, n=52), HbA1c (7.2±0.13-7.3±0.17%, P=0.39, n=53), weight (99.7±2.51-99.2±2.37 kg, P=0.49, n=74). Diastolic BP (79.6±1.7-78.7±1.2 mmHg) or systolic BP (139.5±1.99-138.9±1.81, P=0.48, n=62). In 92.1% T2D medication was unchanged. The CV deaths and events were lower than expected in this morbid population. No adverse effects on routinely assessed CV risk factors were found with a beneficial effect on TC. Only a small number of T2D cases needed a change in

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medication. This safety audit in association with current knowledge of the adverse effect of low testosterone on survival and benefits of TRT on CV risk factors support the need for larger clinical trials.

P177

Mice harbouring the familial juvenile hyperuricaemic nephropathy disease-causing uroromulin (Tamm–Horsfall glycoprotein) mutation Cys125Arg have a urine concentrating defect, progressive renal failure, and altered uric acid handling

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Familial juvenile hyperuricaemic nephropathy (FJHN), an autosomal dominant disorder characterised by raised serum urate, reduced fractional excretion of uric acid (FEUA), a urine concentrating defect, and progressive renal failure, is caused by mutations in the UMOD gene, encoding uroromulin (Tamm–Horsfall glycoprotein). The FJHN-causing UMOD mutations are missense mutations (>90%) or inframe deletions (<10%), and none result in prematurely truncated proteins, indicating the disorder is unlikely to be due to a loss of function. We therefore engineered a knock-in mouse harbouring the FJHN-causing Cys125Arg mutation. Mice were maintained in accordance with UK license restrictions. Wild type, heterozygous (UMod+/–) and homozygous (UMod Knock-in mice, aged 8 weeks or 6 months, were investigated in metabolic cages. UMOD1/2 and UMOD1/2/2 mice had a low urine osmolality (P<0.02) with a normal or high plasma osmolality, and were polyuric (P<0.02), and polydipsic (P<0.001). Eight week old UMod1/2/2 mice had significantly raised plasma urea (P<0.02), whilst by 6 months of age, UMod1/2/2/2 and UMod1/2/2 mice had developed significantly raised plasma urea (P<0.0001), and UMod1/2/2 mice had elevated plasma creatinine (P<0.005), consistent with progressive renal failure. Six month old UMod1/2/2 mice also had significantly raised plasma alkaline phosphatase (P<0.01), and a significantly reduced FEUA (P<0.001), which was not associated with a raised plasma uric acid, due to the presence of the enzyme uricase in mice. Thus, these uroromulin knock-in mice have phenotypic similarities to FJHN patients, in having a urinary concentrating defect, progressive renal failure, and reduced FEUA. Our study opens the way for further in vivo investigations to elucidate the pathophysiological basis of FJHN and its associated renal failure.

P178

Investigating the role of ventromedial hypothalamic glucose-sensing neurons in the response to hypoglycaemia

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Hypoglycaemia and hypoglycaemia unawareness severely limit the optimal management of diabetes mellitus and cause recurrent morbidity and even mortality in intensively controlled patients. Altered hypothalamic glucose sensing has been implicated in the development of defective counter regulatory responses to insulin induced hypoglycaemia and hypoglycaemic unawareness. This change in hypothalamic glucose sensing has been attributed, at least in part, to an increase in glucokine activity in the ventromedial hypothalamic (VMH) following insulin induced hypoglycaemia. An increase in VMH glucokinase is also noted in fasting rats. This suggests that VMH glucokinase may have an important role in mediating physiological responses that restore normoglycaemia during glucoprivation.

To test this hypothesis we stereotactically injected recombinant adeno-associated virus (rAAV) encoding glucokinase (α=11) or GFP (α=9, controls) into the VMH of adult male Wistar rats. Glucokine expression in the VMH was confirmed using in situ hybridisation. We tested the role of glucokinase in gradual-onset hypoglycaemia during fasting. Blood glucose was measured, in mmol/l, in the fed state and following a 24 or 48 h fast. There was no significant difference in blood glucose levels, at baseline (GFP: 5.38 ± 0.38 versus glucokinase: 5.56 ± 0.48, P=0.39), 24 h (GFP: 3.88 ± 0.28 versus glucokinase 3.92 ± 0.66, P=0.83) or 48 h (GFP: 3.87 ± 0.62 versus glucokinase: 3.74 ± 0.61, P=0.64).

Altering glucokinase activity in the VMH, using adeno-associated viral gene transfer, offers an attractive and novel method to study the importance of VMH glucose sensing on responses to hypoglycaemia. Our initial results suggest that an increase in VMH glucokinase activity does not affect glucose homeostasis in response to gradual onset hypoglycaemia induced by fasting. Further work is needed to assess the role of VMH glucose sensing in acute glucoprivation, as observed in insulin induced hypoglycaemia.

P179

Functional muscarinic acetylcholine receptors are expressed in white and brown adipose tissue

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Both brown (BAT) and white (WAT) adipose tissue have neuroanatomically well characterized sympathetic innervation (with activation initiating lipid mobilization), but little evidence to support the presence of a (putatively counter-regulatory) parasympathetic input.

Parasympathetic actions are mediated through muscarinic acetylcholine receptors (mACHR). The mouse 3T-L1 (white fat-derived) cell line expresses M1, M3 and M4 mACHR during differentiation; there was no evidence of either M2 or M5 mACHR. M4 mRNA transcripts are present in the greatest concentrations (up to 7.4±3.0/1000 ARP, compared with 0.5±1000 ARP (M1) and 0.24±1000 ARP (M3) in 3T-L1 cells). M3 expression increases significantly during adipogenesis, whilst M1 and M4 exhibited little variation with time. Western blot analysis confirms the presence of mACHR protein.

Incubation of 3T-L1 cells with carbachol, a non-specific cholinergic agonist, produces a variable enhancement of GPDH (a terminal marker of adipogenesis) mRNA expression in the differentiating adipocytes. This is abolished by the addition of the universal muscarinic antagonist, atropine. Pirenzepine and P-fluoroethoxyl-hydrosylide-dienidipol, specific M1 and M3 antagonists respectively, significantly reduce GPDH transcripts in the presence of carbachol; these effects appear additive when the two are combined. Incubation with tropicamide (an M4 antagonist) has no effect on GPDH expression.

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<table>
<thead>
<tr>
<th>Glucose Sensing neuron type</th>
<th>Expression (percentage of control cells in adipogenic medium; mean ± S.E.M.)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbchol (1 μM)</td>
<td>142.4 ± 27.6</td>
<td>NS (9)</td>
</tr>
<tr>
<td>Carbachol and antagonists</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Atropine (1 mM)</td>
<td>70.5 ± 17.5</td>
<td>0.048 (3)</td>
</tr>
<tr>
<td>Pirenzepine (10 nM)</td>
<td>51.4 ± 6.3</td>
<td>0.007 (3)</td>
</tr>
<tr>
<td>P-fluoroethoxyl-hydrosylide-dienidipol (1 μM)</td>
<td>66.1 ± 5.6</td>
<td>0.018 (3)</td>
</tr>
<tr>
<td>Pirenzepine and P-fluoroethoxyl-hydrosylide-dienidipol (1 μM)</td>
<td>23.6 ± 13.1</td>
<td>0.0016 (3)</td>
</tr>
<tr>
<td>Tropicamide (10 μM)</td>
<td>105.5 ± 17.5</td>
<td>NS (3)</td>
</tr>
</tbody>
</table>

The antagonists have no significant effect on GPDH levels in the absence of carbachol.

Primary cultures of mouse and human BAT and WAT express M1, M3 and M4 mACHR at levels comparable to 3T-L1 cells, validating their use as a model system. Our results suggest the possibility of direct end-organ parasympathetic modulation of fat metabolism, and may represent a novel therapeutic target in obesity.

P180

Impact of glucocorticoids upon lipogenesis and β-oxidation in skeletal muscle

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Glucocorticoid excess is characterized by increased adiposity, skeletal muscle lipoprotein lipase and insulin resistance. Although there is a strong inverse correlation between intramuscular triglyceride (IMTG) levels and insulin sensitivity, the impact of
glucocorticoids upon the processes that regulate skeletal muscle lipid metabolism has not been explored. Mouse C2C12 skeletal myocytes were grown to confluence and differentiated into myotubes in chemically defined media. Expression of key components of the lipogenic and β-oxidation pathways were examined by RT-PCR. Functional impact of glucocorticoids upon de novo lipogenesis (ACC activity) was assessed by measuring [1-14C]acetate incorporation into intramuscular lipids and β-oxidation was assessed by measuring the accumulation of [3H]water following 9.10-3[3H]palmitate treatment. Experiments were performed using synthetic glucocorticoid dexamethasone (DEX) in the presence and absence of insulin.

C2C12 myotubes treated with DEX has reduced expression of the key lipogenic genes: FAS (1.90 ± 0.18 vs 1.25 ± 0.15 AU, P < 0.01), ACC1 (1.03 ± 0.09 vs 0.83 ± 0.06 AU, P < 0.05) and GPAT (0.56 ± 0.06 vs 0.35 ± 0.05 AU, P < 0.05). The effect of DEX was reversed when cells were coincubated with the glucocorticoid receptor antagonist RU38486. Endorsing these findings, de novo lipogenesis was decreased by DEX in a dose dependent manner (2.9 ± 0.04 vs 1.9 ± 0.03 (5 nM) versus 1.3 ± 0.02 p.d.m.×10^5 (500 nM), P < 0.05). DEX reversed the decrease in the rate of β-oxidation (25.8 ± 2.1 vs 27.7 ± 2.9 (5 nM) versus 28.9 ± 3.1 p.d.m.×10^5 (500 nM), P < 0.05). Importantly, an increase PDK4 expression was observed with DEX (0.66 ± 0.08 vs 0.73 ± 0.11 AU, P < 0.001) and reversed by RU3846, suggesting metabolic switching from glucose to fatty acids as fuel.

In the presence of insulin, low dose DEX was without effect upon lipid accumulation (3.1 ± 0.05 vs 3.2 ± 0.06 p.d.m.×10^5 (5 nM), P = NS), but increased β-oxidation (25.8 ± 2.1 vs 27.7 ± 2.9 (5 nM) versus 28.9 ± 3.1 p.d.m.×10^5 (500 nM), P < 0.05). These data highlight the impact of glucocorticoids to decrease lipid accumulation and increase β-oxidation in the absence of insulin. In contrast, in the presence of insulin they act together to promote IMTG accumulation.

P182

Deficiency in 11β-hydroxysteroid dehydrogenase type 1 reduces systemic inflammation and inflammatory cell infiltration in atherosclerotic lesions of ApoE−/− mice

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High plasma levels of glucocorticoids cause metabolic disease (central obesity, hypertension, diabetes) and increase risk of cardiovascular disease. 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) regenerates glucocorticoids in intact cells, converting inactive cortisons (11-dehydrocorticosterone in rodents) into active cortisol (corticosterone in rodents). Recent work has shown the pathological importance of elevated adipose tissue 11β-HSD1 expression in development of obesity, potentially driving metabolic disease and 11β-HSD1 deficiency or inhibition ameliorates type II diabetes and obesity-related disease in humans and animal models. Importantly, 11β-HSD1 inhibition prevents atherosclerotic plaque development in atherosclerosis-prone ApoE−/− mice fed a ‘western’ diet (WD). We have recently shown substantially reduced atherosclerotic lesion development in 11β-HSD1−/− mice fed ‘western’ diet for 16 weeks.

Here we show that the reduction in atherosclerotic lesion size is accompanied by reduced macrophage infiltration and a dramatic reduction in T cell infiltration in lesions of DKO mice (Number of CD3+ T cells/mm²). DKO, 275 ± 8 ± 4.9 vs ApoE−/−, 432 ± 1 ± 24.4; P < 0.01, n = 7/group). DKO mice also have more monocyte progenitors in their bone marrow (number of monocytes/femur; DKO, 9.36 ± 10^5 ± 1.32×10^5 versus ApoE−/−, 4.41 ± 10^5 ± 4.55×10^5, P < 0.01, n = 7/group) but fewer circulating monocytes in blood (DKO, 8.30 ± 10^5 ± 5.16×10^5 versus ApoE−/−, 1.16 ± 10^5 ± 2.12×10^5 monocytes/ml; P < 0.01, n = 21-16/group). Circulating levels of monocyte chemotactic protein-1 (MCP-1) were not different in ApoE−/− and DKO mice fed chow diet (DKO 42.5 ± 7.4 pg/ml versus ApoE−/−, 30.1 ± 3.9 pg/ml, n = 7-16/group). Following WD, plasma MCP-1 levels increased in ApoE−/− mice but not in DKO mice (DKO 31.6 ± 4.0 pg/ml versus ApoE−/−, 50.6 ± 9.3 pg/ml, n = 8-11/group). As a result plasma MCP-1 was significantly lower in DKO fed WD than in ApoE−/− mice. These data indicate that 11β-HSD1 deficiency reduces atherosclerotic lesion formation in ApoE−/− mice by reducing recruitment of inflammatory cells. The mechanisms responsible are currently under investigation.

P183

Short sleep duration is independently associated with obesity in Type 2 diabetes

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Background

From population studies, short and long sleep duration are associated with obesity. This association has not been studied in patients with type 2 diabetes (T2D).

Methods

Design: Cross-sectional study of adult T2D patients recruited randomly from outpatient department of a large UK tertiary centre. Patients with known sleep-related disorders were excluded. Data were collected during one-to-one interviews. Sleep duration assessment: 7-day sleep diary including bedtimes and wake-times, and number and duration of naps. Adiposity was assessed using body mass index (BMI), waist and neck circumferences and fat mass. Obstructive sleep apnoea (OSA) risk was assessed using the Berlin questionnaire. Other data collected: demographics, past medical history, drug history, smoking, alcohol, blue collar industry (BP) and a biochemical profile. Data presented as median (IQR) or percentages.

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Results
Seventy-seven patients returned questionnaires (70% response rate). Participant characteristics: age 61 years (52-68), 59.7% men, 57% white Caucasian, diabetes duration 10 years (6-17), BP 130 (121-141)/80 (74-86) mmHg, BMI 32.8 (29.3-36.1) kg/m², waist circumference (WC) 112 (101-122) cm, neck circumference (NC) 41 (38-43) cm, fat mass 32.4 (25.4-43.9) kg, HbA1c 7.6 (7.8-8.7)%, 48% on insulin, 14.3% taking exenatide, average sleep duration 7.9 (7.2-8.9) hours/night, 60% had naps (number 4 (1-7) naps/week, duration 145 (35-375) minutes/weeks), 79% were at ‘high-risk’ for OSA. 18, 60 and 22% had short (<7), normal (7-9) and long (>9 hours) sleep duration, respectively.

There was a stepwise reduction in BMI and WC between short, normal and long sleepers (BMI: 36.3 (32.4-42.4) versus 32.8 (30.7-35.7) versus 28.8 (25.8–35) kg/m², P=0.08; waist circumference: 119 (109-134) versus 112 (103-122) versus 103 (94-113) cm, P=0.05).

Sleep duration correlated negatively with BMI (r=−0.34, P=0.003), waist circumference (r=−0.31, P=0.007), NC (r=−0.2, P=0.09) and fat mass (r=−0.2, P=0.07). Multivariate linear regression, that included age, gender, ethnicity, HbA1c, diabetes duration, diabetes treatment, alcohol, smoking, napping, OSA risk and sleep duration, showed that short sleep was independently associated with BMI (β=−0.24, P=0.037), waist circumference (β=−0.28, P=0.027) and NC (β=−0.25, P=0.029) but not fat mass (β=−0.19, P=0.08).

Conclusions
Short sleep duration is independently associated with obesity in patients with T2D. Further studies examining the impact of sleep duration on adiposity in T2D are needed.

P184
Vitamin D status relates to premature aortic sclerosis in South Asians – a novel risk factor for cardiovascular disease?
David Webb, Melanie Davies & Kamlesh Khunti
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Background
South Asians are at higher risk of vascular disease than the indigenous UK population. Susceptibility to conventional cardiovascular risk factors does not entirely account for this discrepancy. Vitamin D deficiency within this group may be an important patho-physiological contributor and an overlooked treatable cardiovascular risk factor.

Aims
To determine whether serum 25-(OH) Vitamin D concentration independently associates with a surrogate of arterio-ocular aortic stiffness in UK South Asians and test the hypothesis that Vitamin D status is a determinant of vascular wall pathology.

Methods
A cross-sectional association study. South Asian (SA) and White European (WE) groups were recruited from a multiethnic cardiovascular risk screening programme (ADDITION study: NC18231B302). Volunteers consented to non-invasive vascular measurements (carotid-femoral Pulse Wave Velocity (cPWV) and biochemistry) (25-(OH)ViD, PTH).

Results
Two hundred and thirty-one (SA=110, WE=121) individuals underwent cPWV and 25-(OH)ViD measurements. Groups were matched for age (SA: 55.5 (0.89) vs WE: 55.8 (0.87) years, P=0.97), gender (SA Male 61 versus WE Male 57%, P=0.41), mean arterial pressure (SA: 97.2 (0.9) versus WE: 99.7 (1.0) mmHg, P=0.08), fasting glucose (SA: 5.9 (0.1) versus WE: 5.7 (0.2) mmol/l, P=0.59), smoking (SA: 10 versus WE: 11%, P=0.87) and prescribed anti-hypertensives. Ten-year CVD risk scores were similar and correlated strongly with cPWV in both groups (r=0.8, P<0.001). cPWV was higher (SA: 9.30 (0.16) versus WE: 8.63 (0.13) msl, P<0.01) and 25-(OH) Vitamin D lower in the SA group (SA: 20.31 (1.3) versus WE: 25.12 nmol/l, P<0.01). Univariate-factors independently relating to cPWV were log25-(OH)ViD, age, mean arterial pressure, glucose, BMI, gender, height, SA ethnicity. In linear regression modelling log25-(OH)ViD inversely associated with cPWV in SA after adjustment for significant co-factors (beta co-efficient −0.28 (−1.16-0.02, P<0.01).

Conclusion
Serum 25-(OH)ViD concentration inversely relates to measures of arterial wall pathology in South Asians, the strength of the association being similar to glucose PWV interactions.

P185
Diet-induced obesity in C57Bl/6 mice is associated with sex-specific changes in glucocorticoid metabolism
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Although obesity affects men and women, the risks of associated metabolic disturbances (e.g. type 2 diabetes) differ between the sexes. Altered peripheral glucocorticoid metabolism may underpin the metabolic consequences of obesity; however, most research exploring this has focused on male animals. This study used a mouse model to investigate the hypothesis that alterations in glucocorticoid metabolism caused by diet-induced obesity (DIO) will be more profound in males than in females.

Male and female C57Bl/6 mice were fed obese or control diets for 8 weeks from weaning (n=8/group) and underwent metabolic testing. Enzyme activity and mRNA expression were quantified from snap frozen tissues obtained post-mortem. After 8 weeks, DIO mice were heavier than controls and displayed hyperglycaemia and hyperinsulinemia: these changes were more severe in males. In DIO males, peak corticosterone levels were decreased (DIO 310.6±30.9 versus Con 509.4±66.6, P<0.05) and hepatic expression of 5α-reductase (5αR) and 11β-hydroxysteroid dehydrogenase type 1 (11βHSD1; DIO 1.27±0.10 versus Con 0.92±0.11, P<0.05) were increased. In contrast, in DIO females, peak corticosterone levels were increased (DIO 1017.2±127 versus Con 488.5±75.3, P<0.01), whilst hepatic expression (DIO 0.83±0.06 versus Con 1.43±0.04, P<0.001) and activity of 11βHSD1 were decreased. Furthermore, hepatic 5αR expression was decreased whereas 5α-reductase expression was increased. DIO in males was also associated with decreased adipose 11βHSD1 expression, while no such changes were seen in females. These results indicate that increased 5αR in male mice with DIO is responsible for decreased circulating corticosterone whilst increased 11βHSD1 activity maintains intrahepatic concentrations. The opposite is seen in females, in which decreased 11βHSD1 may compensate for increased systemic corticosterone. Thus, DIO induces sex-dependent changes in the hypothalamic–pituitary–adrenal axis and glucocorticoid metabolism. We suggest that such sexually dimorphic effects may play a role in the differential metabolic responses to obesity in males and females.

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Circadian rhythmlicity is altered in hepatic corticosterone-related genes from diet induced obese mice
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Increased corticosterone (cortisol in humans) in liver has important adverse effects on glucose metabolism and is therefore a target for diabetes treatments. The functional effects of corticosterone depend on circulating levels as well as tissue regeneration by 11β-hydroxysteroid dehydrogenase 1 (11β-HSD1) and inactivation by sterol 5α-reductase type 1 (5αR). This study investigates how obesity affects diurnal changes in circulating corticosterone, the genes controlling tissue bioavailability and glucocorticoid receptor (GR) expression.

Male C57Bl/6Jax mice were fed chow or 60% kcal from diet for 13 weeks before being euthanised at each of 10 time points (n=6) around the predicted peak and nadir of corticosterone. Plasma samples were taken for corticosterone and progesterone in 11β-hydroxysteroid dehydrogenase 1 (11β-HSD1) and inactivation by sterol 5α-reductase type 1 (5αR).

In obese mice, POMC concentrations were increased at both nadir (leak 24.9±3.2 versus obese 395.8±26 pmol/l, P<0.001) and peak (leak 253.2±33 versus obese 295.3±17 pmol/l, P<0.01). Corticosterone was also increased at nadir (leak 78.2±9 versus obese 183.7±29 ng/ml, P<0.01) and peak (leak 149.0±22 versus obese 138.0±25 ng/ml, P<0.001). In lean mice, 11β-HSD1 has a diurnal rhythm, being higher at the nadir, which is lost in obese mice because of decreased levels at the nadir. In contrast 5αR mRNA was reduced in obese mice at both nadir (62%) and peak (74%). GR mRNA was decreased in obese mice by 42% at nadir (P<0.001) and 36% at peak (P<0.05). A similar decrease was observed in its coregulator, PGC-1.

In summary, the HPA axis is upregulated in obese mice. The loss of diurnal rhythm of 11β-HSD1 in liver leads to decreased mRNA expression at the corticosterone nadir implying reduced tissue corticosterone. Conversely decreases in 5αR expression may lead to increased tissue corticosterone. Importantly, the decreased expression of GR and PGC-1 in liver could limit the effects of excess corticosterone in obesity.
Anti-inflammatory salicylates improve insulin sensitivity, however the mechanism remains unclear. We have observed down-regulation of the glucocorticoid repressing enzyme 11β-hydroxysteroid dehydrogenase 1 (11βHSD1) by salicylates in cultured adipocytes and in human adipose tissue after oral salbutamol therapy, consistent with known transcriptional regulation of 11βHSD1 by pro-inflammatory cytokines. Since inhibition of 11βHSD1 improves insulin sensitivity, we have tested whether the insulin sensitising effects of salicylates are underpinned by reducing adipose 11βHSD1 in mice.

Male C57BL/6 mice (8 weeks) were studied after 10 weeks high-fat diet (59% fat with sucrose). Male homozygous 11βHSD1 knockout mice (HS1D1KO) were also studied on the high-fat diet, weight-matched with obese wild type mice. Groups received either sodium salicylate (120 mg/kg per day, 4 weeks subcutaneously) or vehicle (n = 8/treatment). Insulin sensitivity was assessed by glucose tolerance tests (i.p. GTT), after 3 weeks treatment. Plasma biochemical indices were quantified by spectrophotometry and transcripts by qPCR (relative expression). Data are mean ± s.e.m; vehicle versus salicylate, *P < 0.05.

In wild type mice, salicylate decreased transcripts of tumor necrosis factor-alpha (1.19±0.14 vs 0.71±0.16*), 11βHSD1 (1.22±0.14 vs 0.72±0.16*), lipoprotein lipase (1.48±0.17 vs 0.45±0.10*) and adipose triglyceride lipase (1.39±0.22 vs 0.57±0.13*) in omental adipose, caused a redistribution of fat from omental to subcutaneous deposits (ratio subcutaneous:omental 6.74±0.24 vs 11.29±1.07*), improved glucose tolerance (30%* reduction in area under curve for glucose) and resulted in greater post-prandial suppression of non-esterified fatty acids (23.3±3.46 vs 44.26±7.37%). In contrast, in HS1D1KO mice, salicylate did not cause a redistribution of fat (ratio subcutaneous:omental 12.87±1.89 vs 13.91±2.49*) or improve glucose tolerance (no significant change in area under curve for glucose; 34.3±0.73 (gd/min) vs 34.5±1.62 (gd/min)). We conclude that down-regulation of 11βHSD1 is crucial in the insulin sensitising mechanism of salicylate in obesity.

Endocrine tumours and neoplasia

P188

Ovarian Leydig cell tumour in a peri-menopausal woman with severe hyperandrogenism and virilisation

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We present the case of a 50-year-old woman who presented with 12 months of amenorrhoea associated with signs of virilisation. Investigations revealed markedly raised serum concentrations of testosterone (9 mmol/L), whereas sex hormone binding globulin, rat anti-cervical, androstenol, 17-hydroxyprogesterone and dehydroepiandrosterone sulphate concentrations were all within the normal range. Computed tomography scan of the pelvis and abdomen showed a slightly bulky right ovary, and there left ovarian mass. An ovarian source of androgens was suspected. The testosterone level fell to suppress after an overnight dexamethasone suppression test and this support ovarian source of androgen. Surgery was arranged and she underwent total abdominal hysterectomy with bilateral salpingo-oophorectomy. Histopathological examination revealed a Leydig cell tumour within the left ovary. Hyperandro- genaemia normalised post-operatively. The patient showed significant regression of clinical signs and symptoms, including the anxiety disorder.

Conclusion

The present case confirms that androgen-secreting ovarian tumours have to be considered in the differential diagnosis of severe hyperandrogenism even in peri-menopausal women.

P189

Laparoscopic versus open adrenalectomy and adjuvant mitotane in adrenocortical carcinoma

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Introduction

Adrenocortical carcinoma (ACC) is an uncommon aggressive malignancy. Despite surgical resection, recurrence rates are high. Two areas of contention currently involve treatment of ACC: the role of laparoscopic surgery in removal of large, potentially malignant tumours and the role of Mitotane. We present a case of a large adrenal tumour, later confirmed as ACC, where initially laparoscopic adrenalectomy was considered, and adjuvant Mitotane has been given despite RO resection.

Case

A 25 years lady presented with clinical features of Cushings’s syndrome. Cortisol levels failed to suppress with a low-dose dexamethasone suppression test and ACTH was undetectable (<5 <ng/L). An adrenal MRI revealed a 10 cm well- circumscribed right adrenal tumour and no metastatic disease. The departmental default position in the absence of overt signs of malignancy or technically excessive size is a laparoscopic adrenalectomy. However, with a large tumour size, IVC displacement and effacement, and sinster tumour washout characteristics on imaging, open adrenalectomy was deemed appropriate.

Within 8 weeks of initial imaging, open adrenalectomy revealed significant tumour growth and a 17 cm mass was removed in its entirety (macro and microscopic R0 resection). Histopathology showed ACC. Postoperatively, Mitotane treatment was initiated.

Postoperative CT abdominal scan at 4 months is clear of recurrence and metastatic disease. She feels well and plans to marry.

Conclusion

Laparoscopic adrenalectomy is the gold standard approach to small functioning adrenal tumours. While the feasibility of operating laparoscopically on large adrenal tumours is established, the role of minimal access approach for such tumours is controversial due to risk of incomplete resection and capsular breach. This case demonstrates how a combination of clinical and radiological features guide in optimal surgical management of such tumours.

Although, adjuvant mitotane postoperatively remains controversial, a retrospective study showed reduced risk of recurrence and better survival in appropriately resected adrenocortical carcinomas followed by mitotane therapy.

P190

An unusual presentation of insulinoma with postabsorptive hypoglycaemia

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Insulinomas are rare neuroendocrine tumours with an estimated incidence of up to four new cases per mill./p.a. Most tumours are benign, <2 cm and arise from the islets of Langerhans. Typically patients present with Whipple’s triad: symptoms of hypoglycaemia, caused by fasting or exercise, low plasma glucose and symptoms relieved by Food. The diagnosis is confirmed by high insulin and c-peptide levels, inappropriately high for prevailing glucose.

Here we report a case of an insulinoma which presented initially with recurrent unexplained episodes of confusion and disorientation. No medical advice was sought until he presented with Severe hypoglycaemia with a plasma glucose of 1.6 mmol/L. Subsequent investigations showed that he became promptly hypoglycaemic during OGTT. During a 72 h fast, the patient became hypoglycaemic after 16 h. High levels of C-peptide and insulin were evident. The pancreatic CT-scan showed a 2 cm mass in the uncinate process which was of higher attenuation in both arterial and PV phase. The octreotide scan showed uptake in the corresponding area as seen on the CT. Initial treatment with octreotide given subcutaneously was started and ameliorated his recurrent hypoglycaemia. He subsequently underwent successful laparoscopic enucleation of the insulinoma. The patient remains asymptomatic but follow-up CT-scans are indicated as histological findings showed adverse prognostic features with tumour cell nests and vascular and peri-neural invasion. This is an unusual presentation in that no fasting hypoglycaemia was reported and most episodes were late in the postprandial phase. This case reports shows that the diagnosis of insulinoma can often be delayed due to lack of classic presentation. They can also be falsely attributed to psychiatric or neurological disorders. Not seeking medical advice could potentially be fatal. We suggest that patients with history of postprandial hypoglycaemia in the absence of dumped large adenoma should be investigated with a prolonged fast to rule out insulinoma.
approximately 10% of cases. These are part of a rare syndrome characterized by slow-growing tumours derived from paranganglia tissue. Recent years have brought significant progress in identifying the genetic etiology of this syndrome.

Thirty-eight years old female was referred to the endocrine department by ENT after finding a second paraganglioma on a follow up MRI for a previously resected right sided Glomus Jugulare tumour 25 years ago. Routine follow up MRI in 2005 showed a recurrent paraganglioma, which was treated with stereotactic radiotherapy. Following her treatment, a repeat MRI in 2008 showed a recurrent right sided neck paraganglioma. What was of interest was that patient’s brother was also under our care at the current time for investigation into a left sided paraganglioma.

Patient was investigated extensively including screening for catecholamine secretions and for other syndromes associated with paragangliomas. Patient had localizing scans including MBIG scan and MRI skull to pelvis. In view of family history and recurrent paraganglioma patient had genetic testing. Results showed raised normetadrenaline excretion on two occasions. Subsequently, patient developed symptoms of hyperadrenalinism and was therefore a-blocked and b-blocked prior to surgical removal of tumour. MBIG scan prior to surgery confirmed the right sided lesion but also showed increased uptake in midline posterior to the bladder which was not seen on MRI.

Genetic testing confirmed a heterozygous mutation in the SDHB gene. The mutation was also identified in the patient’s brother who has a diagnosis of paraganglioma. Furthermore two of the patient’s children had the SDHB mutation. They are asymptomatic and are under surveillance for neuroendocrine tumours.

The case highlights the importance of family history, family genetic screening and surveillance in patients with recurrent paragangliomas.

P192
Medical management of an insulinoma – a safe long-term alternative to surgery?
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We present a 65-year-old lady initially diagnosed and treated for epilepsy who was subsequently referred 10 years later for the investigation of underlying spontaneous hypoglycaemia. Laboratory plasma glucose concentrations of 2.0 and 1.7 mmol/l were recorded prior to referral and subsequently a fasting challenge provoked hypoglycaemia associated with a plasma glucose of 0.8 mmol/l. C-peptide 371 pmol/l (NR 120-600) and insulin 7.4 mU/l (NR 0-10). CT and MRI imaging of her pancreas revealed a 10 mm vividly enhancing oval nodule within the parenchyma of the mid body of the pancreas consistent with an insulinoma.

Initial treatment with diazoxide was not tolerated due to nausea and she was converted to lanreotide injections (60 mg every 28 days). Her hypoglycaemic episodes resolved and phenytoin was tailed off with resolution of her ‘epilepsy’.

Surgical resection of the insulinoma was advised but the patient did not want to proceed in view of her successful response to lanreotide and fear of surgery and its complications. She has now remained on lanreotide for over 4 years without side effects and is asymptomatic. She maintains her desire to avoid surgical resection. Insulinomas are β-cell tumours of the pancreas, which can present with hypoglycaemia and are often misdiagnosed. Somatostatin analogues are known to be an effective treatment for insulinomas and have been used as a short-term measure. However, whilst they are an established long-term treatment of carcinoid tumours (having been used in individual patients for over 10 years), this case demonstrates that long-term use of somatostatin analogues may also be an effective treatment for insulinomas in those individuals not undertaking surgical resection.

P193
Two cases of meningitis as a complication of pituitary adenoma
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Introduction
Meningitis is a recognized complication of the treatment of pituitary adenoma. It is usually considered as a peri-operative problem but does need to be considered as a potential complication at other points in the treatment pathway. We present two patients with meningitis where the link to pituitary pathology was originally unrecognized.

Case report
Patient 1: 38 years old lady, diagnosed with acromegaly 4 years previously and treated with trans-sphenoidal surgery and radiotherapy with continuing medical treatment (lanreotide). She was admitted by the neurology team with an acute confusional state and sepsis. Diagnosis - pneumococcal meningitis. She made a full recovery and was discharged. Within weeks she was readmitted with a second episode of meningitis. On direct questioning she had noticed a nasal drip for some months. This had followed a severe blow to the face and nose when she walked into a glass door. Cisternogram demonstrated a spheonidic csf leak which was subsequently repaired.

Patient 2: 41 years old lady, presented with a history of nasal drip under investigation by ENT. Before final diagnosis was made she developed an acute confusional state and meningitis. Her MRI showed an extensive pituitary and infrasellar lesion, the biopsy confirmed a pituitary adenoma with strong staining for prolactin. Interestingly her serum prolactin level was minimally elevated at 900 mU/l. Multiple operation was performed to repair the CSF leak. She is currently managed on cabergoline.

Conclusion
Pituitary adenoma, treated or untreated, can potentially cause meningitis when it is complicated by CSF leak. This diagnosis needs to be considered at any stage and not just the immediate post-operative period. The treatment of choice is by surgical repair of the defect to prevent further reoccurrence.

P194
A case of phaeochromocytoma mimicking an acute ischemic limb: an unusual presentation
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Case
A 53-year-old Caucasian lady presented with an acutely painful, cold left leg to the vascular surgeons. She was being treated for respiratory tract infection in the community. She has been investigated for palpitations 7 years ago with no cause identified and headache 3 years ago with normal CT/MRI. Her peripheral vascular examination was unremarkable, including a normal ankle brachial pressure index. She was admitted for further investigations to rule out an underlying ischemic pathology.

She later went into respiratory peri-arrest and was subsequently intubated. Chest radiograph demonstrated acute respiratory distress syndrome, arterial blood gas analysis showed Type I respiratory failure. She required inotropic support in Intensive Care Unit. CT pulmonary angiogram was negative for pulmonary embolism but demonstrated an incidental 7 cm left sided adrenal mass. No other lesions were identified on the CT of thorax, abdomen and pelvis. An echocardiogram showed an ejection fraction of 25% with a dilated left ventricle. The urinary catecholamines, which were sent earlier in view of her labile blood pressure came back grossly elevated. The total urinary metadrenaline level was 51 umol/24h (0.00-2.00) and normetadrenaline level was 33 umol/24h (0.00-4.90).

Despite adrenergic blockade with intravenous phentolamine, she deteriorated haemodynamically in ITU and an emergency left sided adrenalectomy was performed. She made good recovery post-operatively and her post operative urinary total metadrenaline returned to normal 0.86 umol and normetadrenaline to 2.11 umol. Histology established the complete excision of a primary phaeochromocytoma.

Discussion
We describe an unusual case of phaeochromocytoma mimicking an acute ischaemic limb. Phaeochromocytomas have been presented as acute myocardial infarction, cardiomyopathy, stroke and mesenteric ischaemia. Elevated levels of serum catecholamines can cause vasospasms and hemodynamic instability. A high index of suspicion should be maintained for diagnosing phaeochromocytoma.

P195
Ectopic Cushings complicated by paraneoplastic cerebral vasculopathy requiring high-dose steroid therapy
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In October 2006 a 53-year-old, non-smoking woman presented with ectopic Cushings from a small cell, undifferentiated carcinoma (Grade 3 neuroendocrine) from a
P196
Cranioplasty for extensive skull defect in the management of differentiated thyroid cancer
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2Northern Centre for Cancer Treatment, Newcastle Upon Tyne, UK.

Thyroid cancer if associated with bony metastases carries a poorer prognosis. Intensive multimodality treatment is usually necessary in management of these patients.

Case
A 51-year-old female presented with hoarse voice in October 2005. Examination revealed a 3 cm left sided thyroid nodule and left vocal cord palsy. CT neck/thorax showed a multinodular goitre, with a dominant left nodule. FNA showed appearances suggestive of a follicular neoplasm. She underwent left hemithyroidectomy in June 2006. Histology showed poorly differentiated follicular carcinoma with vascular invasion (pt4N0Mx). She had completion thyroidectomy in July 2006 and 6 weeks later she received 3760 MBq radioidine. The post-ablution scan showed intense uptake with ‘starburst’ effect in the left parietal bone, significant uptake in the left clavicle and humerus and minimal uptake in the thyroid bed. A month later she developed a patch of scalp hair loss overlying the area of iodine uptake in the post-ablution scan. Bone scan confirmed bony secondaries. MRI brain showed a large extradural parietal bone deposit. She had a left parietal craniotomy and excision of extradural deposit with acrylic cranioplasty in August 2007, followed by 5060 MBq radioidine in January 2008. US of neck with FNA showed evidence of local recurrence. Further 5200 MBq radioidine was given in July 2008. Post-ablution scan showed no uptake in the skull but some in the left clavicle and shoulder. Repeat MRI scan in May 2008 showed no changes to her left shoulder or clavicle but left parietal region appeared free in metastasis. Her thyroglobulin levels has shown significant improvement but has remained detectable. She is due repeat imaging of her neck and shoulder before further planning of radioidine ablation or radiotherapy to her bony lesions.

P197
Pseudophaeochromocytoma syndrome associated with Modafinil
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A 48-year man, referred with symptoms suggestive of pheochromocytoma and elevated 24 h urinary noradrenaline level. He had a background history of obstructive sleep apnoea, controlled hypertension and obesity. His medications included Amiodpine 10 mg od, Modafinil 200 mg daily, Amitryptyline 10 mg and Rabeprazole.

Obstructive sleep apnoea was diagnosed at 39 years. CPAP was tried and later Modafinil 100 mg daily. A few months prior to referral his Modafinil was increased to 200 mg daily.

Systemic examination was unremarkable. Anterior pituitary function tests, U&E, bone profile, LFT, CRP were normal. Urinary catecholamines measured off Modafinil was normal while a challenge on Modafinil 200 mg showed elevated noradrenaline levels. Urinary metanephrines & CT scan of the adrenals were normal.

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Modafinil acts by blocking the reuptake of noradrenaline by the noradrenergic terminals in hypothalamus. Some studies have shown increased heart rate & bloodpressure whilst on modafinil. In our case on a modest dose of 200 mg of modafinil, patient was symptomatic. The elevated 24 h urinary catecholamines suggest significant adrenomedullary activation by Modafinil rather than a pheochromocytoma.

P198
An unusual case of rapidly progressing pituitary lesion
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Case
An 81-year-old man with type 2 diabetes, cerebrovascular disease and hypertension presented with a sudden onset of headache. CT scan and MRI scan of the head did not show any significant abnormality. Headache improved spontaneously and he was discharged with no cause for his headache being identified. Four months later he was diagnosed to have pan-anterior hypopituitarism at another hospital and was commenced on appropriate replacement therapy. Serum prolactin was not elevated. A repeat MRI scan done did not show any pituitary abnormality. Two months later he again presented to us with worsening headache, nausea and diplopia with a partial third nerve palsy. A repeat unenhanced MRI scan showed a pituitary lesion which was 20 mm x 19 mm x 13 mm in size abutting the optic chiasm with evidence of lateral extension to the left where the soft tissue surrounded the left internal carotid artery. The radiological appearance was not suggestive of metastatic deposits. In view of rapid development of this lesion he went on to have a biopsy of the lesion. Histological examination confirmed the diagnosis of a primary high grade B cell lymphoma. He was deemed unfit for chemotherapy in view of his co-morbidities and he is awaiting palliative radiotherapy.

Discussion
Primary pituitary lymphoma is rare with only a few case reports in the literature. It is predominantly observed in males around the 6th decade of age. Usual presenting features are headache, cranial nerve abnormalities and about half of cases have anterior hypopituitarism at diagnosis. Chemotherapy should be considered as the first line of therapy. Neurosurgical decompression of the mass does provide symptom benefit. Our case was unusual as hypopituitarism preceded the diagnosis at least by a few months.

Conclusion
Primary pituitary lymphoma should be considered as a possible diagnosis in patients presenting with rapidly progressing pituitary lesion.
Cinacalcet treatment of resistant hypercalcaemia due to MEN1-associated primary hyperparathyroidism in pregnancy

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Cinacalcet increases sensitivity of the calcium sensing-receptor, currently licenced for treatment of refractory secondary hyperparathyroidism in patients with end-stage renal disease. It decreases parathyroid hormone, calcium and phosphorus levels. There is no clinical data for the use of Cinacalcet in pregnancy. Hyperparathyroidism is rare during pregnancy, mainly presenting early, when surgery is safely performed. Here we report a patient presenting with a MEN1-associated parathyroid tumour in the third trimester, where surgery was delayed until post-partum.

A 27-year-old Caucasian lady with a positive family history for MEN1 presented with symptomatic hypercalcaemia at 33 weeks gestation. Parathyroid hormone level of 39.1 pmol/l (1.1-4.2 pmol/l) and adjusted calcium of 3.73 mmol/l (2.10-2.59 mmol/l) were elevated. Ultrasound revealed a right-sided enlarged parathyroid gland (30 mm x 18 mm x 8 mm).

Treatment with Cinacalcet was started and parathyroidectomy delayed until post-partum. Pre-term caesarean section at 34+3 weeks was performed; the child required 4 days respiratory support for pulmonary surfeicant deficiency. Infantile corrected calcium at birth was 3.12 mmol/l but normalised within 3 days, still remaining stable.

Parathyroid gland biopsy confirmed parathyroid hyperplasia and the patient underwent complete parathyroidectomy with implantation of part of one gland as an autograft. Due to the background of familiar MEN1-mutation, pittutary and gastrointestinal neoplasia screening was performed but is presently negative.

Hyperparathyroidism during pregnancy can have devastating effects on both mother and foetus. At present there are no evidence-based parameters that reliably predict the outcome of hyperparathyroidism in pregnancy and optimal management remains uncertain. Most authors advocate parathyroidectomy during pregnancy as treatment of choice in second trimester, where safety of surgery in other trimesters is debated.

Treatment with Cinacalcet may be an effective approach in selected patients with hyperparathyroidism in the third trimester before surgery can be safely performed.

Chorangial malignant melanoma in a patient with Carney complex

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We report a case of Carney complex who demonstrated most of the disease-defining features and an unusual manifestation of chorangial malignant melanoma not previously reported in the literature.

A 16-year-old female patient initially presented to the endocrinologists in 1991 with secondary amenorhoea and raised prolactin levels, diagnosed to have a pittutary microprolactinoma. Past history included episodes of vague syncope and palpitations treated as complex partial seizures since childhood. Echocardiogram performed to investigate her recurrent syncopal attacks revealed an atrial myxoma, which was removed in 1998. This led to the diagnosis of Carney complex. Genetic screening showed PRKAR1A gene mutation, which was absent in both her parents, suggesting sporadic origin.

During the course of follow-up she remained supraventricular. Two thyroid nodules were identified on screening which appear to be benign. Loss of cortisol circadian rhythm was noted, and serum cortisol fails to suppress after dexamethasone treatment. However serial 24-h urine free cortisol levels remained normal with no clinical evidence of Cushing’s syndrome, and with normal adrenals on CT imaging. In 2002 she had a local resection of a chorangial melanoma in the right eye. Histology suggested highly pleomorphic aggressive malignant melanoma, but there has been no recurrence over 7 years of follow-up.

Carney complex is a multiple neoplasia syndrome characterised by myxomas at various sites, endocrine, neural tumours and lentiginosis, all of which are usually benign. Endocrine tumours include primary pigmented nodular adrenocortical dysplasia (PPNAD), gonadal tumours, thyroid nodules and pittutary adenomas. Malignant melanoma is not a lesion associated with Carney complex, and only one previous case of a malignant melanoma in Carney complex was reported in the literature. This case highlights the need to remain aware of the multiple tumour types that may emerge in patients with Carney complex, and suggests an additional tumour that may be a part of the syndrome.
Treatment
She was commenced on prednisolone (30 mg once a day) with consequent improvement in hypoglycaemic episodes. Liver and hepatic nodules reduced in size after chemotherapy with doxorubicin.

Conclusion
Our case report serves to highlight a very rare cause of IGF2 producing tumour resulting in non-islet cell tumour hypoglycaemia. In addition, it also demonstrates the role of high dose steroids in this case, as effective medical therapy for hypoglycaemia.

P203
Adrenal venous sampling for catecholamines- a normal value study
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Context
Phaeochromocytomas are rare, but potentially fatal, neoplasms. The diagnosis and localization of phaeochromocytoma can be challenging and recently there has been some debate regarding the role for adrenal venous sampling (AVS). The utility of AVS in this setting is hampered by a lack of normative value data for adrenal vein catecholamine concentrations and the reliability of lateralization ratios. We sought to address these concerns by analyzing AVS catecholamine concentrations from patients who did not have phaeochromocytoma.

Design/setting
Eighteen patients underwent successful AVS for evaluation of possible cortisol-producing adrenal masses. All had normal 24 h urinary excretion of fractionated catecholamines and metanephrines.

Results
There was a wide range of catecholamine concentrations in both the right (epinephrine 389–118 526 pg/ml; norepinephrine 156–11 193 pg/ml) and left (epinephrine 113–9327 pg/ml; norepinephrine 229–2116 pg/ml) adrenal veins. The right adrenal vein to left adrenal vein epinephrine gradient was as high as 83.1 (median, 2.1; 1.1; P<0.02); the majority of right-to-left adrenal vein epinephrine ratios were greater than one. Although less striking, similar findings were also seen for norepinephrine.

Conclusions
This report provides a reference range for adrenal vein catecholamine concentrations in non-phaeochromocytoma patients and illustrates the wide variation in epinephrine and norepinephrine concentrations. Epinephrine and norepinephrine concentrations are statistically significantly higher in the right versus the left adrenal vein; in the case of epinephrine, up to a 83-fold difference was found between the right and left adrenal veins. This wide range in variability limits the utility of AVS in the diagnosis or localization of phaeochromocytoma.

P204
Temozolomide-induced regression of hepatic metastases in a pituitary corticotroph carcinoma with low O6-methylguanine-DNA methyltransferase expression
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Background
Pituitary carcinoma occurs in ~0.2% of resected pituitary tumours, and carries a poor prognosis (mean survival <4 years), with standard chemotherapeutic regimens showing limited efficacy. However, recent evidence suggests that temozolomide, an orally active alkylating agent used principally in the management of glioblastoma, may also be effective in controlling aggressive/invasive pituitary adenomas/carcinomas. Low levels of expression of the DNA-repair enzyme MGMT, as assessed by immunohistochemistry, predicts temozolomide responsiveness. Here, we report a case of a pituitary corticotroph carcinoma with hepatic metastases, which responded clinically, biochemically and radiologically to temozolomide therapy.

Case report
A 65-year-old man presented as an emergency with frontal headache and evolving bilateral 3rd nerve palsies. Imaging showed a sellar-based mass with parasellar and suprasellar extension. At transphenoidal surgery a necrotic ACTH-staining pituitary adenoma was resected. There were no features of Cushings syndrome clinically, biochemically, and hydrocortisone replacement was required post-operatively. No residual tumour was identified on post-operative MRI and the patient elected for surveillance follow-up.

However, 2 years later he developed clinical and biochemical evidence of ACTH-dependent Cushing’s syndrome. Pituitary MRI showed no evidence of tumour regrowth and, although a peripheral CRH test suggested a corticotroph origin, IPSS did not demonstrate a central: peripheral gradient. Further imaging with CT and FDG-PET revealed multiple hepatic lesions, and subsequent biopsy confirmed metastatic ACTH-staining pituitary carcinoma. MGMT expression was very low in both the primary pituitary tumour and hepatic metastases. Temozolomide therapy was commenced (200 mg/m² daily for five consecutive days every 28 days) and has been well tolerated. ACTH levels have fallen from a peak of 5685 to 2318 ng/l after 5 cycles, with CT demonstrating regression of the hepatic lesions. Metyrapone and ketoconazole were also used to help control hypercortisolism and the patient has undergone pituitary radiotherapy to mitigate against the potential risk of local tumour recurrence.

P205
Secretory glomus jugulare tumour treated with stereotactic radio surgery
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Glomus jugulare tumours are rare and often difficult to manage. A small proportion of these tumours (1–3%) can cause symptoms due to catecholamine secretion. Here we describe a patient with secretory glomus jugulare tumour treated with stereotatic radio surgery and its effect on catecholamine secretion.

Case report
A 39-year-old lady was referred to ear specialists with the characteristic symptoms of pulsatile tinnitus and decreased hearing in the left ear. Clinical diagnosis of a left sided glomus tumour was later confirmed with an MRI scan of the skull base which showed a dumbbell shaped avidly-enhancing 32 mm×19 mm lesion of the jugular fossa with intra cranial and middle ear extensions. A 24 h collection of urine showed elevated noradrenaline excretion (3370 nmol/ collection) with normal adrenaline and dopamine excretion. She did not have any symptoms pertaining to catecholamine excess. Genetic analysis confirmed deletion of exon 6 of SDHC gene. She underwent stereotactic radio surgery following which her urinary noradrenaline secretion was reduced by more than 50%. Subsequent MRI did not show further of the growth of the tumour.

Discussion
Stereotatic radio surgery is now an established treatment option for glomus jugulare tumours and has shown to arrest tumour growth and improve symptoms. This case reports illustrates that it could improve endocrine secretory problems as well and add to valuable information to the evidence base for management of these rare tumours.

P206
Case report: multiple endocrine neoplasia type 2a and hereditary haemorrhagic telangiectasia presenting consecutively in a single patient
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1Cork University Hospital, Cork, Ireland; 2Mercy University Hospital, Cork, Ireland; 3South Infirmary Victoria Hospital, Cork, Ireland.

Multiple endocrine neoplasia type 2a (MEN 2a) is an autosomal dominant disorder with an incidence of ~1 in 30,000 of the population. It is characterized by medullary thyroid cancer (MTC), benign or malignant phaeochromocytomas, and parathyroid hyperplasia or tumours. Hereditary hemorrhagic telangiectasia (Osler-Weber-Rendu syndrome), is also an autosomal dominant disorder; this condition occurs with an incidence of ~1-2 per 100,000 of the population. It manifests with primarily with epistaxis,
gastrointestinal bleeding, iron deficiency anaemia, mucocutaneous telangiectasia, and arteriovenous malformations. We present an uncommon case of two familial autosomal dominant conditions diagnosed consecutively in a single patient: MEN 2a, which she inherited from her father, and hereditary haemorrhagic telangiectasia (HHT) which was of maternal inheritance. Our 38 year-old lady was diagnosed with medullary thyroid cancer at the age of 23 after presenting with goitre. A partial thyroidectomy was performed and histology revealed the presence of foci of medullary thyroid cancer. Complete thyroidectomy was then carried out and subsequent review of her family history revealed a positive history of thyroidectomy (later confirmed to contain foci of medullary thyroid cancer) in her father. The diagnosis of MEN 2a was confirmed by genetic testing. More recently, as part of annual screening for MEN 2a, the patient was noted to have pulmonary arterio-venous malformations. Detailed history and physical examination revealed oral telangiectasia and a history of recurrent epistaxis in both the patient and her mother consistent with a diagnosis of hereditary haemorrhagic telangiectasia. Our patient has subsequently undergone embolisa-
tion of these lesions.

P207
Parathyroidectomy has no impact on haemoglobin levels on patients with primary hyperparathyroidism
Siva Sundar, Gregory Salder & Radu Mihai
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Background
Mild primary hyperparathyroidism (PHPT) is frequently diagnosed in patients with minimal hypercalcaemia whose symptoms might remain sub-clinical or even be absent. In contrast, series of patients with severe PHPT report multiple end-organ failures including suppressed haematopoiesis (Horm Metab Res 1997, 29: 387) and marrow fibrosis (Clin Endo 2009; 70: 527).

Aim
To determine the effect of parathyroidectomy on haemoglobin and haematocrit levels in a recent cohort of patients diagnosed with PHPT.

Methods
A retrospective database was created and catalogued perioperative haematol-
ogical parameters, clinical and operative details. Wilcoxon–Signed rank tests were performed comparing pre-operative and post-operative haemoglobin (Hb) and haematocrit levels (Hct).

Results
Seventy-six patients (25M: 51F; 18-89 years old, median 62 years) with biochemical diagnosis of PHPT (hypercalcaemia 2.86±0.25 mmol/l, uninhibited/raised PTH levels 32.7±38.2 pmol/l). Patients underwent scan-directed minimally invasive parathyroidectomy (n=42) or bilateral cervical exploration (n=34) and were disease free at last follow-up between 1–27 months (median 8 months).

Preoperative calcium/PTH and Hb/Hct levels did not correlate. Pre-operative and post-operative haemoglobin levels were higher in males than females (P=0.003 and P=0.004, respectively / Mann-Whitney U tests). There was no difference between pre-operative and post-operative Hb/Hct levels in the population as a whole (P=0.242 and P=0.445 respectively). Male patients with pre-operative anaemia (Hb<11 g/dl) had no significant change in their haemoglobin levels (P=0.859) or haematocrit levels (P=0.594) after parathyroidectomy. Similarly, female patients with pre-operative anaemia (Hb<10 g/dl) had no significant change in Hb (P=0.134) nor Hct (P=0.109) following parathyroidectomy.

Conclusion
In contrast to historical data and recent series of severe PHPT, we found no significant change in haemoglobin/haematocrit levels following parathyroidectomy. This might be due to earlier treatment in patients with less severe disease.

P208
Low incidence of hypocalcaemia after thyroidectomy in patients administered routinely oral calcium supplements
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Background
The national audit from the British Association of Endocrine Surgeons and the Scandinavian Quality Register of Thyroid and Parathyroid Surgery have reported that 7–17% of patients have hypocalcaemia in the first 6 weeks after thyroid surgery. This study is a snap-shot of management of peri-operative hypocalcae-
mia using routine Calcichew supplements.

Methods
Retrospective review clinical and biochemical data on consecutive patients undergoing total thyroidectomy. Harmonic scalpel was used in all operations. Patients were prescribed Calcichew 1 g qds for 2 weeks postoperatively and were discharged on day 1–3 postop with information on how to recognise symptomatic hypocalcaemia and how to decide to reduce gradually the daily dose of Calcichew. A minority of patients (n=12) were discharged same day without immediate biochemical assessment.

Results
Between February 2007 and May 2008, 80 patients (58F:22M, age 15–84 years) underwent total thyroidectomy for Graves disease (n=26), benign pathology (n=30) or thyroid cancer (n=24). In 13 patients (16%) 15 parathyroid glands were removed incidentally. Biochemical hypocalcaemia (Ca <2.1 mmol/l) was observed in 14 of 68 patients (21%) in the first day postop. The gland weight was higher in these patients than higher than in normocalcaemic patients (129±91 vs 102±110 g, P=NS). Parathyroid hormone levels were measured in 28 patients on day 1 postop: four of six patients with <0.3 pmol/l developed permanent hypocalcaemia.

Long-term hypocalcaemia was observed in 6 of 80 patients (7.5%) at 6 months follow-up. Three of these patients underwent total thyroidectomy + radical modified neck dissection for locally advanced thyroid cancer and two had very large goites (>300 g).

Only one patient in this cohort was readmitted with symptomatic severe hypocalcaemia (1.6 mmol/l).

Conclusion
Routine administration of oral calcium supplements allow early discharge from hospital after total thyroidectomy with very low rate of readmission for symptomatic hypocalcaemia.

P209
Utilization of various imaging modalities in the localization of tumours in MEN-1 and insulinoma
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Department of Radiology, Cambridge University Hospitals, NHS Foundation Trust, Cambridge, UK.

Objective
A descriptive study to summarize the experience on diagnosis of NET using various imaging modalities.

Methods
Imaging was reviewed for 25 patients between 2002 and 2009 (22 MEN-1, 3 sporadic insulinoma). Of 13/25 had elevated fasting gut hormones. CT, MRI, endoscopic ultrasound (EUS), somatostatin receptor scintigraphy (SRS) and angio-gramy were reviewed. (CT, MRI examined with arterial enhancement).

Results
Most frequently utilised tests were CT and EUS (16, 15). MRI, SRS and angiography were done in 13, 8 and 8 patients respectively. One test was done in 7 patients. Seven subjects were imaged with 2 > 3 tests were performed in 10. All with biochemically confirmed insulinoma were imaged with at least CT and angiography.

Of 17/25 patients had tumours based on any test. EUS was positive in 11/15, CT 6/16, MRI 5/13, SRS 5/8 and angiography 9/9. EUS detected a minimum of 19 lesions whereas CT, MRI, SRS and angiography 15, 7, 9 and 8 respectively. EUS and CT showed a minimum of 9 and 5 in the head of the pancreas respectively. Angiogram was positive in all with hypervascularinsulinaemia whereas CT only in 2. Minimum detection diameters for CT, MRI and EUS were 1.0, 0.4 and <0.3 cm respectively.

Of 6, 2 and 1 duodenal lesions were localized with EUS, SRS and CT respectively. Three had liver metastasis. SRS showed multiple liver lesions in 1 patient while CT and MRI none.

Conclusion
Imaging plays a vital role in the pre-operative work-up of NET. Ascertainment of the number, site and size of tumours in MEN-1 is important to determine the type of surgery. Angiography identified all lesions, but is invasive. EUS had a high rate of identifying lesions, but not all were detected. We advocate a multimodality strategy in tumour localization in MEN-1.
Our data confirm that HIF-1α plays a key role in thyroid cancer cell migration and metastasis. Blocking HIF-1α by small molecules may be an important therapeutic target in the treatment of thyroid cancer, which will be explored in the metastatic tumour models described above.

P212
Routine central compartment lymph node dissection for papillary thyroid cancer has minimal impact on early postoperative parameters
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1Department of Endocrine Surgery, John Radcliffe Hospital, Oxford, UK; 2Department of Clinical Oncology, Churchill Hospital, Oxford, UK.

Background
Arguably patients undergoing total thyroidectomy for papillary thyroid cancers (PTC) are more likely to have negative radioactive iodine uptake and low/undetectable thyroglobulin levels if the central compartment lymph node dissection (CCLND) is performed routinely irrespective of the macroscopic appearance of the LN. The aim of this study was to determine whether routine CCLND leads to more favourable outcome in the first year after treatment for well-differentiated thyroid cancer.

Methods
Clinical, operative and radiological data on patients operated in a University centre were collected in a prospective database. Parameters in a recent group of patients who underwent routine CCLND were compared with similar values in a historical cohort of patients. Results
CCLND was performed in 54 patients (Table). A further 41 patients who presented with palpable local LN metastases and underwent radical neck dissection ≥ CCLND were excluded from this analysis. All 54 patients analysed had no macroscopic evidence of LN involvement. The yield of LN ranged 1–39 (median 8). Metastatic PTC was identified in local LN in 34/44 patients with percentage of involved LN ranging 26–100% (median 57%). This allowed for accurate lymph node staging in all patients (N0 = 10, N1 = 54). There was no correlation between tumour stage/size and likelihood of positive LN.

There was a trend for lower radioactive iodine (RAI) uptake after first ablative dose of 131I in patients who underwent CCLND (Table). There was a notable difference in the levels of thyroglobulin at 3 months (P = 0.09) and 1 year (P = 0.18) in favour of those who underwent CCLND (Table).

<table>
<thead>
<tr>
<th>CCLND</th>
<th>Historical group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>47 years (15–93 years)</td>
</tr>
<tr>
<td>Gender</td>
<td>37F:17M</td>
</tr>
<tr>
<td>Tumour size</td>
<td>36 mm (2–85 mm)</td>
</tr>
<tr>
<td>Tumour type</td>
<td>46 PTC:4 FTC:4</td>
</tr>
<tr>
<td>Lymph nodes retrieved</td>
<td>Median 8 (range 1–39)</td>
</tr>
<tr>
<td>Lymph nodes positive for disease</td>
<td>Median 4 (range 0–14)</td>
</tr>
<tr>
<td>RAI uptake</td>
<td>After first ablative dose of 131I</td>
</tr>
<tr>
<td>At 3 months after 131I</td>
<td>0.02% (0–0.16%)</td>
</tr>
<tr>
<td>At 3 months after 131I</td>
<td>2.7% (0–35%)</td>
</tr>
<tr>
<td>Thyroglobulin levels</td>
<td>6.9 (5–26.6)</td>
</tr>
<tr>
<td>12 Months postop</td>
<td>8.7 (5–29.1)</td>
</tr>
</tbody>
</table>

Conclusion
Routine CCLND leads to a trend towards lower RAI uptake and lower thyroglobulin levels in the first year after treatment for differentiated thyroid cancer. Whether such patients will have lower rates of local recurrence remains to be determined during long-term follow-up.
P217

Is the anti-proliferative effect of AIP (aryl hydrocarbon receptor interacting protein) via ZAC transcription factor?
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Background
Pituitary adenomas are relatively common in the general population, but the pathogenesis of these tumours remains largely unknown. Recently, germline mutations have been described in the AIP (aryl hydrocarbon receptor interacting protein) gene in several families with familial isolated pituitary adenomas (FIPA). This gene is located on chromosome 11q33 and loss of heterozygosity in this locus, as well as functional data from our group demonstrates a tumour suppressor role for AIP. The mechanism by which AIP exerts its tumour suppressive action in the pituitary remains unclear. A recent study has demonstrated that up-regulation of the AIP gene in the liver of transgenic mice increases the expression of ZAC/PLAGLI, a tumour suppressor gene. We hypothesised that AIP mediates its tumour suppressor role in the pituitary via up-regulation of ZAC. Aims and objectives
To study the effect of wild-type and mutant AIP on ZAC mRNA expression in GH3 cells (rodent somato-mammotroph pituitary cell line).

Methods
GH3 cells were transiently transfected with wild-type and mutant AIP (C238Y and R304X) as well as empty vector plasmids. The expression of ZAC mRNA was assessed by real-time PCR.

Results
Over-expression of wild-type AIP significantly increased ZAC mRNA expression compared to the empty vector and the mutant AIP (C238Y and R304X).

Conclusion
ZAC mRNA expression was significantly increased in GH3 cells transiently transfected with wild-type AIP compared to the empty vector and mutant AIP. Our results suggest AIP may exert its tumour suppressor role in the pituitary by up-regulating ZAC mRNA expression.

References

P218

The role of PBF in NIS and MCT8 cellular trafficking
Vicki Smith, Martin Read, Rachel Watkins, Gregory Lewy, Jim Fong, Robert Seed, Neil Sharma, Gavin Ryan, Kristien Boellaert, Jayne Franklyn & Christopher McCabe
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Ablative therapy for thyroid cancer depends upon the adequate uptake of radioiodine via the sodium iodide symporter (NIS). However, thyroid tumours frequently show reduced iodide uptake, impacting on prognosis. We previously demonstrated that the proto-oncogene PBF binds NIS and modulates its subcellular localisation. We now demonstrate that PBF binds the thyroid hormone transporter MCT8 in GST-pulldown assays, and alters MCT8 subcellular localisation in vitro. To investigate PBF function further we carried out immunohistochemical studies in COS-7 cells. PBF was localised predominantly within intracellular vesicles, whereas a PBF deletion mutant lacking the C-terminal 30 amino acids was targeted to the plasma membrane. We hypothesised therefore that PBF shuttles in and out of the plasma membrane, and that loss of a tyrosine-based internalisation signal (YXXΦ motif) within the deleted region caused the accumulation of PBF within the plasma membrane. Discrete mutation of the critical tyrosine residue (Y174) and the hydrophobic residue (F177) resulted in increased plasma membrane staining, confirming the YXXΦ motif as an active internalisation signal. Further, a functional signal peptide and transmembrane domain were identified when deletion of residues 3–32 and 94–120 respectively resulted in protein retention in the endoplasmic reticulum. PBF has two putative N-linked glycosylation sites at amino acids 45 and 54. Following Western analysis of COS-7 cell lysates, multiple PBF bands between 25 and 37 kDa were all reduced to ~20 kDa through the use of the glycosylation inhibitors and glycosylation mutants. Interestingly, a mutant lacking residues 39–93 showed an increase in membrane staining, suggesting that glycosylation is not essential for membrane targeting, but is required for efficient internalisation. These data further characterise the transmembrane glycoprotein PBF, which given that it regulates the subcellular expression of NIS, may provide a therapeutic target in the enhancement of radioiodine therapy in relatively radioresistant thyroid cancers and their metastases.

P219

MEN2B patients with a RET A883F mutation have less aggressive MTC than those with the common RET M918T mutation
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MEN2B is the most aggressive form of MEN2. Consequently, the new American Thyroid Association guidelines recommend prophylactic thyroidectomy early in the first year of life. Ninety-seven percent of MEN2B cases result from a germline methionine to threonine mutation at codon 918 (M918T) of the RET proto-oncogene. In addition, an exceedingly rare alanine to phenylalanine mutation at codon 883 (A883F) has been reported in 4 unrelated adults. In each case metastatic MTC and the clinical features of MEN2B occurred in the presence of the a novel A883F germline mutation. We describe the first MEN2B family with the A883F mutation and report the results of prophylactic thyroidectomy. The 29-year-old female proband presented with symptomatic, metastatic MTC and features of MEN2B. Her 8-year-old son, who inherited the A883F mutation, also had classical features of MEN2B. Unexpectedly, his basal calcitonin was within the normal range. Remarkably, prophylactic thyroidectomy revealed only C-cell hyperplasia (CCH). The ATA guidance for prophylactic thyroidectomy in MEN2B is based solely on data from individuals with the common M918T mutation and the specific finding that virtually all infants have CCH and MTC by 1 year of age. The finding of only CCH in an 8-year-old with MEN2B suggests the A883F mutation results in a much less aggressive form of MTC. Accordingly, the A883F mutant has a lower in vitro transforming ability than the common MEN2B mutant M918T or even the C634R mutant that causes MEN2A. This unique family provides new insight into the molecular pathology of MTC with important implications for management of MEN2B. The finding of CCH alone in an 8-year-old with MEN2B resulting from an A883F mutation suggests that prophylactic thyroidectomy may be delayed in such families until 5 years of age when surgical complication rates approach those of adults.

P220

First report of SOX2 loss of function associated with a large hypothalamo-pituitary tumour; further insights into the role of SOX2 in pituitary development
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Background
SOX2 is a member of the SOX family of transcription factors (SRY-related high-mobility group (HMG) box). Heterozygous, de novo, loss-of-function mutations were initially reported in patients with bilateral anophthalmia/microphthalmia,
The median operative time was 130 min (78–264) in the LA group and 152 min (98–260) for the OA group (P = 0.088). Median hospital stay was 4 days (2–12 days) for the LA group and 13 days (6–36 days) for the OA group (P = 0.001). Two LAs were converted to OA. All postoperative complications (wound infection 3, PE 1) occurred in OA group. There was no mortality.

Conclusion

In our series, LA took no longer than OA and was associated with fewer postoperative complications and shorter hospital stay. LA is a procedure of choice for most phaeochromocytomas and OA should be reserved for tumours >10 cm and paragangliomas.

<table>
<thead>
<tr>
<th>Steroid ratio</th>
<th>Mitotane + H/cortisol</th>
<th>Hydrocortisone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>Range</td>
<td>Mean</td>
</tr>
<tr>
<td>50/5/4</td>
<td>499</td>
<td>52–2024</td>
</tr>
<tr>
<td>20x20/5/Reduced CM</td>
<td>30</td>
<td>16–46</td>
</tr>
<tr>
<td>Hydrocortisone</td>
<td>tetrahydrocortisol</td>
<td>0.7</td>
</tr>
</tbody>
</table>

A high level of excretion of polar unconjugated steroids during mitotane was also confirmed, with 6β-hydroxycortisol and (previously unrecognised) 6β-hydroxy-20α-dihydrocorticoids predominating. Mitotane treatment thus produces a profound decrease of 5α- vs 5β- and of 20β- vs 20α- reduction, a net decrease of 5-reduction but not of 11- or 20-reduction. No metabolic changes correlated with either dose or blood level of mitotane. Two ACC patients showed relapse during treatment, with increase of previously identified steroid markers, none of which were 5α- or 20β-reduced.

We conclude that mitotane causes consistent, but previously unrecognised, changes in cortisol catalbolism, which do not interfere with early detection by USP of relapse in ACC. These indicators of mitotane use are not concentration-dependent. Induction of 6β-hydroxylation probably explains the observed decrease of cortisol bioavailability.

P225.1

The microRNA let-7a is downregulated in pituitary tumours from a multiple endocrine neoplasia type-1 mouse model

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MicroRNAs are highly conserved non-coding RNAs that regulate diverse cellular processes. Altered microRNA expression is observed in many human cancers and microRNAs may have tumour suppressor or oncogenic properties. One group of putative tumour suppressor miRNAs is the let-7 family whose expression is reduced in several human tumours, and which inhibit the expression of several oncogenes including HMGA2 and K-Ras. Let-7 expression have also been observed to be reduced in human pituitary tumours, including prolactinomas, corticotrophinomas and non-functioning adenomas, although the functional significance of this has not been assessed. We therefore examined the expression of let-7a and its putative targets HMGA2 and K-Ras in anterior pituitary tumours that developed in mice deleted for a multiple endocrine neoplasia type 1 (Men1<sup>−/−</sup>) allele. The anterior pituitary tumours that develop in Men1<sup>−/−</sup> mice include prolactinomas, somatotrophinomas, corticotrophinomas and non-functioning adenomas. Mice were kept in accordance with UK Home Office welfare guidelines and project licence restrictions. Total RNA was extracted from pituitaries of five age-matched wild-type (Men1<sup>+/+</sup>) and five Men1<sup>−/−</sup> mice and quantitative reverse transcriptase-PCR performed to measure microRNA and target mRNA expression. Compared to Men1<sup>+/+</sup> pituitary tissue, Men1<sup>−/−</sup> pituitary tumours showed significant downregulation of let-7a expression (~1.7 fold change, P<0.05). HMGA2 expression was similar in Men1<sup>+/+</sup> pituitaries and Men1<sup>−/−</sup> pituitary tumours but a significant increase in K-ras mRNA expression (~1.5 fold change, P<0.05) was observed in Men1<sup>−/−</sup> pituitary tumours compared to Men1<sup>+/+</sup> pituitary tissue. Thus, our studies, which demonstrate a reduced let-7a expression in Men1<sup>−/−</sup> mouse pituitary tumours, together with an increased expression of the oncogene K-ras provide insights into the role of the microRNA let-7a and its target gene K-ras in pituitary tumours.
Growth and development

Klinefelter’s syndrome often remains undiagnosed or diagnosed late
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Introduction
In spite of great progress in Endocrinology, even today Klinefelter’s syndrome remains an under diagnosed late diagnosed chromosomal disorder as is evident from the three case reports below.

Case 1
A 79 years old man admitted with exacerbation of COPD, was noted to have bilateral Gynaecomastia, feminine features, very small scrotum without testes and a small penis. Throughout his life he remained isolated and single. Scrotal ultrasound showed testicles in inguinal canal.

Case 2
A 62 years old gentleman with diagnosis of PE was noted to have bilateral Gynaecomastia, small testes. He was married, had no children and has extensive investigations for infertility, no cause found. He had history of low trauma ankle fracture.

Case 3
A 75 years who was referred to endocrine clinic with mild hypercalcaemia, which resolved after stopping calcium medication. He had osteoporosis with multiple vertebral fractures. He had typical Klinefelter’s features, bilateral Gynaecomastia and small testicles. He was socially isolated, never had a partner and even could not enjoy swimming because of embarrassment caused by his Gynaecomastia. In all hormone profile revealed primary hypogonadism and 1 and 2 had 47XXY and 46XX karyotype. All were put on testosterone replacement.

Discussion
Klinefelter syndrome is the most common genetic cause of human male infertility, but many cases remain undiagnosed because of substantial variation in clinical features and insufficient professional awareness of the syndrome as in our cases. It is associated with increased risk of germ cell tumours, breast cancer, metabolic syndrome and cardiovascular morbidity and mortality. Early recognition and hormonal treatment can improve quality of life, prevent serious consequences and even many can achieve successful fertility with advanced techniques. And for early diagnosis professionals need to be more aware and keep high index of suspicion.

P227

Altered responses to GH and IGF1 in children born small for gestational age without post-natal catch up growth
Imogen Butcher, Andrew Whatmore, Philip Murray, Melissa Westwood & Peter Clayton
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Background
Infants born small for gestational age (SGA) usually show catch up growth within the first few years of life. However in the UK ~1500 SGA children each year remain small, with no clear endocrine cause with rare genetic syndromes accounting for only a minority of cases. In order to define growth factor activation in these children we have initiated an assessment of cell growth and signalling in response to GH and IGF1 in vitro and cell lines.

Methods
Skin biopsies were obtained with local ethics approval from healthy children (n = 4) and SGA children without post-natal catch up growth (n = 4). Fibroblasts were isolated and cell growth measured by cell counting and BrdU incorporation, and apoptosis by TUNEL staining. Growth factor signalling was assessed by western blotting for both total and phospho isoforms of MAPK, Akt and Stat5b.

Results
Under basal and GH stimulated conditions proliferation in SGA cells is comparable to controls, whereas response to IGF1 is significantly reduced. However when treated with a combination of GH and IGF1 SGA cells grew at a similar rate to controls, with cell proliferation much greater than with GH alone. Apoptosis was also increased in SGA cells compared to controls. Stat5b, Akt and MAPK were present at similar levels in SGA and control cells. Stat5b activation by GH was decreased in SGA cells compared to controls while activation of MAPK was similar. A different pattern of IGF1 induced Akt activation was found with Akt 2 activation occurring in SGA but not control cells.

Conclusion
Decreased IGF1 stimulated cell growth, increased rates of apoptosis and altered GH and IGF1 signalling are associated with a non catch up SGA phenotype. The combination of GH and IGF1 ‘rescues’ poor cellular growth, which may suggest a role for such combination treatment in IUGR in these patients.

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Delayed puberty in children with inflammatory bowel disease may be associated with gonadotrophin resistance
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Background
The aetiology of delayed puberty in children with inflammatory bowel disease (IBD) is unclear.

Methods
Retrospective analysis of 27 children with IBD with growth retardation and/or pubertal delay, who had basal LH and FSH (27) and LHRR stimulation test as part of clinical evaluation (25). Height velocity was converted to SDS for bone age for girls >11 years and boys >12 years to adjust for delayed puberty. Data expressed as median (range).

Results
Twenty-seven children (five females), with IBD (25 Crohn’s disease; two ulcerative colitis), median age 14.5 years (7.7, 17.0); median height (Hh) SDS – 1.9 (~3.6, –0.9); median IVS SDS – 2.4 (~7.7, 2.8); and median bone age delay 1.7 years (~0.6, 2.9) are described.

P229

Familial growth hormone deficiency – response to growth hormone therapy and analysis of the GH-1 and GHRH-R genes
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Familial isolated growth hormone deficiency is exceedingly rare. We present two siblings of non-consanguineous parents with this syndrome and describe their response to growth hormone therapy. We have also carried out a mutational analysis of their GH1 and GHRH-R genes.

A mutation scan of the entire coding region and flanking intronic sequences of GH-1 and GHRH-R was undertaken in both index cases and parents. GH1 and GHRH-R mutational analysis did not reveal any pathogenic mutations in either the coding regions or the intron-exon boundary regions.

We then analysed the 5’UTR of GH-1 and found that the father, siblings 1 and 2 had a deletion SNP in the 5’ UTR of GH-1 – rs11568827. We also found that the mother, siblings 1 and 2 have an SNP in the 5’ UTR of GH-1 – rs11568828.

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The impact of IBD on pubertal growth is most marked in boys with Crohn’s disease

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Background

Whilst puberty is understood to be commonly effected in adolescents with Crohn’s disease (CD) and ulcerative colitis (UC), the extent of this effect as well as related problems with growth have rarely been quantified.

Objective

To determine the impact of CD and UC on Size, Tempo and Velocity of the growth spurt.

Methods

Retrospective study of 142 children with IBD (case notes available, 68) who fulfilled the criteria for describing growth spurt parameters (48). Twelve children who had been treated with growth promoting agents (8 CDM, 2 CDF and 2 UCM) and four children who had coexisting conditions, which may impact on growth were excluded. The remaining groups CDM (11); CDF (8); UCM (5) and UCF (8) had median age at diagnosis of 13.2, 11.9, 11.7 and 11.0 years respectively. Height at diagnosis (HAD) and height at peak height velocity (HPHV), converted to SDS, defined Size; age at HPHV (APHV) defined Tempo; and PHV, converted to SDS adjusted for pubertal stage, defined velocity of the growth spurt. The median interval between height measurements was 0.4 years. Results expressed as median (range).

Results

There was no statistical difference between groups for each of the parameters. However 6/11 in CDM had 1 parameter affected: 1 subject had HAADS < –2, 1 subject had HPHVSDS < –2; 2 subjects had an APHV > 2 years above population mean, and 2 subjects had a PHVSDS < –2. There was no correlation between PHV and either HPHV or APHV in analysis of the whole group (r = 0.29, P=0.11 and r, 0.16, P=0.40) in subgroup analysis.

Conclusion

As a group, disorders of the pubertal growth spurt are more likely to occur in boys with Crohn’s disease.

P231

Phenotypic presentation of P450 oxidoreductase deficiency during puberty

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P450 oxidoreductase (POR) transfers electrons to all micromosomal P450 enzymes including CYP21A2 and CYP17A1, key enzymes of glucocorticoid and androgen synthesis, respectively. Mutant POR results P450 oxidoreductase deficiency (ORD) manifesting with glucocorticoid deficiency and disorders sex development in both sexes. Neonatal presentation with undervirilisation in boys and virilisation in girls is well described. However, there is a paucity of data on the pubertal phenotype in ORD. Here we present the pubertal phenotype in five ORD patients (three females, two males). In all cases, we confirmed ORD by urinary steroid analysis (GC/MS) and direct sequencing of the POR gene. In addition, missense mutations were confirmed as disease causing using a yeast-based co-expression system. Case 1 (46,XX; homozygous p.A287P) developed hypergonadotropic hypogonadism and large bilateral ovarian cysts at 11 years of age that required surgical intervention and resolved only following combined treatment with oestradiol, GnRH superagonist and dexamethasone. Case 2 (46,XX; homozygous p.A287P) showed no pubertal development until 17 years when oestradiol/ progesterone replacement was initiated. However, large bilateral ovarian cysts were observed at 24 years after brief discontinuation of oestradiol, resolving upon reintroduction of HRT. Case 3 (46,XX; p.T142A/p.Y376f,L374T) presented with oligomenorrhea and partially delayed puberty at 19 years, gonadotropins were elevated and a large ovarian cyst was observed before initiation of oestradiol treatment. Case 4 (46,XY; p.R475H/p.S578X) presented at 12 years with manifest hypogonadism and subsequently required induction of puberty by testosterone. Case 5 (46,XY; p.A287Tc.c.330+2dupT) presented with hypogonadism at the age of 12 years and no apparent signs of pubertal development except for a left testicular volume of 12 ml. Pubertal development was initiated by testosterone. In summary, patients with ORD develop symptoms of hypergonadotropic hypogonadism during puberty, resulting in lack of pubertal development in both sexes, further complicated by large ovarian cysts due to gonadotrophin stimulation in affected 46,XX individuals.

P232

Loss of the Golgi localised E3 ubiquitin ligase containing Cullin 7 in the growth disorder 3-M syndrome leads to reduced cell proliferation and reduced IGF1 mediated activation of Akt

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Background

3-M syndrome is an autosomal recessive disorder characterized by pre- and postnatal growth restriction, normal intelligence and dysmorphic facial features.

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Mutations in the genes encoding Cullin 7 (CUL7) and Obsculin-like-1 (OBSL1) have been shown to cause 3-M syndrome.

Aims
To characterize CUL7 production and localization in a primary fibroblast cell line from a patient with a 3-M syndrome due to a CUL7 mutation and to assess cell proliferation, apoptosis and response to growth hormone (GH) and insulin like growth factor 1 (IGF1) in patient versus control fibroblasts.

Methods
A dermal fibroblast cell line was established from a patient with a novel nonsense mutation in exon 22 of CUL7 (c.4198delC p.H1379RfsX11). Immunofluorescence using antibodies to CUL7 and the golgi apparatus was carried out in patient and control fibroblasts, with quantitative RT-PCR being used to measure expression of CUL7 and OBSL1. To assess GH and IGF1 signal transduction, control and patient fibroblasts were stimulated with GH and IGF1 with lysates immunoblotted for activation of ERK1/2, STAT5b, IRS-1 and Akt.

Results
The patient fibroblasts demonstrated decreased expression of both CUL7 (relative expression 0.25, P < 0.001) and OBSL1 (relative expression 0.45, P < 0.001) mRNA compared to control fibroblasts. Western immunoblot demonstrated absence of the 185 kDa band for full length CUL7 with no evidence of truncated protein.

In control fibroblasts CUL7 co-localised with GM130 (a known golgi marker), CUL7 was not identified in the patient fibroblast cell line. Cell proliferation was reduced in patient cells while no difference in apoptosis was observed. Patient fibroblasts displayed normal STAT5b and ERK1/2 signalling following stimulation with rhGH. Activation of IRS1 was normal but reduced levels of activated Akt were seen following IGF1 stimulation.

Conclusions
Loss of CUL7 is associated with reduced OBL1 transcription, reduced cell proliferation and reduced activation of Akt following stimulation with IGF1.

P233
A novel dominant-negative Gilial Cells Missing B (GCMB) mutation (A502H) is associated with autosomal dominant hypoparathyroidism and results in reduced transactivation activity
Michael Bowl1, Samantha Mirzcek1, Carl Fratter2, Treena Cranston2, Jeremy Allgrove3,4, Caroline Brain3, Andrew Neshil5 & Rajesh Thakker3

Gilial cells missing B (GCMB), which is the mammalian homologue of the Drosophila GMC gene, encodes a 506 amino acid parathyroid-specific transcription factor that contains: a DNA-binding domain (residues 21–174); a predicted nuclear localization signal (residues 176–193); an inhibitory domain (residues 258–347); and two transcription activation domains (residues 174–263, and residues 428–506). To date only two dominant GCMB mutations have been reported in three kindreds with autosomal dominant hypoparathyroidism (AD-HPT). We therefore investigated an additional family with AD-HPT for GCMB mutations, after obtaining informed consent, using guidelines approved by the national ethical committee. Leukocyte DNA was used with GCMB-specific primers for PCR amplification of the five exons. DNA sequence analysis of the PCR products revealed an A to C transversion at codon 502, which altered the wild-type asparagine (Asn) to histidine (His). The DNA sequence abnormality was demonstrated to be absent in 101 alleles of 55 unrelated normal individuals, thereby demonstrating that it is not a common polymorphism. Functional studies, utilizing transient transfections of COS7 cells with GCMB wild-type and mutant (A502H) tagged constructs, demonstrated that: the wild-type and mutant GCMB A502H proteins localized to the nucleus; and, electrophoretic mobility shift assays (EMSA) showed that the mutant protein retained the ability to bind the GMC-consensus DNA recognition motif. However, a luciferase-reporter assay, demonstrated that the GCMB A502H mutation resulted in a reduction in gene transactivation. Moreover, co-transfection of the wild-type with mutant GCMB A502H did not lead to an increase in luciferase activity, thereby demonstrating a dominant-negative effect of the A502H mutant which would be consistent with its autosomal dominant inheritance. Thus, our results have identified the first dominant missense GCMB mutation, to help us improve our understanding of the mechanism underlying gene transactivation, that is a prerequisite for the function of this parathyroid gland-specific transcription factor.

P234
Sleep is frequently disordered in patients irradiated for brain tumours
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Unwanted late effects of cranial irradiation frequently includeendocrine and psychosocial impairment, which may be overlooked in routine follow-up. The aim of the present study was to obtain systematic data on the long-term, psychosocial impairments of patients treated with cranial irradiation as compared to a non-irradiated control group. Both groups were fully hormone replaced including growth hormone (GH) substitution.

A compilation of questionnaires assessing quality of life, depression, psychological distress and ability to work was mailed to all patients treated in a specialized single centre late-effects clinic. Patients included had received cranial irradiation at least 36 months ago and were on stable hormone replacement including GH (Ethical approval obtained). A group of similarly replaced but non-irradiated patients served as controls.

QoL was assessed using the QoL-AGHD and SF-36 questionnaire. The BDI-II was used to evaluate depression. Sleep disturbances and daytime sleepiness were assessed by the PQTI and the ESS, respectively. The WPAI-GH was used to assess capability and impairment at work.

Of 54/197 questionnaires from the irradiated group were returned and analyzed. QoL in the QoL-AGHD was severely diminished in 51% of the patients and 40% showed depressive symptomatology in the BDI-II. Impaired mental QoL in the SF-36 was evident in 22%. Sleep disturbances and daytime sleepiness were prominent with 72% exhibiting scores of significant sleep disturbance in the PQTI and 35.2% qualifying for specialist sleep advice according to the ESS. 35 questionnaires from non-irradiated patients analysed for comparison confirmed more severe forms of sleep disturbance including daytime sleepiness but differences between did not reach significance. Expectedly, both brain-irradiated and non-irradiated patients suffer from a high degree of psychosocial impairment, including reduced QoL and depression. The dominant sleep disturbances observed were surprising and warrant intensive screening in this group of patients.

P235
Intra-cerebral haemorrhage in a young woman with phaeochromocytoma
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Introduction
Phaeochromocytoma is a rare neuroendocrine tumour, with serious and potentially lethal cardiovascular complications. Cerebral haemorrhage is an unusual complication of phaeochromocytoma with <0.5 cases reported worldwide.

Case
We report the case of a 29-year-old woman who presented with sudden onset of headache and collapse. CT Brain showed right sided frontal haemorrhage, her BP > 200/100, LVH on ECG and Echocardiography suggesting long standing hypertension. Her cerebral angiography showed no sign of an AVM or other vascular abnormality.

Phaeochromocytoma was clinically suspected and appropriate antihypertensive drugs commenced. Further investigations for possible related endocrinopathies revealed very high urine normetanephrine 44 umol/24 h (<4.3), high urine metanephrine 0.7 umol/24 h (<20), and raised plasma normetanephrine 9.56 (<1.09). CT abdomen confirmed a large left adrenal mass (4.6 x 2.7 x 5 cm) consistent with a phaeochromocytoma.

She has improved neurologically and transferred to neurorehabilitation centre. She would be candidate for the surgery in the next few months. This lady phaeochromocytoma may be sporadic or part of MEN2 or Von Hippel Landau syndrome or familial paraganglioma syndrome (SDH deficiency), MEN2 genetic screen requested.

Conclusion
Hypertensive crises in phaeochromocytoma can become manifest in many different ways, including cerebrovascular accident. Early recognition of the symptoms of phaeochromocytoma and prompt appropriate intervention can prevent a potentially fatal outcome.

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The endocrine and behavioural effects of high fat feeding
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Diet induced obesity is associated with an increased relative risk of developing type 2 diabetes, hypertension, dyslipidaemia and cardiovascular complications. In addition to these co-morbidity factors there is increasing evidence to suggest that obese insulin resistant individuals show impaired negative feedback control of the HPA axis in response to stress. Here we investigate HPA axis response to an acute stress following high fat feeding in rats.

Male Wistar rats (150–175 g; n = 6/group) were randomly assigned to receive either standard rat chow (SC, RM1) or a high fat diet (HF; 45% kcal lard) for 12 weeks. Animals were housed in cages of two and food and water were provided ad libitum. Weight was monitored weekly and blood samples taken every 3 weeks to monitor glucose and insulin. On the test day one animal from each cage was exposed to an elevated plus maze for 15 min and their cage-mate killed immediately. The proportion of entries and time spent in open and closed arms were recorded as a measure of anxiety. Animals were killed by cervical dislocation. Tissues and trunk blood collected for analysis.

HF animals gained significantly more weight and had higher circulating plasma glucose compared to controls. Adrenal mass was greater in HF animals with higher SC and HF groups compared with unstimulated controls, and higher than SC rats. HF animals made more total arm entries and spent more time in the open arms.

These findings suggest that high fat feeding raises basal corticosterone levels, which may be associated with increased activity and decreased anxiety related behaviour in the elevated plus maze.

Profilng the metabolic clearance and enzymatic degradation of the gut hormone Peptide YY as a tool to design long-acting PY3-36 analogues in treating obesity
Melisande Addison, Emily Thompson, James Minnion, Kevin Murphy, Samar Ghourab, Klara Hostomska, Mohammad Ghaedi & Stephen Bloom
Imperial College London, London, UK.

Peptide YY (PY) is a satiety hormone that communicates nutritional status to the central nervous system. PY is released postprandially from enteroendocrine L-cells in proportion to calories consumed. It is processed to generate the principle bioactive form PY3-36, which acts on Y2 receptors in feeding centres within the brainstem and hypothalamus to reduce appetite. Chronic intravenous infusion of PY3-36 reduces weight loss in rodents, and obese humans display low plasma PY levels, suggesting PY3-36 may be a useful anti-obesity drug target. However, PY3-36 has a short biological half-life. Previously, zinc metalloendopeptidase concentrations in kidney brush border (KBB) have been implicated in PY degradation. Renal failure patients exhibit high levels of PY3-36 and reduced appetite. Thus we hypothesised that the kidney may be a primary site of PY3-36 clearance. This was investigated by comparing the pharmacokinetics of exogenous PY3-36 in nephrectomised and sham-operated rats. The half-life of PY3-36 was increased from 25.9 ± 0.03 to 45.4 ± 0.7 min in nephrectomised rats versus sham-operated controls. To further investigate the metabolic profile of PY3-36, both purified metalloendopeptidase meprin β and KBB preparations were incubated with PY3-36 in vitro and the cleavage products were assessed by high performance liquid chromatography (HPLC) and mass spectrometry. Both meprin β and KBB membranes appeared to cleave PY3-36 between the negatively charged residues Glu10-Asp11. When KBB was co-incubated with the meprin β, the breakdown of PY3-36 by KBB was entirely prevented. Furthermore, mice administered acxinon with PY3-36 displayed a significant increase in PY3-36 plasma levels at 20 and 60 min post-injection compared to mice administered PY3-36 only. These experiments have begun to elucidate the mechanisms responsible for the breakdown of PY3-36 in vitro, providing data useful in the development of long-acting PY analogues for the treatment of obesity.

Characterising the role of 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) in viral meningitis
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Meningitis is associated with high mortality and morbidity predominantly driven by meningeal infection. Herpes Simplex virus (HSV) is a major cause of viral meningitis typically progressing to HSV encephalitis. Numerous studies highlight the importance of glucocorticoids in lowering infection in bacterial meningitis, but very limited data exists on the use of glucocorticoids in viral meningitis. We aimed to characterise 11β-HSD1 (which interconverts inactive cortisone to active cortisol) in meningeal cells, and evaluate the role of glucocorticoids in modulating inflammation.

An in-vitro model of meningeal infection was established using human meningeal fibroblasts (HMF) primary cells. Treatments with tofli receptor (TLR) ligands, whereas immune pathogen associated infection, were used to initiate an immune response. RT-PCR analysis of HMF defined mRNA expression of 11β-HSD1, glucocorticoid receptor, mineralocorticoid receptor, heat shock-phosphate dehydrogenase and TLRs 1-6 and 8-10. Following 24 h treatment with TLR ligands (1-10), the TLR ligand polyIC, which mimics HSV infection, significantly up-regulated the pro-inflammatory cytokine IL-8 (60672±1967 versus control 10.596±1980 pg/ml, P<0.001). In addition, polyIC significantly increased 11β-HSD1 ono-reductase activity (0.59±0.01 pmol/ng per h, P<0.001). Twenty-four hours treatment with IL-1β produced a similar result, significantly increasing IL-8 production (26279±3455 versus control 10.596±1980 pg/ml, P=0.05) and upregulating 11β-HSD1 activity (0.84±0.01 pmol/ng per h, P<0.001). Twenty-four hours incubation with polyIC and glucocorticoids (dexamethasone (1×10−5−1×10−7)M, cortisol (1×10−5−1×10−7)M) and cortisone (1×10−7M) significantly reduced IL-8 production (P<0.001, P<0.01 and P<0.001 respectively).

We have identified HMF as a novel glucocorticoid target tissue which up-regulates local cortisol production, via 11β-HSD1, in the presence of HSV ligand. Additionally, glucocorticoids significantly reduce TLR3 (HSV) driven inflammation in HMF. 11β-HSD1 may have a protective role in viral meningitis and further evaluation of the therapeutic role of glucocorticoids is now warranted.

Alarin stimulates food intake and the hypothalamic-pituitary–gonadal axis in male rats
Charlotte Boughton, Michael Patterson, Gavin Bewick, John Tadross, James Gardiner, Faizan Chaudhry, George Hunter, Mohammad Ghaedi, Stephen Bloom & Kevin Murphy
Imperial College London, London, UK.

Alarin is the most recently discovered member of the galanin peptide family, and is encoded by a splice variant of galanin-like peptide (GALP) mRNA. Galanin and GALP are known to regulate fever, reproduction and reproduction. Galanin is thought to mediate its effects via the three-galanin receptors. GALP also binds to these known galanin receptors, but evidence suggests that it may also act via an as yet unknown GALP specific receptor. The role of alarin in energy homeostasis and reproduction is currently unknown. The objective of these studies was thus to determine the effects of alarin on food intake and the hypothalamic-pituitary–gonadal axis.

Intracerebroventricular (ICV) administration of alarin (30 nmol) to unanaesthetised ad libitum fed male rats significantly increased food intake 1 h after injection (0–1 h food intake/g: saline 0.60±0.28, alarin 30 nmol 3.06±0.55, P<0.01). ICV administration of alarin (6 nmol) to unanaesthetised adult male rats significantly increased plasma LH levels 30 min after injection (plasma LH ng/ml: saline 0.7±0.1, alarin 1.2±0.1, P<0.05). In vitro, alarin stimulated Neuropeptide Y (NPY) and GnRH release from hypothalamic explants from male rats, and GnRH release from GT1-7 cells. In vivo, pre-treatment with the GnRH receptor antagonist cetorelix blocked the alarin-induced stimulation of plasma LH levels in unanaesthetised adult male rats. Rceptor binding studies confirmed that alarin does not bind to galanin receptors 1 or 2, and demonstrated that alarin does not compete with radio-labelled galanin for hypothalamic binding sites. These results suggest that alarin is a novel orexigenic peptide, and that it stimulates the HPG axis via hypothalamic GnRH. Further work is now required to determine the receptor that mediates the biological effects of alarin.

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Society for Endocrinology BES 2010, Manchester, UK
P240
Transgenic disruption of 5α-reductase 1 in mice results in a blunted stress response
Dawn Livingstone, Yang Chenjing, Di Rollo Emma, Kara Madina, Mathews John, Codrington Lucy, Kenyon Christopher, Walker Brian & Andrew Ruth
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5α-Reductase1 (5αR1) catalyses glucocorticoid metabolism, potentially modulating glucocorticoid clearance and therefore affecting the activity of the hypothalamic-pituitary-adrenal (HPA) axis. Here we investigated if inhibition of 5αR1 impairs glucocorticoid clearance and suppresses the HPA axis.

Age-matched male and female mice homozygous for transgenic disruption of 5αR1 (KO) and wild type littermates (WT) were studied under basal (0700 h) and stress stimulated (15 min restraint) conditions (n = 8-12/group). Mice were culled (0800-1100 h), at least 1 week later. In further groups of male mice, corticosterone was chronically infused (50 μg/day, 4 weeks). Plasma corticosterone (radioimmunoassay) adrenal morphology (in tissue sections stained with haematoxylin and eosin), and mRNA for pro-opiomelanocortin and CYP11B1 in pituitary and adrenal respectively (real-time PCR) were assessed.

Indices of basal HPA activity (basal corticosterone concentrations, adrenal mass, adrenal CYP11A1 expression or pituitary POMC expression) did not differ between genotypes in either gender. Adrenal morphology (cell size or number) was unaffected. However, the corticosterone rise in response to stress was blunted in KO compared to WT male (peak: 202.2±18.9 vs 268.7±28.75 nm; P = 0.04; AUC suppressed by 44%; P = 0.002) and female (peak: 915±62 vs 1150±115 nm; P = 0.08; AUC suppressed by 36%; P = 0.009) mice. Circulating concentrations of corticosterone were not different following chronic infusion (176±48 vs 166±21 nm).

In summary, global 5αR1 KO is not associated with changes in glucocorticoid clearance or basal activity of the HPA axis, suggesting that mechanisms exist to compensate for lack of this enzyme. However, the central drive to the HPA axis in response to stress is attenuated in 5αR1 KO.

P241
Stress hormone response in men at high altitude: effect of ethnicity
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Background and objective
Adaptive process to hypoxia of altitude involves changes in the homeostatic steady state of several endocrine variables which precede and contribute- to many physiologic adaptations. Physiologic responses to altitude-stress exhibit ethnic variation. However, there has been limited characterization of 'ethnicity effect' on endocrine responses to high-altitude stress. This is true for enzyme-immunoassay method (EIA) of plasma hormone analysis. Little is known in this regard about the Indian population. Ethnic variation in physiologic and psychological performance during altitude-stress has been reported in this population. The objective of this study was to examine the effect of ethnicity on plasma stress hormones during high-altitude exposure in lowlanders as compared to sea-level, and with high-altitude natives (HAN) at high-altitude in the Indian population.

Methods
Healthy male soldiers (n = 115) between 20-50 years were enrolled for the study. Lowlanders belonging to Rajput (n = 25), Gorkha (n = 30) and South-Indian (n = 35) ethnicities were studied at sea-level and after 3-4 weeks of stay at ~4500 m. Ladakhi (HAN, n = 25) were studied at ~4500 m only. Estimation of plasma cortisol-CORT, testosterone-T, prolactin-PRL, arginine vasopressin-AVP and proenkephalin natucretic peptide-proANP, 1-84 was measured by EIA.

Results
In lowlanders, there was a significant (P < 0.001) change in PRL, AVP and proANP, 1-84 (within physiological-range) during high-altitude exposure. Also, ethnicity of the subject was found to have a significant (P < 0.001) effect on hormonal changes at high-altitude. Among lowlanders, difference in plasma hormones was observed between north-(Rajput, Gorkha) and South-Indians at high-altitude.

<table>
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<tr>
<th>Hormones</th>
<th>Rajput</th>
<th>Gorkha</th>
<th>South-Indian</th>
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<tr>
<td>CORT</td>
<td>↓</td>
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<td>proANP, 1-84</td>
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P242
Hypopituitarism secondary to pituitary apoplexy- is it due to high dose Aspirin?
Daniel Kannappan, Doros Polydorou, Sam Kenz, Angela Paisley & Tara Kearney
Salford Royal Hospital, Manchester, UK.

Seventy six year old man presented with sudden onset headache for 3 days. He was seen by the GP and treated for migraine. But no improvement in his headache and he developed drooping of left eyelid and blurring of vision. No other neurological symptoms.

On examination left ptosis with normal visual fields to confrontation method. Initial differential diagnosis was isolated 3rd nerve palsy probably due to posterior communicating artery aneurysm. He was on aspirin 300 mg for last 10 years as suggested by cardiologist, which was reduced to 75 mg.

His CT Head with Angiogram showed large supra sellar mass measuring 2.5X1.7 cm with no evidence of aneurysm, MRI showed marked expansion of pituitary fossa with macro adenoma and evidence of apoplexy and chiasmal compression.

Pituitary hormone profile

<table>
<thead>
<tr>
<th>Hormone</th>
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<tr>
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<td>&lt; 1.0 U/l</td>
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<tr>
<td>FSH</td>
<td>1.5 U/l</td>
</tr>
<tr>
<td>Testosterone</td>
<td>&lt; 0.5 nmol/l</td>
</tr>
<tr>
<td>TSH</td>
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</tr>
<tr>
<td>FT3</td>
<td>9.0 nmol/l</td>
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<tr>
<td>Prolactin</td>
<td>&lt; 10 mU/l</td>
</tr>
<tr>
<td>GH</td>
<td>0.1 U/l</td>
</tr>
<tr>
<td>IGF1</td>
<td>5.4</td>
</tr>
<tr>
<td>Cortisol</td>
<td>45 nmol/l</td>
</tr>
</tbody>
</table>

Patient was reviewed by Neuro surgeon and in view of his eye symptoms he warrants surgical intervention. He was commenced on Dexamethasone and underwent Tran’s sphenoidal resection. He was reviewed by Endocrinologist, who has suggested his apoplexy could be secondary to high dose aspirin. Commenced on Thyroxine and Testosterone replacement.

Histopathology showed mononuclear cell infiltrates with necrotic and haemorrhagic fragments suggestive of Pituitary Apoplexy.

Patient was reviewed in the outpatient clinic and his visual symptoms have improved dramatically.
In conclusion, pituitary apoplexy is often the initial manifestation of a pituitary adenoma, and the diagnosis should be considered in all patients with acute severe headache, particularly in the presence of neuro-ophthalmic signs. The majority of these patients have hypopituitarism at presentation, and steroid replacement (in deficient patients) during the acute stage could decrease morbidity and mortality.

P243
Not every pituitary apoplexy is caused by pituitary haemorrhage
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1Colchester Hospital, Colchester, Essex, UK; 2Colchester Hospital, Colchester, Essex, UK.

Introduction
Pituitary apoplexy is essentially bleeding into a necrotic area of the pituitary tumour, which has presumably outgrown its blood supply and caused infarction. Usually this is a haemorrhagic infarction but rarely a non-haemorrhagic infarction can cause marked pituitary swelling and presents as pituitary apoplexy. Our case represents a typical case of pituitary apoplexy with rapid evolving neurological deficit due to pituitary necrosis rather than haemorrhage.

Case
A 66-year-old taxi driver who was previously fit and well presented with seven days history of severe frontal headache associated with vomiting and photophobia. He also reported blurred vision around ‘edges’ of vision and dizziness on standing. Urgent CT head was done in pursuit of subarachnoid haemorrhage showed a large iso-dense pituitary mass with supra-sellar extension and bony erosion of the sella. There was no evidence pituitary haemorrhage. Visual field testing by 120 point Humphrey fields revealed bitemporal hemianopia. Next day MRI pituitary showed a 3.6 cm pituitary mass extending superiorly to indent and displace the inferior surface of the frontal lobe. The optic chiasm was compressed by the tumour, which was also extending laterally and abutting both internal carotid arteries. Again there was no evidence of bleed within the tumour. On the second day of admission he developed right third nerve palsy with diplopia and palsy of the right eye. Later on during the same day his visual acuity deteriorated to fingers counting. He was operated urgently by transphenoidal debulking of pituitary tumour. The tumour capsule was tough (unusual for an apoplexy) and difficult to remove. Preoperative hormonal profile: random cortisol 80 nmol/l, FT3 8.6 pmol/l, TSH 0.39 mu/l, prolactin 203 mu/l, FSH 1.6 iu/l, LH 0.3 iu/l and testosterone 0.66 nmol/l. Necrotic and barely viable pituitary tissue was seen on pituitary histopathology. Retinol stained showed expanded efaaced reticulum and hormonal stains were negative for all hormones. He made an excellent recovery with complete resolution of his visual acuity and field defect. His 3rd nerve palsy had almost recovered with only subtle mild medial rectus weakness. He was started on hormonal replacement therapy with hydrocortisol, thyroxine and testosterone and he is back to his normal life, driving his car without any problem. Two follow up MRIs showed small remnant tumour with no evidence of change in size or compression of adjacent tissue.

P244
Management of non-functioning pituitary adenomas in a University Hospital: a retrospective analytical audit
Lakshminarayanan Varadan, Arjun Mukherjee, Maureen Brown, Richard Clayton & Fahmy Hanna
University Hospital North Staffordshire NHS trust, Stoke on Trent, UK.

Introduction
Pituitary adenomas account for 15% of all intracranial neoplasms and could remain asymptomatic for a significant period. We conducted a retrospective analysis to assess pattern of referrals and management of non-functioning pituitary adenomas at our tertiary referral centre.

Methods
Data was collected on referrals for NFA received at our university hospital from 2005 to 2008. Medical records, radiology reports and endocrine department data were analysed to gather relevant data.

Results
Fifty-one patients were referred to our centre during this period, aged between 31 and 89 years, with 30% being females. Of 74.5% of referrals were from within the hospital, the rest being referred from other DGHS. Ophthalmologists were the predominant origin of referrals (33%), followed by general physicians (29%) and GPs (20%). Visual defects were the main presenting symptom (63%), hypogonadism and headache being next. Initial assessment was carried out in 53 days (0-357 days), mainly by endocrinologist (50%) or neurosurgeon (39%). Seventy-eight percentage had proven visual field defect by perimeter. Forty-two patients had MRI (seven further had CT) and all had pituitary function tests (Table 1). Forty-seven patients underwent surgery, 27 by neurosurgery and the rest by ENT, in 99 days from initial assessment (1-749). Seventy-six percentage were proven as null cell adenoma on histology, followed by Rathke’s cyst and normal pituitary tissue. The mean duration of inpatient stay was 11 days. Visual fields improved in 70% and one patient’s vision deteriorating. Post operative MRI was organised on 94% in 92 days, showing substantial improvement (Table 1).

Conclusion
The delay between the initial referral and treatment is still substantial. Pituitary surgery improves the visual defects significantly; however the pituitary function could deteriorate.

P245
Lymphocytic hypophysitis or Sheehan’s syndrome?
Helen Partridge & Darryl Meeking
Queen Alexandra Hospital, Portsmouth, UK.

A 34-year-old lady attended clinic-requesting information on recombinant prolactin to assist with breast feeding as she was 32 weeks gestation with her third pregnancy having failed to lactate after her first two pregnancies. In 2003 she developed pre-eclampsia during her first pregnancy and required delivery by Ventouse extraction. She had a significant post-partum haemorrhage of 1200 mls with haemoglobin 7g/dl but no evidence of cardiovascular compromise. Despite early colostro she was unable to breastfeed. She had 1 reported normal period after the birth but then restarted the OCP.

She was later referred to the rheumatologists with arthralgia, which was investigated with routine bloods, auto immune profile and thyroid function. On the grounds of her TFT’s she had a pituitary profile performed.

<table>
<thead>
<tr>
<th>Test</th>
<th>Normal Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>T4</td>
<td>9.2 pmol/l</td>
</tr>
<tr>
<td>TSH</td>
<td>0.15 mIU/l</td>
</tr>
<tr>
<td>LH</td>
<td>0.35-5.5</td>
</tr>
<tr>
<td>FSH per oestradiol</td>
<td>On OCP</td>
</tr>
<tr>
<td>Prolactin</td>
<td>5 mIU/l</td>
</tr>
<tr>
<td>Cortisol</td>
<td>&lt;28 nmol/l</td>
</tr>
<tr>
<td>GH/IGF1</td>
<td>Normal</td>
</tr>
<tr>
<td>ACTH</td>
<td>&lt;5</td>
</tr>
</tbody>
</table>

A likely diagnosis of possible Sheehan’s syndrome was made and the patient was started on hydrocortisone replacement. There had been no headaches or visual field abnormalities. Her combined TSH/GnRH test showed an adequate response of thyroid and gonadotrophin axis but her prolactin remained unrecordable as did her adrenocortical response. MRI at the time and on repeat was normal. She went on to have two further uncomplicated pregnancies but was unable to breastfeed.

In this lady MRI was normal acutely and at follow up and we found a combined persistent ACTH and prolactin deficiency but our patient has gone on to have two successful pregnancies. This is an unusual constellation of findings which probably represents lymphocytic hypophysitis rather than Sheehan’s Syndrome. Lymphocytic hypophysitis and Sheehan’s diagnosis can be difficult to distinguish but there may be clinical reason to do so as spontaneous resolution of lymphocytic hypophysitis has been documented as has progressive pituitary failure.

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P246
Management of diabetes insipidus during pregnancy
Aif Munir, Anjali Santhakumar & Vincent Connolly
James Cook University Hospital, Middlesbrough, UK.

Introduction
Diabetes insipidus can complicate up to 1 in 30 000 pregnancies and has a variety of causes, some that predate the pregnancy and others that begin during gestation. In addition, women can experience diabetes insipidus de novo in pregnancy through the actions of placental vasopressinase, which causes accelerated degradation of vasopressin. This form of diabetes insipidus may be associated with increased complications of pregnancy, including pre-eclampsia. Management of central diabetes insipidus and transient diabetes insipidus of pregnancy can be achieved with desmopressin (DDAVP), a vasopressin analogue.

Case history
We report the management of a 36-year-old lady who was diagnosed with central diabetes Insipidus at the age of 26. MRI Brain at the time of diagnosis showed Rathke’s cleft cyst for which she underwent transphenoidal decompresion. Her Diabetes Insipidus remained well controlled on a steady dose of desmopressin (2 mcg twice a day subcutaneous injections as nasal route proved ineffective & patient did not tolerate tablets) for many years but deteriorated during her pregnancy & desmopressin dose required titrating up to 16 mcg (4 mcg four times a day) to achieve control with strict monitoring of fluid balance. Vasopressin requirements can increase markedly during pregnancy through placental action of vasopressinase. Patients should have pre pregnancy counselling about the potential for a deterioration in their condition and be reviewed early in the course of their pregnancy. Vasopressin and the structurally related synthetic polypeptides, desmopressin and lypressin, have been used during pregnancy to treat diabetes insipids. No reports linking the use of vasopressin with congenital defects have been located. Oxytocic effects and increased risk of pre-eclampsia in the third trimester has been mentioned in literature with use in pregnancy.

P247
Thyrotropin-secreting pituitary tumour causing high output cardiac failure
Julie Kyaw Tun1, Haliza Hannif2 & Cornelle Parker3
1Airedale General Hospital, Keighley, UK; 2Leeds General Infirmary, Leeds, UK.

A 51-year-old asymptomatic man presented in 1998 with elevated thyroid stimulating hormone (TSH) 6.34 mIU/l (0.5-5.6 mIU/l), FT4 54.1 pmol/l (7.5-21.1 pmol/l), FT3 20.1 pmol/l (3.0-5.0 pmol/l) and TSH-α subunits 3.6 mcg/l (<2.0 mcg/ml). After the first visit, he was lost to follow-up. He presented 10 years later in 2008 with 1-week history of symptoms of heart failure and hyperthyroidism. Clinically he was thyrotoxic with no goitre, in fast atrial fibrillation and biventricular failure. Visual fields were normal. Chest radiograph showed pulmonary oedema and echocardiogram, moderate left ventricular systolic dysfunction (ejection fraction 49%). His TSH was 8.2 mIU/l, FT4 48.3 pmol/l, FT3 14.42 pmol/l and TSH-α subunits 4 mcg/l. MRI revealed a 16x14-mm left sided pituitary tumour. The provisional diagnosis was high output cardiac failure secondary to thyrotoxicosis from a thyrotropin-secreting pituitary tumour (TSH-oma).

His peak cortisol and GH were 615 nmol/l and 47.1 mIU/l following glucagon stimulation. His IGFI was 13 nmol/l (11.3-30.9) and prolactin 81 mIU/l (60-278). Interestingly, he had raised gonadotrophins: FSH 6.0 IU/l (1.27-10.26) and LH 9.47 IU/l (1.2-8.6). Testosterone was 40.6 nmol/l (6.1-27.2) and SHBG 145 nmol/l (13-71), which was in keeping with the effect of excess thyroxine on SHBG. Unfortunately, he failed to attend his glucose tolerance test for GH levels although notably he had normal IGFI.

He continued to have poor attendance but underwent transphenoidal surgery in March 2009. Surprisingly, histology revealed a pituitary adenoma with immunohistochemistry positive for GH only. Post-operatively, thyroid status improved: TSH 4.7 mIU/l, FT4 24.2 pmol/l and FT3 3.4 pmol/l. Repeat glucagon stimulation showed adequate cortisol (545 nmol/l and GH (11.9 mcg/l) responses. Repeat MRI showed a small residual pituitary adenoma.

This case illustrates indolent clinical progression of a TSH-oma over a 10 years interval. We can also assume from the size of the adenoma on MRI scan that it was slow-growing. Although this was clinically and biochemically a TSH-oma, immunohistochemistry stained for GH alone.

P248
Is it pituitary adenoma or metastasis?: an unusual presentation of carcinoma prostate
Daniel Kannappan, Sami Kenz, Doros Polydoro, Tara Kearney & Kanna Gnianalingham
Salford Royal Hospital, Manchester, UK.

Sixty seven year old gentleman presented to eye clinic with blurring of vision in the left eye for 2 weeks. Patient was seen by Ophthalmologist and discharged home. Presented 3 weeks later with headache and blurring of vision. On examination no perception to light in the left eye and bitemporal hemianopia. His past history includes carcinoma prostate, diagnosed a year ago and had surgery and Radiotherapy. His recent PSA was normal. Bones scan showed no evidence of bony metastasis. His MRI brain showed mass lesion within the pituitary fossa with chiasmal compression. The MRI was reviewed by NeuroRadiologist and the diagnosis was pituitary macro adenoma. His CT chest and abdomen was normal. His prolactin was 1059 mIU/l. His TSH was 0.81 mIU/l, FT3 of 7.7 pmol/l. GH was <0.1 U/l and cortisol was 35 nmol/l suggestive of non functioning pituitary macro adenoma.

He underwent Tran’s sphenoidal resection of adenoma. Unfortunately only subtotal resection was achieved. Intra operative smear has been reported as possible meningioma. Repeat MRI (4 days post-op) showed significant residual tumour with chiasmal compression. He underwent left eyebrow craniotomy and debulking of residual tumour. Histology showed metastatic adenocarcinoma and immuno phenotype suggestive of Prostate primary. His case was discussed in the MDT and the plan was to give Cranial Radiotherapy in view of metastasis. Reviewed in the clinic after 2 months. His vision has improved significantly visual field was normal to confrontation method.

This case is very interesting due to the rarity of brain metastasis in Carcinoma of prostate. Usually it is <1%. Brain metastasis signifies late stage of the disease and the mean survival is 9 months. Only three cases have been reported in the literature so far. Endocrinologist should consider a metastatic lesion in the differential diagnosis of a non-functioning pituitary tumour.

P249
Sub-optimal testosterone replacement in acromegaly
Mohammed Choudhury & Tristan Richardson
Royal Bournemouth Hospital, Bournemouth, UK.

A 62-year-old male was referred by his GP querying acromegaly. His past medical history included sleep apnoea, hypertension, distalgiapnea and gout. Serum IGFI was elevated at 827 µg/l (normal range 100-300 µg/l). Prolonged oral glucose tolerance did not show suppression of GH with a nadir of 9.0 mU/l. A pituitary MRI demonstrated a 5x5mm microadenoma. There were no visual field defects. The patient was pre-treated with somatostatin analogues and proceeded to transphenoidal hypophysectomy which was performed without complications. Post-operatively he developed fatigue and sweats and hypogonadotropic hypogonadism was confirmed biochemically (LH 2.2 µIU/ml, FSH 4.0 µIU/ml, Testosterone 7.9 nmol/l). The patient was prescribed Testogel 5 g/50 mg once a day. The remainder of his pituitary function was assessed and was within normal limits.

The patient’s replacement was sub-optimal on 5 g/50 mg daily (LH 1.5 µIU/ml, FSH 3.6 µIU/ml, testosterone 3.6 nmol/l) and so the dose was increases to 10 g/100 mg daily. This improved symptoms and normalised serum testosterone (LH 0.4 µIU/ml, FSH 1.4 µIU/ml, testosterone 18.8 nmol/l).

The addition of newer methods in prescribing testosterone has been associated with an increase in the frequency of prescribed topical testosterone replacement regimes. In acromegaly, increased skin thickness is almost pathognomonic of the condition and may cause reduced absorption of topical testosterone. One patient’s skin thickness was increased on plain heel X ray (at 34 mm in thickness, normal range <33 mm). This abstract demonstrates that modern treatment of hypogonadism with topical agents in patients with acromegaly may need revising with regard to the potential for larger doses being required for decreased absorption through thickened dermal layers.
P250
TSH-secreting pituitary adenoma: potential benefits of pre-operative octreotide
Ian Wallace1, Estelle Healy2, Steve Cooke2, Roy Harper2 & Steven Hunter2
1Regional Centre for Endocrinology and Diabetes, Royal Victoria Hospital, Belfast, UK; 2Department of Neuroendocrinology, Royal
Victoria Hospital, Belfast, UK; 3Department of Neurosurgery, Royal
Victoria Hospital, Belfast, UK; 4Department of Endocrinology and
Diabetes, Ulster Hospital, Dundonald, UK.
TSH-secreting pituitary adenomas are rare and the optimal investigation and management is uncertain. A 42-year-old lady presented with a three-month history of three stone weight loss, palpitations, heat intolerance and tremor. Her sister was being treated for Graves’ disease. Visual fields were intact. Thyroid function tests showed free T4 concentration 29.5 pmol/l (9.0–19.0) and TSH concentration 3.672 mU/l (0.4–4.5): TSH concentration remained elevated after repeat measurement using an alternative assay. Alpha-satuban concentration was 1.1 IU/l (<1.0) and Sex Hormone Binding Globulin concentration 90 nmol/l (20–115). MRI showed a pituitary macroadenoma. There was no clinical or biochemical evidence of co-secretion of growth hormone, prolactin or gonadotropins. A TRH test revealed a flat response and TSH administration (80 µg daily for 8 days) failed to induce suppression of TSH. TSH suppressed following a TSH stimulation test. Post-operatively this lady was treated with octreotide, resulting in a reduction in maximal tumour diameter from 22 mm to 11 mm and normalisation of thyroid biochemistry. She proceeded to trans-sphenoidal resection of the pituitary adenoma, with histological examination confirming a TSH-adenoma. Post-operatively she is clinically euthyroid with TSH in the normal range, but again failed to completely suppress following administration of TSH. The optimal management of patients with this rare pituitary adenoma is uncertain. We describe a case where pre-operative octreotide therapy normalised thyroid biochemistry and was associated with a significant reduction in tumour size, possibly enhancing surgical outcome.

P252
Herpes encephalitis causing hypopituitarism
Krishnamurthy Chakravartrapa & Ian MacFarlane
Aintree University Hospitals, Liverpool, UK.
We report an interesting and important case of hypopituitarism secondary herpes encephalitis and available literature evidence.
Case history
Twenty-one year old gentleman admitted to tertiary neurosurgical unit with excessive water intake, feeling tired, increased sleepiness, a few weeks history of confusion and ‘strange behaviour’. He was oriented in person but not in place, had high temperature. He had lumbar puncture, CT, MRI, Brain biopsy, and positive PCR for herpes virus. He subsequently diagnosed with Herpes encephalitis and treated with Acyclovir. He made gradual slow recovery over several months, during which was also diagnosed with diabetes insipidus. He was treated with vasopressin. Three months after the discharge, he was re-admitted to hospital with hypernatremia. Further investigations revealed hypopituitarism with diabetes insipidus. He was started on hormone replacement therapy and being closely monitored. Further course He is currently taking replacement therapy with hydrocortisone, thyroxin, testosterone, growth hormone and vasopressin.
Conclusion
This case illustrates an important but a rare cause of hypothalamic damage secondary to herpes encephalitis causing hypopituitarism along with diabetes insipidus. Only few case reports of similar condition have been reported in the literature.

P253
The effects of 3 year growth hormone replacement on the lipid profile of adults with growth hormone deficiency using a fixed graded initiation, followed by a titration phase method: 3-year retrospective analysis
Victor Oguntoluo & Simon Aylwin
Kings College Hospital, London, UK.
Objective
Growth hormone (GH) deficiency in adult patients is associated with poor quality of life and increased cardiovascular morbidity and mortality. We studied the effects of GH replacement on the lipid profile in adult patients with GH deficiency treated over a 3-year period.
Method
A retrospective analysis of 70 adult patients, who had completed 3 years of GH therapy. Growth hormone is initiated at a dose of 0.3 mg/day and titrated to 0.5 mg/day over the first 3 months period. Titration is made at each visit based aiming for a target IGF1 level. Fasting lipids and IGF1 were measured at every visit.
Results
Analysis of results was focused on baseline, 6 months, 12 months, 24 months and 36 months. Significant improvement was observed in all lipid parameters except HDL-cholesterol, this was evident after 12 months for total and LDL-cholesterol and after 24 months for triglyceride.
Total cholesterol (mean ± s.d.: 6.1 ± 0.8; 5.8 ± 1.0; 5.49 ± 0.93; 5.2 ± 0.82; 5.07 ± 0.93); LDL-cholesterol (mean ± s.d.: 3.6 ± 0.9; 3.4 ± 0.8; 2.92 ± 0.95; 2.73 ± 0.77; 2.58 ± 0.88) and triglyceride (mean ± s.d.: 2.6 ± 1.1; 2.32 ± 1.0; 2.23 ± 0.97; 2.06 ± 0.44; 1.98 ± 0.98). There was minimal elevation of HDL-cholesterol (mean ± s.d.: 1.2 ± 0.5 vs 1.4 ± 0.4).
Conclusion
Treatment with GH in GH deficient adults results in significant improvement in all lipid parameters with modest elevation in HDL-cholesterol. Reduction in total and LDL-cholesterol was observed after 12 months of GH therapy, but reduction in triglyceride level was only evident after 2 years.

P254
Adverse diagnosis in breast cancer patients who develop pituitary metastases
Simeen Akhtar, Simon J Howell & Kalpana Kaushal
Royal Preston Hospital, Preston, UK.
Pituitary metastases are uncommon, with breast and lung carcinomas being the most common primary sites. Of the minority that are symptomatic, diabetes
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P255
High incidence of survivors of childhood malignancy in an endocrine transition service
Anna-Louise Dennis1, Carol Acrimori2 & Helen Simpson3
1School of Clinical Medicine, University of Cambridge, Cambridge, UK;
2Department of Paediatrics, University of Cambridge, Cambridge, UK;
3Institute of Metabolic Science, CUH NHS Foundation Trust, Cambridge, UK.

Objective
To audit GH deficiency in the transition service at our institution.

Methods
Patients between 19–25 years old were identified from the database of patients having GH replacement. Data were obtained from case notes and electronic records.

Results
Twenty-four patients in the transition service were identified to be GH deficient, 13 female and 11 male. 14/24 had CNS or pituitary tumours treated in childhood, 11 having been treated with radiotherapy, six having undergone chemotherapy and nine undergoing surgery. 6/24 had idiopathic/congenital hypopituitarism, and four had RT as part of treatment of leukaemia. Of 16/24 patients had their GH axis retested on completion of linear growth. GH doses for females was 0.41 (range 0.3–0.8 mg/day) and for males 0.39 (0.2–0.8 mg/day). 18/24 have central hypothyroidism, 18/24 required sex hormone replacement (12/13 females on oestrogen replacement and 6/11 males requiring testosterone replacement), 11/24 were ACTH deficient, and 4 patients had cranial D.I. 1 patient had a diagnosis of isolated GH deficiency. IGFI were in the age and gender related normal range in 19/24 patients. However 5 had an IGFI of <10 ng/l suggestive of poor compliance/concordance.

Metabolic indices: All patients were normotensive and had normal HbA1c. 14/24 patients had a BMI >25 kg/m2. 15/24 patients had a cholesterol >5 mmol/l, with 6 of these having a cholesterol >6 mmol/l. This was not related to weight or any of his other anterior pituitary hormone replacement.

Conclusions
The majority of patients in this cohort are survivors of childhood malignancies. Hypercholesterolaemia was an unexpected finding, aetiology of which is unclear. Another unexpected finding was the lack of difference in GH dose between female and male patients, especially as the majority of females were taking oestrogen replacement.

P256
Hypopituitarism following Russell’s Viper bite: a case report
Charles Antonympillai1, John Wast2 & Henry Rajaratnam2
1Oxford Centre for Diabetes, Endocrinology and Metabolism, Oxford, UK;
2Nawaloka Hospital, Colombo, Sri Lanka.

Introduction
Russell’s Viper is a venomous snake found in South and South East Asia. The snake bite causes coagulopathy, neurotoxicity, renal failure, local effects and even death. But hypopituitarism is an extremely rare complication1. There are only a few case reports from India and Burma and we report the first case from Sri Lanka.

Case report
A 49 year old man from a remote part of Sri Lanka was bitten by a Russell’s viper 3 years ago. He felt faint and started vomiting and also noticed swelling of the bitten foot and bleeding from the varicose veins. He recovered following treatment with antivenom serum. Over the next 3 years he was feeling unwell with generalised weakness of the body, lethargy, sleepiness and reduced libido. He did not have sinister headache or visual disturbance. On examination he had an apathetic face but with preserved secondary sexual characteristics. The visual fields and fundi were normal. The pituitary function tests revealed panhypopituitarism. FT4 0.87 ng/dl, TSH 1.85 mIU/ml, 9 am Cortisol 2.5 mcg/dl, IGF1 145 ng/ml, Proctolin 2.7 ng/ml, FSH 2.6 µIU/mL, LH 1.5 µIU/ml, Testosterone 1.9 ng/ml. The MRI of the pituitary showed normal sized pituitary gland without evidence of pituitary adenoma. He had a history of Hydrocortisone, Levithroxine and Testosterone which brought about marked improvement in his symptoms and general well being.

Discussion
Viper bite could lead to hypopituitarism. This is probably due to the procoagulants in Russell’s viper venom that cause pituitary damage through Disseminated Intravascular Coagulation. As unrecognized hypopituitarism can be potentially fatal physicians should have a high degree of suspicion. Chronic ill health in those who apparently recover from the acute envenomation should alert the physician regarding the possibility of hypopituitarism.

Reference

P257
Carcinomatous change in a cranioopharyngioma: a case report
Simeen Akhtar, Aprajay Golash, Windsor Gunawardena, Andrew Hindley & Simon Howell
Royal Preston Hospital, Preston, UK.

Introduction
Cranioopharyngiomas are usually benign intracranial tumours which can sometimes be locally aggressive. Malignant transformation is extremely rare and has a poor prognosis. We report a case of a patient who developed carcinomatous changes in a cranioopharyngioma which was first excised almost 55 years ago.

Case report
Mr AM was diagnosed with a cranioopharyngioma at the age of 5 years. He underwent surgery in 1952 but was lost to follow up and presented again in 1997. MRI at this time showed a large residual cranioopharyngioma. He remained stable for several years but in 2001 his visual fields deteriorated and repeat MRI confirmed an increase in the size of the tumour. He underwent transsphenoidal excision of the cranioopharyngioma followed by radiotherapy. Histology did not show any features suggesting malignancy.

Things remained stable until September 2008 when he started having severe headaches. MR scan showed a stable appearance of the tumour. However he then developed diplia associated with a right sixth nerve palsy. MR scan demonstrated a significant increase in the solid component of the tumour. Further scans in February and March 2009 showed a rapid increase in tumour size with optic chiasmal compression, extension into the nasopharynx and left temporal lobe, and destruction of the skull base and clivus. Biopsy of the lesion confirmed this to be a recurrent adamantominatous cranioopharyngioma with foci of malignant transformation. As the tumour was not considered suitable for further surgery he received radiotherapy which resulted in some tumour shrinkage and improvement of his symptoms initially but later deteriorated and died in October 2009.

Discussion
Malignant transformation in adamantominatous cranioopharyngioma is relatively rare and is usually associated with previous multiple recurrences. The case we have reported demonstrates carcinomatous change in a patient who had previously followed a relatively stable course for over 50 years.
P258
Outcome of treatment for patients with acromegaly in a single referral centre
Sanjaya Dissanyake, Kate Millar, Kalpana Kaushal & Simon Howell
Royal Preston Hospital, Preston, UK.

Introduction
To assess outcome for patients with acromegaly treated at Royal Preston Hospital since 1st January 2000.

Results
Out of 22 patients (12 presenting in the last 2 years) 20 had endoscopic transphenoidal hypophysectomy and two were managed medically. Headaches, visual disturbance and characteristic morphological features were the main presenting problems. 4 were microadenomas and 18 macroadenomas. Exact dimensions were only available in 15 of 18 macroadenomas of whom 10 had tumours > 2 cm in size. In 50% (9/18) of these macroadenomas, chiasmal compression was demonstrated on MRI scan, although visual field defects were present in only 33% (6/18) and visual symptoms in only 22% (4/18). Pre op hormone deficiencies were present in 7/21 (33%); TSH deficiency in 9.5% (2/21), ACTH deficiency in 14% (3/21) and Gonadotrophin deficiency in 24% (5/21).

Visual fields improved in all six patients who had pre-operative defects. Second surgery was undertaken in eight patients (only 2 with tumour <2 cm) due to inadequate biochemical control of acromegaly. Twelve patients were treated with somatostatin analogues, five patients with cabergoline and three patients with pegvisomant. Attainment of ‘safe’ GH levels (mean GH <2 ng/ml during GH day curve) after surgery was achieved in 10/20 patients (3 of 3 microadenomas and 7 of 17 macroadenomas) and normalisation of IGF1 in 5/20. Following treatment with addition medical therapy normalisation of IGF1 has been achieved in 64% (14/22). Of the remaining 8 patients treatment has reduced IGF1 to less than 1.5x upper limit of normal in 6 patients (75%) and all of these patients are still undergoing titration of medical therapy.

Conclusion
Transphenoidal pituitary surgery has been effective in correcting visual field defects. After adjustment for the relatively high number of patients with large tumours, achievement of biochemical control of acromegaly following surgery is similar to other published series.

P259
An interesting case of intrasellar cavernous carotid aneurysm mimicking pituitary adenoma
Cynthia Mohandas & Ian Scobie
1Kent and Sussex Hospital, Tunbridge Wells, UK; 2Medway Maritime Hospital, Gillingham, UK.

A 34-year-old lady presented with irregular periods but no galactorrhea. A prolactin level was 1326 mU/L. Clinical examination—Normal. BMI 26 and visual fields were full to confrontation. MRI scan of pituitary was reported as large pituitary macroadenoma measuring 11 × 11 × 12 mm denoting the optic chiasm. Results of other endocrine tests were as follows; cortisol 265 nmol/l, TSH 0.32 mU/l, FT4 8.5 pmol/l, FSH 4 U/l, LH 1.6 U/l, oestradiol 104 pmol/l, GH 8.4 mU/l, IGF1 = 125 nmol/l.

Although it was very suspicious of a non-functioning pituitary macro adenoma, a decision to treat her with Cabergoline 500 mcg once weekly was commenced due to high levels of prolactin. Interestingly there was prompt reduction in her prolactin levels to 21 mU/l after 6 months. Her thyroid function tests normalised and her menstrual cycles that was always six-weeks length reduced to a regular 28-days cycle. However she still had persistent headaches, so her MRI scan was repeated and she was referred to a combined pituitary clinic. A pituitary radiologist now described it as an 11 mm lesion with intermediate T1 waited signal. So CT angiogram was performed the same afternoon. This showed a 9 mm right-sided intrasellar cavernous carotid aneurysm.

The case was then referred to joint neurovascular clinic. The risk of rupture was relatively low, so the opinion was that; the risk of interference at this stage was greater than the risk of pursuing a conservative line. From endocrine prospective she remains on Cabergoline 250 mcg once weekly which maintains normal pituitary function.

Conclusion
This is a really fascinating case and is intriguing. Although we did not make a correct diagnosis initially, the combined pituitary clinic was reassured they did not intervene at that point which could have let to permanent stroke and death. This patient still remains stable in her recent clinic visits.

P260
Solitary pituitary metastasis from carcinoma of the prostate: a case report
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Mr GC aged 67 presented to the ophthalmologists in December 2008 with blurring vision and headaches. Visual acuity was 6/9, 6/24 and reasured. Two weeks later he returned as an emergency with severe visual loss of left eye to finger movements and temporal field defect right eye.

Past history included cancer prostate, Gleason score 7 (T2 N0). TURP performed in January 08 followed by hormonal therapy and external beam irradiation. Neuroradiological studies confirmed a large enhancing mass arising from the pituitary fossa with suprasellar extension and chiasmal compression suggesting a possible pituitary macro adenoma.

Initial pituitary function tests revealed a non-functioning tumour with slightly raised prolactin at 1059 suggesting a stalk effect. He was deficient of thyroxine, growth hormone and testosterone. Perioperative steroid cover and thyroxine was commenced.

Follow-up imaging studies revealed no other secondary with a normal sized prostate. Postoperative pituitary radiotherapy was uneventful. Growth hormone and testosterone therapy was contraindicated with active prostate malignancy.

Pituitary metastasis from prostate cancer is unusual. Widespread metastasis is usually noted at the time of diagnosis. Pituitary lesion presenting as the solitary site of metastasis has never been reported. PSA levels which are usually a reliable marker of disease activity has remained normal throughout. Histologically a coexistent meningioma could be explained by a tumour in tumour phenomenon where metastatic deposits shows a predilection to seed on pre-existent benign tumours in the region facilitating local spread. With improving life expectancy in cancer, the incidence of pituitary metastasis is on the rise.

P261
Glucocorticoid replacement therapy and fibromyalgia in hypothyroidism
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Hypothyroidism is associated with increased cardiovascular mortality. It has been suggested that hypogonadism, hypothryroidism, growth hormone deficiency (GHD), or indeed unphysiological hormone replacement regimens, might contribute to this excess cardiovascular risk. The adverse effect of hypercortisolism on insulin resistance, carbohydrate metabolism and hypertension is well recognised. It is also known that glucocorticoids adversely affect the coagulation-fibromyalic system, with an increased risk of thromboembolism in Cushing’s syndrome. GHD reduces fibromyalic activity and might predispose to a pro-coagulant state reversed by growth hormone replacement. We examined the hypothesis that short term higher dose hydrocortisone replacement might adversely affect the fibromyalic system and be an additional factor leading to the excess cardiovascular mortality in hypothyroidism. We measured serum cortisol, plasminogen activator inhibitor-1 (PAI-1), tissue plasminogen activator (tPA) and fibrinogen levels in 10 hypothyroid adults whilst treated with either a fixed high dose hydrocortisone regime of 30 mg / day (HD) or a patient-tailored lower dose (mean dose 18 mg / day) regimen (LD). We also studied 10 controls. PAI-1; [median (range)] HD 25 (5–53) ng/ml versus LD 21 (4.5–56) ng/ml; P = 1.0; tPA; HD 10 (5–5) ng/ml versus LD 10 (4–13) ng/ml; P = 0.3; and fibrinogen; HD 2.5 (1.8–3.5) g/l versus LD 3.0 (2.3–4.4) g/l; P < 0.001. Short term higher dose hydrocortisone replacement dose did not affect PAI-1 or tPA levels and suggests that such differences in hydrocortisone replacement doses are unlikely to lead to significant effects on the fibromyallic system, at least in the short term. The small but significant increase in fibrinogen during long dose therapy was unexpected and might not be clinically significant. In terms of fibromyalic activity, this study does not does support the suggestion that traditional higher dose hydrocortisone replacement regimens might adversely affect cardiovascular risk in hypothyroid patients.
P262

Prolonged remission of acromegaly after cessation of somatostatin analogue treatment
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Introduction
The concept of life-long medical therapy for acromegaly has recently been challenged. Hormonal remission for up to 48 months after stopping somatostatin analogues in acromegalic patients has recently been reported. Herein, we present a further case showing the longest remission yet in growth hormone levels after somatostatin analogue treatment.

Case
In 1987, a 43 year old man was diagnosed with acromegaly (nadir GH on oral glucose tolerance test (OGTT) 33.1 mU/l) attributed to a somatotroph macroadenoma (MRI: L-sized 11 mm intrasellar adenoma). Immediately, he proceeded to TSA; post-operative OGTT showed nadir GH at 4.8 mU/l. At that time, the patient had no further treatment, as he was clinically well. In the following years, radiotherapy was discussed, but the patient declined. In 1995, his nadir GH on the OGTT was 2.7 mU/l. In July 1998, he finally agreed on medical treatment with octreotide LAR (20 mg/every 4 weeks); before this, he had a mean GH on the GH day curve was 5.4 mU/l and his IGF-I was 25.6 nmol/l (7.5–30). He achieved ‘safe’ mean GH levels 1.1 mU/l and his IGF-I remained normal 18 (7.5–30). During the next 3 years, the dose interval was extended successfully up to 12 weeks. In October 2001, he had his last octreotide injection and his mean GH was 1.3 mU/l with IGF-I 16.7 nmol/l (7.5–30) level. He had his mean GH assessed regularly until 2003, which remained at ‘safe’ levels. Annual evaluations demonstrated normal IGF-I 9.7 nmol/l (7−28) and its OGTT in 2005 and 2008 showed nadir GH at 0.35 mU/l and 0.45 mU/l, respectively.

Conclusions
This is the first reported case of a patient with active acromegaly remaining cured for 8 years following withdrawal of long-acting somatostatin analogue therapy demonstrating that life-long requirement of somatostatin analogues needs further evaluation.

P263

Spontaneous remission of Cushing’s disease: apoplexy of a pituitary microadenoma?
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The spontaneous remission of Cushing disease (CD) might be a phase of cyclic disease, but could also be explained by an ACTH-microadenoma infiltration or hemorrhage, with clinical pictures ranging from asymptomatic hypocortisolism to dramatic hypocortisolism. We describe the case of a 23-years-old woman, referred for amenorrhea lasting for 5 months, overweight and acne. Physical examination showed mild hirsutism, acne, round face with a hint at rububes, abdominal striae rubaeas, normal BP. Biochemical examination showed IGT; lipids, kalaemia, thyroid function, PRL and androgens were normal. Hypercortisolism was confirmed by elevated UFC (143-361 μg/24 h, < 20-90), supranormal h24-cortisol(P), lack of cortisol suppression after LDLT. Cortisol suppressed normally after HMDT, ACTH increased by 36% after hCRL, ACTH and cortisol increased by 350 and 80% after DDAVP-test. Pituitary MRI was not conclusive for a microadenoma. BIPSS, performed 40 days later, did not show a C-P/ACTH gradient, but UFC value in the day before the procedure turned out to be normal. In the following months UFC was normal and menses resumed in spite of persistent cushingoid features. Two months later the patient was hospitalized with severe headache, fatigue, diffuse unspcific pain. Unequivocal hypocortisolism was found (morning F 0.1 μg/dl, no response to ACTH-1 iug). Symptoms remitted under substitutive therapy, DDAVP-test turned out to be negative. MRI showed an hypointense area smaller than that previously seen, suggesting a progressive disappearance of a microadenoma. After five months all cushingoid features had disappeared, weight had decreased by 7 kg, menses regularized. After six months the patient stopped substitutive therapy, with a normal response to ACTH 1 μg two months later. MRI after one year was normal. We concluded for spontaneous apoplexy in ACTH-microadenoma, not related to BIPSS or dynamic tests. Nowadays we cannot still exclude a cyclic CD: a prolonged follow up is mandatory to discern between these possible conditions.

P264

Both acetylcholine and choline stimulate externalisation of annexin I from S100-positive folliculo-stellate cells of the pituitary gland
Damon Lees, John Morris, Susan Greenfield & Helen Christian
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Annexin-I (ANXA1) is a 37 kDa calcium- and phospholipid-binding protein expressed abundantly in S100-positive folliculo-stellate (FS) cells in the anterior pituitary gland. ANXA1 is localised both intracellularly and on the cell surface in high density foci appearing at points of contact between the FS cells and the neighbouring secretory cells, where it has been demonstrated to mediate the glucocorticoid-induced negative feedback on the secretion of ACTH from corticotrophs. Glucocorticoids stimulate the externalisation of ANXA1 to the surface of FS cells (and the subsequent replenishment of intracellular ANXA1), which in turn act on cell surface receptors on adjacent secretory cells to inhibit release of ACTH. The cholinergic system has been implicated in inhibitory pituitary responses to immune or inflammatory stress and FS cells play an important role at the interface of the immune and endocrine systems. In addition, acetylcholine (ACh) has been demonstrated to increase intracellular Ca2+ in FS cells in the pituitary via activation of muscarinic M3 receptors and phospholipase C activation but it is not known if this stimulates release of FS bioactive mediators or if nicotinic receptors are involved. By use of immunofluorescence microscopy and western blot analysis to measure ANXA1 we have investigated the effects of ACh on the cellular distribution of ANXA1 in the TdT/GF FS cell line. We also investigated the effects of choline, an agonist at alpha7-nicotinic receptors. TdT/GF cells were incubated for 3 h with either ACh (0.05-200 μM), choline (0.05-200 μM) or positive control dexamethasone (100 nM). Both ACh and choline concentration-dependently stimulated the externalisation of ANXA1 from TdT/GF cells. Furthermore, we have also demonstrated the expression and secretion of the enzyme acetylcholinesterase (AChE) from TdT/GF cells. All these findings suggest a participation of a cholinergic mechanism in the control of ANXA1 release from FS cells.

Reference

P265

Gender differences in presentation and response to treatment for prolactin-secreting adenoma
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Context
Prolactinomas are the most common functioning pituitary adenomas & it is recognised that gender has an influence on presentation and management of this condition.

Objective
To examine the effects of gender on presentation and response to treatment in a large cohort of adults with confirmed prolactinoma (MRI performed and macroprolactin excluded).

Design & patients
This retrospective cohort study design used an electronic database (Diabetes3®) to identify adult patients with prolactinoma attending the endocrinology department of a University Teaching Hospital from October 2007-February 2008. Data including tumour size, prolactin levels, age at diagnosis, current management and gender were recorded.

Results
Three hundred and sixty-nine patients (326 females, 43 males; 7.3:1 females to males) with prolactinoma were included in the study. In the total cohort the ratio of microprolactinoma-to-macroprolactinoma was 8:2:1. Males presented significantly later in life than females (43.7±16.5 vs 31.4±8.5 years; mean±s.d., p=0.0001), with higher prolactin levels (53 339±12 420 vs 3799±11 023 μU/l, p<0.0001) and a larger tumour size at diagnosis (16.4±15.3 vs 7.7±4.33 mm, p<0.0001). The majority of patients were receiving cabergoline therapy (51.5%). A higher proportion of males than females achieved normoprolactinaemia (62.8% vs 44.2%, p=0.03) with men receiving a higher dose of cabergoline per week (1000 μg (250–4000) versus 500 μg (250–3000) (median (range)), p=0.0003).

Conclusions
Prolactinomas occur more frequently in females but males present at a later age with higher PRL levels and larger tumour size at diagnosis. In this study the overall rates of biochemical normalisation were low, although males had higher
rates of normoprolactinaemia during treatment. This may reflect more aggressive treatment of the relatively larger prolactin-secreting adenomas seen in males and a ‘tolerance’ of modest hyperprolactinaemia in females without clinical symptoms from prolactin excess.

P266
Cabergoline therapy is associated with successful abolition of abnormal cycles of excess steroid excretion in a case of pituitary dependent Cushings’s disease
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A 27 years old girl presented with weight gain, hirsutism, fatigue, bruising and striae. On examination she was Cushingoid. On initial assessment, 3 out of 4 24 h urinary free cortisol collections were elevated. 0800 h serum cortisol was 280 nmol/l after 1mg dexamethasone given at 2300 h. At formal 48 h low and high dose dexamethasone suppression tests cortisol values were 164 and 34 nmol/l respectively. Basal 0800 h ACTH was 33 ng/l. Given the clinical features and the discrepant biochemical results, cyclical Cushings’s syndrome was suspected. A 28 day collection of early morning urine cortisol to creatinine ratios showed a clear cyclical pattern of 4 days in length. MRI pituitary and pitrosal sinus sampling were arranged. To ensure pitrosal sinus sampling was performed during a period of cortisol excess, 0800 h serum cortisol was measured and available immediately before sampling proceeded. MRI pituitary showed an 8 mm adenoma. Pitrosal sinus sampling confirmed a pituitary source of ACTH with elevated left pitrosal sinus to IVC ratio (14 basally and 18 after CRH). Cabergoline was commenced and titrated to a dose of 2 mg twice weekly across 4 weeks. After 2 weeks on this dose a repeat 28 day collection demonstrated normal daily cortisol to creatinine ratios and loss of the cyclicity. A mean 24 h serum cortisol profile on therapy was 310 nmol/l. Repeat MRI at 3 months showed no change in adenoma size. At present no drugs have been shown to be effective in cyclical disease. Corticotroph adenomas express the dopamine receptor D2 subtype to which cabergoline binds with high affinity. This is the first reported case of successful use of cabergoline therapy in a patient with confirmed cyclical Cushings’s disease. There is still the very rare possibility that this patient may have 2 simultaneous cycroid cycles thus making therapy assessment difficult. Currently continuing use of cabergoline appears worthwhile.

P267
Endocrine outcomes of pituitary surgery
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Transphenoidal surgery is an effective treatment option in patients with pituitary tumours associated with compression of the optic chiasm or hormone hypersecretion. Surgery carries with it a risk of the development of new pituitary hormone deficits but also the potential for recovery of existing pituitary hormone deficits. We have examined data concerning pituitary function in 80 patients who underwent endoscopic transphenoidal pituitary surgery at Royal Preston Hospital. The majority of patients had non-functioning pituitary tumours (44), 25 were GH secreting, 2 ACTH secreting, 1 FSHoma, 1 prolactinoma, 1 gonadotrophin secreting adenoma and 6 cranio-pharyngiomas. Visual field defects were present in 47. Preoperative assessment revealed ACTH deficiency in 52%, TSH deficiency in 33%, and gonadotrophin deficiency in 57%. 1 patient had diabetes insipidus pre-operatively and testing for GH deficiency was not routinely done prior to surgery. The proportion developing new post-operative hormone deficits in those with a normal axis before surgery was 56%, 37% and 12% for ACTH, TSH and gonadotrophins respectively, whilst recovery of hormone production was observed in 18.2%, 3.8% and 9.5% of those with preoperative ACTH, TSH or gonadotrophin deficiency. In addition new persistent postoperative diabetes insipidus was seen in 12.5% of patients. GH stimulation tests were performed in symptomatic post-operative patients and confirmed severe GH deficiency in 16 of 25. Of the 47 patients with visual field defects, information on postoperative visual fields was available in 44. Of these patients 37 (84%) improved, 4 (9%) remained stable and 3 (7%) deteriorated.

Baseline tumour size significantly correlated with the likelihood of pre-operative hormone deficiencies (TSH P=0.004, ACTH P=0.0005, gonadotrophins P=0.05), visual field defects at presentation (P=0.001) and the risk of developing new postoperative ACTH deficiency (P=0.007).

These findings highlight the importance of postoperative hormone assessment and are useful when counseling patients regarding the risks of surgery.

P268
Recurrent meningitis secondary to an invasive TSHoma
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Fifty year-old year old gentleman presented unwell whilst on holiday with headache and vomiting. He also reported a clear fluid trickling down his nose. On examination he was pyrexial, photophbic and had demonstrable neck stiffness. Lumbar puncture and a subsequent culture confirmed streptococcal meningitis which responded to antibiotics. His CT scan head suggested a pituitary mass and a subsequent MRI revealed an unusual massive pituitary tumor extending into the sphenoid sinus, the clivus and the cavernous sinus with a necrotic centre communicating with the supra sellar cisterns.

Pituitary screening bloods was indicative of a TSH oma with FT4 3.24 pmol/l (11–22.7), FT3 8.2 pmol/l (0.9–2.8) and TSH 2.4 mlU/l (0.5–5.5). Prolactin was 2820 mlU/l (45–375), testosterone 9.9 mlU/l (10–20) and cortisol 391 mlU/l. Synacthen test was normal.

He had a transphenoidal biopsy and the CSF leak was repaired. Biopsy confirmed a pituitary adenoma and on immunocytochemistry it showed reactivity with TSH and prolactin.

Alpha subunit was elevated at 24 µl confirming the diagnosis of a TSH oma. The tumor was deemed unsuitable for debunking surgery or medical treatment and he had radical radiotherapy. Although asymptomatic after radiotherapy he had two further admissions with headache. First one with pneunoecephalus after his GP gave him intra nasal steroids for blocked sinuses. It was thought that the intra nasal spray had led to pressure changes causing the pneumo encephalus. Second admission was with recurrent CSF leak and meningitis. This was treated with antibiotics and he had a further leak repair done. At a recent follow up MRI demonstrated 33% reduction in the tumor size.

Discussion
TSH omas are rare forming less than 1% of pituitary adenomas and can be invasive. To our knowledge this is the first reported case of recurrent meningitis secondary to an invasive TSH oma.

P269
When should an MRI pituitary scan be performed in hypogonadal men with low or low normal LH?
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Background
There is no published guidance on when to request a pituitary MRI in hypogonadal men. The presence of low or low normal gonadotrophins is common in men with symptomatic testosterone deficiency and metabolic syndrome and/or type 2 diabetes. Our practise is to perform pituitary MRI scans in men with isolated hypogonadotrophic hypogonadism who have LH < 4 lu/l.

Method
One hundred and eighty-one patients that had a pituitary MRI scan between January 2005 and October 2009 were identified using the hospital database. These patient’s notes were reviewed and those that had an MRI requested because of hypogonadotrophic hypogonadism but otherwise normal pituitary function were included (n=96). The MRI reports were reviewed to ascertain if there were any focal pituitary abnormalities.

Results
Mean age was 52 years (range 20–80). LH < 2 lu/l n=37, LH 2–4 lu/l n=49 and LH >4 lu/l (4.1–7.5) n=40. Overall 15/96 (15.6%) patients had an abnormal scan of which LH <2 lu/l 9/37 (25.7%) (3 pituitary cysts, 2 Rathke’s cysts, 3 empty or partially empty sella, 1 microadenoma); LH 2–4 lu/l 5/49 (8%)
(4 partially empty or empty sella, 1 microadenoma); LH > 4 IU/l (3/10) (empty sella LH 4.7 IU/l). The mean total testosterone for subjects with abnormal scans was 7.38 nmol/l (2.2–11.1). An analysis of variance indicated no significant difference in levels of LH, FSH and age between patients with abnormal and normal scans.

Conclusion
A substantial proportion of the scans analysed contained abnormalities. No MRI scan identified a lesion which required interventional treatment of the structural abnormality. Some abnormalities may have been incidental findings however some give the patient a diagnosis. This shows that pituitary MRIs should be performed in men with below normal or low normal LH and remains a valuable diagnostic tool for patients with hypogonadotropic hypogonadism. The lack of correlation with age, LH and FSH shows that these serve as poor indicators for pituitary disease in hypogonadotropic hypogonadism.

P270
Hypothalamic resistance to signals of energy reserve, rather than excess appetite stimulation, may contribute to obesity in CP patients
Beaumont Hospital, Dublin, Ireland.

Obesity is a recognised complication of craniopharyngioma (CP), but its aetiology is not understood. Leptin, which signals energy reserve, and ghrelin, which regulates appetite, are implicated in the development of obesity. Our aim was to assess leptin and ghrelin levels in CP patients and to compare these to normal and BMI-matched controls.

CP patients were identified from the local database. All CP patients were on hormone replacement therapy where clinically indicated. Fasting blood samples were taken from 13 CP patients and from seven obese or overweight controls and 11 normal weight controls at 0800 h. Ghrelin and leptin levels were assessed using radioimmunoassay kits (Mediagnost). Results were compared using the t-test or Wilcoxon’s test, as appropriate. Statistical significance was accepted at P < 0.05.

Diagnosis
<table>
<thead>
<tr>
<th>CP (n=13)</th>
<th>Overweight (n=7)</th>
<th>Normal (n=11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI kg/m² (median, IQR)</td>
<td>32.3 (31.35)</td>
<td>28.2 (25.0–34.7)</td>
</tr>
<tr>
<td>Ghrelin pg/ml (median, IQR)</td>
<td>910 (749–1117)</td>
<td>931 (683–1249)</td>
</tr>
<tr>
<td>Leptin ng/ml (median, IQR)</td>
<td>20.9 (10.3–39)</td>
<td>17.4 (7.3–27.1)</td>
</tr>
</tbody>
</table>

Data were described with median and interquartile range (IQR).

*Difference between normals and CP / overweight, P < 0.05.

**Difference between CP and normals only, P < 0.05.

Ghrelin levels did not differ among the 3 groups, which implies that abnormal appetite signalling is not the cause of obesity in CP. However, leptin levels were higher in CP patients, which suggests that there may be hypothalamic resistance to signals of energy reserves in the CP group.

P271
The effect of pituitary surgery on VEGF, MMP 2 and MMP 9 levels in acromegaly and non-functioning pituitary adenomas
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Background
Serum biomarkers which correlate with pituitary tumour growth would be valuable in the treatment and follow-up of patients with pituitary adenomas, particularly non-functioning adenomas. Vascular Endothelial Growth Factor (VEGF) mRNA is upregulated in almost all tumours, whereas matrix metalloproteinases 2 and 9 (MMP 2 and 9) have been demonstrated to be important in tumour vascularisation and invasion, particularly in prolactinomas.

Aim
The objective of this study was to investigate VEGF, MMP 2 and MMP 9 levels before and after pituitary surgery in both patients with acromegaly and non-functioning pituitary adenomas. This would help ascertain whether these biomarkers could be used to assess response to treatment.

Methods
Blood samples were obtained from 29 patients with acromegaly and 12 patients with a non-functioning pituitary adenoma an average of 84 days before and 101 days after surgery. Paired t-tests were used to compare VEGF, MMP 2 and MMP 9 levels before and after surgery.

Results
Mean VEGF levels (± snt) before and after surgery respectively were 129.5 ± 62 and 134.7 ± 50 pg/ml in acromegaly, 100.1 ± 10.7 and 107.7 ± 14.5 pg/ml in non-functioning adenomas. Mean MMP 2 levels before and after surgery were 176.6 ± 9.7 and 154.5 ± 7.7 pg/ml in acromegaly, 178.8 ± 14.7 and 176.5 ± 10.8 ng/ml in non-functioning adenomas. Mean MMP 9 levels before and after surgery were 160.5 ± 19.5 pg/ml and 186.3 ± 23.9 pg/ml in acromegaly, 337.5 ± 49.6 ng/ml and 369.5 ± 50 ng/ml in non-functioning adenomas. No significant difference was seen between VEGF, MMP 2 or MMP 9 levels before or after surgery.

Conclusions
The serum levels of VEGF, MMP 2 and MMP 9 were not altered by debulking of pituitary adenomas, which suggests that these markers do not correlate with changes in tumour size or surgery.

P272
Obesity and cardiovascular risk factors in adult patients with acquired structural hypothalamic damage
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Background
Obesity is a common sequel to tumours of the hypothalamic region and their treatment. Weight gain occurs at a rate faster than any expected age-related increase and despite treated hormone deficiencies.

Methods
Retrospective review of patients with hypothalamic-pituitary tumours attending a large neuroendocrine clinic in UK.

Results
Initial review in 2002 had identified 42 adults with tumours causing hypothalamic damage. 24% were obese [body mass index (BMI) > 30 kg/m²] at diagnosis, but after a median of 5 years follow-up, 52% had become obese and 24% had BMI > 35. Review 7 years on, in 2009, of 68 patients (40 from original cohort, 28 new patients) demonstrated increased weight from diagnosis (mean BMI 28.4) to latest follow-up (mean BMI 33.4). Weight gain was fastest in the first year after diagnosis and treatment of the hypothalamic tumour, with a mean increase in BMI of 2.1 kg/m². Despite increased awareness since 2002 of the problem of obesity in these patients, and advice on weight management, dietary review and anti-obesity treatments consistently offered in clinic (15 treated with orlistat, 4 had multiple weight loss medications; 5 attended a multidisciplinary hospital weight management clinic), the prevalence of obesity in this group further increased, with 63% of patients now obese and 37% with BMI > 35. Moreover, other cardiovascular risk factors are highly prevalent: 57% have treated dyslipidaemia, mean cholesterol is 5.3 (range 2.6–9.8) mmol/l, 29% have hypertension, 13.8% type 2 diabetes, 11% have ischaemic heart disease and 3.7% cerebrovascular disease.

Conclusions
Weight gain and obesity are common, difficult to prevent and treat in patients with hypothalamic damage and the high prevalence of other cardiovascular risk factors and diseases can further contribute to increased morbidity and mortality. Increased awareness, active monitoring and aggressive treatment of these complications by clinicians is vital.
P273

The R304X mutation of the Aryl hydrocarbon receptor interacting protein (AIP) gene in familial isolated pituitary adenomas: A functional Hot-spot or founder effect?

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Background

Familial isolated pituitary adenomas (FIPA) is a rare inherited disorder accounting for about 20% of pituitary adenomas. Mutations in the Aryl hydrocarbon receptor Interacting Protein (AIP) gene have been described in about 15% of FIPA families and rarely in early onset sporadic pituitary adenomas. Among the AIP mutations reported so far, the R304X represents, after the Finnish founder mutation Q14N, the second most common one.

Methods

Three Italian families carrying the R304X mutation, including a newly reported kindred, have been genotyped for 12 genetic markers surrounding the AIP gene on chromosome 11 in order to look for a potential founder effect in Italy. Disease penetrance was studied by familial screening and genotype-phenotype correlation in affected patients has also been performed.

Results

Analysis of chromosome 11’s genetic markers revealed that two out of three R304X kindreds shared a common haplotype. Overall, seven patients and ten healthy mutation carriers were identified among subjects, indicating a disease penetrance of 41% in AIP mutation-positive subjects. Mean age at diagnosis was 19.1 ± 6.7 and even though most R304X pituitary adenomas were somatotropinomas (6/7), a great variability in disease severity was observed, also between the subjects sharing the same at-risk haplotype. All patients received pharmacological therapy, but only two experienced disease control.

Conclusions

Despite convincing evidences support the hypothesis that AIP codon 304 represents a mutational hot-spot, the presence of a common haplotype surrounding the AIP gene between two Italian FIPA families carrying the R304X mutation provides strong evidences for a new founder effect in a region of central Italy. We therefore suggest that special attention should be paid to young acromegalics in this region in order to determine the magnitude of this founder effect and favour precocious diagnosis, given the potential aggressiveness and common pharmacological resistance of pituitary tumors harbouring this mutation.

P274

Rathke’s cleft cysts in need of regular follow-up in case of recurrence

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Background

Rathke’s cleft cysts (RCC) are benign, cystic lesions arising from remnants of Rathke’s pouch and reported in 13–22% of normal autopsies. Their prognosis after surgical intervention is not clearly defined.

Aim

To analyse the outcome of patients who presented to our Department with RCC between 1/1977–3/2009.

Patients and methods

Thirty-three cases were identified [13 males/20 females, median age at diagnosis 43 years (range 9–87)]. In 4 cases, the diagnosis was established from combination of pathology, imaging and operative evidence, whereas in the remaining ones, the pathology was consistent with this diagnosis.

Results

Suprasellar component was detected in 29/33 (87.9%) of cysts. At presentation, 17/33 (51.5%) of subjects had at least quadrantanopia, 16/33 (48.8%) had gonadotrophin, 11/31 (34.5%) ACTH, 11/31 (34.5%) TSH deficiency and 5/32 (15.6%) had DI. The patients were treated by cyst evacuation combined or not with biopsy/removal of cyst wall (3/33 via craniotomy and 30/33 transsphenoidal). One patient received adjuvant radiotherapy. All, but one, patients had follow-scans during a mean observation period of 45.3 months (2–267).

Cyst relapse was detected in 6/32 of the cases at a mean interval following surgery of 29 months (range 3–48 months). Kaplan Meier analysis showed relapse rates 7.9 and 24.5% at 12 and 24 months, respectively. At last assessment, at least quadrantanopia was found in 6/31 (19.4%), ACTH deficiency in 14/33 (42.4%) of cases and 13/33 (39.4%) of patients were on Desmopressin.

Conclusions

In this series of RCCs observed for one of the longest periods following surgical intervention, we have shown considerable recurrence rates, as well as endocrine/visual morbidities. These data advocate careful long-term monitoring of these patients.

P275

Recurrence rates in patients with non-functioning pituitary adenomas presenting with acute apoplexy: a long-term follow-up study

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Background

Pituitary apoplexy has been reported in around 2% of surgically treated adenomas. Around 45% of all pituitary tumours presenting with apoplexy are non-functioning ones (NFAs). Currently, no data exist on recurrence rates in patients with NFAs who have had classical apoplexy.

Aim

We therefore, put together our data aiming to provide the first reliable series on recurrence rates in patients with NFA and classical pituitary apoplexy.

Patients and methods

All patients with NFA presenting with acute apoplexy to our Department between 1985 and 2008 and treated by surgery combined or not with radiotherapy were studied (inclusion criteria: sudden onset of clinical features including headaches, visual disturbance, ophthalmoplegia, nausea/vomiting, alteration of mental status combined with signs of pituitary haemorrhage on imaging and/or histological evidence of haemorrhage or necrosis in the pituitary gland). Recurrence was diagnosed on the basis of radiological appearances and the follow-up was estimated form the time of surgery until last available imaging.

Results

Thirty-two patients were identified [23 males, median age 58.5 yrs (29–85)]. Mean follow-up was 65 months (3–211). Five subjects received adjuvant radiotherapy after surgical removal. No patient offered radiotherapy had tumour recurrence. Three patients treated by surgery only (327, 11.1%) were diagnosed with relapse at a median time 51 months after resection (12, 51, 86 months – further managed by surgery, surveillance and radiotherapy, respectively). All had had partial removal of their tumour. Kaplan Meier analysis for those treated surgically only showed recurrence rates 4.3% and 13.9% at 48 and 72 months, respectively.

Conclusions

In this first study to assess pure classical apoplexy and recurrence rates, there is a significant degree of recurrence. This seems to be less than non-irradiated NFAs, but it is still significant and therefore, patients with pituitary apoplexy need regular surveillance imaging, in order to detect recurrence early.

P276

Pituitary gonadotrophinoma and ovarian-hyperfunctioning: link or coincidence?

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A 40-year-old woman, who had developed oligomenorrhea after discontinuing the contraceptive pill at age 30, presented with a 6-month history of intermittent vomiting associated with abdominal discomfort and distension. Ultrasound, followed by laparotomy, revealed multiple bilateral ovarian luteal cysts consistent with hyperoestrogenism and ovarian-hyperfunctioning. Only 9 months post-operatively her bilateral theca ovary cysts had recurred. Over 4 months monitoring her estrogen levels fluctuated between 9923, 20 616 and 18 506 pmol/l (110–850) with FSH levels of 5.9–9.6 IU/L (1–9) and LH levels of 1.9–2.1 IU/L (4–10). Tumour markers HCG, CEA and AFP were not elevated. Inhibin-B levels (67 pg/ml (<45)) and thyroid and adrenal function were normal. α-subunit and 17-OHP were slightly raised at 1.4 IU/L (<1) and 29.2 nmol/l.

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P277
Somatotroph adenoma subtype and responsiveness to somatostatin analogues in patients with acromegaly
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Background
Data on the relationship between the hormonal response to somatostatin analogues and morphological subtype of somatotroph adenomas is sparse. We have previously shown that nadir GH < 1.75 μg/l on the octreotide suppression test (OST) has positive and negative predictive value 94 and 100%, respectively in predicting achievement of ‘safe’ GH levels following treatment with octreotide LAR (Karivsitsi et al. 2005).

Aim
To investigate the responsiveness to OST according to somatotroph adenoma phenotype.

Patients and methods
Patients with pathologically confirmed somatotroph adenoma presenting between 2001 and 2008 were studied. Those who received medical treatment for their acromegaly prior surgery were excluded. The histological subtype was defined according to the presence of antibody Cam5.2-positive fibrous bodies (i.e. markers of sparsely granulated tumours, representing cytoplasmic filamentous globular structures).

Results
One hundred patients were identified; 44 had received medical treatment pre-operatively, 14 had no OST and 5 had no histological subtype assessment. The remaining 27 subjects were included (median age 46 years (21–80), 12 males, 7 mixed/10 densely/10 sparsely granulated). There was no difference in the GH at time 0 min (morning fasting sample) or nadir GH between the three subtypes or between densely and sparsely. There was significant difference in the percentage fall of GH on the OST between all subtypes and between densely and sparsely granulated (median: mixed 88.8% – densely 88.1% – sparsely 67.0%, P < 0.05). There was no difference in the number of patients achieving nadir GH < 1.75 μg/l between the subgroups. Logistic regression showed no significant effect of granularity on achieving nadir GH < 1.75 μg/l after adjusting for age, sex and basal GH levels.

Conclusions
This is the first series showing that achievement of ‘safe’ GH levels following treatment with octreotide LAR, as predicted with the OST, is not associated with somatotroph adenoma subtype. Nevertheless, GH reduction is significantly less in sparsely than in densely granulated.

P278
Remission rate in microprolactinomas treated with dopamine agonists
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Introduction
Remission rate in microprolactinomas after treatment with dopamine agonists (DA) is variable, from about 25% in bromocriptine-treated patients to 46% in those treated with cabergoline.

Patients
We retrospectively studied the remission rate in 98 patients with prolactinomas ≤ 1 cm evaluated in 1982–2009 in our department and treated with DA for at least 1 year.

Results
Mean age of patients was 26.5 ± 8 years, 99% were women. Defining resistance to DA as lack of serum prolactin (PRL) normalization after bromocriptine (BRC) ≥ 20 mg/day or cabergoline (CAB) ≥2 mg/week, 11 patients (11%) were resistant to DA (all to BRC, four also to CAB, one was resistant to CAB). Resistance to lower DA doses occurred in other 14 patients (14%), in 9 of them it was overcome by high DA doses. Normal prolactin was obtained after pituitary radiotherapy in two patients unequivocally resistant to DA. In patients responsive to DA, the pituitary tumour decreased by ≥50% in only 16% of patients. Remission could be evaluated after DA withdrawal in 31 DA responsive patients, treated for medium 3.9–4.7 years (range 1–21.6 years): four (13%) had normal PRL (normal pituitary imaging in one) at 1.2–3.8 years after DA withdrawal, other two had normal PRL at <6 months; six patients (20%) had a functional remission normal PRL ± normal pituitary imaging in one) at 0.5–6.6 years after DA withdrawal; 19 patients (61%) had remission of hyperprolactinaemia at median 3.5 months (0.5–5 years). The remission rate was evaluated by the type of DA, age, PRL or tumour size at diagnosis, or duration of treatment.

Conclusion
Dopamine agonists induced a long-term clinical remission only in 16% of patients with microprolactinomas (38% of DA – responsive patients) treated more than 1 year.

P279
Echocardiography in patients with hyperprolactinaemia treated with dopamine agonists: what happens in daily clinical practice and what are the findings?1,2
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Background
Ergot-derived dopamine agonist therapy (EDDAT) is associated with cardiac valvulopathy in Parkinson’s disease. The risk to patients with prolactinomas is uncertain. The EMEA/MPHA and BSE recently issued guidelines for surveillance echocardiography (ECHO) of patients receiving treatment with EDDAT. Our policy is to arrange opportunistic ECHO screening at first clinic visit guidelines.

Objective
We investigated the uptake and findings of echocardiography in this cohort.

Methods
From our endocrine database we identified patients with hyperprolactinaemia receiving treatment with bromocriptine or cabergoline who attended between 01/07/2008 and 31/10/2009. We audited their treatment, ECHO request, ECHO findings and communication of results to the GP.

Findings
One hundred and thirty-five patients received treatment with either cabergoline (110) or bromocriptine (25). A number of patients had used bromocriptine before cabergoline. The average weekly dose of cabergoline was 0.83 mg (0.125–5), average daily dose of bromocriptine was 3.35 mg (0.5–7.5). Average treatment duration was 7.6 years (0–39). ECHO was requested for 75 (55%) patients. Fifty-five ECHOs were performed. Results were communicated to the GP in 28 (51%) of patients. Thirteen ECHOs are pending or done locally. ECHO was normal in 40 (73%). Fourteen patients (25%-3) had trivial/mild regurgitation of at least one valve, eight (15%) had mild tricuspid regurgitation and 11 (20%) mild mitral regurgitation, one (2%) pulmonary regurgitation and four (7%) had minimal valve leaflet thickening. One patient (2%) had moderate mitral regurgitation.

Conclusion
In daily clinical practice in a specialised pituitary unit the cardiac surveillance guidance was followed in only half of all eligible patients and results were communicated in only half of those. Systematic call-back may increase uptake. We did not find clinically significant valvar heart disease in this cohort. Over a quarter of patients exhibited mild valve abnormalities that will warrant revaluation to ensure they do not worsen on continued EDDAT.
P280

Treatment experience in 11 patients with gigantism
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Gigantism is an extremely rare condition and hence the relevant literature is largely a series of case reports. We present data on patients with gigantism <20 years of age identified from Pfizer’s Acrostudy registry of patients treated with pegvisomant.

Eleven patients (5M) were identified: IGFI at diagnosis was 1.6×ULN (1.15–3.3), height >5 SDS (1.1–3.8) and age 14.5 years (4–19). The three youngest (4.7 and 14 years) had pituitary hyperplasia secondary to NF-1 (n=1) or McCune-Albright (n=2). The remaining eight had macroadenoma and had transphenoidal surgery (TSS) as first line treatment. Two patients had a second TSS, and one had two TSS followed by a craniotomy. Four subsequently had radiotherapy. Four patients had hyperprolactinemia at diagnosis, seven were treated post-surgery with cabergoline (median dose =1.75 mg/week (0.25–7)). All patients received octreotide LAR (median duration of 12 months (6–120), dose 30 mg/month (10–40). IGFI was elevated in all patients prior to starting pegvisomant. On pegvisomant (median dose 20 mg/day (10–30 mg), duration 4.5 years (2.5–6)) monotherapy (n=8) or in combination with cabergoline (n=2) or octreotide (n=1) all patients achieved IGFI within the reference range. There have been no significant adverse events reported or increases in tumour size measured during pegvisomant therapy.

Syndromic gigantism is more common in patients with early presentation and in such patients surgery may be avoided. Vigorous and prompt treatment is required to minimise the harm from excessive GH, particularly tall stature. Radiotherapy may be more rapidly effective than in adults (data not shown) although the long-term risks are unknown. Somatostatin analogues and dopamine agonists are effective but despite all conventional treatment a significant number of patients remain uncontrolled. Experience from 11 patients treated with pegvisomant suggests that it is a safe and effective treatment in this group.

P281

The actions of long-term opioids for persistent pain on anterior pituitary hormones
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Objective
Exogenous and endogenous opioids affect the hypothalamic-pituitary-gonadal axis through binding to receptors centrally and by peripheral inhibition of testosterone synthesis, thereby altering the release of hormones. Approximately 12% of patients with persistent non-cancer pain use strong opioid analgesics in the UK, but the action on endocrine function may not be recognised. The aim of this study is to establish the incidence of gonadotrophin and other endocrine dysfunction in patients using opioids for persistent non-cancer pain who attended an inner city pain clinic.

Methods
We randomly analysed 34 patients on long-term opioids. All patients were using oral, transdermal, or intrathecal opioids; the duration varied between 6 months and 20 years.

Early morning blood samples were obtained measuring: LH, FSH, testosterone, cortisol, prolactin, oestradiol, and thyroid function.

Results
Eighty percentage of the male patients were found to have low testosterone with a mean testosterone of 3.5 nmol/l, range 0.5–7 nmol/l; the LH and FSH were disproportionally low in all these patients. Mean LH 2.8 U/l, range (0.1–5.1), s.d. of the mean 2.4, mean FSH 4.3 U/l, s.d. of the mean 4.7.

Seventy-one percentage of the female patients had undetectable oestradiol <60 pmol/l with a mean of 75 pmol/l and s.d. of mean 26.

Abnormal (0900 h cortisol was observed in 30% of patients; range 30–92 nmol/l. No thyroid or prolactin abnormality was detected.

Conclusion
We demonstrated that long-term opioid use is associated with pituitary hormone disturbance. The effect is more profound on gonadotrophins through the inhibition of hypothalamic GnRH. We also observed that opioid use may be associated with a decrease in cortisol level as seen in 30% of our patients. It is imperative that patients on long-term opioids have routine anterior pituitary hormone assessments.

P282

Cortisol but not GH responses are dependent on symptoms during the glucagon stimulation test
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The glucagon stimulation test is commonly used to assess the hypothalamic-pituitary axis when the insulin stress test (IST) is contraindicated. The mechanism behind glucagon induced secretion of GH and cortisol is unclear. To determine whether these responses are dependent on symptoms during the glucagon test, a retrospective case note study of glucagon tests performed over 3 years in patients with pituitary disease was undertaken.

Methods
Fifty six patients underwent a glucagon test. Patients with held glucocorticoid treatment prior to the test on the day of the test. Acrromegalic patients were excluded from the GH analysis.

Results
Patients of 78.6% were asymptomatic during the test. Amongst the symptomatic, nausea (50%) and lightheadedness (41.7%) were the most common symptoms. There was no statistical difference in glucose levels between those with and without symptoms during the test.

Peak GH and cortisol occurred at 150-180 min. Glucose nadir was at 240 min. The peak and increment in cortisol secretion from baseline was significantly greater in symptomatic than asymptomatic patients, but no difference was noted with respect to GH between the two groups (Table). IGFI levels correlated significantly with peak GH level (r=0.38, P=0.008).

Conclusion
The GH and cortisol response during an IST depends on hypoglycaemia and symptoms. Initial conclusions suggest that the GH response during the glucagon test does not appear related to symptoms whereas cortisol does. Cortisol secretion may be in response to either physiological stress or pharmacological effect of glucagon. Baseline IGFI levels may provide an indication of GH response to glucagon.

P283

Presentation, management and outcomes in acute pituitary apoplexy: a single centre experience from the United Kingdom
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Background
Pituitary apoplexy is rare resulting from acute haemorrhagic infarction of a pituitary adenoma. Optimal management in the acute stage still remains a matter of debate.

Methods
Retrospective analysis of casenotes of patients presenting with acute apoplexy at a single neurosurgical centre between 1984 and 2009 in the United Kingdom.

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Results
Fifty-five patients (35 males, mean age 52.4 (range 14–78) years, mean years of follow up 7.9 (range 0.5–25)) were identified; 45/55 (81%) had nonfunctioning adenomas, 4/55 (7.2%) acromegaly and 6/55 (11%) prolactinomas. Two patients had MEN-1 syndrome. The commonest presenting feature was acute headache (48/55, 87%), followed by diplopia (26/55, 47.2%), and visual field (VF) defects (20/55, 36%). The most frequent ocular palsy involved the 3rd nerve (21/60, 80%), followed by 6th nerve palsy (9/26, 34.6%) and multiple palsies (5/26, 19.2%). Of 11/55 patients had hypertension, five were on anticoagulants, five had recent major surgery and four cases occurred in patients with a known pre-existing pituitary adenoma. Forty-two percentage (23/55) of patients were treated conservatively and the remaining 32/55 (58%) had surgery; indication for surgery was deteriorating visual acuity and field defects. Patients presenting with VF defects (n=20) were more likely to undergo surgery (15/20, 75%) than to be managed expectantly (n=5/20, 25%). There was no statistically significant difference in the rates of complete or near-complete resolution of VF deficits and cranial nerve palsies between those treated conservatively and those who underwent surgical decompression. Endocrine outcomes (cortisol, thyroid hormone, GH, sex hormone deficiencies and requirement for desmopressin) were also similar in the two groups.

Conclusions
This is the largest series from a single centre in the UK of patients presenting with acute apoplexy. Patients without VF deficits or whose visual deficits are stable or improving can be managed with a conservative approach without a negative impact on neuro-ophtalmological and endocrine outcomes.

P284

Validity of the new pituitary apoplexy guideline group scoring system (PAGGS) in the management of pituitary apoplexy: a large retrospective review of 46 patients with classical pituitary apoplexy

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Background
There is lack of consensus in the management of pituitary apoplexy. Following the 11th Clinico pathological Pituitary Conference at the RCP (2009), a multidisciplinary group produced evidence-based guidelines for management of apoplexy. A patient with apoplexy and significant neuro-ophtalmic signs or reduced level of consciousness will usually undergo surgical decompression. It is unclear what defines a significant neuro-ophtalmic deficit. There is no objective tool to monitor conservatively managed patients. A pituitary apoplexy scoring system (PAGGS) has been designed to quantify the neuro-ophtalmic deficits and serve as a monitoring tool. This study evaluates the validity of PAGGS in apoplexy.

Methods
Retrospective analysis of case records of 46 patients with classical pituitary apoplexy treated in two centres from 1979 to 2009. Level of consciousness and neuro-ophtalmic signs (visual acuity, visual fields and ocular paresis) were assessed. PAGGS was internally validated by six clinicians and was retrospectively applied to 46 patients to assess usability.

Results
Fifty-four percentage of patients had surgery within 8 days of presentation (mean PAGGS 3.16); 11% had surgery within 9–14 days (mean PAGGS 3.80) and 35% of patients were managed conservatively (mean PAGGS 1.43). Mortality rate was 0%. No patient had any deterioration in visual symptoms after surgery. Final visual and endocrine outcomes were similar to published observational studies.

Conclusion
The PAGGS has been demonstrated to be usable in a large series of patients. In addition to being a uniform monitoring tool, the score quantifies the severity of neuro-ophtalmic deficits of apoplexy. Based on the clinical criteria in PAGGS, the current management of patients with pituitary apoplexy appears arbitrary. We suggest that this score should be validated in prospective studies comparing the outcome in conservatively and surgically managed patients. This would help in formulating an effective clinical assessment tool to optimise the management of this rare neuroendocrine emergency.

P285

Electrocardiographic features in Cushing’s disease: are there specific ECG changes associated with hypercortisolism?

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Introduction
Hypercortisolism is characterised by an increased risk of cardiovascular disease (CVD), either through a direct action on the myocardium or by affecting traditional cardiovascular risk factors. The electrocardiogram (ECG) is the initial examination to assess the structural and functional characteristics of the myocardium.

Aim of the study
To investigate whether the metabolic and cardiovascular features of Cushing’s disease (CD) are associated with alterations in the ECG indices as a measure of cardiac abnormality.

Methods
Seventy-nine patients (61 females; age: 40.76 ± 13.8 years) with a diagnosis of CD, retrospectively recruited, were compared with 42 healthy subjects (29 females; age: 41.76 ± 0.84 years) with similar age and BMI. The ECG features: QT dispersion (QTcd), left (LVH) and right (RVH) ventricular hypertrophy, and systolic (SBP)/diastolic (DBP) blood pressure were assessed in both groups. In patients with CD biochemical (fasting glucose, lipids), hormonal parameters (cortisol day curve), carbohydrate abnormalities (CHAS impaired fasting glucose, impaired glucose tolerance, diabetes mellitus), CVD (angina, myocardial infarction, heart attack, arrhythmias, stroke, thromboembolic disease, acute pulmonary oedema), hypertension (HTN) presence were recorded.

Results
LVH (b = 12.41, P = 0.01) and RVH (b = 12.33, P = 0.04) were predictors of QTcd; QTcd (OR = 1.08, CI = 1.02–1.14, P = 0.006) and DBP (OR = 1.05, CI = 1.01–1.10, P = 0.02) were predictors of LVH; QTcd (OR = 1.09, CI = 1.02–1.17, P = 0.02) and age (OR = 0.88, CI = 0.80–0.98, P = 0.02) were predictors of RVH. No ECG parameter was correlated with hypercortisolism. In patients with CD metabolic syndrome (39%), HTN (81%), CVD (21.5%), dyslipidaemia (36.7%), CHA (40.5%), arrhythmias (1.3%) were observed. Patients had longer QTc (P < 0.001), prevalent LVH (P < 0.001), RVH (P = 0.05), and higher SBP and DBP (P < 0.001) compared to controls. Patients without HTN, CVD were younger females (P < 0.001) but still more obese (P = 0.05), with longer QTc (P < 0.001), prevalent RVH (P = 0.05) and higher DBP (P < 0.001) compared to controls.

Conclusions
QTcd seems to be directly related to the presence of CD independently from the cardiovascular risk factors or the level of hypercortisolism. Since CD is associated with increased risk for CVD, ECG features might represent an easily assessed cardiovascular risk marker present early in the natural history of CD.

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substitution. A single pre-prandial blood sample was taken between the hours of 0830 and 0930 from each patient for the measurement of serum IGF1, serum total cholesterol, serum total triglycerides, serum HDL-cholesterol and serum LDL-cholesterol. The relevant demographic, anthropometric and clinical (e.g. exogenous GH requirement) data were also recorded.

Results
The mean daily GH requirement was 90% higher in the women receiving oral oestrogen compared with the control population (P<0.001) and 53% higher in those receiving transdermal oestrogen compared with the control women (P<0.01). The mean daily weight-corrected GH dose required to achieve target IGF1 levels was higher for oral versus transdermal subjects (12.0±3.7 vs 8.3±3.0 µg/kg per day, respectively, P<0.01). Despite this higher GH dose the mean IGF1 levels were lower in oral versus transdermal patients (24.2±12.6 vs 35.0±16.1 nmol/l, respectively, P<0.05).

Conclusion
This study shows that oestrogen replacement per se reduces sensitivity to exogenous GH in hypopituitary women. The route of oestrogen replacement is an important influence on GH requirement and those on oral oestrogen are clearly more GH resistant than women using transdermal preparations: The route of oestrogen administration is of practical and economic importance in the management of the hypopituitary woman receiving GH replacement.

Keywords: GH replacement; GH sensitivity; hypopituitarism; oral oestrogen; transdermal oestrogen.

P287
Patients with pituitary disease are at risk of under-replacement with levothyroxine
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Introduction
Achieving optimal levothyroxine replacement is more difficult in THS deficiency compared to primary hypothyroidism because of the inability to be guided by TSH. A combination of clinical symptoms and free thyroid serums levels (T4, T3) are typically used to monitor replacement. We reviewed adequacy of levothyroxine replacement in our patients with pituitary disease, and compared with T4 levels in patients with primary thyroid disease.

Methods
We identified all 525 patients with a diagnosis of any type of pituitary tumour in our department’s clinical information system who had been seen in 2007 and 2008. A free T4 (T4f) value was found for 514 (97.9%). Two hundred and twelve were at high risk of THS deficiency with macroadrenomas and/or surgery and/or radiotherapy. We compared T4f values in patients with primary thyroid disease in our thyrotrocytosis shared-care scheme and thyroid register within the same timescale – assessing T4f only in samples with a normal serum TSH = 3777 samples euthyroid off treatment, 11 805 on levothyroxine and 5074 on carbimazole.

Results
T4f levels overall were lower in pituitary patients than in equivalent controls. Of high risk group not taking levothyroxine 17% had a free T4 <11 pmol/l (100th centile for T4f in untreated controls) compared to only 8.4% of untreated controls. Furthermore, 38.9% of patients on levothyroxine had a T4f <13 pmol/l (100th centile for T4f in treated controls) compared to 9.5% of controls on levothyroxine with previous thyrotrocytosis and 13.4% of controls with primary hypothyroidism. Median T4f in controls on levothyroxine was 16 pmol/l and 20th–80th centile range was 14–19.

Conclusion
In our pituitary population we have found that levothyroxine doses are under-replaced compared to primary thyroid disease and that some untreated patients may actually be TSH deficient. The distribution of T4f in patients with primary thyroid disease on levothyroxine may guide optimum replacement levels.

P288
Anti-allergic cromolyn drugs facilitate annexin I secretion from pituitary folliculo-stellate cells
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Annexin I (ANXA1) was first identified as a glucocorticoid (GC)-inducible protein in macrophages and is a mediator of the powerful anti-inflammatory actions of these steroid hormones. In the pituitary ANXA1 plays a critical role in mediating the early-onset negative feedback effects of GCs on the release of ACTH. ANXA1 is expressed in abundance in the anterior pituitary gland where it is localized specifically to the S100-positive folliculo-stellate (FS) cells. GCs act on FS cells to cause translocation of ANXA1 to the plasma membrane, with particular accumulation at points where the cells make contact with adjacent secretory cells. The released protein then acts via cell surface receptors on the adjacent secretory cells to suppress stress-evoked ACTH release. ANXA1 lacks a cleavable signal sequence and its secretion is not affected by inhibitors of the classical secretory pathway. We have demonstrated a role for the ATP binding cassette transporter, ABCA1, as a ‘non-classical’ transport pathway for ANXA1 export from FS cells. In monoocytes a class of anti-allergic drugs, the cromolyns, enhance the release of ANXA1 when stimulated with glucocorticoids. Using western blot analysis, immunofluorescence and electron microscopic techniques in a FS model system, TGF/FGFs, we investigated the effect of the cromolyn anti-allergic drugs di-sodium cromoglicate (4 µM–4 mM) and sodium nedocromil (4 µM) on the trafficking and release of ANXA1 when triggered by dexamethasone treatment (3h, 100 nM). When administered alone, cromoglicate or nedocromil had no effect on ANXA1 release however, in the presence of a fixed sub-maximal concentration of dexamethasone, increasing amounts of the cromoglicate-like drugs caused a significant (P<0.001, n=3) enhancement of ANXA1 release. Therefore, although control of peripheral ANXA1 secretion is thought of as the mechanism of action of cromolyn anti-allergy drugs, in the pituitary such drugs enhance the negative feedback actions of GCs.

P289
A fall in serum prolactin level after a 2-h rest predicts a normal MRI in patients using dopamine antagonist treatment
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Introduction
Hyperprolactinaemia associated with antipsychotic drug use is commonly encountered clinical problem, yet no decision-making tools exist to guide whether to scan the pituitary gland.

Methods
A 10-year data series was reviewed of 246 patients who were evaluated for prolactin excess with a cannulated prolactin study. All patients had a prolactin drawn immediately following the insertion of a forearm cannula (P1) and a second sample drawn after 120 min bed rest (P2).

Comparison was made between patients with a confirmed microprolactinoma (galactotheora and/or eilisemenorrhoea with resting hyperprolactinaemia and microadenoma on MRI) against those on dopamine antagonists as well as normal controls. The presence of macroprolactin was excluded in all samples.

Results
Sixteen patients out of 246 were on dopamine antagonists. In eight patients the prolactin normalised following discontinuation of the drug for at least 3 weeks.

<table>
<thead>
<tr>
<th>Number of subjects</th>
<th>P1 (mU/L)</th>
<th>P2 (mU/L)</th>
<th>ΔP (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal controls</td>
<td>25</td>
<td>170.4±36.2</td>
<td>170.7±22.7</td>
</tr>
<tr>
<td>Microprolactinoma</td>
<td>54</td>
<td>187.8±179.3</td>
<td>171.6±163.9</td>
</tr>
<tr>
<td>Dopamine antagonist</td>
<td>6</td>
<td>2918.2±672.3</td>
<td>1588.0±257.5</td>
</tr>
<tr>
<td>Normal controls</td>
<td>2</td>
<td>1201.0±235.0</td>
<td>1229.5±270.5</td>
</tr>
</tbody>
</table>

*P<0.001 versus microprolactinoma; †P<0.04 versus dopamine antagonist and microadenoma; ‡P>0.001 versus normal controls; §P<0.05 versus normal controls.

Conclusion
In our series, prolactin normalised after 2 weeks of cessation of dopamine antagonists, when treatment could safely be discontinued. A fall after 2 h rest predicted a normal MRI in patients continuing on dopamine antagonists.
P290

Adult GH replacement and risk of tumour recurrence: 15 years experience from a single large centre

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GH replacement (GHR) has resulted in a significant improvement in quality of life for many adults with GHR deficiency. Many of these patients are previously irradiated survivors of malignancy, and as such are at high risk of recurrent (RN) or secondary neoplasms (SN). There is a particularly strong association between CNS irradiation and subsequent development of meningiomas, which are known to express GH receptors. There is uncertainty as to whether GHR increases the risk of development of RN or SN. We present our experience from a single centre demonstrating the incidence of tumour recurrence in a large cohort of patients undergoing GHR replacement.

An electronic database was used to identify 358 adult patients who had been on GHR for at least 12 months over the period 1994–2009. Two hundred and sixty-three (73.5%) of patients had a history of cranial irradiation. Data was gathered from case records and the electronic database. Regular surveillance MR or CT brain imaging was carried out. RN were identified in 10 (2.8%) of patients, resulting in death in seven patients. SN were seen in 19 (5.3%) of patients, 17 of which were meningiomas. No SN resulted in death. A history of cranial irradiation was present in 29 (69.7%) of patients with RN/SN. Of the 95 non-irradiated patients, SN was seen in only one patient (1.1%).

Our experience extends a previous report focussing primarily on childhood onset GHR deficiency. Interestingly, all SN under GHR were found in previously irradiated patients with 17(19%) being meningiomas. The incidence of RN/SN is comparable to that previously demonstrated in large series of cancer survivors not treated with GH. It can be speculated that any effect of GHR on tumour recurrence is likely to be small, although controlled studies would be required to confirm this.

P291

Fish and chicks: C-type natriuretic peptide and the development of the pituitary gland in Gallus gallus and Danio rerio

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Mammalian pituitary gland development is well established on a molecular and morphological level. In developmental biology the use of chick and zebrafish models is common but despite chicken pituitary gland development being characterised in 1952 these models have only recently been regularly employed in pituitary studies. The third member of the natriuretic peptide family, C-type natriuretic peptide (CNP), has been investigated in rodents embryos but little is known about its role in chicken and zebrafish development. In our study we have used Gallus gallus and Danio rerio embryos to investigate the role of CNP in pituitary gland development. RNA in situ hybridisation probes were generated to detect expression of chick CNP3 gene and zebrafish CNP3 and Nppc-like (NPPCL) genes. The chick CNP3 probe was synthesised from a CNP3 EST (Univ. Manchester Chick EST Database). Primers were designed against zebrafish CNP and NPPCL RT-PCR was carried out on whole mixed-age zebrafish CDNA and PCR products purified and cloned using pGEM-T Easy vector. All plasmids were linearised, reverse transcribed and labelled with digoxigenin for detection with anti-digoxigenin antibody during in situ hybridisation. We have detected expression of CNP3 at days 4, 5 and 6 of Gallus gallus development (Hamburger Hamilton stages 24–29), which was especially marked at day 4 in the developing chick pituitary gland. In Danio rerio CNP3 expression was detected at 48 h post fertilisation (hpf) in the brain and pituitary. NPPCL expression was widespread in zebrafish from 24 hpf but became more specific along the midline of the head up to 72 hpf, including expression in the pituitary. Our findings suggest CNP may be important in the crucial stages before and after Rathke’s pouch formation in the chick. In zebrafish CNP appears to be important after internalisation of the pituitary and during the later stages of pituitary patterning.

P292

Circadian and pulsatile secretion of copeptin, an arginine vasopressin (AVP) marker, argues against a physiological role of AVP in cortisol release

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Copeptin is a stable AVP marker and stoichiometrically released with AVP. It closely reflects changes in water balance. Its stimulation in severe stress has recently been suggested for the early diagnosis of myocardial infarction but clear definition of the physiological variability is necessary. Here, we studied the pulsatile and circadian variation in healthy individuals and compared copeptin to cortisol rhythms.

Copeptin levels were sampled every 20 min for 24 h starting at 0900 h in a series of seven healthy subjects (one female; age 18–37 years, mean BMI 22.6 kg/m²). Serum copeptin was measured with a specific immunoassay (detection limit of 1.7 pmol/l), cortisol by a commercially available assay (Bayer Corp., Pittsburgh, PA, USA). The maximal inter- and the intraassay CV are 6.5% for copeptin and 7.9% for cortisol. Cluster algorithm was used to analyse copeptin profiles.

No consistent circadian copeptin rhythm was detected in either individuals over the 24 h with no evidence of synchronization among subjects or a clear relation to the light–dark cycle or to cortisol secretion. Particularly the cortisol peaks found during the second half of the night is not anteceded or otherwise reflected in copeptin release.

AVP is known to acutely stimulate cortisol in stress situations. However, our present findings argue against any important physiological role of AVP in the generation of early morning cortisol rise (circadian rhythm). Our data will further help to better define this diagnostic grey zone for using copeptin measurements in the prediction of stress conditions. Particularly, in the light of the very attractive idea to use low copeptin levels as a very early negative predictor in myocardial infarction this physiological variation of copeptin have be considered.

P293

Long-term unsynchronised transcriptional cycles in individual living pituitary cells

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Gene expression in living cells is dynamic and unstable, and fluctuations in transcription may be subject to stochastic regulation of processes including transcription factor and polymerase recruitment, and chromatin remodelling. One gene that has been shown to display dynamically variable transcription and marked heterogeneity between cells is prolactin (PRL). Time-lapse imaging of PRL-reporter gene expression in single rat pituitary GH3 cells revealed distinct long-term transcription cycles (~11 h) with a shorter off-phase (~4 h) and a longer off-phase including a refractory period (~3 h). Regular transcriptional cycles were also observed in primary rat pituitary cells expressing a 160 kb PRL bacterial artificial chromosome containing the firefly luciferase gene.

A systems biology approach was implemented to enable the direct comparison of two different reporter genes (luciferase and d2EGFP) driven by identical PRL promoters within the same single pituitary cell over time. Mathematical reconstruction (MCMC) of transcription rates showed that transcription cycles from the two PRL promoters were out-of-phase, and hence there was no significant co-ordination between the behaviour of two loci in the same cell. Synchronisation between the timing of expression peaks of the two promoters in the same cell was induced by treating the cells with the histone deacetylase inhibitor, trichostatin A, suggesting that histone acetylation has a key role in the co-ordination of the temporal peaks of PRL transcription.

Our data indicate that cycles of PRL transcription are longer than the transcription cycles previously observed in other systems, and are largely asynchronous between cells as well as between identical transcription units in the same cell. If such stochastic and cyclical patterns of gene expression occur in living cells in the context of intact tissue, this might explain how tissues mount widely differing acute or chronic responses to environmental cues while maintaining a controlled average level of gene expression.
P294
Can we ever stop imaging in surgically treated and radiotherapy naïve patients with non-functioning pituitary adenoma?
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Background
Non-functioning pituitary adenomas (NFAs) are slow growing tumours with reported 5-year recurrence rates following resection up to 51%. The time point that it is safe to stop surveillance imaging is not clearly defined.

Aim
To clarify the time spectrum of recurrence in patients with NFAs offered solely surgery as primary treatment and to estimate the safe time to stop surveillance pituitary imaging.

Methods
Case-note analysis of all patients who underwent surgery for NFA between 01/1984 and 2/2007 was undertaken. Patients treated only by surgery and not adjuvant radiotherapy with a minimum follow-up of 1 year were included. Surveillance imaging was performed by scans every 1–2 years for the first 10 years and every 2–3 years thereafter. Recurrence was diagnosed on the basis of radiological appearances (tumour detection after total resection or tumour growth after partial resection) with or without associated manifestations.

Results
One hundred and fifty-two patients (95 males, median age at diagnosis 59.5 years (range 18–88)) met the inclusion criteria. Mean observation period following surgery was 6 years (median 4.3 (range 1–25.8)). Recurrence was documented in 57 cases (37.5%). Kaplan-Meier analysis showed relapse rates of 25.4, 45.5, and 62.1% at 5, 10, and 15-years, respectively. 50% of the recurrences were detected by 4.8 years. 76% by 8.8 years and 95% by 17.2 years (range 1–25.8). 22.7% of the patients had recurrence 10 years after the surgery.

Conclusion
In this large series of subjects with NFA (treated solely by surgery) and followed-up for an extensive period, we have shown that significant number (22.7%) of patients develop recurrence 10 years after the surgery. These results importantly suggest that these subjects need to be closely monitored following surgery with yearly imaging for the first 5 years and 2 years thereafter, to pick up recurrences early. Exact frequency of imaging is yet to be determined.

P295
Effects on insulin action of dehydroepiandrosterone sulphate replacement in hypopituitary females
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Hypopituitary patients are at increased vascular risk. This may be partly attributable to changes in insulin action. It has been suggested that the addition of dehydroepiandrosterone sulphate (DHEAS), which is low in patients with secondary hypoadrenalism, to routine replacement may have beneficial effects on glucose metabolism. Previously, patient populations and techniques used to assess insulin action varied and overall results have been conflicting. We assessed effects on insulin action of DHEAS replacement in female patients with hypopituitarism on stable replacement therapy using the hyperinsulinaemic–euglycaemic clamp. A randomised double blind placebo control crossover design was used. Fourteen patients were assigned to either DHEAS 50 mg daily or placebo for 12 weeks with 4 weeks washout between treatments. Insulin action was assessed at the end of each treatment period. Thirteen patients completed the study. DHEAS (DHEAS 5.4 ± 0.8 versus placebo <0.8 ± 0.0 μmol/l, P < 0.001) and androstenedione (DHEAS 4.1 ± 0.8 versus placebo 1.2 ± 0.2 μmol/l) levels rose to within the normal range after DHEAS. There were no differences between treatments in testosterone, SHBG or IGFI concentrations. There were no differences between treatments in fasting glucose, serum insulin concentrations or HbA1c. Triglyceride concentrations were lower following DHEAS (DHEAS 1.24 ± 0.18 versus placebo 1.41 ± 0.19 mmol/l; P < 0.05) but other lipid parameters were the same. Following treatment with DHEAS, there was no statistical difference in glucose infusion rates required to maintain euglycaemia when compared with placebo (DHEAS 21.9 ± 2.5 versus placebo 24.5 ± 2.1 μmol/kg per min; P = 0.4).

In summary, there were no differences in insulin action following DHEAS replacement therapy across 12 weeks. These results do not provide any evidence for a positive effect on insulin action or support the addition of DHEAS for this purpose to hypopituitary replacement therapy.

P296
Cabelergone suppression test: assessment tool for management of hyperprolactinemia
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Cabelergone (CAB) is a selective dopamine D2 receptor agonist with long-lasting action, highly effective in treating micro- and macroprolactinoma. However, the clinical response to cabergoline can be seen only after several months of treatment, allowing the tumor shrinkage and decrease of prolactin. Despite most prolactinomas are responsive to CAB, there are up to 8% of cases in which tumor responsiveness is limited. In order to assess the sensitivity to CAB, we aimed to develop a fast PRL suppression assay in patients with hyperprolactinemia, in a prospective interventional study.

Patients and methods
A total of 55 cases with hyperprolactinemia, naïve for dopamine agonists were included in a prospective study: 38 patients with prolactinoma and 17 with hyperprolactinemia from other causes, who served as controls. From the prolactinoma cases, eight proved in a longitudinal follow-up of 1 year to be resistant to dopamine agonists treatment. The test consist in assays of serum prolactin at basal, 12, 24 and 48 hours after a dose of 0.5 mg CAB was given p.o. In addition, in order to assess the plasma levels of cabergoline, we used a mass-spectrometry based method developed for cabergoline assay. PRL levels were measured in serum samples using an automated immunoassay. Cabergoline measurements were performed using mass-spectrometry: instrumental analysis were performed on an HPLC tandem mass-spectrometer in the multiple-reaction monitoring method (MRM).

Results
Cabergoline determined the decrease of PRL of more than 50% of basal value in all sensitive prolactinoma cases, while in resistant, the decrease was much lower. The highest decrease is in the first 12 h after a dose of 0.5 mg Cab. In sensitive prolactinomas, basal PRL values decreased from 713.7 to 396.75 ng/ml at 12 h, 295.6 ng/ml at 24 h and 233 ng/ml at 48 h, while in resistant prolactinomas, basal PRL values decreased from 1508.7 ng/ml at 1060.34 ng/ml at 12 h, 755.33 ng/ml at 24 h and 600.84 ng/ml at 48 h. However, the decrease of PRL in control group was much smaller. Cabergoline pharmacokinetics showed the highest value at 12 h, of 8.72 gg/ml, with a decrease at 5.64 gg/ml at 24 h and 4.23 gg/ml at 48 h.

Cabergoline suppression test can give information about the sensitivity to dopamine agonists in newly diagnosed prolactinomas, allowing a closer follow-up and a better management of patients with tumor hyperprolactinemia.

P297
Should we take macroprolactinoma patients off dopamine agonists at 3 or 5 years as they almost invariably recur?
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Department of Endocrinology, OCDEM, Churchill Hospital, Oxford, UK.

Objective
Our objective was to examine recurrence of hyperprolactinæmia following discontinuation of dopamine agonist (DA) therapy in patients with macroprolactinoma who have had treatment for 3–15 years.

Methods
We identified retrospectively adult patients (n=15) attending OCDEM (Churchill Hospital, Oxford, UK) with a confirmed diagnosis of macroprolactino-ma (established during the last 25 years), who had been treated with DA therapy for at least 3 years and had had a trial off DA therapy. None had any alternative treatment modalities and none were pregnant. Data collected included: age at diagnosis; sex; initial tumour dimensions and shrinkage with therapy; length of DA therapy; prolactin levels at baseline, during DA therapy and at recurrence (defined by prolactin higher than 375 mU/l (men) and 620 mU/l (women)); and time to recurrence.

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Results
Fourteen patients (93%) had a recurrence of hyperprolactinemia, 9 within 6 months and 13 within 1 year of discontinuing DA therapy. Mean time to recurrence was 8.8 months (s.d. 8.7). Mean time on DA therapy was 7.5 years (s.d. 3.4). All 15 patients achieved suppression of prolactin levels to normal during DA therapy. Mean initial tumour diameter was 2.0 cm (s.d. 0.6). The vast majority of patients (n = 14) had tumour shrinkage with DA therapy (in nine patients down to a thin rim). In the 14 patients who recurred, mean prolactin level at baseline was 28 246 mU/l (s.d. 24 569). Prolactin levels during DA therapy and at recurrence were 144 mU/l (s.d. 105) and 2236 mU/l (range 411–12847) respectively (P = 0.05). Linear regression analyses revealed no predictors for recurrence.

Conclusions
In patients with macroprolactinoma who achieve normal prolactin levels and whose tumours shrink with DA therapy administered for 3–15 years, the vast majority (93%) experience a recurrence following discontinuation of DA therapy, most (64%) within 6 months. Our data argue against a trial off DA therapy in patients with macroprolactinoma.

P298
Onset of prolactin gene transcription in nascent pituitary lactotroph cells
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Pituitary development, in particular the differentiation of anterior pituitary endocrine cells, remains to be fully understood, and may have implications for adult pituitary plasticity and hyperplasia. We have used prolactin-reporter transgenic rats to characterise prolactin transcription during the appearance of newly formed lactotroph cells during fetal development. Transgenic rats with the firefly luciferase reporter gene inserted into exon 1b of a 160 kb human prolactin BAC enabled real-time bioluminescence imaging of fetal pituitary glands ex vivo. Luminescence signal was detected as early as ED16.5, with cells appearing to exist initially either as isolated single cells or as small isolated cell clusters. RT-PCR analyses confirmed the presence of Pit-1, endogenous rat prolactin and hPRL-luciferase transcripts at this gestational age. RT-PCR indicated that low-level prolactin transcription may occur as early as ED15.5. These data indicate that lactotrophs appear earlier than previously suggested. This has implications for the differentiation of lactotroph cells, which has been hypothesised to occur via a somatotroph differentiation step from the Pit-1 cell lineage. Our discovery of a limited time frame between the initiation of Pit-1 expression at ED15.5 and prolactin expression suggests that transdifferentiation from the somatotroph lineage is unlikely to be a major pathway for lactotroph differentiation. This is consistent with previous data showing that only a small proportion of lactotrophs ever express GH.

P299
Immuno-modulatory role of prolactin in immune tissues driven by the alternative promoter
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Prolactin (PRL) is a hormone mainly produced by the lactotroph cells of the anterior pituitary gland. Besides its pivotal role in reproduction, PRL has also been found to have immuno-modulatory properties. The pituitary is the main source of circulating PRL, however it is also expressed in humans at extra-pituitary sites. In extra-pituitary tissues, PRL mRNA contains an extra exon (exon 1a), and expression is regulated by an alternative promoter upstream of the pituitary transcript. Previous studies have shown that PRL is upregulated in inflammatory settings, and that PRL has immunosuppressive properties. We explored the role of the alternative PRL promoter in the expression of PRL in the immune system.

P300
Genetic screening for variability in regulatory regions of SOX2 and implications for hypothalamo-pituitary development
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Background
SOX2 is a member of the SOX (SRY-related HMG box) family of transcription factors, and shares homology with SOX1 and SOX3 which are members of the SOX13 subfamily. Heterozygous, de novo, loss-of-function mutations in SOX2 were initially reported in patients with bilateral anophthalmia/microphthalmia, developmental delay and male genital tract abnormalities, with variable manifestations including defects of the corpus callosum, oesophageal atresia and sensorineural hearing loss. We have recently reported a number of SOX2 mutations in patients with anterior pituitary hypoplasia and hypothalamic-pituitary hypogonadism, which highlight the role of SOX2 in hypothalamo-pituitary development. Five regions that may be implicated in SOX2 regulation have been identified, four upstream (4.9-1 kb) and one downstream of SOX2, based on sequence conservation and previously published data on SOX2 regulation.

Aim and methods
The aim of the study was to screen a cohort of 200 patients, who did not have changes within the SOX2 coding sequence, for variations within these regions. This cohort included patients with a) eye phenotype of varying severity and b) patients with an ectopic posterior pituitary, without eye phenotype, who had been initially screened for SOX3.

Results
Direct sequencing showed that these regions were highly conserved in our cohort. However, in one patient with anterior pituitary hypoplasia, undescended posterior pituitary and GH, ACTH and TSH deficiency, we had identified a single base change (C>T) in a region ~4.5 kb upstream of SOX2. We did not find this change in 100 matched controls. Transient transfection of NT3/D1 cells that constitutively express SOX2 has shown no difference compared with the wild type (w) construct. However, the lack of an effect may be explained by the cell- and tissue-specific regulation of SOX2 expression. Search for transcription factor binding sites using Genomatix Mat-Inspector, showed that this change is predicted to affect binding of transcription factors including FoxO3 and SF-1. Conclusion
This variation in a highly conserved region may provide further insight into the phenotypic consequences of mutations affecting regulation of SOX2 expression.

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**P301**

**Adults with partial GHI deficiency (GHD) show phenotypic dichotomy related to the timing of onset of the deficiency**

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Considerable dichotomy exists in the phenotype of adults with severe GHD of childhood (AO-GHD) and adult-onset (AO-GHD). Those with AO-GHD show immaturity. Adults with partial GHD (GH insufficiency (GHI), peak GH 3.1–7.0 μg/l) show a similar, but milder phenotype to adults with severe GHD. Whether this dichotomous relationship relating to timing of onset is observed in CO-GHI and AO-GHI adults is not known.

We studied 24 adults with GHI (CO-GHI n = 13, 22 ± 5.6 years; AO-GHI n = 11, 42.3 ± 11.1 years), 32 with GHD (CO-GHD n = 14, 27.6 ± 6.7 years; AO-GHD n = 18, 41.9 ± 10.4 years), and 27 age-matched controls (young normal (YN) n = 17, 21 ± 3.0 years and old normal (ON) n = 15, 40.5 ± 7.8 years).

The study was approved by the local REC.

CO-GHI adults, compared with YN controls, were shorter (163 ± 0.09 vs. 175 ± 0.08 m, P < 0.001) and weighed less (62.1 ± 9.5 vs. 70 ± 11.3 kg). DNA analysis of body composition showed reduced truncal LBMI (20.1 ± 3.7 vs. 25.5 ± 4.1 kg, P < 0.001) in this group: CO-GHI adults had raised cholesterol (5.41 ± 1.15 mmol/l, P = 0.019) and LDL-cholesterol (3.13 ± 1.51 mmol/l, P = 0.053). Apolipoprotein B (94.3 ± 25.46 vs. 75.43 ± 19.69 mg/dl, P < 0.05) and PAI-1 (83.15 ± 17.93 vs. 91.94 ± 31.31 ng/ml, P < 0.05) levels. CO-GHI adults also had reduced lumbar spine T score (−1.73 ± 0.92 vs. −0.60 ± 0.95, P < 0.05).

In contrast, AO-GHI adults, compared with ON controls, were heavier (74.0 ± 18.1 vs. 69.0 ± 12.5 kg) with increased waist circumference (89.4 ± 17.4 vs. 80.9 ± 11.1 cm) and truncal fat mass (13.1 ± 7.5 vs. 9.0 ± 4.1 kg). They exhibited abnormal cardiovascular profile with reduced HDL-cholesterol (1.58 ± 0.29 vs. 1.60 ± 0.25 mmol/l) and significantly increased carotid IMT (0.084 ± 0.041 vs. 0.053 ± 0.080 mm, P < 0.05). Serum leptin levels were elevated in AO-GHI compared with controls (46.82 ± 33.10 vs. 23.15 ± 16.87 ng/ml, P < 0.05). Both CO (338.1 ± 104 vs. 426 ± 117 μg/l) and AO-GHI (244 ± 80 vs. 309 ± 89 μg/l) adults had lower IGF-I levels compared to their respective controls. These data confirm that CO-GHI leads to adults who have failed to optimise their skeletal and somatic development, reflecting the phenotype of adults with CO-GHI.

Both AO-GHI and CO-GHI adults show an adverse metabolic profile.

**P302**

**The effect of the familial pituitary adenoma gene AIP on apoptosis**

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Background: Pituitary adenomas usually occur as sporadic tumours, but familial cases are increasingly identified. Patients of 15–40% with familial-isolated-pituitary adenoma (FIPA) harbour germline mutations in the aryl-hydrocarbon receptor interacting gene (AIP). AIP is thought to act as a tumour suppressor gene, with loss of heterozygosity in pituitary tumour samples at the 11q13 locus, where AIP is located. Previously we have shown AIP has properties consistent with a tumour suppressor role, with wild-type AIP attenuating cell proliferation, whereas mutant AIP losing this effect and siRNA knock-down of endogenous AIP resulting in increased cell proliferation. However, it is unclear whether the reduced cell proliferation is due to increased activation of the apoptosis pathway.

Aims and objectives

To study the effect of AIP on apoptosis.

Methods

GH3 cells were transiently transfected with wild-type AIP, mutant AIP and empty vector. Caspase assays specific for the common (Caspase-Glo™ 3, 7, 11), intrinsic (Caspase-Glo™ 2, 9) and extrinsic (Caspase-Glo™ 8) caspase pathways were performed to determine the level of apoptosis. Nuclear staining with DAPI was used as a second method to confirm the effect of AIP on apoptosis. GH3 cells were treated with a caspase inhibitor to assess whether the attenuated cell proliferation induced by AIP could be reversed.

Results

Overexpression of AIP resulted in significant increase in caspase 3, 7, 8 and 9 activity compared to empty vector (P < 0.0001) and mutant AIP (P < 0.05). Nuclear staining confirmed an increase of apoptosis in cells transiently transfected with wild-type AIP compared to mutant AIP and empty vector. Addition of a caspase inhibitor reversed the attenuated cell proliferation.

Discussion

In a rodent somato-mammatroph pituitary cell line overexpression of AIP resulted in the activation of caspase cascade via both the intrinsic and extrinsic pathways. Our results suggest that AIP may express its tumour suppressor effect via the apoptosis pathway.

**P303**

**Oncogene-induced senescence occurs in human pituitary adenomas**

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Pituitary adenomas are benign tumours, which do not involve mutations in tumour suppressor genes or oncogenes. We have shown that the canonical Raf/MAPK and PI3K/Akt pathways are over-activated in these tumours, but their downstream effectors are modified to a much lesser extent. It is probable that the oncogenic mutations responsible for the initiation of these tumours therefore lies proximal to the convergence of these pathways, at or even upstream to the growth factor receptor(s). However, the ability of these tumours to grow extremely slowly or, in many cases, to stop growth entirely has been difficult to understand. Oncogene-induced senescence (OIS) is a process whereby tumours can undergo growth cessation or attenuation in the presence of oncogenic mutations or over-expression. We have speculated that such OIS also occurs in pituitary adenomas, and that loss of this mechanism may account for the very low prevalence (~ 0.2%) of carcinomatous change in pituitary tumours. We therefore have compared the expression of β-galactosidase, as a marker of OIS, in pituitary adenomas, and normal pituitary tissue using quantitative immunohistochemistry. Pituitary adenomas (n = 42) and normal pituitary (n = 7) were stained for β-galactosidase immunostaining and scored semi-quantitatively in a blinded manner. Senescence-associated β-galactosidase expression was significantly increased in GH-secreting and NFPAs compared to normal pituitary, but its expression in prolactin- and ACTH-secreting tumours was similar to the normal tissue. All staining was cytoplasmic, and was absent when non-specific anti-serum was used as a control. We conclude that two major subgroups of pituitary tumours showed over-expression of β-galactosidase, suggestive of OIS, although this was not seen in the two subgroups, prolactin- and ACTH-secreting, which are most likely to undergo malignant transformation. We are currently exploring the expression of β-galactosidase in pituitary carcinomas. It seems probable that OIS plays a significant role in maintaining the benign nature of most pituitary adenomas.

**P304**

**Pituitary tumours of mice deleted for a multiple endocrine neoplasia type 1 allele have alterations in apoptotic pathway components**

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Multiple endocrine neoplasia type 1 (MEN1) is an autosomal dominant disorder characterized by the occurrence of anterior pituitary, pancreatic islet and parathyroid tumours. Mice (Mene−/−) deleted for an MEN1 allele develop these tumours. The MEN1 gene encodes a 610 amino acid protein that has been reported to upregulate caspase 8 expression and promote apoptosis. To characterize the functional effects of menin loss in vivo, we assessed apoptosis using a TUNEL assay utilizing sections from eight pituitary tumours of Mene−/− mice and eight normal pituitaries from Mene+/− (wild-type) littersmates. This demonstrated a significant increase in apoptotic cells in the Mene−/− pituitary tumours when compared to Mene+/− normal pituitaries (0.22 ± 0.03 vs 0.12 ± 0.03%, P < 0.02). To gain mechanistic insights into the role of menin in apoptosis, we determined the cDNA expression profile of pituitary tumours from five Mene−/− mice and in normal pituitaries from five Mene+/− littermates by extracting total RNA and by hybridizing to Affymetrix Mouse Genome arrays. Pituitary tumours were found to have significant alterations of several anti-apoptotic pathway components including down-regulation of B-cell leukemia/lymphoma 2 (Bcl2) and neuronal apoptosis inhibitory proteins 2 and 5 (Iap2, Iap5) (~2.7–2.3- and ~2.1-fold respectively, FDR<0.05) and up-regulation of Kirsten rat sarcoma viral oncogene homolog (Kras) and Survivin (Birc5)
MicroRNAs, miR-15a and miR-16-1, are implicated in pituitary tumourigenesis via regulation of cyclin D1
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MicroRNAs (miRNAs) are small non-coding RNAs of ~22 nucleotides that negatively regulate gene expression through imperfect base pairing to the 3’ untranslated regions (UTRs) of target mRNAs. We have investigated the role of the miR-15a-miR-16-1 cluster in pituitary tumourigenesis, as it functions in other cancers as a tumour suppressor via regulation of the cell-cycle regulator cyclin D1. We have used two approaches: 1) in vitro studies examining for altered expression of miR-15a, miR-16-1 and cyclin D1 in pituitary tumours, mainly somato-lactotrophinomas, that develop in mice deleted for a multiple endocrine neoplasia type 1 allele (Men1-mice); and 2) in vivo studies examining the effects of antagonists, which are modified oligonucleotide miRNA inhibitors, on mouse pituitaries. Mice were kept in accordance with UK Home Office welfare guidelines and project licence restrictions. Use of total pituitary RNA from wild-type (Men1+/-) and Men1-/- mice for quantitative reverse transcription-PCR (qRT-PCR) revealed significant downregulation of miR-15a (~4.2-fold change, P<0.005) and miR-16-1 (~3.8-fold change, P<0.005) expression in pituitary tumours compared to Men1+/- pituitary tissue. Furthermore, cyclin D1 mRNA (+2.9-fold change, P<0.005) and cyclin D1 protein (>10-fold change) were increased in Men1-/- pituitary tumours, and the greater cyclin D1 protein expression is consistent with miRNA-mediated post-transcriptional regulation of cyclin D1 mRNA. In addition, miR-15a and miR-16-1 expression were strongly correlated, consistent with transcription from the same genomic locus (r²=0.89), and there was an inverse correlation between miR-15a and miR-16-1 with cyclin D1 mRNA expression (r²=0.86 and r²=0.63 respectively). In vivo, direct transarticular injection of antagonists specific for miR-15a and miR-16-1 into the pituitaries of Men1-/- mice resulted in a twofold increase in cyclin D1 mRNA. Thus, these findings, which support a role for the miR-15a-miR-16-1 cluster in pituitary tumourigenesis via regulation of cyclin D1, may facilitate the development of novel therapeutic strategies.

P307
Androsterone glucuronidase does not differ between nonobese and obese Caucasian women with polycystic ovary syndrome despite a higher DHEAS in nonobese subjects
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Objective
Androsterone glucuronidase (ADTG) concentrations have been suggested as a more reliable marker of the effects of androgen at the target tissue level and is significantly elevated in hirsute compared to non-hirsute women with PCOS. As the mechanism for hyperandrogenemia in obese and nonobese PCOS may differ, this study compares the different precursors of testosterone, including DHEAS, ADTG, and androstenedione in nonobese compared to obese women with PCOS and their implications on cardiovascular risk.

Method
Eleven nonobese (BMI 22.9 ±1.4 kg/m²) and 40 age-matched obese (BMI 36.8 ± 4.8 kg/m²) Caucasian women with PCOS were recruited using the Rotterdam criteria. Diabetes was excluded in all subjects by a 75 g oral glucose tolerance test. All subjects gave their informed consent and the study was approved by the local ethics committee. Fasting serum was collected at the same time each day and DHEAS, ADTG, androstenedione and total testosterone were analysed by isotope dilution liquid chromatography–tandem mass spectrometry (Waters Corporation, Manchester, UK).

Results
Both nonobese and obese PCOS were equally hyperandrogenic as measured by total testosterone level (1.70 ± 0.66 vs. 1.60 ± 0.71 nmol/l, P = 0.074), but DHEAS and androstenedione were significantly higher in nonobese compared to obese women with PCOS (7.64 ± 2.33 vs 5.51 ± 1.76 µmol/l, P = 0.0001 and 7.25 ± 2.82 vs 6.87 ± 3.62 µmol/l, P = 0.009 respectively). SHBG was significantly reduced in obese women with PCOS. Despite the much higher DHEAS in the nonobese group, ADTG levels were similar (213.7 ± 82.3 vs 216.9 ± 121.7 nmol/l, P = 0.186). HOMA-IR significantly higher in obese PCOS compared to nonobese PCOS.

Conclusion
This study compared the different components of androgen levels between nonobese and obese women with PCOS and showed that nonobese PCOS patients have higher DHEAS and androstenedione concentrations compared to obese PCOS women, but both had surprisingly similar levels of ADTG.

P308
In vivo validation of potential natural products’ abortifacient property mediated through modulation of uterine protein and vascular endothelial growth factor-C expression in foetal–maternal interface in albino rats
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Arunachal Pradesh is one of the northern eastern provinces of India situated on the Eastern Himalayan mountain range. Enriched with diversified flora, fauna and ethnic population, this zone has been recognised as one of the world’s Mega Biodiversity hot spots. The people of this state use bark powder of a plant Dyssoxylum alliarium for pregnancy control of mated females especially dogs and pigs.

Objective
Validation of this bark’s abortificient property was carried out in pregnant female albino rats. The aim was to study the effect of the bark extract on number of implantation sites, uterine protein profile and expression of VEGF-C in the foetal–maternal interface during early gestation.

Methods
Thin layered chromatographic fraction of the bark extract (methanolic) has been orally administered in a dose of 500 mg/kg per day during 0700-0900 h from day 1 of pregnancy.
Females were killed with cervical dislocation on the last day of treatment from day 5 to 8 of gestation during 1800–1900 h. Uterine protein profile was studied by one dimensional reducing SDS-PAGE (12%), while VEGF-C has been localised using anti-VEGF antibodies in paraffin sections.

Results
Gradual reduction of implantation sites from day 6 to 8 of gestation and degenerated embryo was observed in the treated females. While some of the proteins failed to express some are newly synthesized in the treated females uterus. Increased VEGF expression in the decidual zone and foetal–maternal interface was a significant observation on the effect of bark extract.

Conclusion
The TLC fraction of bark extract of *Dysosclium alliarius* carries potential antireproductive compound capable of pregnancy disruption mediate through endometrial protein expression. Increased expression of VEGF following extract treatment may be due to required internal stimuli to restore fast changing uterine activity during embryo implantation that has been disrupted by the bark extract.

P309

Alteration of uterine receptivity and modulation of uterine protein expression during perimplantation by natural product from *Piper species* leads to pregnancy disruption in albino rats

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*Piper betuloides* is a creeper grown wildly in the tropical forests of the north east part of India. The ethnic people of this region, especially in Arunachal Pradesh use the secondary roots of this creeper for fertility control in women. The traditional preparation has been reported to be used orally during post coital period.

Objectives
The anti-reproductive property of the secondary aerial roots has been tested on female albino rats during early gestation period. Potentiality of the roots’ pregnancy termination property has been studied by counting litter size at the end of pregnancy, protein profile of the receptive uterus (day 3–4 of gestation) and during perimplantation period. Ovarian and uterine histoarchitecture along with expression of TGF-β in uterus was studied during the experiment.

Methods
Methanolic crude extract of the roots was administered through oral route in a dose of 500 mg/kg body weight/day from day 1 to 6 of gestation. Subsequently females were killed by cervical dislocation on the respective day from day 3 to 6 of treatment. Uterine proteins were studied by SDS-PAGE, while growth factor (TGF-B I and II) were localized in paraffin sections using anti-TGF-β antibodies.

Results
A drastic change in the protein profile of receptive uterus (day 3–4 of gestation) as well as during perimplantation period showed the potentiality of the root extract for blocking implantation process. Altered expression of growth factor in uterus and reduced number of litter size on completion of full term gestation suggest the pregnancy disruption by the root extract.

Conclusions
The secondary roots of *Piper betuloides* contain potential phyto compound(s) for pregnancy disruption in rats. Changed milieu of receptive uterus during day 3–4 of gestation induced by root extract treated postnatally is a significant control mechanism of pregnancy by natural product.

P311

A possible cardioprotective role for metformin in PCOS: a study of clot structure and fibrinolysis

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Introduction
Polycystic ovary syndrome (PCOS) is associated with known cardiovascular risk factors, consequently predisposing to premature cardiovascular disease. Blood clot structure has been shown to predict predisposition to atherothrombosis and the aim of this study was to investigate the effects of rimonabant and metformin on clot structure/function in women with PCOS.

Methods
Twenty women with PCOS were recruited in a randomised open labelled parallel study of metformin (1.5 g/day) and rimonabant (20 mg daily) treatment for 3 months. After this period all individuals received metformin treatment for another 3 months. Clot structure and fibrinolysis was investigated *ex vivo* using a validated turbidimetric assay. Maximum absorbance (MA), a measure of clot density, and time from full clot formation to 50% lysis (LT), an indicator of fibrinolysis potential, were subsequently calculated.

Results
Treatment with metformin or rimonabant for 3 months had no significant effect on clot MA. However, metformin reduced LT from 2450±469 to 1678±425 s (P=0.01) at 3 months, whereas rimonabant had no effect. Switching all patients to metformin had no effect on MA but there was a non-significant decrease in lysis time in the group previously treated with rimonabant from 1925±7:11 to 789±41 s (P=0.1), whereas a further decrease in LT to 668±39 s (P<0.01) was observed in the group initially treated with metformin.

Conclusion
This study demonstrates that metformin use in PCOS is associated with a favourable effect on clot lysis, which is evident 3 months after initiation of treatment and becomes more pronounced at 6 months. In contrast, rimonabant treatment for 3 months has no significant effect on clot structure or fibrinolysis. These preliminary data suggest that metformin reduces thrombosis risk in PCOS and future clinical studies are warranted to clarify the potential cardioprotective role of metformin in women with this condition.
P312
Clinical experience of Nebido; monitoring the efficacy and safety of testosterone undecanoate in men over 60 years of age
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Aim
In normal men testosterone levels fall with age. We wished to assess the efficacy and safety of Nebido in men over the age of 60 years in order to assess dose frequency and other potential complications.

Subjects and methods
Ten men over 60 years of age (range 60–77 median 64) with primary or secondary hypogonadism were treated with Nebido 1000 mg i.m. Each was given a loading dose of two injections 6 weeks apart (our normal protocol for all patients regardless of age). Third and fourth injections were titrated with the aim of a trough testosterone level in the lower third of the normal range (range 8.7–28.4 nmol/l). Regular measurements of trough testosterone, PSA and FBC were taken.

Results
Baseline mean serum testosterone was 8.2 nmol/l (range 1.5–12.1). One out of ten patients had an elevated through testosterone pre-2nd injection (24.2 nmol/l) and had next injection interval extended to 24 weeks. Three out of ten patients had elevated through testosterone levels pre-3rd injection (23.9, 16.1, 17.5) and had next injection interval extended to 16, 15 and 14 weeks respectively.

Two out of ten patients stopped Nebido due to intervals exceeding > 24 weeks. Three out of ten patients developed polycythaemia, two requiring venesection.

There was no significant rise in PSA.

Conclusion
Men over 60 years of age frequently (40%) require extended intervals of > 12 weeks between administrations of Nebido. The percentage of patients over 60 years of age who developed polycythaemia was 30%. This highlights the importance of monitoring through testosterone levels prior to administration of Nebido, particularly in patients over 60 years of age.

P313
A simple modification of a commercial RIA to enable the more accurate measurement of serum testosterone in women
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The measurement of serum testosterone in women is difficult with most automated immunoassays because of poor sensitivity, accuracy and precision at low concentrations. Mass spectrometry is the gold standard for these measurements but it is slower and requires special expertise and equipment.

We investigated whether the modification of a commercial RIA allowed improved testosterone measurements in laboratories without access to mass spectrometry.

A commercial RIA (Siemens coat-a-count) was modified by increasing the volume of serum and diluting the low calibrator to extend the lower limit of the standard curve from 0.70 to 0.35 nmol/l. The new method had a functional sensitivity of 0.22 nmol/l and showed recoveries of 93–122% when measuring specimens made up by adding testosterone to blank serum (range 0.27–4.3 nmol/l). The between batch imprecision was 12.7% at a testosterone concentration of 0.39 nmol/l (n = 21).

The results of the new assay were compared with those of an automated immunoassay (Siemens Centaur) using a variety of serum specimens referred to the laboratory for testing. The RIA gave lower results than the Centaur by ~0.5 nmol/l. In women with raised serum sex hormone binding globulin as a result of oestrogen therapy the difference was increased to ~1.0 nmol/l suggesting that the automated assay was adversely affected by changes in the hormone binding environment. In 56 women referred because of low libido, serum testosterone by RIA was reduced in 34% although it was typically normal in this group when measured using the automated immunoassay.

In conclusion, a simple modification of a commercial RIA enabled more reliable measurements of serum testosterone in women. The changed results from the modified assay could have important therapeutic implications in different groups of patients.

P314
GLUT4 expression and translocation in immortalised mouse granulosa cells
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Polycystic ovary syndrome (PCOS) is associated with insulin resistance. Abnormally low glucose transporter 4 (GLUT4) expression has been shown in adipocytes from subjects with PCOS2 and skeletal muscle from type 2 diabetes3. Although GLUT4 expression is mostly restricted to classic insulin target tissues such as skeletal muscle, cardiac muscle and adipose, we have found it in granulosa cells (GCs) of mice. Glucose uptake and metabolism by GCs is impaired in PCOS4. Intracellular trafficking of GLUT4 has been studied in muscle and adipose tissue, but little work has been done on investigating GLUT4 expression and translocation in granulosa cells.

We studied GLUT4 expression in an immortalised luteinizing mouse granulosa tumour cell line (kK1 cells). Immunocytochemistry was used to analyse the response of the KK1 cells to insulin 100 ng/ml and forskolin 10 μmol. GLUT4 protein expression increased in response to insulin. The KK1 cells were then transfected with an HA-GLUT4-GFP plasmid (a gift from J Tavare, Bristol) and, using live confocal imaging2, we analysed intracellular trafficking of GLUT4-GFP in response to either insulin or IGF (IGF upregulates GLUT4 in mouse GCs4). GLUT4 was seen to move toward the cell membrane after 5 min of adding insulin 100 ng/ml and after 6 min of the addition of IGF 20 ng/ml. These results support the hypothesis that, in common with classic insulin target tissues, GLUT4 has a key role in glucose uptake by GCs and is regulated by both insulin and gonadotrophins.

Acknowledgements: Wellbeing of Women.

References
Conclusions
Like primary ETVs, HTR-8/SVneo cells become more invasive with T4 treatment. MCT8 and MCT10 may have both T3-dependent (suppress proliferation) and T3-independent (reduce apoptosis) effects in ETVs. This suggests an important role for MCT8 and MCT10 in human placental development.

P316
The tyrosine phosphatase, SHP-1, acts on multiple tyrosine kinase receptors to negatively regulate human cytotrophoblast proliferation
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Pregnancy complications that lead to fetal growth restriction are associated with abnormal placental cell (cytotrophoblast) proliferation and apoptosis. Regulation of these events is unclear but recently we have used a placental explant model to demonstrate that IGFs influence cytotrophoblast kinetics and demonstrated that the protein tyrosine phosphatase (PTP) SHP-2 is required for IGF actions in the placenta. However, SHP-2 accounts for only 20% of total PTP activity, suggesting other PTPs may also be important. mRNA for a closely related PTP, SHP-1, has been reported in the placenta but its actions are unknown; in other systems it functions as a negative regulator of signalling events. We examined the hypothesis that SHP-1 negatively regulates IGF actions by measuring cytotrophoblast proliferation following siRNA-mediated knockdown of SHP-1.

SHP-1 siRNA or non-targeting siRNA (500 nM) was delivered to BeWo cells or first trimester villous tissue explants. Cells and tissue were maintained in culture for 72 h, then treated with IGF1 or IGF2 (10 nM) for a further 24 h before western blot, immunohistochemical (IHC) and QPCR analysis. Following exposure to SHP-1 siRNA, SHP-1 expression was reduced in both BeWo cells (~85% knockdown) and in first trimester explants (~73%). IHC analysis for cell proliferation (Ki67) demonstrated that SHP-1 siRNA had no effect on IGF-induced proliferation but significantly enhanced levels of basal (serum-free) proliferation in both BeWo cells (from 19.7 ± 2.6 to 52.3 ± 2.9%; P < 0.05, n = 4) and first trimester explants (from 22.3 ± 3.7 to 63.8 ± 2.7%; P < 0.05, n = 4). To elucidate the mechanism(s) by which SHP-1 regulates basal but not IGF-induced proliferation, the activation status of multiple receptor tyrosine kinases (RTKs) was examined using antibody arrays and IHC following depletion of SHP-1. Following SHP-1 knockdown there was enhanced activation of EGF and Trk, suggesting that under basal conditions SHP-1 may interact with these molecules to inhibit proliferation.

This study demonstrates a role for SHP-1 in human trophoblast and establishes SHP-1 as a negative regulator of multiple RTKs that regulate placental growth.

P317
Localisation of adiponectin receptors in normal and polycystic ovaries
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Background
Polycystic ovary syndrome (PCOS) is the most common endocrine disorder in women. It is characterized by hyperandrogenism, which results primarily from excess androgen production by ovarian theca cells (TCs). Obesity contributes to androgen excess in PCOS and previous studies in vitro using bovine TCs have shown that adiponectin (production of which is inhibited in PCOS) is a repressor of P450c17 activity. However, little is known about expression of adiponectin receptors in the human ovary and no data to indicate whether adiponectin receptor expression is altered in PCOS.

Aims and methods
The aim of this study was to compare protein expression of adiponectin receptors 1 and 2 in normal and polycystic ovaries. We used specific antibodies to analyse, by immunohistochemistry, localisation of adiponectin receptors 1 and 2 in archived ovarian tissue from ovulatory PCOS (n = 11), anovulatory PCOS (n = 5) and normal women (n = 10).

Results
Immunostaining for both ADIPOR1 and ADIPOR2 was observed in preantral and antral follicles of the human ovary, whether in PCO or control tissue. Expression of ADIPOR1 was more prominent in GCs whilst that of ADIPOR2 labelling was more intense in theca cells. Compared with normal ovaries, granulosa cells in primordial and transitional follicles from PCOS ovaries showed a lower proportion of positive staining for ADIPOR1 (P < 0.03 Fisher’s exact test), whereas, in theca cells, there was a higher intensity of labelling for ADIPOR2 (P < 0.03, Fisher’s exact test). However, no differences were distinguished when only ovaries from lean patients (BMI < 25) were analysed.

Conclusion
We have reported, for the first time, expression of adiponectin receptor protein in the human ovary. ADIPOR1 receptors were more concentrated in GC layer and ADIPOR2 receptors in TC layer. There were no major differences between normal and polycystic ovaries in distribution or abundance of adiponectin receptors but the presence of these receptors in steroidogenic cells of the ovary suggest that circulating adipokines such as adiponectin can directly affect steroidogenesis and that any disturbance of adiponectin secretion (e.g. as a result of obesity/PCOS) may have implications for the pathogenesis of hyperandrogenism in PCOS.

Acknowledgements
MRC Caps

P318
Expression of adhesion molecules in preantral mouse follicles
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Initial follicle growth results in a number of distinct morphological changes, such as cuboidalisation and proliferation of the granulosa cells (GC) as well as an increase in oocyte size. Cell adhesion molecules and intercellular junctions play a pivotal role in changes in cell shape. We hypothesize that change in GC shape is key, and that increased understanding of changes in cell adhesion and associated junctions will provide insight into the regulation of initiation of follicle growth and help identify key regulators, and their mechanisms of action.

Follicles were isolated from day 12 and 21 mouse ovaries for mRNA analysis of proteins associated with cell adhesion. RT-PCR was used to screen for cell adhesion and junctional proteins in isolated follicles of similar sizes. Isolated oocytes, pure GCs and immortalized mouse GCs (KK1 cells) were also included in the PCR screen.

Molecules involved in cell adhesion including afadin, nectin and ZO-1 were detected in growing follicles. Interestingly, E-cadherin mRNA was not detected in any GC samples, however was detected in both oocyte and follicle samples. Immunohistochemistry for E-cadherin confirmed protein expression was restricted to the oocyte membrane at all stages of follicle development.

Ocigospermomised follicles confirmed that removal of the oocyte was associated with ablation of E-cadherin expression. Further analysis using immunohistochemistry and RT-PCR suggested that N-cadherin is intermediately involved in adhesion between GCs, associated with the linker protein β-catenin.

Since E-cadherin is normally associated with adherens junctions between epithelial cells, these results suggest GCs are not truly epithelial, and that E-cadherin may have a unique role in oocyte/GC interaction.

We can now assess the role of the oocyte in paracrine regulation of GC adhesion during early follicle development.

P319
Establishment of an in vitro model to study oocyte regulation of gene expression in granulosa cells of preantral follicles
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In rodents and other mammals, it is well established that the production of certain TGF-β members including Gdf9 and Bmp15 by oocytes is important for follicle development to proceed. It is believed that these growth factors form part of a short loop paracrine system to regulate gene expression in neighbouring follicle cells. We propose that one aspect of this feedback loop may involve the genomic modulation of TGF-β antagonists in granulosa cells by oocyte-secreted factors, yet simple models to directly test this concept in preantral follicles are currently

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limited. Precausal follicles with <3 complete layers of granulosa cells were freshly isolated from the ovaries of 12-day-old mice and imaged. Follicle size was related to the number of layers of granulosa cells as assessed by 3D confocal imaging. Each follicle was either mechanically oocyte-centred (OOCX), or manipulated under a microscope using glass micropipettes (control) and maintained in vitro. After 24 h, individual follicles were snap frozen for RNA isolation and RT-PCR analysis. Expression of Gd92 was detectable in control follicles but was completely absent in all OOCX samples. Transcripts for factors normally expressed by granulosa cells such as AMH receptor 2, inhibin-α and Kit ligand were detectable in all samples. The TGF-β antagonists Twsrg1, Prdc and Htra1, which are normally highly expressed in preantral follicles, were still readily detectable in granulosa cells after oocyteectomy, indicating that the oocyte does not play a major role regulating their expression. These results demonstrate that the microsurgical removal of preantral oocytes still enables the integrity and phenotype of the remaining granulosa cells to be preserved, thus providing a useful model for dissecting the effects of oocyte secreted factors on the granulosa cell transcriptome during early stages of follicle development.

P320
Activation of testicular melanocortin 3 receptors (MC3-R) inhibits LH-stimulated testosterone production

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MC3-R expressed in the brain are well characterized however the role of MC3-R in the periphery is unclear. Recently, we described immunopositive staining for MC3-R in the testes of wild-type mice and reported that the testicular histology of the MC3-R null mouse was abnormal. The aims of this further work were to confirm that MC3-R is expressed in testes and determine if ACTH1-39 affects testicular steroidogenesis in vitro. RNA and protein were extracted from the testes of adult wild-type mice (C57 Bl/6) for use in RT-PCR and western blotting, respectively. Testes were also hemi-sected and incubated ± ACTH1-39 (10^-7-10^-5 M) ± LH (2 ng/ml) for 5 h at 35 °C in air. The media were removed and frozen until assayed for testosterone by RIA. Treatments were done in quadruplicate and each experiment was repeated 2 or 3 times. The results are presented as the mean of the means of each experiment ± s.e.m.

A single band of the expected PCR product size, 820 bp, was obtained: this band was absent in the negative control. A band, as expected at 40 kDa, was detected by western blotting. Testes incubated in the absence of LH, released 150±40 pg testosterone/mg of tissue (n = 3) into the media over 5 h and this was not modified by the addition of ACTH1-39 regardless of the concentration used. Addition of LH significantly increased the amount of testosterone released into the media (620±80 pg/mg of tissue: P < 0.001) whilst the addition of ACTH1-39 resulted in the inhibition of steroid production (55% inhibition in the presence of 10^-7 M ACTH1-39, P < 0.05).

Both mRNA and protein for MC3-R have been detected in adult mouse testes. ACTH1-39 has no effect on the basal production of testosterone but appears to inhibit LH-stimulated production.

P321
Study of serum visfatin in Egyptian women with polycystic ovary syndrome

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Background

In polycystic ovary syndrome (PCOS), insulin resistance (IR) might be involved in the development of endocrine and metabolic abnormalities. Visfatin, a protein secreted by adipose tissue, is suggested to play a role in pathogenesis of insulin resistance. However, the biological activity and regulation of this novel adipokine are still unknown.

Objectives

To study the visfatin level in PCOS in Egyptian women and its relationship with IR and markers of hyperandrogenism.

Subjects and methods

This study included 50 Egyptian women aged from 20 to 35 years old. They were divided into two groups: Group I: 30 women with PCOS (11 lean, as group Ia and 19 obese, as group Ib). Group II: 20 healthy, normally menstruating women (8 lean as group IIa and 12 obese as group IIb: control group). All individuals were subjected to full medical history and thorough clinical examination, measurement of fasting and 2 h postprandial plasma glucose, lipid profile, fasting insulin for calculation of HOMAIR, serum LH, serum level of free testosterone, serum uric acid, serum visfatin and pleviobiomolecular ultrasound.

Results

Our study revealed that visfatin was found to be significantly higher in PCOS than control (P < 0.001) and in lean patients with PCOS than the obese PCOS group (P < 0.001). Also, serum visfatin was positively and highly significantly correlated with BMI, FPG, serum insulin, HOME IR, TG, cholesterol, LDL-c, uric acid, LH and testosterone and positively significantly correlated with PPGP (P < 0.05).

Conclusion

Our findings might suggest that visfatin could play a role in the pathogenesis of PCOS more in lean than obese subjects.

P322
Interaction between fibroblast growth factor-8 and bone morphogenetic proteins in regulation of ovarian steroidogenesis by rat granulosa cells

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Bone morphogenetic proteins (BMPs) have been recognized as crucial molecules as a luteinizing factor but BMPs have differential actions in FSH-induced estradiol production in a ligand-dependent manner. We recently reported the presence of oocyte-granulosa cell communication through BMP actions by regulating MAPK. To approach the oocyte factors that modulate steroidogenesis controlled by BMPs, we here investigated the effects of FGF-8 in rat granulosa/oocyte co-cultures. FGF-8 potently suppressed FSH-induced estradiol production, while it did not affect cAMP-induced estradiol levels by rat granulosa cells. FGF-8 had no effects on progesterone and cAMP production induced by FSH. The inhibitory effects of FGF-8 on FSH-induced estradiol production were not altered by BMPs. However, in the presence of FGF-8, BMPs significantly suppressed FSH-induced progesterone with reducing cAMP, suggesting that FGF-8 and BMP independently regulate FSH-R signaling. Notably, FGF-8 induced ERK and SAPK/JNK phosphorylation in granulosa cells, which was further enhanced by FSH. Inhibitions of ERK and SAPK/JNK commonly reduced FSH-induced progesterone and cAMP levels, suggesting that the activation of these pathways enhances FSH-induced cAMP signaling. In addition, ERK inhibition upregulated FSH-induced estradiol synthesis, suggesting that ERK pathway is also involved in suppressing aromatase activity in granulosa cells. Interestingly, FGF-8 enhanced BMP-induced Smad1/5/8 and Id-1-promoter activities with decreased expression of Smad6/7. Since a SAPK/JNK inhibitor restored the FGF-8 effects upregulating Id-1-Luc activity, SAPK/JNK appears to be involved in the mechanism by which FGF-8 enhances BMP-Smad signaling. Furthermore, in the presence of oocytes, the inhibition of endogenous FGF-8 FGF-8 signaling by SUS402 suppressed FSH-induced progesterone and cAMP, implying that endogenous FGF-8 activates FSH-induced cAMP-PKA signaling via ERK and SAPK/JNK pathways. Thus, an oocyte factor FGF-8 not only suppresses FSH-induced estradiol production by activating MAPK, but also enhances BMP-Smad signaling in granulosa cells. This interaction between FGF-8 and BMPs may play a key role in regulating steroidogenesis through oocyte-granulosa cell communication.

P323
Kiss1 mRNA and kisspeptin immunoreactivity are differentially regulated in hypothalamic and visceral fat in a rat model of polycystic ovary syndrome (PCOS)

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Introduction

The hypothalamic kiss1/kisspeptin system is pivotal in controlling fertility. However, kiss1 transcripts were also quantified in rat fat, where expression was

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regulated by oestradiol (Brown et al. 2008) and by dihydrotestosterone (DHT; Brown et al. 2009). In human fat, microarray analysis revealed abnormal, multiple gene expression in obese PCOS patients (Corton et al. 2007). In the present experiments we used a rat model (Manneras et al. 2007) to determine the possible influence of PCOS on adipose and brain kiss1 expression. Methods Weanling female rats (PD 21) were anaesthetized and given subcutaneous pellets that continuously released DHT (Innov. Res. of America: 83 μg/day; 60 days). Controls received sham surgery. Rats were killed at PD47 and PD81 by: a) decapitation, for RNA samples, or b) intracardiac perfusion with paraformaldehyde. RNA was isolated from basal hypothalamus (HYP), pituitary (PIT) ovary and visceral fat (FAT), and kiss1 mRNA was quantified by realtime RT-PCR. Kisspeptin immunoreactivity (KIP-ir) was localized by immunohistochemistry. Results At PD47, HYP kiss1 mRNA was largely undetectable in DHT rats compared to controls, whereas significant increases were seen in FAT (+ ninefold; P<0.01) and ovary (+ threefold; P<0.05). PIT kiss1 expression was unaffected. At PD81 kiss1 mRNA remained elevated in FAT (fourfold; P<0.05) but levels had reverted to normal in HYP and ovary. Hypothalamic KIP-ir partially reflected kiss1 mRNA levels, i.e. fibre and cell body staining was severely reduced by DHT at PD47. However at PD81 KIP-ir in nerve fibres, but not cell bodies, was essentially restored to control levels. Conclusions These data suggest that extended DHT treatment differentially affects kiss1 expression in female rats tissues, but is reversible to some extent by PD81, when the DHT pellets are likely to be depleted. We conclude that atypical kiss1 expression may contribute to the multiple tissue abnormalities observed in PCOS. Funded by IWR, Alice Endowment and UMRRI/Capital Health.

P324
Apparent under-reporting of polycystic ovary syndrome in primary care
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Introduction
Polycystic ovary syndrome (PCOS) is the most common endocrine abnormality in women with an estimated prevalence of 6–8%. Women with PCOS have a known increased prevalence of prediabetes (30–40%), type 2 diabetes (T2DM 10%), and metabolic syndrome (40%). We determined the prevalence of recorded PCOS through a structured primary care based T2DM screening programme.

Methodology
Eligibility criterion for a structured and systematic screening programme for T2DM in 19 general practices included age between 25–75 years for South Asian and 40–75 years for White European people. Out of 30 080 eligible women, 3527 (11.7%) attended the study. Response to a questionnaire including a question on ‘previous history of PCOS’ was analysed in this cohort of 3527 women. Computer database in all related GP practices were searched for a recorded diagnosis of PCOS.

Results
Of 3527 women attending screening, 47 (1.3%) (mean age 47 years, range 26–75 years) answered ‘yes’ to having a past history of PCOS. Of these only 10 (21.2%) had a record of the diagnosis in their GP practice. Out of 30 080 eligible women from 19 practices only 151 (0.5%) had a recorded diagnosis of PCOS (mean age 36.6 years, range 25–60 years).

Discussion
Although, we could not attempt to confirm the diagnosis in our cohort, PCOS appears to be both insufficiently diagnosed and under-recorded in primary care with a prevalence of 0.5% compared to the expected 6–8%.

Women with PCOS have multiple risk factors for increased cardiovascular morbidity and mortality. There is a clear need to increase awareness of this condition in the public and primary care setting to facilitate more proactive cardiovascular and glucose monitoring in this high risk group.

P325
Pregnancy adversely affects ability to recall previously seen spatial locations
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Background
Female sex steroids influence learning and memory and the neurobiology of brain regions involved in memory processing such as the hippocampus. Pregnancy allows overriding of regulatory feedback loops leading to substantial elevation of endogenous serum hormone levels, depending on concentration; oestradiol can be either neurologically protective or toxic. This investigation aimed to increase understanding of the influence of sex steroids on memory and attention during pregnancy.

Method
Participants were tested each trimester and at 3 months following birth, some were also tested preconceptually and at 12 months, a non-pregnant control group were also included. Memory and attention were examined using the Cambridge Neuropsychological Test Automated Battery, a computer based assessment tool. Edinburgh Postnatal Depression (EDP5) and General Health Questionnaire12 (GHQ12) scores were collected; the National Adult Reading Test (NART) a measure of verbal intellectual ability was also administered. Steroid/peptide analysis was carried out on a subset of participant’s plasma. Data were analysed using STATA version 10 and SPSS version 16. Antenatal/postnatal and control group and preconception and control group scores for each test session outcome measure were compared. The study received ethics approval.

Results
Data reveal a significant pregnancy group deficit in mean spatial recognition memory (SRM) % correct score compared to the control group during the second (70 vs 82%, P = 0.001) and third trimesters (73 vs 80%, P = 0.033) and at 3 months following birth (68 vs 80%, P = 0.0001). There was a also a significant reduction in antenatal SRM score between first and subsequent testing occasions. The pregnant group also had significantly higher mean EPDS and GHQ12 scores in the first and second and first, second and third trimesters respectively. Control group scores were stable across all testing occasions and on all measures apart from intra/extra dimensional shift adjusted errors which indicated a learning effect. There were no group differences when NART, BMI and age were compared.

Conclusion
Data support the hypothesis that pregnancy adversely affects ability to perform certain cognitive tasks, specifically memory for previously seen spatial locations. Increased EPDS and GHQ12 scores indicate pregnant women have lower mood and greater risk of depression.

P326
Female mice expressing constitutively active FSH receptor present with a phenotype of premature follicle depletion, premature aging and teratomas
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Strong gain-of-function mutations have not been identified in humans in the FSH receptor (FSHR), while such mutations are common among many other G-protein-coupled receptors. In order to predict consequences of such mutations on humans, D580 (D64) mutant forms of mouse (m) FSHR were expressed under the human anti-Mullerian hormone promoter in transgenic mice. Transgenic expression of mFSHrD580 mutant genes led to abnormal ovarian structure and function in the form of hemorrhagic cysts, accelerated loss of small follicles, augmented granulosa cell proliferation, increased estradiol biosynthesis, and occasional luteinized unruptured follicles or teratomas. While the litters of the females with low transgene expression were larger than those of wild type, the most affected mFSHrD580 females were infertile with disturbed estrus cycle, and decreased gonadotropin and increased prolactin production. Increased estradiol and prolactin apparently underlay the enhanced development of the mammary glands, adenomatous pituitary growth and lipofuscin accumulation in the adrenal gland, also detected in the transgenic females. The influence of the mFSHRD580 mutation was milder, mainly causing hemorrhagic cysts. The results demonstrate that gain-of-function mutations of the FSHR in mice bring about distinct and clear changes in ovarian function, informative in the search of similar mutations in humans.

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Sexual dimorphism in experimental endotoxaemia
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Sexual dimorphisms have been observed in numerous diseases, particularly those associated with inflammation. Generally, males are more at risk of developing infection and subsequent mortality1, whilst women are more prone to develop autoimmune disorders2. Hormones, particularly oestrogens, are thought to play a significant role effecting these dimorphisms, and oestrogens have been shown to reduce the severity of sepsis3. In order to investigate the protective effect of oestrogens in sepsis, a murine model of experimental endotoxaemia was used. Intact male C57BL/6 mice or ovariectomised female C57BL/6 mice treated for 8 days 17β-estradiol (40 ng/mouse, s.c.) or a corresponding volume of the vehicle were used. Mice were treated with LPS (10 µg/mouse) or saline vehicle. After 2 h, they were anaesthetised, the mesentery was exteriorised and leukocyte-endothelium interactions in post-capillary venules were quantified by intravital microscopy. LPS caused an inflammatory response in male animals characterised by increased cell flux and adherent and emigrated leukocytes, and reduced leukocyte rolling velocity. In females, LPS caused no change in cell flux, rolling or emigration. Ovariectomy caused an increase in adherent cells, with both control and LPS-treated groups showing significantly more adherent cells than male counterparts. In addition, baseline levels of emigrated cells in oestrogen-deficient females were increased above males. Females given oestrogen replacement showed no alterations in baseline compared to males, and additionally had no significant increase in adherent cells following LPS. These novel data demonstrate a protective effect of oestrogen in murine experimental endotoxaemia may further help explain why the incidence of sexual dimorphism in inflammatory disease reduced after the age of the menopause.

References

Steroids
P329
Metapyrone interference in serum cortisol immunosay
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2University of Cambridge Metabolic Research Laboratories, Cambridge, UK.
3University of South Manchester NHS Foundation Trust, Manchester, UK.

Metapyrone (MT) is used in the medical management of Cushings syndrome as it decreases serum cortisol (CT) levels by inhibiting adrenal β-hydroxylation of 11-deoxycortisol, the final step in CT synthesis. CT precursors, in particular 11-deoxycortisol (DOC), increase following MT therapy. Monitoring glucocorticoid replacement in patients taking MT could therefore be confounded as DOC cross-reacts in commonly used immunosays (IA) for serum CT. Serum CT results from two patients taking MT and hydrocortisone replacement are presented (Table 1). In both patients, insufficient glucocorticoid replacement was suspected on clinical criteria. In each case serum CT was measured by three commonly used IA; all showed positive bias compared to a liquid chromatography tandem mass spectrometric method (LC/MSMS). 11-DOC, the CT precursor, was elevated in both cases.

Table 1

<table>
<thead>
<tr>
<th>Patient</th>
<th>A</th>
<th>B</th>
<th>Cross reactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metapyrone dose</td>
<td>1 g qd</td>
<td>1 g qd</td>
<td></td>
</tr>
<tr>
<td>Hydrocortisone dose</td>
<td>1.5 mg/h iv</td>
<td>2 mg tid</td>
<td></td>
</tr>
<tr>
<td>ACTH (mg/l)</td>
<td>68</td>
<td>2166</td>
<td></td>
</tr>
<tr>
<td>CT (nmol/l) LCMSMS</td>
<td>86</td>
<td>32</td>
<td></td>
</tr>
<tr>
<td>IA Siemens centaur</td>
<td>272</td>
<td>263</td>
<td>0.151* (DOC)</td>
</tr>
<tr>
<td>IA Beckman</td>
<td>195</td>
<td>204</td>
<td>0.181* (DOC)</td>
</tr>
<tr>
<td>IA Roche</td>
<td>173</td>
<td>169</td>
<td>0.035* (DOC)</td>
</tr>
<tr>
<td>11-DOC (nmol/l) LCMSMS</td>
<td>235</td>
<td>700</td>
<td></td>
</tr>
</tbody>
</table>

Cross reactivity of DOC in each immunosay was determined by spiking cortisol-free serum with DOC. The observed discrepancy between CT levels measured by IA versus LC/MSMS could not be explained by the degree of 11DOC cross reactivity in any of the CT IA. However the observed positive bias of IAs was proportional to serum (DOC) and (ACTH) levels. This suggests that another ACTH-regulated steroid precursor might cross-react in CT IA, contributing to their positive bias. Immunosay methods cannot be relied upon to provide reliable estimates of serum CT in MT treated patients. The precise basis for the positive bias of IAs remains to be elucidated.

P328
Twice-weekly administration of kispeptin leads to long-term stimulation of reproductive hormone release in infertile women with hypothalamic amenorrhoea
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2Imaging Department, Imperial College Healthcare NHS Trust, London, UK.
3Assisted Conception Unit, Department of Reproductive Medicine and Surgery, Imperial College Healthcare NHS Trust, London, UK.

Background
Hypothalamic amenorrhoea (HA) accounts for over 50% of cases of amenorrhoea in women of reproductive age. Current treatments have limited success rates and side effects. We have recently shown that a single injection of the novel hormone kispeptin potently stimulates reproductive hormone release in women with HA. However, twice-daily kispeptin administration to women with HA, results in tachyphylaxis due to desensitisation of the kispeptine receptor. This suggests that less frequent administration of kispeptin may lead to sustained reproductive hormone release in women with HA, which would have therapeutic implications.

Aim
To determine if long-term twice-weekly kispeptin administration chronically stimulates reproductive hormone release in women with HA.

Methods
We performed an ethically approved prospective, randomised, single-blinded, parallel design study. Patients with HA received twice-weekly s.c. injection of either kispeptin (6.4 nmol/kg) or saline (n = 5/group) for 56 days. On days 0, 14, 28, 42 and 56, blood was sampled at regular intervals for 4 h post-injection for measurement of plasma LH and FSH.

Results
Women were more responsive to kispeptin injection on day 0 than day 14 (mean maximal LH increase in IU/L: day 0, 21±5.5; day 14, 10±4.3; P<0.001). However there was no further significant drop in responsiveness to kispeptin beyond day 14 (mean maximal LH increase in IU/L: day 28, 9.0±4.1; day 42, 8.9±3.5; day 56, 8.9±3.5; P=0.05 versus response on day 14). On the last (56th) day, women with HA were still 16 times more responsive to kispeptin than saline. No adverse effects following kispeptin administration were observed during the study.

Conclusion
In this first long-term study of kispeptin administration to women with HA, we have demonstrated that twice-weekly kispeptin administration stimulates reproductive hormone release in a sustained manner. Thus kispeptin may be a future novel therapy for reproductive disorders.

P330
Cyclical epilepsy associated with testosterone replacement
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Introduction
High testosterone level lowers the seizure threshold in patients previously well controlled on medication. Clinical scenario
A 59-year-old male, with a history of complex partial seizure, having 12 years of seizure free period on phenytoin and lamotrigine, presented with short history of vomiting, positional headache, and visual impairment. A diagnosis of pituitary macroadenoma was made following a MRI scan of his head. He underwent a transphenoidal hypophysectomy followed by radiotherapy in 2005.

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His testosterone levels before the surgery was 8 nmol/l (9.9–27). One year later, in March 2006, he returned with the history of loss of libido and sexual dysfunction. This time his serum testosterone level was 7.0 nmol/l (normal range – 9.9 to 27). He was treated with testosterone ester (Sustanon) injection 250 mg once every 3 weeks, as per manufacturer of sustanon, it is a mixture of four different testosterone esters with different half life and peak testosterone levels in 24–48 h. This treatment improved his libido, but in last week of October he started getting 2–3 episodes of facial twitching for the first few days of sustanon injection and recovered for every month when he was on injections. His testosterone level was 27.4 nmol/l during the follow-up and peaked to 51.5. This peak coincided with episodes of facial twitching each lasting about 30’s. We changed him to buccal testosterone preparation to give smoother blood levels of the drug all through out the day. He had no further recurrence of seizures on this preparation.

Conclusion
This case shows that higher testosterone levels will lower seizure threshold in epileptic patients.

P331
Group education improves patient confidence in managing steroid sick day rules
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Background
Group education for patients with diabetes is a well validated method of enhancing self-management skills. More recently some departments have used group education to teach endocrine patients how to administer IM hydrocortisone.

Innovation
Patients on steroid replacement, identified by the endocrine nurse and GPs, were invited to attend a group session. The aim was to ensure every relevant patient in the locality had the opportunity to be educated about dose adjustment in times of illness, etc. and supplied with an emergency hydrocortisone injection.

Method
The first 57 patients who attended the groups were asked to complete a questionnaire, including a measurement of their level of confidence regarding steroid medication adjustment during intercurrent illness. This was assessed using a likert scale pre and post group education.

Results
Fifty-seven patients: 21 Addison’s, 30 hypopituitarism, 3 Cushing’s, 2 CAH, 1 isolated ACTH deficiency.

Likert scale 1 = not confident, 10 = very confident.

<table>
<thead>
<tr>
<th>Pre group average confidence level</th>
<th>Post group average confidence level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Addison’s</td>
<td>6.19</td>
</tr>
<tr>
<td>Hypopituitarism</td>
<td>4.73</td>
</tr>
<tr>
<td>Cushing’s</td>
<td>4.0</td>
</tr>
<tr>
<td>CAH</td>
<td>5.5</td>
</tr>
<tr>
<td>ACTH det.</td>
<td>7.0</td>
</tr>
</tbody>
</table>

Conclusion
Patients who have participated in a group education session have higher levels of confidence regarding managing their steroid replacement therapy (P=0.003).

The increase in confidence is demonstrated irrespective of their diagnosis. The challenge now is to maintain patient self management skills which may require development of a new group education programme.

P333
Reference range data on androsterone glucuronide in healthy male and female volunteers and clinical uses of the assay
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Androsterone glucuronide (ADG) is a major metabolite of the androgen dihydrotestosterone and has also been shown to arise from the intracrine conversion of other adrenal androgens such as androstenedione and androsterone. ADG has been shown to be raised in some women with clinical signs of hyperandrogenism such as acne and hirsutism, even when levels of androgens, e.g. testosterone or DHEA-S are normal. This indicates that raised ADG levels may be an early indication of hyperandrogenism therefore measurement could be helpful in patients with clinical symptoms. Reference range data is essential before an assay can be used clinically, in order to distinguish between normal and abnormal levels. In order to produce this data, serum samples from 104 healthy female and 105 healthy male volunteers aged between 16 and 74 were analysed for ADG by liquid chromatography–tandem mass spectrometry. The data was found not to be normally distributed, with a skewness of 1.90 for the female data and 1.63 for the male data. The reference ranges were therefore determined non-parametrically. The reference range for the female population was 15–322 nmol/l. The lower limit of quantitation of the assay is 20 nmol/l, but as low levels of ADG have not been shown to be of clinical significance, this means that the female reference range can be given as <20 to 322 nmol/l. The male reference range was 56–377 nmol/l. This reference range data will enable the ADG assay to be used in aid of the identification of androgen disorders such as hyperandrogenism in women. It may allow earlier diagnosis as ADG is produced from the intracrine metabolism of androgens therefore may better reflect tissue concentrations of androgens than traditional markers. Further work could be carried out to determine whether the assay is of use in other situations, e.g. detection of hypogonadism.

P334
A rare case of massive bilateral adrenal enlargement complicating management of congenital adrenal hyperplasia
Sath Nag, Atif Munir, Arif Ullah & Anjali Santhakumar
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A 33-year-old lady presented to the surgeons with history of abdominal pain. CT scan abdomen done revealed massive adrenal enlargements reported as adrenal myelolipomas (left gland measuring 8.0×10.9×11.8 cm and multifocal lesions on the right with the larger one measuring 5.2×4.3×3.6 cm). She was referred to the endocrinology team where further history was elicited. She had been diagnosed as a child to have congenital adrenal hyperplasia (CAH) but was lost to follow up. Her main complaints were severe hirsutism and amenorrhoea and her current medication was hydrocortisone (10 mg daily on a 2/3/1 alternate day regimen). Systemic examination demonstrated marked obesity and hirsutism with no features of Cushing’s syndrome.

Serum testosterone was 3.4 nmol/l (0.5–2.6), 17-hydroxy-progesterone level 378 nmol/l (0–14) and ACTH 537 ng/l (10–80). Plasma catecholamines were normal.
The hydrocortisone was increased to 20 mg twice a day but caused weight gain and bloating. Despite concordance with medication (established with suppressed ACTH and 17-hydroxy-progesterone), she continued to be troubled by androgen excess. She was subsequently switched to dexamethasone 1 mg to be taken at 10 pm at night. However, her symptoms persisted with only a marginal reduction in the size of the adrenal masses. She was referred for bilateral adrenalectomy as along with sub optimally controlled CAH, she remained at risk of spontaneous rupture and hemorrhage into the adrenal masses.

Discussion

Adrenal myelolipomas are rare non-functioning benign adrenal tumors with a reported incidence between 0.08 and 0.4%. To our knowledge <20 cases have been reported in association with CAH to date. Most are asymptomatic but nonspecific abdominal pain secondary to compression or mechanical compression may occur. Bilateral adrenalectomy for CAH patients poorly controlled on medical therapy is still little explored as a treatment with very little data available for adults.

P335

Diagnostically difficult Cushing’s syndrome in a pharmacy student
Haris Marath & Ketan Dhatariya
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Twenty-two years old pharmacy student was urgently referred from primary care with a history of recent weight gain of 15 kg and an elevated 24 h urinary free cortisol (UFC) of 4517 nmol/l (<330). Clinically she described increasing hirsutism, low mood, loss of libido and symptoms suggestive of proximal myopathy. Her past medical history included recurrent low impact wrist fractures, asthma, PCOS, depression and epilepsy.

On examination her BMI was 34 kg/m² with abdominal striae, hirsutism, truncal and nuchal fat pads, acanthosis nigricans and proximal myopathy. She was admitted urgently for a full Cushing’s workup. She had two 24 h UFC measurements which were 5633 and 3401 nmol/l respectively. Surprisingly her 0900 h and midnight cortisol levels were normal, with continuously undetectable ACTH levels. She also had normal diurnal variation in her cortisol secretion. She went on to have a high dose dexamethasone suppression test which showed complete suppression (10 µg/day) by 48 h.

A low dose dexamethasone suppression was not done as she was on anti-convulsant medication. Her CT adrenals and MRI pituitary were also normal.

Because of the discrepancies in these tests we wondered if she had factitious Cushing’s syndrome and her 24 h urine sample was sent for further analysis. This showed that the sample contained prednisolone, confirming our suspicion.

This case illustrates the complexities that arise when dealing with patients who have access to prescription drugs. This patient had classical clinical features of Cushing’s syndrome probably due to exogenous steroid use, but more importantly her urine cortisol results were also high as she managed to add prednisolone to the urine sample. A high index of clinical suspicion and extensive biochemical testing may be needed in this situation especially when the initial results are discordant.

P336

Post-traumatic hypoadrenalism case report
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Direct trauma to the adrenal glands resulting in adrenal gland failure is relatively rare. We present two cases of post-traumatic hypoadrenalism.

Case 1

A 53-year-old male who was trapped under a lorry trailer. He was found to have fractures of T6 (unstable), T12, rib and sternal fractures. He was transferred to the orthopaedic ward and required a spinal splint. He was slowly recovering until day 20 of his admission when he became hypotensive and tachycardic. Blood tests demonstrated hypomagnesaemia and hyperkalaemia. Despite fluid resuscitation and emergency treatment for hyperkalaemia he remained hypotensive with major electrolyte abnormalities.

Endocrine opinion was requested and he was found to have an abnormal short Synacthen test. His hypertension and electrolyte abnormality rapidly responded to glucocorticoid and mineralocorticoid treatment. A repeat short Synacthen test has confirmed hypoadrenalism.

Case 2

A 57-year-old man was injured when a lorry fell on him. He had fractures of the T2–T4 thoracic vertebrae which were unstable, rib fractures and a pneumothorax. He had a long admission to ITU and then HDU and made slow clinical progress. Eventually several weeks after admission, due to ongoing problems with hypotension and hyperkalaemia the endocrine team were asked to review him. A short Synacthen test was performed which was grossly abnormal. After starting steroid replacement good clinical progress was made and he was subsequently discharged home having made a full recovery but with hypoadrenalism.

Both patients had been well prior to their traffic accidents and had no family history of autoimmune disease. The other hormone axes were tested and found to be normal and neither patient had adrenal autoantibodies. We think both patients had sustained critical damage to their adrenal gland circulation due to the major trauma and developed hypoadrenalism several weeks into their illness.

P337

Determination of tandem mass spectrometry specific reference ranges for testosterone, androstenedione and DHEAS
Philip Macdonald1, Federick Wu2, Laura Owen1 & Brian Keevil1
1University Hospital of South Manchester, Manchester, UK; 2Central Manchester and Manchester Children’s University Hospitals, Manchester, UK.

Testosterone, androstenedione and DHEAS are commonly measured by immunoassays. Variations in antibody specificity and calibration of assays results in non-commutability of measurements. Even more specific mass spectrometry (LC-MS/MS) assays still exhibit differences in calibration. As the use of mass spectrometry for measuring steroids is becoming more common in the clinical laboratory, the development of LC-MS/MS reference range for these analytes is essential to help accurately identify patients with abnormal serum concentrations, indicating possible disease states.

In order to generate reference ranges, serum samples from 92 healthy female and 94 healthy male volunteers aged between 16 and 74 were analysed for testosterone, androstenedione and DHEAS by LC-MS/MS. The data was analysed using Analyse-it for Microsoft Excel. The data showed testosterone, androstenedione and DHEAS were not normally distributed in both male and females and therefore ranges were determined non-parametrically with 95% confidence intervals. The female reference ranges for testosterone, androstenedione and DHEAS were 0.3–1.68 nmol/l, 0.85–6.0 nmol/l and 0.4–5.8 nmol/l respectively. The male reference ranges for testosterone, androstenedione and DHEAS were 8.4–30.9 nmol/l, 1.5–7.4 nmol/l, and 1.3–13.4 µmol/l.

As the majority of clinical laboratories use immunoassay, cascade testing is common when investigating the androgen status of an individual, therefore it is essential to use accurate reference ranges. The reference ranges found in our LC-MS/MS assays are lower than those found in immunoassays highlighting the lack of specificity of these assays. Mass spectrometry has the flexibility to allow the measurement of multiple analytes in a single assay, negating the need for cascade testing. We have developed accurate reference ranges to quickly and comprehensively assess the androgen status of an individual.

P338

A sensitive and specific tandem mass spectrometry assay for the measurement of salivary testosterone
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Saliva contains the free unbound fraction of testosterone, which has been shown to correlate with the free circulating serum testosterone, and may better reflect the physiologically active form. Recently, measurement of free testosterone has gained international recommendations for the diagnosis of hypogonadism in the ageing male. Saliva collection is a non-invasive technique, offering more flexibility to the patient, e.g. the collection of samples at home. Liquid chromatography–tandem mass spectrometry (LC-MS/MS) offers a specific, sensitive technique for measuring this analyte.

Saliva (200 µl) and D5-testosterone internal standard (10 µl) were extracted with 1 ml methyl-tet-butyl-ether. The supernatant was evaporated and reconstituted with 100 µl of 50:50 mobile phase; water (A) and methanol (B), each containing 2 mmol/l ammonium acetate and 0.1% (v/v) formic acid. Extract (30 µl) was injected onto an ACQUITY HSS T3 2.1×50 mm, 1.8 µm column. Testosterone and D5-testosterone were eluted from the column at 1.3 min using an increasing linear gradient of 50–90% mobile phase B over 1.5 min. The column was then washed with 98% B for 0.75 min followed by re-equilibration with starting conditions. The flow rate was maintained at 0.6 ml/min throughout and the total

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run time was 3.5 min injection to injection. The transitions for testosterone and D5-testosterone were m/z 289.2 > 108.8 and m/z 294.1 > 99.8. The lower limit of quantitation was 25 pmol/l, which was linear to at least 52 pmol/l. The mean recoveries were 96, 100 and 96% ± 0.15, 0.57 and 2.23 nmol/l respectively. Intra-assay and inter-assay imprecision were 8.4, 6.2, 3.5 and 4.3% and 7.8, 5.9, 2.2 and 2.4% respectively at concentrations of 0.1, 0.25, 1.0 and 4.0 nmol/l.

We have developed a simple, robust assay, which has sufficient sensitivity to measure testosterone in male saliva. This assay may be used to assess condition such as hypogonadism in males.

P339
Analysis of cortisol by stable isotope dilution liquid chromatography–tandem mass spectrometry (LC–MS/MS): pitfalls of rapid LC–MS/MS analysis of clinical samples
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The glucocorticoid hormone, cortisol, regulates fuel metabolism, inflammation and stress-responses. Its circulating concentrations are tightly controlled by the hypothalamic–pituitary–adrenal axis. However, 11β-hydroxysteroid dehydrogenase 1 (11βHSD1) generates additional cortisol in tissues, by reduction of cortisone. Using a tracer (9,11,12,13[3H]cortisol; d4-cortisol), the velocity of 11βHSD1 can be determined as the rate of appearance of 9,12,13[3H]cortisol (d3-cortisol), generated via the intermediate, 9,12,13[3H]cortisone (d3-cortisone).

Historically, quantitation of cortisol has been performed by gas chromatography–mass spectrometry, achieving limits of detection (LODs) of ~ 100 ng/ml in biological fluids, following derivatisation and with run times of 45 min. Increasingly LC–MS/MS assays are being developed, with improved selectivity and more rapid throughput. To quantify cortisol, cortisone and their isopropenols, an LC–MS/MS assay was developed. Plasma steroids (extracted in chloroform) were resolved in 11.5 min on an Alltech Eclipse column (50×2.1 mm; 3.5 μm) prior to MS detection. The protonated analytes gave the mass transitions; cortisol (m/z363→121), d3-cortisol (m/z366→121), d4-cortisol (m/z367→121), cortisone (m/z361→163) and d3-cortisone (m/z364→164). The assay had superior LOD (2 ng/ml), compared with GC/MS, with inter-assay precisions (5.5, 8.6%) and accuracies (~4.5, ~3.8%) for cortisol and cortisone respectively. While this assay achieved improved performance and throughput, unexpectedly a further substance co-eluted with d3-cortisone plasma extracts, yielding an ion undergoing the same mass transition. To resolve d3-cortisone from the interferent, prolonged chromatography (30 min), on a longer column, was necessary. Further investigation revealed that the precursor ion was the mass -2 isopropenol of an ion with m/z362. The accurate mass (362.11703 amu), determined by Fourier Transform-MS, identified the interfering substance as omeprazole sulphone. Three of the four participants from whom samples contained this interferent recalled recently taking recent omeprazole.

Thus LC–MS/MS as an excellent tool to enhance throughput and specificity of analysis of glucocorticoids. However care is required in screening of pre-existing medication in clinical studies, before executing rapid chromatography with biological samples.

P340
Steroid replacement: an unusual alternative to oral therapy via 24-h s.c. infusion device
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Background
Adrenal insufficiency is a well-recognised feature of congenital adrenal hyperplasia (CAH). CAH is commonly treated with oral steroid replacement, taken 2–3 times a day, at doses that aim to reproduce normal diurnal variation. Though acceptable for most patients, this does not control others, resulting in high levels of 17-hydroxyprogesterone-aceate (17OHP), ACTH, and the need for increased doses of steroid replacement, with associated co-morbidities.

Insulin, too, has a diurnal rhythm, which can be difficult to replace physiologically. In some diabetic patients, continuous s. c. insulin infusion (CSII) has been used to good effect. Here we discuss a patient with CAH who was successfully treated with continuous subcutaneous steroid replacement therapy delivered through a CSII pump.

Methods
We present a 39-year-old lady with classical CAH poorly controlled on oral steroid replacement (hydrocortisone 10 mg mane and prednisolone 4 mg nocte). Body mass index 35.13, elevated 17-hydroxyprogesterone levels and long standing fertility problems. Oral steroids were replaced by a hydrocortisone solution delivered via a CSII pump at a rate of 1.5 mg/h (0000–0800 h), 0.8 mg/h (0800–1600 h), and 0.4 mg/h (1600–2400 h). Total daily dose remained the same.

Education re. escape therapy and care of pump and lines was taught, and the patient had open access to specialist nurses.

Biochemical assessment with plasma and urinary steroid day-profiles was undertaken prior to, and regularly after, commencement of therapy.

Results
CSII pump delivery was associated with an improved physiological steroid day-profile, suppression of 17OHP and ACTH levels, and a one-third reduction in daily steroid dose.

Conclusion
CSII devices can be successfully used to deliver hormones other than insulin. This provides an alternative option for specific adrenal insufficiency patients.

P341
Moderate liquorice consumption enhances salivary DHEA and testosterone levels in healthy volunteers
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Liquorice root has been used medicinally for many years to treat dry cough, asthma, hunger, thirst, sterility of women, fever, digestive problems and pathologies of organs such as lungs, stomach, intestines and kidney. Glycyrrhetinic acid (one of the active constituents in liquorice) has diverse in vitro effects as an inhibitor of 11β-hydroxysteroid dehydrogenase (11βHSD). 5x reductase and hormone receptor binding. The aim of this study is to investigate the effects of moderate daily consumption of liquorice sweets on salivary DHEA and testosterone levels in healthy individuals. Ten men and 10 women (18–30 years) were given 100 g liquorice sweets (containing 3 g of liquorice extract) per day or non-liquorice containing confectionary for 7 days in a crossover study. Sensitive and specific ELISA methods were used to measure total (free plus conjugated) steroid levels following either extraction of saliva samples collected at 0800–0900, 1100–1300 h and 1700–1900 h of the final day of each arm of the study. Free and conjugated levels were also measured separately in a second aliquot of saliva after fractionation using C18 Sep-Pak cartridges (Waters). Conjugated steroids were first hydrolysed by incubation with extracts of Helix pomatia. Other steroid hormones such as aldosterone, cortisol and testosterone were also estimated. Changes in cortisol, cortisone and aldosterone confirmed expected effects on 11βHSD activity. Liquorice increased total and free DHEA levels in both males and female saliva (P<0.01) by 48.3 and 56.9% respectively. Testosterone levels were also higher perhaps due to peripheral conversion of DHEA. However, conjugated DHEA levels were decreased by liquorice. Since conjugated DHEA is predominantly DHEA sulphate, which is synthesized in the adrenal gland, we suggest that the opposing effects of liquorice on free and conjugated DHEA reflect inhibition of DHEA sulphation. GA or another component of liquorice could be an inhibitor of the adrenal sulphotransferase enzyme SULT2A1. This represents a novel target in the control of adrenal steroid hormone secretion.

P342
Is a raised DHEAS significant when analysed as part of an androgen profile
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Aim
The investigation of female androgen status in our laboratory is measured as a profile. Androstenedione, testosterone and DHEAS are analysed by liquid

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chromatography–tandem mass spectrometry. SHBG is analysed by immunoassay, and all results are reported simultaneously. The main aim was to investigate the management of patients who have a raised DHEAS level.

Method

Ethical approval was granted for this study. Females with DHEAS levels >6.9 nmol/l were selected, questionnaires were sent to the corresponding general practitioner or hospital clinician. The information gathered from the questionnaire included: medication, and prescribed medication at presentation, initial diagnosis, results of follow up investigations, final diagnosis and treatment. Results

One hundred and seventy-four questionnaires were sent to GPs, 22% replied, the remaining data were gathered from hospital case notes for referred patients. One hundred female patients were investigated, data were analysed on 84 patients. Most patients (53%) were aged between 21 and 30 years, the commonest presenting sign was hirsutism (43%). Eleven percent had only raised DHEAS levels. Forty-nine percent of the patients had an USS as part of follow up; 54% were identified as having cysts on their ovaries. Eight-two percent of the patients with cysts on their ovaries identified by USS, had a raised androstenedione level, compared to only 36% with a raised testosterone level. T-test data suggest that androstenedione and testosterone combined together can differ between those patients with a positive versus a normal USS for ovarian cysts. DHEAS and androstenedione were shown to have a significant correlation with patient’s age.

Conclusions

An isolated elevation of DHEAS was shown not to be clinically significant. Data suggest that age related reference ranges should be determined for androstenedione, whilst raised androstenedione and testosterone levels do relate to the presence of ovarian cysts when identified by USS.

P344 Application of a highly specific and sensitive ELISA for the estimation of cortisone in biological fluids

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It is now generally acknowledged that local tissue concentrations of cortisol and cortisone are modulated by site-specific actions of 11β-hydroxysteroid dehydrogenase (11β-HSD) isoenzymes 1 and 2. Cortisone, the inactive metabolite of cortisol is produced by type 2 11β-HSD. To assess 11β-HSD type 1 and type 2 activity, the cortisone/cortisol ratio has to be accurately determined. Immunoassays to measure cortisol levels are not widely available and tend to lack specificity. The aim of this project was to develop a highly specific and sensitive ELISA method for the estimation of free cortisone levels in urine, saliva, and in vitro media samples without chromatographic separation. Antibodies against cortisone were raised in rabbits by using cortisone-3-CMO-KLH as immunogen. HRP-goat anti-rabbit IgG conjugate (Upstate, UK) was used as enzyme tracer. Urine and cell incubation media samples were either extracted with dichloromethane or assayed directly following a 10X dilution. Saliva were extracted with ether and reconstituted in assay buffer. Aliquots were then assayed using the ELISA technique previously described after minor modifications (Al-Dujaili et al. 2009 Steroids 74 456–462). Cross-reactivities of the untreated cortisone antiserum with major potential interfering steroids were minimal except for cortisol (3.15%). However, following an immunodiffusion purity of the antibodies (incubation with CNBr-activated Sepharose-cortisol-3-CMO-BSA for 24 h), cross reactivity of the purified cortisone antibody with cortisol was reduced to 0.6%. Minimum detection limit of cortisone ELISA was 28 pg/ml (77.7 pmol). The validity of the cortisone ELISA was confirmed by the good correlation obtained before and after an HPLC fractionation step. Inter-assay imprecision was 8.7–12.8% CV. Using this assay, salivary cortisone levels showed a circadian rhythm (11.2 ± 7.3 nmol) at 0800 h and 5.1 ± 3.6 nmol at 1800 h, n = 20, and the levels were reduced following lowcarbohydrate ingestion. In adrenal H295 cell line incubations, basal cortisone levels were 4.24 ± 0.02 nmol that went up to 7.62 ± 2.2 nmol post forskolin stimulation. Urinary free cortisone excretion in healthy volunteers was 56.62 ± 36.9 nmol/day, n = 32). In human volunteers following ingestion of green coffee bean extract for 2 weeks, urinary free cortisone excretion reduced significantly from 58.38 ± 9.3 to 34.74 ± 4.9 nmol/day (P = 0.016) and cortisone/cortisol ratio (1.44 ± 0.33 to 0.96 ± 0.21). In conclusion, a simple and highly specific and sensitive ELISA has been developed to estimate cortisone levels in biological fluids.

P345 Dexamethasone testing and visceral/subcutaneous fat ratios in patients with adrenal incidentalomas

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Background

The Endocrine Society clinical practice guidelines suggest use of the 1 mg overnight dexamethasone suppression test (ONDST) to screen for cortisol excess in patients with adrenal incidentalomas, followed by the 48-h, 2 mg/day, low-dose dexamethasone suppression test (LDDST), to confirm a positive result. Visceral fat accumulates in cortisol-excess and accounts for increased cardiovascular risk. In this study, we investigate whether the LDDST offers additional information to the ONDST, and whether there is an association between cortisol excess and the visceral/subcutaneous fat (V/S) ratios in patients with adrenal incidentaloma.

Method

A retrospective, observational study was performed to collect demographic and clinical data on 137 patients diagnosed with incidentalomas. A full clinical and endocrine work up was performed, including either an ONDST or LDDST, or in some patients both tests. The V/S ratio was calculated by two observers in duplicate on a CT work-station by subtraction of the subcutaneous from visceral fat area. Inter-observer reproducibility was 98%.

Results

Of 76/137 (55%) patients had either a positive ONDST and/or LDDST (cortisol > 50 nmol/l). Of 60/103 (58%) had a positive ONDST whilst 45/63 (71%) patients had a positive LDDST. Twenty-nine patients had both tests done of which 20/21 patients with a cortisol level > 70 nmol/l after the ONDST had a positive LDDST, whilst the other eight patients, all of which had a cortisol level < 70 nmol/l after ONDST, had a negative LDDST. Correlation analysis revealed a significant positive correlation between cortisol levels for both tests (r = 0.78; P < 0.001). There was a significant increase in V/S ratio with increasing cortisol levels (≤ 50 nmol/l) and those who did not, 0.8 vs 1.4 (95% CI 0.15–0.98; P = 0.008).

Conclusion

In patients with adrenal incidentaloma and a cortisol level of > 70 nmol/l after an ONDST, the LDDST is usually positive and does not offer more information. Furthermore, we show, for the first time, that patients with a positive ONDST have a significantly increased V/S ratio that very likely contributes to the worsening cardiometabolic profile that is well-described in these patients.
P346
Salivary aldosterone is a useful marker of serum aldosterone in normotensive individuals
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Primary aldosteronism is an important cause of secondary hypertension though its diagnosis can prove challenging. In normal individuals aldosterone release follows a diurnal pattern, with a morning peak and low levels in the evening. Aldosterone is present in saliva and due to its lipophilic nature it passes into saliva along a concentration gradient. Salivary steroid testing is well established for cortisol and testosterone, but not yet for aldosterone.

We sought to determine the relationship between salivary and serum aldosterone in normotensive individuals and to examine the effect of time of day on salivary aldosterone concentration.

We developed a salivary aldosterone assay by modifying the Siemens ‘Coat-At-Count’ aldosterone solid phase RIA. Saliva was collected on three occasions during a 24 h period from 100 normotensive volunteers; at 2300 h following 30 min sitting at rest, at 0730 h the next morning while recumbent and at 1200 h after a morning of normal activity. Serum aldosterone was measured to coincide with the saliva sample given at 1200 h. Approval was obtained from the local research ethics committee.

Male to female ratio was 36–64, median age 33 years (range 19–65), mean blood pressure 118/74 mmHg (s.d. ± 156). Salivary aldosterone at 1200 h correlated strongly with the paired serum aldosterone sample (r=0.78, P<0.001). A repeated measures ANOVA showed a marked difference in mean salivary aldosterone concentration between 1200 and 2300 h (47.3 vs 11.0 pmol/l, P<0.001).

We have demonstrated that salivary aldosterone correlates well with serum aldosterone at 1200 h and that in normal individuals late night salivary aldosterone is significantly lower than at noon. Salivary sampling was non-invasive and acceptable to subjects. Further studies of salivary aldosterone in hypertensive patients are warranted.

P347
Regulation of glucocorticoid receptor activity by cellular stress
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Cofactors of the nuclear hormone receptors are crucial in regulating their transcriptional activity. The glucocorticoid receptor (GR) is a member of the nuclear hormone receptor superfamily, involved in the regulation of metabolism, inflammation and stress–responses and many GR cofactors are known, including p300. Here, we identify a new cofactor for GR: TTC5/Strap, tetrasarcosine/ repeat domain S/ stress–responsive activator of p300. Strap is a TPR motif containing protein, which forms a complex with p300 histone acetyl transferase. This complex regulates p53 in response to DNA damage. Given the role of TPR motif containing proteins as co-chaperones of nuclear receptors, here we investigate the effects of TTC5 on GR function. We have shown that GR and Strap interact through multiple binding motifs and that this interaction is modulated by cellular stress such as DNA damage and heat shock. TTC5 stabilises GR and regulate the transcriptional activity of GR, in a target gene specific manner. TTC5 also plays a role in the regulation of oestrogen receptor. In conclusion, our results suggest an important role for TTC5 in the regulation of nuclear hormone receptor function in response to cellular stress.

P348
–344 CT polymorphism of aldosterone synthase gene (CYP11B2) and cortisol synthesis in heart failure patients
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Background
Increased levels of cortisol are associated with worse prognosis in heart failure (HF). The final step in cortisol production is catalysed by 11β-hydroxylase encoded by CYP11B1, which is highly homologous with CYP11B2. A common polymorphism in the aldosterone synthase gene (CYP11B2 –344T) is associated with a raised ratio of 11-deoxycortisol/ cortisol (S/F). We examined the relationship between –344CT polymorphism and corticosteroid levels in decompensated and chronic HF.

Methods
Four hundred and fifty patients were studied during admission at hospital with decompensated HF and at a study visit 4 weeks after their discharge. Blood samples were collected on each occasion for corticosteroid and DNA analysis. Data were compared by the Wilcoxon and Mann-Whitney test between and within hospital and study visit respectively.

Results
Plasma cortisol concentration was higher and S/F was lower in the hospital than in the study visit (Table). Cortisol levels were not different between genotypes at hospital but 11-deoxycortisole levels and S/F were higher in TT than CC subjects. At study visit, cortisol levels were lower in TT subjects than other genotypes. 11-Deoxycortisol levels were not different between genotypes but the S/F ratio tended to be higher in TT patients reflecting the lower cortisol levels.

P349
Urinary steroid metabolite profiling in 11β-HSD1 and H6PDH transgenic mice
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11β-Hydroxysteroid dehydrogenase type 1 (11β-HSD1) converts inactive glucocorticoids to their active form (cortisone to cortisol in humans, 11-dehydrocorticosterone (11-DHC) to corticosterone in mice), and is dependent upon the presence of cofactor NADPH generated by the enzyme hexose-6-phosphate (H6PDH) for its activity. The 11β-HSD1/H6PDH system is implicated in the pathogenesis of the metabolic syndrome by generating tissue specific glucocorticoid excess. The use of selective 11β-HSD inhibitors therefore offers a novel therapeutic approach. By breeding 11β-HSD1/H6PDH double heterozygous mice on a mixed C57BL/6J background we have generated 11β-HSD1-/-/H6PDH-/- double KO and all conceivable intermediate genotypes (n=5) and assessed their urinary steroid metabolome using GC/MS. This enables us to further understand the role of these enzymes in glucocorticoid metabolism and hypothalamo-pituitary–adrenal axis drive. In H6PDH-/- mice the direction of 11β-HSD1 is reversed and glucocorticoid is inactivated, whereas 11β-HSD1-/- mice only fail to re-activate. Both 11β-HSD1-/- and H6PDH-/- mice have reduced corticosterone metabolites compared to WT animals (% corticosterone metabolites: WT 96.1 (11β-HSD1-/-), 67.8 (H6PDH-/-) 2.6), with the levels from H6PDH-/- mice being more exaggerated, consistent with increased inactivation of corticosterone. Double KO mice exhibited similar levels to 11β-HSD1-/- mice, consistent with lack of 11β-HSD1-/- (% corticosterone metabolites: 11β-HSD1-/-/H6PD 58.2). Interestingly, urinary metabolites from the intermediate genotypes 11β-HSD1-/-H6PDH+/-, H6PDH-/- and 11β-HSD1-/- H6PDH-/- were comparable to those of WT mice (% corticosterone metabolites: 11β-HSD1-/- 96.8 (H6PDH+/-), 98.8 (11β-HSD1-/-H6PDH+/-) and 100.0). Therefore, loss of one allele from either 11β-HSD1-/- and/or H6PDH does not alter the urinary steroid profile of 11β-HSD1 and corticosterone in mice. Further studies are required to determine the relationship between 11β-HSD1 gene expression and urinary metabolites, and whether this can be applied as a therapeutic tool to assess levels of 11β-HSD1 enzyme activity.

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Epitopes of pterin-dependent hydroxylases in autoimmune polyendocrine syndrome type 1
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Background
Autoimmune polyendocrine syndrome type 1 (APS1) is a disorder caused by mutations of the autoimmune regulator (AIRE) gene, that controls central tolerance. Tetrahydrobiopterin (BH4)-dependent hydroxylases, consisting of tryptophan hydroxylase (TPH1 and TPH2), tyrosine hydroxylase (TH) and phenylalanine hydroxylase (PAH) are commonly targeted autoantigens. Nevertheless, detailed characterization of their epitopes and independent roles of TPH isoforms has not been systematically studied. We aimed to localize the epitopes of these enzymes and explore the independent of TPH2 in APS1.

Materials and methods
We obtained sera from 51 APS1 patients. The chimeras were composed of N-PAH fused with the catalytic domain of the respective antigen. Recombinant antigens for wild-type enzymes, truncated fragments and chimera were utilized in RIA. The ethical clearance was granted by Health West Region, Norway.

Results
The prevalence of antibodies against wild-type enzymes was 65% (33/51) and for individual antigens were 24/51 (47%), 24/51 (47%) and 21/51 (41%), respectively for TPH1, TPH2 and TH. One patient reacted exclusively against TPH2. N-PAH did not show any immunogenicity. The N-TPH1 exhibited low reactivity (n=8, median 14%, range 2–43%) while its chimera preserved reactivity in most sera (n=8, median 52%, range 35–62%). The N-TPH2 displayed significant reactivity in a half of the sera while others showed complete loss (n=10, median 57%, range 0–83%). The chimeric TPH2 appeared less immunogenic (n=10, median 28%, range 0–82%). The N-TH seemed more immunogenic (n=9, median 48%, range 0–55%) while TH chimera retained low reactivity (n=9, median 14%, range 10–45%).

Conclusion
Autoantibodies targeting BH4-dependent hydroxylases are frequent in APS1 and TPH2 is a novel autoantigen in APS1.

P351

Responses of putative adrenocortical stem/progenitor cells and transiently amplifying cells to ACTH
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Steroidogenic cells of the adrenal cortex are thought to originate from a peripherally-located self-renewing population of undifferentiated stem or progenitor cells. These cells divide infrequently to give daughters, which proliferate transiently (termed transiently amplifying (TA) cells), migrate and differentiate to replenish the functionally-differentiated zones of the adrenal cortex, or remain in situ as undifferentiated stem cells. Theoretically such stem cells can be identified as taking up and retaining a bromodeoxyuridine (BrDU) label during infrequent cell divisions, but which, unlike terminally differentiating steroidogenic cells, do not migrate. After one week’s BrDU infusion into female mice, immunostaining of adrenocortical tissue sections showed that the majority of BrDU-labelled cells were located in the zona glomerulosa (ZG) and outer zona fasciculata (ZF). Six weeks following infusion, BrDU-labelled cells were distributed throughout the cortex, though a proportion remained in the ZG.

Co-staining for aldosterone synthase (ZG-specific) and 21-hydroxylase (expressed by all steroidogenic cells) showed that very few of the BrDU-labelled cells in the ZG stained for aldosterone synthase, indicating that most were relatively undifferentiated. However, most BrDU-labelled cells in the inner ZF co-stained for 21-hydroxylase suggesting that they were at least partially differentiated. The propensity of adrenocortical BrDU-labelled cells to proliferate further was tested by injecting mice with ACTH. Four hours after treatment, BrDU-labelled cell numbers were increased significantly in the ZG and in the outer and inner ZF; however additional staining for Ki67 (a marker of actively proliferating cells) indicated that ZF cells had responded more actively to ACTH.

Conclusions
(1) BrDU-labelled/non-steroidogenic/ACTH-responsive cells in the ZG/outer ZF may represent stem/progenitor cells.
(2) BrDU-labelled/steroidogenic/ACTH-responsive cells in the inner ZF may represent partially-differentiated late TA cells.

P352

Assessing adrenal status in patients before and after coronary artery bypass graft surgery
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Background
Cortisol is an essential stress hormone and deficient patients suffering a systemic inflammatory response (SIR) will rapidly die if not replaced. However, controversy remains on the definition for a normal adrenal response in critically ill patients. We investigated cortisol status in patients undergoing coronary artery bypass surgery (CABG), surgery frequently associated with a SIR, varying in severity from sub-clinical, to life-threatening.

Methods
A prospective study was performed to analyse tests for adrenal insufficiency pre- and post-operatively. Prior to CABG 30 patients had a basal ACTH and a short Synacthen test (250 μg, i.v.). After being weaned off cardiopulmonary bypass, patients were transferred to CICU, and had a post-op ACTH and Synacthen test around 4 h from time of induction. A 50 min cortisol post-Synacthen < 550 nmol/l was taken as an abnormal response. Intensive care monitoring parameters were described.

Results
Prior to surgery all patients had a normal response to Synacthen with a peak cortisol > 550 nmol/l. In contrast, post-op, eight patients (26.7%) did not obtain stimulated-cortisol levels > 550 nmol/l. 11/22 in those with a response to Synacthen > 550 nmol/l and 5/8 in those with a response < 550 nmol/l needed inotropes with a significant difference in time on inotropes (8.4 vs 21.0 h, P=0.05) and time to extubation (5.6 vs 11.7 h, P<0.001). Notwithstanding, all patients had a good final outcome from surgery. Interpretation
The results show that up to a quarter of patients with a normal pre-operative ACTH and cortisol response to Synacthen show a raised ACTH and apparent deficient cortisol response post-operatively. In conclusion, caution needs to be taken interpreting endocrine tests post major surgery. Future studies need to focus on the ability of tests to predict outcome from steroid intervention.

P353

Ongoing symptoms in treated Addison’s disease significantly impair health-related quality of life
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Impaired quality of life in patients with treated Addison’s (primary adrenal insufficiency) has been identified in several recent studies, which have also identified high rates of working-age disability. However, causation in patients whose replacement endocrine medications appear adequate remains unclear. To try and identify factors influencing this reduced quality of life, we analysed demographic information reported by a UK patient sample (n=485), drawn from an international survey conducted in 2003, and compared them to a well-matched control group (n=327).

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Among Addison’s patients, those in paid employment (n = 219) typically report fewer ongoing symptoms than those outside the paid workforce, with those reporting they are unable to work through disability (n = 51) reporting the highest rates of symptoms such as fatigue, dizziness, nausea, hyperpigmentation, joint pains, diarrhoea, headaches, difficulty concentrating and difficulty in recovering from illness. Those unable to work through disability also reported a greater frequency of adrenal crises requiring hospital treatment. Those in skilled occupations typically reported fewer ongoing symptoms and fewer adrenal crises than those in unskilled occupations, although these differences were not statistically significant.

Associated autoimmune conditions such as diabetes and asthma contributed to a greater frequency of ongoing symptoms, as did a body mass index above 30. However, Addison’s patients with no associated conditions and a body mass index below 30 (n = 175) also reported rates of ongoing symptoms that were significantly higher than all controls (n = 316). Patients who self-identified with above-average fitness (n = 29) reported the fewest ongoing symptoms, but at significantly higher levels than very fit controls (n = 35). These findings suggest that current steroid therapy offers only a partial solution to the demands of physiological replacement and that patient education regarding medication management and crisis prevention remain important responsibilities for the endocrinologist.

**P354**

Low testosterone and androgen receptor insensitivity results in decreased AMP-activated protein kinase activity (AMPK) in the liver in the testicular feminised (Tfm) mouse

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There are controversial data on the beneficial/detrimental effects on the cardiovascular as well as on the metabolic system by the sex hormone testosterone. Recent data support the hypothesis that low levels of androgens are associated with adverse cardiovascular risk factors including an atherogenic lipid profile, obesity, insulin resistance and hypertension. AMPK is a sensor of energy balance at both the cellular and whole-body level. Once activated by low energy status, it switches on ATP-producing catabolic pathways and switches off ATP-consuming anabolic processes. Hormones are known to influence AMPK. The mechanism by which testosterone exerts its beneficial effects on both the cardiovascular system and the metabolic system are still unclear. We are hypothesising that testosterone produces its beneficial effects via AMPK activation. For this purpose we utilised the testicular feminised mouse (tfm) which have a non-functional androgen-receptor, low endogenous-testosterone and reduced levels of 17α-hydroxylase. All animal groups including XY-placebo, tfm-placebo, XY-castrate, tfm-sham operated and tfm-receiving physiological testosterone replacement were fed a cholesterol-enriched diet for 28 weeks. Serum levels of total cholesterol (TC) were elevated in the tfm-placebo mice compared to XY-placebo. No significant differences in TC were detected between tfm mice receiving testosterone replacement and tfm-placebo. However, the levels of high-density-lipoprotein-cholesterol were significantly raised in tfm mice receiving testosterone replacement compared to tfm-placebo mice. Liver, heart and abdominal fat tissues were collected to investigate the AMPK activity and mRNA expression of genes that are regulated by AMPK such as sterol regulatory element binding protein-1c (SREBP-1c, a transcription factor that regulates lipogenic genes) and phosphoenolpyruvate carboxykinase (PEPCK, a key enzyme in gluconeogenesis).

In the liver, AMPK activity was low in the testosterone-deficient animal groups (tfm-placebo, XY-castrate and tfm-sham operated animals). This effect was possibly mediated via the AR pathway, since AMPK activity was also decreased in tfm mice receiving physiological testosterone replacement. No significant changes on the AMPK activity were observed in the other tissues. Surprisingly, the mRNA expression of SREBP-1c and PEPCK was also decreased in the low testosterone animal groups. Taken together, reduced liver AMPK activity was found in testosterone deficient mice but lipogenic enzymes were also down-regulated possibly due to lipid influx from the circulation or via alternative mechanisms.

**P355**

Salivary annexin 1 has a diurnal rhythm but does not share an awakening response with cortisol at 30 min

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Several hormones, including cortisol, have a circadian rhythm in plasma and saliva. Salivary cortisol is now extensively used for screening and following patients with various conditions where the regulation of hypothalmo-pituitary–adrenal axis is abnormal, for example in major depression where salivary cortisol levels show a disruption or loss of the circadian rhythm.

Annexin 1 is a glucocorticoid-induced protein, which is responsible for mediating several anti-inflammatory actions of glucocorticoids as well as being involved in glucocorticoid negative feedback inhibition. We have previously demonstrated that annexin 1 is present is produced by the submandibular salivary gland and is present in human saliva. We also presented pilot data from six control subjects demonstrating a positive correlation with salivary cortisol, suggesting that cortisol may be regulating salivary annexin 1.

Our objectives were to i) to determine whether it has a diurnal rhythm in secretion which correlates with salivary cortisol and ii) determine if salivary annexin 1 has an awakening response.

We sampled the salivary of 23 healthy subjects at i) time of waking (Time 0), ii) 30 min after waking (30 min) and iii) immediately before sleep (Bed) and measured annexin 1 using an in-house sandwich ELISA and cortisol by commercial EIA (Salimetrics, UK).

Salivary cortisol values demonstrated a significant increase between waking (0.326 ± 0.0436 μg/dl) and 30 min (0.591 ± 0.0645 μg/dl) indicating an awakening response. The confirmation of a diurnal rhythm in cortisol release was determined by a significant decrease in salivary cortisol at the ‘Bed’ sample (0.0521 ± 0.0067 μg/dl) compared to ‘Waking’ sample P < 0.05. Salivary annexin 1 levels in the same set of subjects also demonstrated a diurnal rhythm with waking levels ranging 51.63 ± 11.04 ng/ml falling to 15.16 ± 2.94 ng/ml before sleep P < 0.05. However, an awakening response was not evident in salivary annexin 1, as mean levels measured 42.32 ± 11.14 ng/ml 30 min post awakening. This suggests that salivary annexin 1 possesses a diurnal rhythm, which correlates positively with cortisol over 24 h. However, the awakening response observed with cortisol is not reflected in levels of salivary annexin 1 possibly due to a lag time between cortisol and annexin 1 secretion from the salivary glands.

We are currently exploring the physiological significance of salivary annexin 1 in the oral cavity and suspect that annexin 1 fulfils a local anti-inflammatory role.

**P356**

Use of biofluorescence resonance energy transfer to reveal the structural dynamics of the ACTH receptor complex

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The melanocortin 2 receptor accessory protein (MRAP) is essential for the functional expression of the ACTH receptor/melanocortin 2 receptor (MC2R). The pituitary hormone ACTH acts via this complex to stimulate glucocorticoid production in the adrenal cortex. Using the bioluminescence resonance energy transfer (BRET) system we investigated the formation of MC2R-MRAP homo/heterodimers in living cells and the influence of ACTH on these interactions. ACTH was found to have a significant effect on the BRET signal in cells expressing MC2R and MRAP. However co-immunoprecipitation of the two proteins showed that ACTH does not increase the physical interaction between the MC2R and MRAP. Real time analysis revealed two distinct phases of the ACTH dependent BRET increase. The antagonist ACTH 11–24 only showed the first rapid phase whilst unstimulated cells and cells with ACTH 1–13 (non binding peptide) did not show either. This led to the suggestion that the first phase may be due to receptor binding leading to a transient conformational change of the MC2R, whilst the second phase of BRET increase, which is unique to ACTH 1–39 stimulation may result from the signalling by the activated MC2R. This was further supported by the observation that the ACTH-induced BRET signal was significantly reduced in the presence of the pKA inhibitor KT5720 suggesting that the phosphorylation of the receptor may play a significant role in the rearrangement of the pre-existing receptor-MRAP complex.

We have also shown that the MC2R is able to form homodimers with the BRET signal being further enhanced in the presence of MC2R-MRAP was only found to exist as antiparallel homodimers. We found no BRET signal in cells expressing
parallel MRAP dimers although the signal appeared enhanced in the presence of the MC2R, which suggested that the MC2R was able to homodimerise bringing the MRAP monomers to closer proximity.

P357
6-Phosphogluconate dehydrogenase: an NADPH-generating enzyme in the lumen of the endoplasmic reticulum
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6-phosphogluconate dehydrogenase (6PGDH) is the third enzyme of the oxidative phase of the pentose phosphate pathway. The cytosolic form of this pathway is well characterised, but the details and significance of the endoplasmic reticulum (ER) version are only beginning to be understood. We have previously identified hexose-6-phosphate dehydrogenase (H6PDH), which catalyses the first two steps of this pathway within the lumen of the ER, as a pivotal regulator of ER reductases such as HSD1. The cortisone-reductase activity of HSD1 is dependent on a high NADPH/NADP+ ratio, which in turn is dependent on the NADPH-generating activity of H6PDH. Activity of 6PGDH also generates NADPH, and hence its presence within the ER would be of potential significance. 6PGDH activity has previously been reported in microsomes, but neither the protein nor the corresponding gene has been characterised. Significant 6PGDH activity was observed in microsomes isolated from mouse liver. This activity was lower than the cytosolic activity (44.6 ± 10.6 vs 300.1 ± 15.4 nmol NADPH/min per mg protein, respectively), and was only measurable upon solubilisation – indicative of a true microsomal form. There was no detectable cytosolic contamination of the microsomes, as indicated by either western immunodetection or activity measurements, using the known cytosolic marker, lactate dehydrogenase. On gel filtration and ion-exchange chromatography, the microsomal activity behaved identically to the cytosolic activity. FT-ICR mass spectrometry of tryptic digests of the chromatographically and electrophoretically-separated microsomal form, indicated a protein sequence identical to the cytosolic form. In addition, in western blot analyses, antibody against the cytosolic form of 6PGDH cross-reacted with the active microsomal fractions. These data indicate that microsomal 6PGDH is likely to be derived from its cytosolic counterpart, and suggest the cytosolic 6PGDH can be shuttled into the ER where it can additionally contribute to the NADPH pool for luminal redutases such as HSD1.

P358
Hepatic vein cannulation and stable isotope tracer infusion reveals that liver cirrhosis regeneration by 11β-HSD1 is sustained in obese men with type 2 diabetes mellitus, providing a target for enzyme inhibition
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Inhibitors of 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) are being developed to prevent cortisol regeneration from cortisone in liver and adipose tissue in type 2 diabetes (T2DM). However, the target patient group is uncertain. In obesity 11β-HSD1 activity is increased in adipose tissue but decreased in liver, as judged indirectly by plasma cortisol levels after oral cortisone administration. However, in T2DM, urinary steroid ratios suggest liver 11β-HSD1 may be preserved. To quantify cortisol regeneration in the liver precisely, we cannulated the hepatic vein during stable isotope tracer infusions in obese men with T2DM and lean controls.

Ten obese men with diet- or tablet-controlled T2DM and seven lean healthy controls (BMI 35.0 ± 10 vs 23.5 ± 1.1 kg/m²) were given 1 mg oral dexamethasone the night prior to infusion of cortisol (600 mg) and 91,12,12-3H2-cortisol (46%) at 1.74 mg/h. Blood was obtained from the hepatic vein and an arterial hand vein at steady state. Cortisone (5 mg) was then administered orally and appearance of cortisol in hepatic vein quantified by tracer dilution. Indocyanine green was infused to measure hepatic blood flow. Local ethical approval was obtained. Data are mean ± s.e.m.

Whole body d-cortisol appearance (a specific measure of 11β-HSD1 activity) was increased in obese T2DM (35.0 ± 2.2 vs 28.9 ± 1.4 nmol/min, P < 0.05). Although basal splanchic d-cortisol release was similar (28.8 ± 0.9 vs 29.5 ± 5.9 nmol/min), cortisol appearance in the hepatic vein after oral cortisone was increased in obese T2DM (27.5 ± 13 vs 20.3 ± 23 nmol/min, P < 0.05).

In contrast with down-regulation of liver 11β-HSD1 and lack of change in whole body cortisol regeneration which occurs in euglycaemic obesity, in obese men with T2DM liver 11β-HSD1 is sustained and whole body cortisol regeneration is increased. This further implicate insulin-dependent mechanisms in the regulation of liver 11β-HSD1 in humans, and highlights the potential therapeutic benefit of hepatic 11β-HSD1 inhibition in obesity-associated diabetes.

P359
Variation in the aldosterone synthase gene may alter gene transcription via altered transcription factor binding
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The aldosterone synthase gene encodes the final step in the production of aldosterone. The aldosterone synthase gene is polymorphic and variants within the gene and the regulatory region have been associated with hypertension and a phenotype of relatively higher level of aldosterone and its metabolites. However to date; none of the polymorphisms in the regulatory region of CYP11B2 have been shown to alter transcription. Seven novel polymorphisms in the promoter region of CYP11B2 have been identified and the aim of this study was to identify those that may cause a change in binding of transcription factors, leading to altered gene expression and the phenotype of hypertension with an increased aldosterone to renin ratio. Polymorphisms were screened for putative transcription factor binding sites using a bioinformatics database (Transfac®). Based on this data, a polymorphism at position –1651 (C/T) was selected for initial binding studies. Electromobility shift assays performed using nuclear extracts from human adrenal cell line (H295R). The C (wild-type) and T (mutant) showed different patterns of binding and nuclear extracts were incubated with 5’biotinylated double-stranded DNA probes and streptavidin-agarose beads in order to identify the bond proteins. The protein–DNA complexes were separated on SDS-PAGE gel and following trypsin digestion peptides were analysed by tandem mass spectrometry. Two peptides were identified which bound to the T oligo only; Apel which functions as a repressor factor, maintaining transcription factors in an active reduced state, and HNRNPK, which interacts with RNA polymerase II transcription machinery, and stimulates transcription. We have confirmed differential binding of adrenal nuclear protein extracts to a polymorphism in the promoter of CYP11B2 and identified candidate proteins. This suggests a mechanism by which polymorphisms in the regulatory region of CYP11B2 may produce a phenotype of relative aldosterone excess and hypertension.

P360
Replicated association of regions at CYP11B1/B2 locus with hypertension in Caucasians
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The locus comprising the genes that catalyse the final steps of cortisol and aldosterone synthesis (CYP11B1 and CYP11B2 respectively) is a plausible candidate risk region for hypertension and other cardiovascular diseases. Nevertheless, there remains uncertainty as to the strength of the relationship between polymorphisms at this locus and increased blood pressure. In this study, association with hypertension at the CYP11B1/CYP11B2 locus in a Caucasian case-control population was tested and replicated in an independent Caucasian population. In the discovery phase, 8 SNPs were genotyped and 29 SNPs imputed in 1643 hypertensive cases and 1697 controls (BRIGHT Study). The SNPs significantly associated with hypertension (rs4110, rs4471016 (~1859A/G), rs43131369 (~1889G/T), rs4711851, rs4546, intron conversion and rs179999 (~344TC)) were tested for replication in 1612 hypertensive cases (NORDIL Study) and 1317 controls (MDC Study). Only the intron conversion showed significant association with hypertension in the replication study, and rs179999 (344TC) analysis. Subjects with the conversion allele in intron 2 of CYP11B2 had a higher risk of hypertension.
Thus, for the first time, the regions most strongly associated with hypertension in Caucasians – one between intron 2 and intron 3 of CYP1B1 and another between the promoter and exon 1 of CYP1B1 – have been replicated. The variability in the strength of these associations suggests the locus is involved in hypertension in a complex manner. Additional functional studies are required to elucidate the mechanism by which these genetic variations lead to alterations in aldosterone and cortisol production and subsequent hypertension.

P361  
Anti-inflammatory effects of SGRMs: are they dependent on DUSP1?  
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Glucocorticoids (GCs) are steroid hormones, products of the hypothalamic-pituitary- adrenal axis. Synthetic GCs, such as dexamethasone, have been widely used in the treatment of inflammatory diseases like rheumatoid arthritis or asthma, but their long-term use causes severe side effects (osteoporosis, diabetes, hypertension, etc.) The ligand-bound GC receptor (GR) can activate gene expression via binding to GC response elements (GREs), a mechanism known as transactivation; or it can inhibit gene expression via interactions with other transcription factors such as NF-kB (known as transrepression). It is commonly thought that transrepression is responsible for the therapeutic effects of the GCs, whereas transactivation accounts for most side effects. Ligands that preferentially induce the transrepression rather than the transactivation function of GR are expected to retain anti-inflammatory properties whilst causing fewer side effects. Such compounds are known as selective glucocorticoid receptor modulators (SGRMs).

We investigated the ability of SGRMs to exert anti-inflammatory effects and to upregulate expression of dual specificity phosphatase 1 (DUSP1), a putative mediator of anti-inflammatory effects of GCs. Although SGRMs were relatively poor activators of a GRE reporter construct, they were able to increase the expression of DUSP1 in different human and murine cell types. The efficacy and potency of this induction varied with cell type and context (absence or presence of a pro-inflammatory stimulus). Moreover, we established a strong correlation between the capacity of GR ligands to induce DUSP1 and their ability to inhibit the expression of the inflammatory mediator cyclooxygenase 2 (COX-2). In conclusion: 1) simplified reporter constructs may not be a reliable guide to the transactivation properties of SGRMs at endogenous GC-responsive genes; 2) transactivation properties of SGRMs may be modulated in a cell type- or context-dependent manner and 3) therapeutic effects of SGRMs may be partly dependent on the upregulation of anti-inflammatory effects such as DUSP1.

P362  
MicroRNA expression profiling of non-tumorous adrenal tissue and modulation of 11β-hydroxylase (CYP11B1) and aldosterone synthase (CYP11B2) genes by miR-24  
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The CYP11B1 and CYP11B2 genes encode 11β-hydroxylase and aldosterone synthase, which catalyse the production of cortisol and aldosterone, respectively, and have been implicated in the development of hypertension. For this study, we wished to investigate the role of microRNAs (miRNAs), a novel class of post-transcriptional gene regulators. To that end, we generated a profile of human adrenal miRNAs to study, and then investigated the action of one adrenal miRNA, miR-24, on CYP11B1 and CYP11B2 genes in vitro. miRNA expression was measured in four non-tumourous human adrenal glands using µParaffin technology microarray. Putative miRNA-binding sites in the CYP11B1 and CYP11B2 genes were identified using four bioinformatic databases. The human adrenocortical cell line, H295R, was transfected with pre-miR-24 (50 nM) and a control, scrambled pre-miR. 48 h Post-transfection mature miR-24, CYP11B1 and CYP11B2 miRNA was measured by qRT-PCR and steroid secretion was quantified by liquid chromatography with tandem mass spectrometry. Cross-referencing of microarray expression and bioinformatic data identified 23 adrenal miRNAs predicted to bind putative sites in CYP11B1 and 16 predicted to bind CYP11B2; 13 of these miRNAs were common to both genes. Transfection of H295R cells with pre-miR-24 produced a significant increase in mature miR-24 expression (P<0.01). CYP11B1 mRNA levels were reduced by 36.1±5.6% (P<0.05) and CYP11B2 mRNA by 36.4±7.3% (P<0.05). The level of secreted cortisol was reduced by 19.6±4.7% (P=0.07) and aldosterone by 15.9±3.1% (P<0.05). The microarray and bioinformatic data have identified several candidate miRNAs that may be involved in regulation of the CYP11B1 and CYP11B2 genes, including miR-24: our in vitro data confirm its regulation of these genes. CYP11B1 and CYP11B2 are therefore subject to regulation by miRNAs and, given the role of these genes in adrenal pathophysiology and hypertension, may prove to be an important therapeutic target.

P363  
Is increased 11β-HSD1 expression a key factor underpinning intrinsic and extrinsic skin aging?  
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Glucocorticoids are highly detrimental to skin integrity and function both when used locally for anti-inflammatory treatments and during conditions of raised systemic concentrations such as Cushing’s syndrome. Many of the adverse effects of glucocorticoids on skin are also symptoms associated with natural intrinsic aging and extrinsic photoaging. Locally, glucocorticoid availability is regulated independently of circulating levels by 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) which activates cortisol from cortisone. Surprisingly, studies investigating 11β-HSD1 activity in a dermal context are limited.

We aimed to characterize the localization of 11β-HSD1 in skin and analyze differential expression between old, young, photoprotected and photoexposed dermal fibroblasts. Immunohistochemistry of full-thickness human skin (Hs) sections (obtained following local ethical approval, n=6) localized 11β-HSD1 to epidermal keratinocytes and dermal fibroblasts. Mouse skin (Ms) sections (n=6) also revealed specific staining in these cell types and in outer root sheath cells. Negligible staining was observed in in vitro control-treated sections or those obtained from 11β-HSD1 knock-out (KO) mice (n=6).

Using radioactive substrate conversion assays, 11β-HSD1 oxidoreductase activity was identified in skin tissue explants from both species (mouse; 150±30 fmol/mg per h, n=4; human, 96±3.6 fmol/mg per h, n=15). Activity was undetectable when samples were co-incubated with an 11β-HSD1 inhibitor or when tissue used was obtained from 11β-HSD1 KO mice. In Hs tissue, there was a positive correlation between 11β-HSD1 oxidoreductase activity and age (P<0.05). This was endorsed by real-time PCR data on primary Hs dermal fibroblasts. Fibroblasts derived from both photoexposed and photoprotected sites indicated that 11β-HSD1 expression increased with age (photoprotected, r²=0.49, P<0.05, n=10; photoexposed, r²=0.78, P<0.01, n=10). Additionally, donor-matched photoexposed fibroblasts displayed 2- to 18-fold higher 11β-HSD1 expression than photoprotected fibroblasts (mean ΔCt, 23.1 vs 20.8 respectively, P<0.05, n=7).

We conclude that age- and site-dependant increases in dermal 11β-HSD1, through increasing local GC activation, may play an etiologic role in the aging skin phenotype.
P364

Functional characterisation of 21-hydroxylase gene mutations is a valuable tool for genetic counselling: in vitro and in silico analysis of six novel CYP21A2 sequence variants

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Congenital adrenal hyperplasia due to steroid 21-hydroxylase deficiency (21OHD) is the commonest inborn error in steroid biosynthesis. It is caused by mutations in the 21-hydroxylase gene (CYP21A2). A good genotype-phenotype correlation exists allowing for prediction of the expressed adrenal phenotype. We performed functional and structural analysis of six novel CYP21A2 variants (p.Try228Cys; p.Asp184Ser; p.Leu198Phe; p.Val305Gly; p.His310Asn; p.Thr443Asn), identified in one individual suspected to suffer from 21-hydroxylase deficiency, two men related to patients with 21-hydroxylase deficiency and in three individuals from the random sample of the general population from Northwest Spain. The novel variants were characterised in vitro using a yeast microsomal assay and in a computational, three-dimensional CYP21A2 protein model. Kinetic assays constants were determined for the conversion of 17-hydroxyprogesterone and progesterone at 0.5, 1, 2 and 5 μM, respectively; p.Try228Cys and p.Asp184Ser reduced wild-type activity to 70-80%; p.Leu198Phe and p.Val305Gly showed enzyme activities similar to the wild-type. The variants p.His310Asn and p.Thr443Asn had 65-70% residual activity. The in silico analysis was consistent with the in vitro findings. In vitro expression analysis revealed that p.Leu198Phe and p.Val305Gly are rare allelic variants not associated with a 21OHD phenotype. The activity of variants p.Try228Cys and p.Asp184Ser are in a borderline area, which has been previously associated with non-classic CAH. Residual enzyme activities of p.His310Asn and p.Thr443Asn are compatible with non-classic 21OHD if homozygous or compound heterozygous. It is unlikely that either of the variants is associated with classic CAH (salt wasting/simple virilising). Thus, our findings demonstrate the importance of CYP21A2 in vitro analyses in the molecular diagnosis of 21OHD to facilitate correct genetic counselling.

P365

Chronic glucocorticoid treatment causes de novo methylation of Tp53 and causes continued reduction in POMC expression after glucocorticoid withdrawal

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Introduction

Long-term repression of the HPA axis is a major side effect of chronic glucocorticoid administration, even after attempted withdrawal of therapy, and complicates management in patients. We have previously shown that the effect of chronic glucocorticoid treatment in vitro is in part mediated by inhibition of expression of the POMC transcription factors Neuro D1 and Tp53, and that this is sustained on treatment withdrawal. We have now further investigated the methylation state of Tp53 after chronic glucocorticoids and using RNA interference have analyzed directly the contribution that it makes to POMC expression.

Method

AT20 cells were cultured with dexamethasone at a dose of 10-6 M for 40 weeks. DNA was extracted at 5, 20 and 40 weeks and bisulphite converted. The methylation of Tp53 was assessed by bisulphite sequencing. siRNAs to Tp53 were used to assess the specific contribution made by Tp53 to the expression of POMC

Results

Tp53 undergoes de novo methylation after chronic treatment with dexamethasone after 40 weeks 46% of CpG sites were methylated in the treated group compared with 0% in the non treated groups; at 20 weeks 43% and at 5 weeks there is very minor areas of methylation (only 3 CpG sites in one clone). In separate experiments knockdown of Tp53 by RNA interference caused a 60% reduction in expression of POMC, confirming the critical importance of this factor.

Conclusion

We have now shown, for the first time, that glucocorticoid exposure causes de novo methylation of Tp53, associated with silencing of this gene and a dramatic reduction in POMC expression. To our knowledge this is the first example where hormonal exposure causes a DNA epigenetic imprint. Moreover, this may have clinical implications for use of demethylating agents in the context of steroid withdrawal.

P366

Spectrum of thyroid disorders in Ohiafe University Teaching Hospital Complex, Ile-Ife

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Introduction

Thyroid dysfunctions are relatively common presentations in Endocrine Practice. They are second to diabetes mellitus (DM) among endocrine disorders. However, management often involves the endocrinologists and surgeons.

Aims

The aim of this review is to determine the spectrum of thyroid disorders and the pattern of presentation seen in our hospital.

Method

It is a five (5) years study (June 2004–April 2009) that looked at the records of patients presenting with thyroid disorders in the medicine and surgery departments. The review excluded the pediatrics and obstetrics and gynaecology departments.

Results

Ninety-two case notes out of one hundred and one patients with thyroid disorders were reviewed. Females were 79 (85.9%) and males 13 (14.1%). Female to male ratio was 6:1. Age range 40–60 years has highest number of cases, 44 cases (47.8%) followed by the age range 21–40 years, 39 cases (42.4%). The general age range was between 17 and 79 years with mean age of 41.1 years. However female age range was between 17 and 79 years while male age range was 29 and 71 years showing that females present earlier. Osun state has largest number of cases, 37 patients (40.2%) followed by Ondo, 25 (27.1%) patients and Ekiti, 9 patients (9.8%) in that order. Of the occupation group of those that presented, traders had highest number with 41cases (44.5%) followed by civil servants 24 cases (26.1%).

The commonest symptom at presentation was anterior neck swelling, 89 cases (96.7%) while 6 cases (6.5%) presented with proptosis, palpitation and other thyrotropic features. Simple multinodular goitre is the commonest thyroid disorder at diagnosed with 76 cases (82.6%).

Conclusion

Simple multinodular goitre is the commonest form of thyroid disorders presenting in our hospital.

P367

Expensive but unreliable: Wayne’s index to the rescue!

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Background and objectives

Thyroid function tests (TFTs) are expensive in Nigeria. The reliability of the test is sometimes called into question when at variance with clinical findings. The Wayne’s clinical index is an old diagnostic index to facilitate objectivity and improve the accuracy of clinical assessment of thyrotoxicosis. This report highlights the unreliability of a TFT result in a case of thyrotoxicosis and the usefulness of the clinical index.

Subject

A 40-year-old cleaner and mother of three presented with 9 years history of anterior neck swelling. There was heat intolerance, excessive sweating, palpitations, generalized weakness and weight loss despite an increased appetite. She had a nodular thyroid swelling. No bruit or eye signs. She had tremors of the hands with moist palms. The pulse rate was 104 beats/min. A diagnosis of toxic multinodular goitre was entertained.

Results

Table 1: Thyroid function test

<table>
<thead>
<tr>
<th>Test type</th>
<th>Results</th>
<th>Reference values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Free T3</td>
<td>1.8</td>
<td>3-5.2 μg/ml</td>
</tr>
<tr>
<td>Free T4</td>
<td>1.4</td>
<td>0.7-1.7 μg/ml</td>
</tr>
<tr>
<td>TSH</td>
<td>0.1</td>
<td>0.54-3.7 μU/ml</td>
</tr>
</tbody>
</table>

Electrocardiogram: Normal sinus rhythm with ventricular rate of 92 beats/min.

Thyroid ultrasound: Conclusion: multinodular goitre.

Fine needle aspiration Cytology: conclusion: toxic diffuse hyperplasia.

Wayne’s clinical index: Total score = 25.
Conclusions
The Wayne’s index remains a useful diagnostic tool in resource challenged clinics in instances when TFT results are at variance with clinical suspicion. Quality control checks should be ensured in Endocrine function test laboratories.

P368
A case of severe hypothyroidism treated with supervised weekly thyroxine
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A 39-year-old lady with severe primary hypothyroidism was referred to the endocrine clinic. Her past medical history includes intermittent colitis and Irritable Bowel syndrome. There was no history of diarrhoea for the last 1 year. TSH 97.2 mUI (0.3-5.0) and free thyroxine (T4) was 5.0 pmol/l (10-22). The dose of levothyroxine was increased gradually from 100 to 300 mg/day. Despite this, TSH level remained high at 96.1 and freeT4 was 4.0. She felt generally unwell, tired and weak. She denied missing her medications. Weekly supervised thyroxine therapy was started at 1000 µg/week for 1 month. TSH, T4 and T3 were monitored closely at 0, 1 and 4 h. Thyroid functions improved dramatically within 1 month (see Table 1). She is now maintained on 150 µg of thyroxine/day and compliance is retested.

Table 1: Thyroid functions at 0 h

<table>
<thead>
<tr>
<th>Week</th>
<th>T4 (8–19 pmol/l)</th>
<th>T3 (2.1–6.0 pmol/l)</th>
<th>TSH (0.3–5.0 mUI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>3.2</td>
<td>45.40</td>
</tr>
<tr>
<td>2</td>
<td>11</td>
<td>3.7</td>
<td>21.9</td>
</tr>
<tr>
<td>3</td>
<td>13</td>
<td>4.0</td>
<td>13.6</td>
</tr>
<tr>
<td>4</td>
<td>16</td>
<td>4.4</td>
<td>6.26</td>
</tr>
</tbody>
</table>

Discussion
Serum T4 rose significantly in the first hour but started to drop at 4 h. T3 did not change much but the TSH levels improved significantly within a month.

Studies were done with weekly and weekly thyroxine supplementation and doses up to 3 mgms per week were well tolerated. In one study a slightly larger dose than 7 times the normal dose was used for biochemical euthyroidism however in our patient <50% of the total weekly dose was used with good response. Poor compliance is not uncommon in patients with hypothyroid disorder as depression may be a co-morbid state. Supervised weekly thyroxine therapy involves careful monitoring of thyroid functions. Cardiac ischaemia and arthralgies should be ruled out before starting this treatment.

Conclusion
Weekly supervised thyroxine supplementation may improve compliance in selected group of patients with unexplained severe hypothyroidism.

P369
A difficult case of very aggressive thyroid eye disease
Saravanan Balaguruswamy, W M H S Chandrasekara & S McNulty
St Helens and Knowsley NHS Trust, Merseyside, UK.

Thyroid eye disease (TED) is clinically evident in 25–50% of patients with Graves’ disease and 3–5% of cases develop severe eye disease. We present a case of very aggressive TED. A 46-year-old gentleman developed Graves thyrotoxicosis and was blocked and replaced with carbimazole and thyroxine. He continued to smoke 20 cigarettes/ day despite several advices. He developed marked exophthalmos (>2 cm), chemosis, with reduced conven- gence. He was commenced on prednisolone. MRI Orbits showed the medial, inferior and superior recti were enlarged on the left side but no significant compression at the conus. On the right the superior and inferior recti were mildly dilated with well-maintained conus. He developed a few acute episodes of compressive optic neuropathy and proptosis particularly affecting the left eye. He was treated with intravenous methylprednis- olone. His vision improved and the optic nerve heads that were initially swollen with hyperemia began to settle. Azathioprine was commenced. Despite this his left optic nerve head became increasingly swollen. His left eye was touching the lens of his glasses.

CT Orbits showed proptosis, the eyes appeared to have convergent squint. Thickening of conal musculature on both eyes (>2 cm), the abnormality affecting the inferior, medial and superior recti. Towards the optic apex the muscular thickening crowding the optic nerve.

He had left orbital decompression and his optic neuropathy resolved. A few days later he developed pigment epithelial detachment in the left eye as a result of polypoidal choroidal lesion. This was treated with photodynamic therapy. He was clinically and biochemically euthyroid. His vision in left eye is poor 2/60 (6/18 with pinhole), has little vision in right eye 6/12 and restricted movements in all directions.

This case illustrates the difficulties with managing TED, importance of cessation of smoking and highlights the options of surgical management and photodynamic therapy in aggressive conditions.

P370
A case of non-Graves thyroid eye disease
Leila Faghfahati & Ritwik Banerjee
Luton and Dunstable Hospital, Luton, UK.

A 29-year-old pregnant lady known hypothyroid for 5 years, presented with thyroid eye disease (TED). She is a non-smoker. Her TFT in the past years has been stable. Her exophthalmos was noticed at early pregnancy. Her anti-thyroid peroxidase antibodies (TPO-Ab) were >1300 mUI (ref. range <1.4) and TSH-R antibodies (TRAB) were 18.6 U/l (ref. range <5.0).

On presentation she was clinically and biochemically euthyroid with no ophthalmic complaints. She had bilateral exophthalmos. There was lid retraction but no lid lag, diplopia, peri-orbital oedema or visual field deficit. She had a smooth goitre.

Her levothyroxine was increased to 125 µg and dropped back near delivery. She delivered a healthy baby in August 2009 with normal TFT and negative TPO-Ab (TRAB not tested by the obstetricians).

Discussion
Thyroid eye disease is technically known as Graves’s ophthalmopathy. Clinical TED with TRAB positive is likely autoimmune. However there are reports of TED in Hashimoto thyroiditis, thyroid cancer and euthyroid eye disease. The pathophysiology of TED likely involves genetic and environmental factors, which may potentiate cellular and humoral-mediated inflammation within the orbit. A unifying hypothesis of TED pathophysiology is elusive. Our patient has Hashimoto thyroiditis with TRAB positive TED. It is likely that similar autoimmune mechanisms, as in Graves, are behind the Hashimoto’s thyroiditis eye disease, contributed in part by the TRAB. It is possible that other antibodies have a major role to play in TED, irrespective of the thyroid status. This would explain the dichotomy seen in TED with and without TRAB positivity and TED with various thyroid diseases.

Our case illustrates the notion that TED could be present in any autoimmune thyroid disorder, irrespective of the nature of the antibodies present. Further research is needed to look at other etiological factors, which might explain the dichotomy.

P371
The unrestricted use of baseline thyroid function tests in elderly and female patients is justified in the diagnosis of thyroid dysfunction
Ravi Popat, Edward Kearney & Stenny Joseph
Queen Elizabeth the Queen Mother Hospital, Margate, Kent, UK.

Introduction
The symptoms and signs of thyroid disease can be subtle and non-specific resulting in the indiscriminate use of thyroid function tests (TFT) for diagnosis. The resulting rise in the number of TFTs has raised issues about cost-effectiveness of such a practice. We set out to identify whether using specific clinical indications was an effective way to identify patients with abnormal TFTs and to determine any demographic data that would support unrestricted TFT requests in individual patients.

Methodology
Case notes and hospital laboratory data were analysed retrospectively in a 2 months period in 2008 for TFT requests. A request was considered to be justified if the patients met pre-determined clinical criteria. Based on this the groups were divided into justified (J) and unjustified (UJ). Thyroid illness was defined by abnormal FT3 and TSH levels while an abnormal FT4 level with normal TSH defining sick euthyroid syndrome (SES). The number of abnormal TFTs and patient demographics in both groups were determined. Group difference was analysed by Students t-testing with a P value of <0.05 significant.

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Results
Two hundred and sixty-five frontline requests were identified. The proportion of abnormal TFTs and SEIs in the J and UJ groups was similar. Abnormal TFTs was three times more likely in the female and elderly (above 80) patient irrespective of group.

<table>
<thead>
<tr>
<th></th>
<th>Thyroidal illness</th>
<th>Sick euthyroid syndrome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Justified tests</td>
<td>127 (47.9%)</td>
<td>5 (1.8%)</td>
</tr>
<tr>
<td>Unjustified tests</td>
<td>138 (52.1%)</td>
<td>5 (1.8%) P=0.89</td>
</tr>
<tr>
<td>Male</td>
<td>100 (38%)</td>
<td>2 (0.7%)</td>
</tr>
<tr>
<td>Female</td>
<td>165 (62%)</td>
<td>8 (3.0%) P&lt;0.02</td>
</tr>
<tr>
<td>Age &gt;80 years</td>
<td>107 (40.4%)</td>
<td>8 (3.0%)</td>
</tr>
</tbody>
</table>

Conclusion
Our study demonstrates that the use of specific clinical indications may not be a reliable measure for abnormal TFTs in the elderly and female patients. There is a suggestion that the unrestricted request of TFTs in this group of patients will result in a higher diagnostic rate compared to when specific criteria are applied before testing.

Methods
We selected a list of 25 patients who had FNA of thyroid nodule over a 6 months period between July 2008 and December 2008 and analysed if the procedure had been done in accordance with current Royal College of Physicians guidelines for FNA Thyroid.

Results
1. None of the cytopathology reporting were done by histopathologist interested in thyroid disease.
2. 96% of procedures were done by physician interested in thyroid disease.
3. Only 24% of cytopathology requests had clinical details; 12% had details of aspiration, and 12% had details about site of aspiration. None conveyed if there was any resolution of mass.
4. 100% of all reports from Histopathology Department included description and numerical coding.
5. 56% of first attempts of FNAs were non-diagnostic. Still among them only 35% had repeat FNA with ultrasound guidance.
6. 12% were found to have follicular cells in FNAC. One among them did not have lobectomy. Rest were non malignant.
7. None of them had clear evidence of malignancy.

Conclusion
1. It is clear from the analysis that majority of flaws are in the area of entering clinical details, and details of aspiration.
2. Histopathology reports are done by any histopathologist and it is not always possible to get histopathologist interested in thyroid disease.
3. All reports have description of cellular picture and numerical coding.
4. Although initial procedure is non-diagnostic in 56%, there was still reluctance in using ultrasound guidance.
5. Care need to be taken when dealing with FNAs showing follicular cells as one patient in our audit was not referred to surgeon immediately. These patients need to be referred urgently.

P372
Technetium pertechnetate scanning in the differential diagnosis of benign thyroid disease
Caryn Wujanto, Edward Wallitt & Khalid Ahmed
West Middlesex University Hospital, Middlesex, UK.

Background
Radionuclide thyroid scanning using technetium pertechnetate (Tc99m) is a useful aid in the differential diagnosis of benign thyroid disease. It is particularly useful in the differentiation of the causes of a thyrotoxic biochemical picture, e.g. viral thyroiditis, toxic nodular disease or Grave’s disease. This has implications for long-term management plans. However, its cost and exposure to radiation necessitate the need to justify its use in the investigation of thyroid disease.

Methods
A retrospective study of 70 Tc99m reports was performed. Indications for the scan and the results were recorded. All Tc99m scans were performed in the same nuclear medicine department.

Results
The indications for requesting a Tc99m scan included the differential diagnosis of thyrotoxicosis in 39 (55.7%) patients, subclinical thyrotoxicosis in 22 (31.4%) and 9 (13%) further scans were performed but the indications were not clear and probably unjustified.

Of 39 scans performed to define the cause of thyrotoxicosis, 16 (41%) showed multinodular disease, 15 (38.5%) Grave’s disease, 1 (2.5%) thyroiditis and 7 (18%) were indeterminate.

Of 22 scans performed to define the cause of a persistently suppressed TSH with normal free T4 and free T3, 14 (63.6%) showed features of nodular autonomy, 3 (13.6%) were consistent with Grave’s disease, 1 (4.5%) showed a medistinal ectopic hot spot and 4 (18.2%) were reported as showing normal uptake. Of the 14 patients suspected of having Grave’s disease clinically, 9 (64.3%) were confirmed to have features consistent with Grave’s disease on Tc99m scanning.

Conclusion
Technetium scan can be useful as a supplementary investigation to clinical, biochemical and ultrasound examination in aiding the differential diagnosis of thyroid disease and subsequent planning of therapy. There should be clear guidelines on the appropriate use of this modality to avoid unnecessary exposure to radiation and cost. We intend to set such guidance locally.

P374
The many faces of hyperthyroidism in primary care
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1Department of Endocrinology, St Heiler Hospital, Carshalton, Surrey, UK; 2Shadbolt Park House Surgery, Worcester Park, Surrey, UK.

Introduction
Hyperthyroidism may mimic many conditions, and there is potential for under-diagnosis if testing is considered only in patients presenting with classical symptoms.

Aim
To describe the range of presentations in patients found to have hyperthyroidism and referred from primary care.

Method
Consecutive patients diagnosed with hyperthyroidism and referred to the endocrine clinic between Jan 2009 and Nov 2009 were studied. Patients were asked to describe their reasons for seeking medical help and any symptoms at presentation using a standard questionnaire. Clinical information including diagnosis and results of investigations were recorded. ‘Typical symptoms’ were defined as those described in a standard on-line GP reference (GP Notebook).

Results
Eighty hyperthyroid patients (F/M 57:23, age 25-91) were studied. Diagnoses were Graves’ disease (58), toxic goitre (14), thyroiditis, (4) hashitoxicosis (2) and amiodarone-induced (2).

For the whole group, the most common presenting symptoms were weight loss (21%), fatigue (15%), palpitations (10%), incidental finding (9%), breathlessness (6%) and eye problems (6%). On direct questioning, weight loss, palpitation, tremor, fatigue and heat intolerance were present in nearly half of all patients.

Presentations in 47 (59%) patients were classified as typical. In nine patients, thyroid function tests were ordered as part of routine screening (on amiodarone, well-person check, diabetes, family history); five of these were asymptomatic. Toxic nodular goitre was present in six of these patients.

In 24 patients, the main presenting symptoms were not typical of hyperthyroidism; routine thyroid tests had been performed in the work-up for non-specific complaints. The most frequent symptoms were non-specific headache (5), weight gain (4), swollen ankles (3) and vomiting (2). Other unusual presentations included anorexia, hyperosmololence, psychosis, menorrhagia, depression, ‘out of body experience’ and impotence. Hyperthyroidism was an unexpected finding in four cases presenting with other pathologies.

P373
Audit on fine needle aspiration of thyroid
Kumar Thulasidas & Penny Hyatt
North Middlesex University Hospital, London, UK.

Aim
To audit if fine needle aspiration of thyroid nodules are done as per the Royal College of Physicians Guidelines.

Endocrine Abstracts (2010) Vol 21
Conclusion
Hypothyroidism may present in many guises and detection requires a high degree of clinical suspicion.

P375
A case of spousal abuse secondary to thyrotoxicosis
Darshna Patel & Jackie Gilbert
King’s College Hospital NHS Foundation Trust, London, UK.

A 24-year-old female make-up artist presented with a 6 months history of emotional lability, heat intolerance, weight loss and shaking of the hands. She described a deteriorating relationship with her spouse and increasing difficulties performing her professional role. On examination, she demonstrated marked agitation, tremor, sweating and tachycardia. Biochemistry confirmed severe thyrotoxicosis (FT3 84 pmol/l, TSH <0.01 mU/l). Subsequent clinic attendance and compliance with carbimazole were extremely poor. After 6 months, the patient’s thyroid function tests were unchanged. She had been made redundant (her employer assumed she was abusing illegal substances) and she reported that several friends had broken down irretrievably. The frequency of conflict between the patient and the thyroid specialist nurse was subsequently increased significantly. A combination of ‘carrot and stick’ strategies were employed including daily reassurance, reminders and encouragement as well as frank consideration and discussion of the evident deleterious medical and psychosocial effects of uncontrolled thyrotoxicosis. This resulted in improved medication compliance and facilitated the completion of definitive treatment (a total thyroidectomy). Post-operatively, the patient’s partner reported that in the months prior to treatment adherence, the patient had been increasingly abusive towards him: verbal abuse had escalated to threatening behaviour and had culminated in an attempt to inflict severe physical injury. There was no preceding history of violent behaviour, which stopped following restoration of euthyroidism. Psychiatric symptoms may occur in up to 10% of patients with thyrotoxicosis. Features include anxiety, depression, mixed mood disorders, mania and cognitive impairment. Paranoid and schizophreniform manifestations are less common. The frequency of violent behaviour against a spouse, precipitated by thyrotoxicosis, is unknown and likely to be under-reported. Asking open questions regarding changes in behaviour in the presence of attending family members may encourage the provision of this highly sensitive information and inform more timely planning of treatment.

P376
A 10-year retrospective analysis of variable-dose radioactive iodine therapy for hypothyroidism
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1Department of Chemical Pathology, St James’s Hospital, Dublin, Ireland; 2Department of Endocrinology and Diabetes, St James’s Hospital, Dublin, Ireland; 3Department of Nuclear Medicine, St James’s Hospital, Dublin, Ireland.

Introduction and methods
Controversy exists regarding the optimal dosing regimen of radioactive iodine (RAI) for the treatment of hypothyroidism. The dose of RAI was individualised, based on the size of the thyroid gland and 24-h RAI uptake. We performed a 10-year retrospective analysis of patients with hypothyroidism treated with variable-dose RAI, with a cure defined as euv- or hypothyroidism.

Results
One hundred and forty-nine patients with hypothyroidism were treated with RAI from 1998 to 2008. Of these, complete data are available on 97 patients (mean ± st.dev age, 52 ± 14 years; 57% female). The causes of hypothyroidism were Graves’s disease (61%), toxic multinodular goitre (TMN) (41%) and solitary toxic nodule (TN) (3%). Mean (± st.dev) administered activity of RAI was 237 ± 179 MBq; patients with TMN received over twofold higher dose than patients with Graves’s disease (366 ± 200 and 150 ± 93 MBq, respectively). The mean time from diagnosis to treatment was 6 years. At 10 years follow-up, 63% of patients were cured (34% euthyroid, 29% hypothyroid). More patients with TMN, than Graves’s disease, were cured (82%, 49%, respectively). All patients with TN were euthyroid. Of those 39 patients (37%) who required treatment, nine received a second dose and one a third dose. Of those 10 patients, 8 were cured.

RAI was generally well tolerated. In those patients with pre-existing ophthalmopathy (5%), no worsening of eye disease with RAI occurred.

Conclusions
RAI is not routinely used as first-line therapy in our institution. Using variable dose RAI, we found that at 10 years, 63% of patients were cured, with more success in those patients with TMN and TN.

P377
An unusual presentation of hypothyroidism
Simon Holmes & V K B Prabhakar
Pinderfields General Hospital, Wakefield, West Yorkshire, UK.

An 83-year-old gentleman was referred to the endocrine clinic with incidentally found abnormal thyroid function tests (TFT): TSH <0.02 (0.2–4.0 mU/l), free T3 20.4 (9.19 pmol/l), and free T4 7.6 (2.5–5.7 pmol/l). His T3T done 6 months previously were normal with TSH 1.21 mU/l and FT3 11.8 pmol/l. His past medical history included BPH, peripheral vascular disease and chronic kidney disease (CKD), and medications were tamulosin, finasteride, aspirin, and lansoprazole. He denied having symptoms of hypothyroidism, or family history of thyroid dysfunction. He was clinically euthyroid with no goitre or dysthyroid eye disease, but had bilateral gynaecomastia, normal body hair and testicular volume. Direct enquiry revealed on-going breast enlargement for 2–3 months; there was no recent change in his drug history. Subsequent tests confirmed hypothyroidism with TSH <0.02 mU/l, FT3 20.4 pmol/l and FT4 6.9 pmol/l. Other tests included negative thyroid peroxidase antibodies, normal liver function tests, stable CKD stage 3, normal HCG, LH 13.4 (2.10 IU/l), FSH 12.6 (2.12 IU/l), testosterone 10.2 (8–30 nmol/l), estradiol 98 (0–150 pmol/l) and SHBG 116 (14–71 nmol/l). A diagnosis of gynaecomastia due to hypothyroidism was made and carbimazole-therapy initiated. He rapidly developed tiredness, albeit with normal FBC and unchanged TFT, and was changed to propylthiouracil 150 mg/day. After 10 weeks, his biochemical abnormalities and gynaecomastia improved. Thyroid ultrasound revealed an 8×6 mm nodule. Radioactive-iodine is planned as the definitive treatment for hypothyroidism.

Gynaecomastia as the sole manifestation of hypothyroidism is very rare, although a well-documented clinical feature, reported in 2–4% of male patients with thyrotoxicosis. Altered oestrogen-to-androgen ratio, increased SHBG, enhanced peripheral aromatase activity, all contribute to hypothyroidism-related gynaecomastia, but exact mechanism is unknown. Gynaecomastia is common in men over 70 years (up to 55% at autopsy); could be due to ageing-related endocrine/metabolic changes, drugs, medical conditions, etc. Aging, finasteride, lansoprazole, CKD, hypothyroidism – multiple causes of gynaecomastia in our patient but improvement was noted on achieving euthyroidism. It is important to recognise hypothyroidism as a reversible cause of gynaecomastia in the increasing elderly population.

P378
Incidence and predictors of transient hypothyroidism or euthyroidism following radioactive iodine therapy for hypothyroidism
Lakshminarayanan Varadhan, Anantli Nayak, Vijaynandni Cherukuri, Varadharajan Baskar & Harit Buch
Royal Wolverhampton Hospital NHS Trust, Wolverhampton, UK.

Objectives
Transient thyroid hypofunction during the initial 3 months following radioiodine (RAI) therapy is well recognised with a reported incidence of 10–15%. There are no clear diagnostic criteria, often leading to management uncertainty, inappropriate diagnosis of ‘cure’ and institution of life-long thyroxine therapy. The aim of our audit was to assess the incidence and identify predictors for transient euthyroidism or hypothyroidism following administration of a standard 400 MBq RAI therapy.

Methods
Retrospective audit of database maintained on all patients who have received RAI for management of hypothyroidism over the past 6 years.

Results
Fifteen of the 161 (9%) patients developed transient hypothyroidism or euthyroidism at the stage of 6 weeks or 3 months post-RAI although this is likely to be an underestimate. Nine (5.5%) had spontaneous conversion from hypothyroidism to either euthyroidism or hypothyroidism and six (3.7%) from euthyroidism to hypothyroidism. Patients who developed transient
hypothyroidism or euthyroidism and those who did not, were comparable in terms of age (mean 49 vs 52 years respectively) gender (% male -13 vs 21) and aetiology (% Graves’ disease - 61 vs 60) (P=NS for all). There was no difference in the proportion of patients who developed transient hypothyroidism in our current cohort (fixed RAI dose of 400 MBq) as compared to our earlier cohort who received calculated dose RAI therapy (mean RAI dose 267 MBq). Patients who developed transient hypothyroidism or euthyroidism were more likely to have a large goitre (40 vs 19%), had a higher 24-h radioactive uptake (mean 65 vs 55%) and a higher pre-RAI free T4 level (60 vs 34 pmol/L) (P<0.05 for all).

Conclusion
A significant proportion of hyperthyroid patients develop transient euthyroidism or hypothyroidism within first 3 months post-RAI. Patients with positive predictors should be made aware of this and delaying thyroxine supplementation should be considered.

P379
The safety and efficacy of iopanoic acid in the pre-surgical management of thyrotoxicosis
Ravi Sankar Erudugulati, Srikantth Mada & Simon Ashwell
James Cook University Hospital, Middlesbrough, UK.

Background
Iopanoic acid is an iodinated oral cholecystographic contrast agent, which can be used for the rapid blockade of thyroid hormone production in preparation for thyroidectomy, especially in patients who are intolerant of thionamides. However, appropriate timing of thyroid surgery is vital.

Project
In this retrospective audit, we identified all patients with thyrotoxicosis who received pre-operative preparation with iopanoic acid from 2006 to 2009 and evaluated appropriateness of patient selection, administration, monitoring, time to normalisation of FT3 and FT4, levels, timing and complications of thyroid surgery.

Outcomes
We identified five patients. All patients had Graves’ disease. The indication for iopanoic acid-assisted thyroidectomy was hypothyropenia on thionamides (n=1), and uncontrolled thyrotoxicosis despite thionamides (n=4). The mean time from commencement of iopanoic acid to thyroidectomy was 13.6 days (range 10-20 days, median 12 days). By the time of thyroid surgery, 100% patients achieved FT3 levels within the reference range and 40% achieved normal or near-normal FT4 levels. Time to normalisation of FT3 levels correlated with baseline FT3. There were no anaesthetic or surgical complications.

Conclusions
Preoperative rapid blockade of thyrotoxicosis with iopanoic acid is safe and efficacious in a supervised environment. The optimum duration of treatment is 12-14 days. However, there is a positive correlation between the pre-iopanoic acid FT3 and FT4 levels and time taken for their normalisation. FT3 levels appear to be a better indicator of safety for surgery than FT4. This audit assists in the planning of the timing of thyroidectomy when iopanoic acid is used.

P380
Audit of TSH-receptor antibodies and 99m technetium pertechnetate scintigraphy in the diagnosis of thyrotoxicosis aetiology
Anjali Amin 1, Louise Newlands 1, Ranju Dhawan 1, Jeremy Cox 1
1 Imperial College AHSC Trust, London, UK; 2 Imperial College London, London, UK.

Aim
In the thyrotoxicosis local investigation protocol, Tc99m pertechnetate scintigraphy technetium scan is used to assess the presence of thyroid uptake and both the degree and pattern of uptake. TSH receptor antibodies are used to demonstrate the presence and activity of autoimmune thyroid disease. We audited the utility of these investigations in a series of thyrotoxic patients.

Methods
Forty-nine patients (11 men, 38 women, mean age 47.5 ± 38) were studied, who were in first presentation of biochemically confirmed thyrotoxicosis. Patients were assessed clinically and a clinical diagnosis made based upon history and examination. TSH receptor antibodies and TPO antibodies were measured; a Tc99m pertechnetate technetium uptake scan was performed to assess degree and pattern of uptake.

Results
Of the 49 patients studied, uptake scans were performed on 39 patients; of these, 7 demonstrated low uptake, 1 showed a toxic multinodular goitre, 5 showed an autonomously functioning thyroid nodule (AFTN) and 26 had diffuse pattern of increased uptake. TSH receptor antibodies were measured in 38 patients, and TPO antibodies measured in 46. Our results showed that, when assessing the aetiology of thyrotoxicosis, the TSH receptor antibody has 90.5% specificity but only 75.0% specificity when technetium uptake scan was used as definitive diagnosis. TPO antibodies were found to be more specific (100%), but less sensitive (53.8%) in predicting autoimmune thyroid disease.

Conclusion
TSH receptor antibodies have been shown to have a useful role in the monitoring of autoimmune thyroid disease. This test may be positive with AFTN and should be interpreted with other investigations in thyrotoxic patients.

P381
Do we warn patients about the risk of neutropenia associated with the use of anti-thyroid drugs?
Muhammad Butt, Itopa Abedo, Fong Chau & Andrew Johnson
Southmead Hospital, Bristol, UK.

Audit standard
All patients should receive written and verbal advice about the risk of neutropenia associated with the use of anti-thyroid drugs. Compliance rate 100%.

Background
Our Endocrinology Department has a detailed patient information sheet that is provided to the patients at the time of initiation of anti-thyroid drugs.

Methods
We retrospectively reviewed the clinic letters and notes of all new patients who were seen in our endocrine clinics between January and December 2008 (n = 69) and prescribed anti-thyroid drugs.

Results
Anti-thyroid drugs were started by the general practitioners in 50 patients (72%) and by the endocrinologists in 14 patients (21%). It was not possible to identify where anti-thyroid drugs were started in five patients (7%). Neutropenia advice was documented in clinic letters and notes in 30 (43%), and 18 patients (26%) respectively following their first clinic appointment. There was no documentation of neutropenia advice in clinic letters and notes in 21 patients (30%). In 18 patients (19%), there was no documentation of advice either in clinic letters or notes even after their third clinic appointment.

Conclusion
Our audit confirms poor documentation of neutropenia advice in both clinic letters and notes.

Action plan
1. We have developed a system with the help of IT department that allows our secretaries to automatically add the warning information into the clinic letters whilst typing.

We have prepared a medic-alert card that is given to all patients during their first consultation. This card provides them precise information about the side effects of anti-thyroid drugs and actions to undertake should they experience any side effects.

P382
Severe thyrotoxicosis due to metastatic differentiated thyroid carcinoma
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Differenitated thyroid cancers function less well than normal thyroid tissue but continue to secrete thyroglobulin (Tg) which can be iodinated to form thyroxine (T4) and triiodothyronine (T3). Functioning metastases causing thyrotoxicosis are rare. The majority of reported cases have large volume, metastatic follicular tumours. A significant proportion develop T3 toxicosis with normal T4 levels. T3 toxicity is often mild. Clinical presentation is similar to that in other patients with thyrotoxicosis but metastatic disease is present. We describe a case of
metastatic follicular thyroid cancer causing severe thyrotoxicosis and the clinical course following treatment. An 82-year-old man with a large thyroid nodule was treated for thyrotoxicosis with carbamazepine for 12 years. He subsequently developed widespread bone metastases including disease of his cervical spine. Pelvic biopsy confirmed metastatic, well differentiated, follicular thyroid cancer. Following total thyroidectomy at which a 7 cm widely invasive follicular thyroid carcinoma was removed carbamazepine was discontinued. Thyrotoxicosis failed to resolve following surgery. He received propranolol and dexamethasone therapy in preparation for radioactive iodine (150 mCi) 4 weeks later. Whole body scan following radioactive iodine showed intense iodine uptake into bone metastases and no neck uptake. He was overtly biochemically thyrotoxic immediately before receiving radioactive iodine, free $T_3$ 75.2 pmol/l ($12-22$), free $T_4$ 32.6 pmol/l (3.1-6.8), TSH <0.03 mU/l (0.27-4.2), TSH receptor antibody <1 UI/l, Tg 8795 ng/ml with no significant interference from anti-Tg antibodies, but had significantly improved 6 weeks later, free $T_3$ 27.1 pmol/l, $T_4$ 10.1 pmol/l, TSH <0.03 mU/l, Tg 3704 ng/ml.

A patient with metastatic follicular thyroid cancer causing severe thyrotoxicosis is described who following total thyroidectomy improved after radioactive iodine therapy. Medical management immediately before radioactive iodine included dexamethasone and propranolol but carbamazepine was withdrawn. Further radioactive iodine therapy will be administered and when euthyroid may follow recombinant human TSH administration.

P383
Pancytopenia and nephrotic syndrome related to autoimmune hypothyroidism: a case report
Manjusha Rathi & Steve Peacey
Bradford Royal Infirmary, Bradford, UK.

Thyroid hormone exerts direct effects on almost every organ or tissue, and thyroid deficiency produces a wide range of metabolic disturbances. Hyperthyroidism is readily recognized in an individual presenting with characteristic clinical signs and symptoms. However involvement of the haematologic and renal systems is less commonly acknowledged making the diagnosis less apparent and, therefore, the initial focus of attention is on a diagnosis other than hyperthyroidism.

Case report
A 64-year-old woman presented with bilateral ankle oedema and dry skin. Initial investigation revealed heavy proteinuria (4+ on urinalysis), full blood count revealed pancytopenia (Hb - 10.2, WBC - 2.9 and platelets - 127); impaired renal function - serum creatinine 129 µmol/l and eGFR – 38. She was investigated by nephrology services and no primary renal pathology was identified. Subsequently primary autoimmune hypothyroidism was diagnosed and thyroid hormone replacement commenced. This led to complete recovery of the pancytopenia, renal function and resolution of proteinuria.

Discussion
In hypothyroidism, mild degree of anaemia is common and seen in about 30% cases. It is associated with reduced plasma volume, RBC mass and plasma erythropoietin levels. However white blood cells and platelets are usually unaffected in hypothyroidism. Besides involvement of haematologic system, hypothyroidism can cause significant changes in kidney function with reduction in glomerular filtration rate and renal plasma flow, leading to rise in serum creatinine and glomerular involvement causing mild proteinuria.

Pancytopenia has been reported in patients with hypopituitarism however we are not aware of any report of pancytopenia associated with primary hypothyroidism.

Conclusion
This case highlights two rare complications of hypothyroidism – nephrotic syndrome and pancytopenia. It is important to recognize that other organ systems may be involved and that the resulting disease states can dominate the clinical picture. As with the classic manifestations of hypothyroidism, these unusual manifestations respond to thyroid hormone replacement therapy.

P384
Radioactive-iodine therapy: a patient satisfaction survey
Sarah Ali1,2, Lina Puntiello1, Sanjeev Mehia1, Daniel Darko3 & Stuart McHardy-Young1
1Central Middlesex Hospital, London, UK; 2Charing Cross Hospital, London, UK.

Radioactive iodine (RAI) therapy is the usual treatment of choice for hyperthyroidism. We have demonstrated a success rate of 88% (euthyroidism or hypothyroidism) over a 5-year period. We are very happy to recommend RAI, however patients still express concerns.

There is little literature available about patients’ reactions to RAI to our knowledge, only two studies. We performed a survey of our RAI treated patients. Of 143 patients contacted, 87 replied (60.8%).

In addition to verbal information, 43 (49.4%) recalled receiving written information; 42 of whom (97.7%) were happy with this information and found it easy to understand. Fifteen used the internet; 73.3% finding this useful. Using this information (verbal, written or internet), 76 patients (87.4%) were aware of why RAI had been suggested for their treatment. Approximately 70% of patients reported awareness of the radiation and safety aspects of RAI.

Eighty-one patients (93.1%) found the treatment process easy with few difficulties reported. Overall, 76 of 87 patients (87.4%) were satisfied with their RAI treatment and management.

There was a noticeable lack of patient knowledge of their thyroid condition; only 68 patients (78.2%) were aware of their original diagnosis and need for treatment. Only 52 patients (59.8%) were clear about complications of hypothyroidism, particularly heart disease.

Only 59 patients (67.8%) were aware of possible outcomes and 54 (62.0%) aware of a possible need for long-term therapy following RAI. Sixty-one patients (70.1%) knew that thyroxine was life-long treatment; however, of those treated with replacement thyroxine such knowledge was better (85.2%).

Our results show general satisfaction with treatment and follow-up arrangements. However, there was a surprising lack of knowledge by patients of their original thyroid condition, complications and an understanding of thyroxine replacement. Provision of structured education for thyroid disease, as in diabetes, may help address this problem.

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P385
Atypical presentation of Riedel’s thyroiditis: multifocal nodular fibrosis and resolution with levothyroxine
Sampath Satish Kumar1, Fraser Sheils2, Andrew Scarsbrook3, Ken Maclellan1, Mark Landsdown1 & Robert Murray1

In patients presenting with a diffusely enlarged hard thyroid gland the differential diagnosis lies between thyroid carcinoma, lymphoma, and Riedel’s thyroiditis. We present a case of Riedel’s thyroiditis with multifocal nodular sclerosis, which improved with levothyroxine replacement.

A 40-year-old woman presented with a 3 months history of neck swelling, dysphagia and breathlessness on exertion. Examination revealed a hard, fixed, diffuse goitre. TFTs revealed $T_3<$ 5.2 pmol/l (9.0-24.0), TSH 62.3 mU/l (0.20-4.0), TPO antibodies >1300 IU/l and CRP 32 µg/l. Repeat TFTs confirmed hypothyroidism and levothyroxine was commenced. Ultrasoundography showed a large nodular goitre suspicious of malignancy. A CT scan revealed a large thyroid mass, encasing the oesophagus, compression of the trachea with a minimum diameter of 5 mm, infiltration of the carotid sheath, multiple bilateral pulmonary nodules, multiple suspicious lesions in the liver and enlarged para-aortic lymph nodes.

Core biopsy revealed dense fibrous tissue, with mixed chronic inflammatory cells focally infiltrating muscle and walls of veins consistent with Riedel’s thyroiditis. Immunohistochemistry revealed no epithelial cells effectively excluding papillary carcinoma. CT guided and thoracoscopic biopsy of a lung lesion revealed byalinalising granuloma. Auto-antibody screening and inflammatory markers were negative.

Four months later, symptoms improved without specific intervention other than levothyroxine. Clinical examination and imaging 6 months later revealed a significant decrease in size of the goitre, and no significant narrowing of the trachea with a minimum diameter of 12 mm. The lung nodules had significantly reduced in size.

Our patient presented with a history and imaging consistent with a diagnosis of disseminated thyroid carcinoma. Repeated attempts to obtain confirmatory histology showed only fibrous tissue. Furthermore, resolution of the inflammatory process occurred after institution of thyroxine replacement therapy. To our knowledge this is the first description of Riedel’s thyroiditis presenting with widespread nodular fibrosis, which improved following initiation of levothyroxine therapy.
P386
Interpreting adrenal status in thyrotoxicosis
Louise Brown, Barbara McGowan, Paul Carroll & Stephen Thomas
Department of Diabetes and Endocrinology, Guy’s and St Thomas’ NHS Foundation Trust, London, UK.

A 19-year-old, female of West African descent presented with a 5-months history of thyrotoxicosis. The GP had commenced carbimazole. She had continuing clinical and biochemical thyrotoxicosis TSH < 0.01 (0.3–5.5 mU/L), FT4 68.0 (9–20 pmol/l) and FT3 18.9 (3.4–5.6 pmol/l). Thyroid antibodies were present at elevated titre and triiodothyronine uptake scanning showed toxic diffuse hyperplasia with an uptake function of 37%, confirming Graves’ disease.

A random cortisol was measured and reported as <30 nmol/l (mid-morning sample). A further 0900 h measurement was 184 nmol/l with simultaneous ACTH of 16 ng/l. Hydrocortisone therapy was commenced and a short synacthen test (SST) arranged within a week of presentation. Hydrocortisone was discontinued 24 h prior to the SST.

<table>
<thead>
<tr>
<th></th>
<th>SST: baseline</th>
<th>SST: month 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time</td>
<td>0</td>
<td>30</td>
</tr>
<tr>
<td>Cortisol (nmol/l)</td>
<td>59</td>
<td>380</td>
</tr>
<tr>
<td>ACTH (ng/l)</td>
<td>13.2</td>
<td>13.9</td>
</tr>
<tr>
<td>Plasma renin activity (p/ml per H)</td>
<td>1.6</td>
<td>1.8</td>
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</table>

Hydrocortisone was continued following the SST. We repeated the SST 1 month later when the patient was clinically euthyroid (TSH 0.01, FT4 5.8 pmol/l and FT3 6.1 mol/l). Adrenal cortex antibodies were negative. Hydrocortisone was cautiously withdrawn with monitoring of random cortisol levels which were around ~400 nmol/l. She had prolonged periods of non-adherence with carbimazole. After 2 months presentation she had a FT4 67 pmol/l, FT3 35.4 pmol/l. ACTH <10, cortisol 117 nmol/l and cortisol binding globulin 33.6 (31–53 ng/l). Eventually after a short period of greater adherence she underwent total thyroidectomy having declined treatment with radioiodine.

Conclusions
We report low cortisol levels with inappropriately low ACTH during a prolonged episode of hyperthryroidism. Interpretation of cortisol values in the setting of hyperthyroidism is not straightforward.

P387
Bulbar myopathy as a rare presenting feature of severe thyrotoxicosis in an elderly man
Allison Martin1,2, Kenneth Foster1 & Sumil Zachariah1,2
1East Surrey Hospital, Surrey, UK; 2Crawley Hospital, Surrey, UK.

A 74-year-old gardener developed progressive dysphagia for solids and liquids over 15 months. He lost five stone in weight and became increasingly weak. There was nothing significant in his past medical and drug histories. He was never smoked and drank little alcohol.

He had an acute medical admission via A&E in April 2008 for profound dehydration and cachexia. His weight was 41.5 kg. There were no gross neurological deficits but he had hoarseness and his cough and swallow reflexes were impaired. His blood investigations showed raised liver enzymes and severe hyperthyroidism (TSH <0.01, T4 91.5) with a high thyroid stimulating hormone receptor antibody titre of 7.7 (0.0-0.4). Videofluoroscopy showed failed attempts to initiate swallow and a barium swallow was abandoned due to marked dysphagia and aspiration. A thyroid uptake scan revealed diffuse increased uptake in both lobes 14.15 (0.4-4.0) compatible with Grave’s thyrotoxicosis.

Given his low body weight and poor swallow, a PEG was inserted. He was initially treated with propranolol as well as carbimazole to which he had worsening liver function. Substituting propylthiouracil led to steady improvements in both liver and thyroid function tests. Most of his nutrition and drugs were given via the PEG for a year. His weight in July 2009 was 67.1 kg, BMI 23. The PEG was removed in August 2009. His recurrent thyroid function tests suggest mild hyperthyroidism (T4 17, T3 7.3 and TSH <0.01) and he is being prepared for radioactive iodine therapy.

This case describes a rare presentation of bulbar myopathy due to Grave’s thyrotoxicosis. Weight loss was his only classical manifestation of thyrotoxicosis, which perhaps contributed to a delay in his diagnosis. This emphasizes the importance of measuring thyroid function in all patients with illnesses associated with unexplained weight loss, muscle wasting and/ or weakness.

P388
Radioactive iodine (RAI) therapy for benign thyroid disease: joint RAI clinic
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Introduction
A joint RAI clinic, led by an endocrinologist and nuclear physicist, was introduced in our centre, in July 2007.

Aims
To compare our practice since the introduction of the joint RAI clinic against guidelines from the Royal College of Physicians of London.

Methods

Results
Seventy-six episodes of RAI therapy, patients’ average age 56 years, 75% females, 55.2% had Grave’s disease and 43.4% received primary RAI. On comparison with the previous audit there was a 200% increase in the number of patients who were treated with RAI (36 vs 72 per year). Cure, defined as hypo or euthyroidism at 6 months was similar at 80.2% vs 82%. Duration from diagnosis to cure for primary RAI was 16.2 vs 41.6 months for secondary RAI.

Since the introduction of the Joint clinic more patients received adequate information prior to treatment in the form of leaflets (100 vs 66%) and warning cards (100 vs 1%). Duration from referral to RAI administration has significantly reduced from 4.8 months to 18.4 days. Other main differences are highlighted in Table 1.

<table>
<thead>
<tr>
<th>Table 1</th>
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<tbody>
<tr>
<td>Number of patients</td>
</tr>
<tr>
<td>Primary RAI</td>
</tr>
<tr>
<td>Signed consent form</td>
</tr>
<tr>
<td>Pregnancy test documented</td>
</tr>
<tr>
<td>FU in clinic within 4–8 weeks post RAI therapy</td>
</tr>
<tr>
<td>Letters sent to the GP within 2 weeks</td>
</tr>
</tbody>
</table>

Conclusions
The joint RAI clinic has led to promotion of radioiodine therapy; better informed patients and subsequently more adherence to radiation protection precautions; reduction in patients’ treatment journey; and better communication between secondary and primary care.

Primary RAI therapy is associated with reduced patients’ journey from diagnosis to cure.

P389
TSH receptor antibody assay: its use in a tertiary centre
Aikaterini Theodoraki1, Gareth L Jones1, Sithara Perera1, Darshna Patel1, Jennifer C Parker1, Chris C Brun1, Mike Thomas1, Pierre-Mark Bouloux2 & Mark Vanderpump3
1Department of Endocrinology, Royal Free Hampstead NHS Trust, London NW3 2QG, UK; 2Department of Clinical Biochemistry, Royal Free Hampstead NHS Trust, London NW3 2QG, UK; 3Department of Clinical Immunology, Royal Free Hampstead NHS Trust, London NW3 2QG, UK.

Background
The BTA guidelines for the use of thyroid function tests recommend the measurement of TSH receptor antibodies (TRAb) when investigating hyper-thyroidism of uncertain aetiology, in suspected Graves’ ophthalmopathy and in
pregnant women with Graves’ disease. An in-house TSH receptor autobody ELSA assay (TRAb) was introduced in 2008. This study has audited the assay performance and evaluated its clinical usefulness in a tertiary centre.

Methods
From May 2008 until July 2009 the Clinical Immunology Department received 251 requests for TRAb. Samples with inadequate clinical information (12%) or duplicates (3%) were excluded. Hospital medical records were available and reviewed for 94% of the processed samples (n=200; mean age 46 years, women 80%). FT₄, FT₃, TSH, TPOAb were also measured. The TRAb assay titre cut off for a positive sample was ≥0.4 UI.

Results
Of the 200 patients identified, 63% (n=125) had Graves’ disease. From the remaining 75 patients: 10% had a toxic multinodular goitre (MNG), 7.5% thyroiditis, 3.5% hypothyroidism, 3% hyperemesis gravidarum, 0.5% toxic nodule and 11.5% other diagnosis. TRAb antibodies were detectable in 83% patients with Graves’ disease. In those without Graves’ disease, TRAb were positive in 9% (three patients with autoimmune thyroiditis, one post-partum thyroiditis, one hypothyroidism and two toxic MNG). With a cut-off point of TRAb ≥0.4 UI the positive predictive value to diagnose Graves’ disease was 95%, sensitivity 83%, specificity 91% and negative predictive value 76%. ROC curve analysis determined an optimal cut-off point of TRAb ≥3.5 UI with a 99% specificity to diagnose Graves’ disease.

<table>
<thead>
<tr>
<th>TRAb cut-off (UI)</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>PPV (%)</th>
<th>NPV (%)</th>
</tr>
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<tbody>
<tr>
<td>0.4</td>
<td>83</td>
<td>91</td>
<td>94</td>
<td>77</td>
</tr>
<tr>
<td>1</td>
<td>72</td>
<td>94</td>
<td>95</td>
<td>67</td>
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<td>2</td>
<td>56</td>
<td>95</td>
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<td>57</td>
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<td>3.5</td>
<td>42</td>
<td>99</td>
<td>98</td>
<td>52</td>
</tr>
<tr>
<td>7</td>
<td>24</td>
<td>100</td>
<td>100</td>
<td>45</td>
</tr>
</tbody>
</table>

Conclusion
The assay studied has a high positive predictive value for diagnosing Graves’ disease and a high specificity when TRAb ≥3.5 UI. However the sensitivity is less than optimal and a negative result does not exclude the presence of Graves’ disease.

P391
Practical value of ¹³¹I whole body scan in follow up of patients with differentiated thyroid cancer
Agnieszka Pazderska₁, Michael Cullen¹, Geraldine O’Reilly².
¹Department of Endocrinology, St James’s Hospital, Dublin, Ireland; ²Department of Medical Physics and Bioengineering, St James’s Hospital, Dublin, Ireland.

Historically, the ¹³¹I whole body scan played a central role in the assessment of disease status in patients with differentiated thyroid cancer. In 2006, the European Thyroid Cancer Taskforce published a new Consensus Statement. It favoured the use of stimulated serum thyroglobulin measurement and neck ultrasound for follow up of disease activity. The use of diagnostic ¹³¹I whole body scan was no longer recommended as a routine test.

Our objective was to assess if the use of ¹³¹I whole body scan provided additional clinical information beyond the combined use of neck ultrasound and stimulated thyroglobulin measurement in follow up of patients with differentiated thyroid cancer.

We retrospectively analysed the data from 41 patients who entered our surveillance programme between February 2007 and July 2009.

¹³¹I whole body scan and serum thyroglobulin measurements were performed after recombinant TSH administration (n=35) or thyroxine withdrawal (n=6). All patients underwent simultaneous diagnostic neck ultrasound.

Disease activity was suggested by stimulated thyroglobulin levels > 1 μg/l in four patients. Ultrasound of the neck was abnormal in two of the four. Further investigations with computed tomography revealed pulmonary metastases in two of the four.

¹³¹I whole body scan showed abnormal uptake in three of these patients, failing to discriminate pulmonary metastases in one patient. Conversely, ¹³¹I whole body scan showed residual uptake in six patients with no other evidence of disease activity.

The concordance between ¹³¹I whole body scan and combined use of neck ultrasound and stimulated thyroglobulin measurements was demonstrated in only 83% of patients.

In our clinical practice, the routine use of ¹³¹I whole body scan is an inaccurate determinant of disease status in follow up of patients with differentiated thyroid cancer.

This retrospective study confirms that the exclusion of this investigation from our surveillance programme would not have resulted in loss of clinical information.

P390
Thyrotoxicosis presenting as intussusception
Hala Alsalfadi, David Dutton & Sailer Sankel
University Hospitals Coventry and Warwickshire, Coventry, UK.

Case report
A previously fit 53-year-old lady presented to A&E with abdominal pain and vomiting. The pain has been present for 2 months and associated with weight loss. She neither smoked nor drank alcohol. On admission her BP was 160/85, pulse 70/min. Abdominal examination revealed generalized tenderness and a right iliac fossa mass with scanty bowel sounds. IBC, U&E, LFT, CRP, amylase, bone were normal. AXR revealed gas filled small bowel loops. Urgent abdominal CT scan confirmed dilated small bowel loops converging to the caecum suggesting intussusception as the cause of sub acute bowel obstruction. Further blood tests revealed TSH <0.02 (0.35–6.00), free T₃, 8.7 (11.0–26) and a diagnosis of thyrotoxicosis was made. She was started on carbimazole and propranolol via NG tube and intravenous hydrocortisone. The anaesthetic team was alerted to the risks of thyroid storm and atrial fibrillation. At emergency laparotomy the intussusception has resolved and full examination of the small and large bowel was normal. She made a good recovery and was discharged home to be followed up in the endocrine clinic. Outpatient small bowel follow through and colonscopy was normal.

Discussion
Thyrotoxicosis, in its classical form, is easily diagnosed and treated. Many hyperthyroid patients have gastrointestinal complaints such as increased frequency of bowel movements and nausea. However, some case reports described atypical presentations with vomiting, abdominal pain or raised liver enzymes. There is also evidence of increased incidence of gastroperesis, gastritis, achlorhydria, and peptic ulcer, in hyperthyroid patients. This is the first reported case of thyrotoxicosis presenting as intussusception.

Conclusion
Abdominal symptoms can be the first and only manifestations of thyrotoxicosis; therefore we emphasize the importance of including thyroid function tests in the evaluation of patients with prolonged, unexplained gastrointestinal symptoms.

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A surveillance programme for assessment of disease status was established in 2006. To date stimulated thyroglobulin has been measured in 46 patients with imaging in 65 patients. The audit highlights the areas in which we must continue to improve in accordance with the best practice guidelines. Further protocols and additional workforce are required in order to improve the standard of care.

P393
Radiodine for Graves’ disease: what do patients really think?
Rebecca Fletchert, Kenneth Muir2, John Bevan2 and Prakash Abraham1,2
1University of Aberdeen, Aberdeen, UK; 2Aberdeen Royal Infirmary, Aberdeen, UK.

Introduction
Radioactive iodine (RAI) is a commonly used treatment modality in Graves’ hyperthyroidism. Patient choice is a major factor in advising radioiodine but there are few data on patient satisfaction following treatment.

Method
Questionnaire
An anonymised questionnaire was distributed to 100 individuals on the thyroid register with RAI treated Graves’ hyperthyroidism. The survey included questions relating to side effects, effectiveness and overall satisfaction with treatment.

Audit
An audit was also carried out on 83 of these individuals to determine the effectiveness of RAI treatment, time from diagnosis to treatment, time to hypothyroidism and effects of RAI on body weight.

Results
Questionnaire
Fifty-eight (58%) questionnaires were completed and returned. Thirty-eight (65%) respondents were satisfied with RAI treatment. Thirty-eight (65%) would have chosen RAI earlier and 30 (52%) would recommend it to a friend. Fifty-five percent (32/58) patients experienced a side effect relating to the treatment. Tiredness (36%) was the most common adverse event followed by neck pain (9%) and eye symptoms (5%).

Audit
From the audit results the average time from first diagnosis to RAI was 31.6 months. Family and workplace contacts led to an average delay of 19.2 and 7.2 months respectively as compared to those without these restrictions. All 83 patients were cured from hyperthyroidism following a single dose of RAI 555 MBq. Ninety-two percent (76/83) developed hypothyroidism with the average time from diagnosis to hypothyroidism being 6.3 months. The average weight gain in the 6 months post RAI was 2.9 kg.

Comments
Satisfaction with RAI treatment was high in those who responded to the questionnaire and the majority stated they would have chosen it earlier and would recommend the treatment to a friend. RAI is a highly effective therapy for Graves’ disease and better perceived by patients than might be generally appreciated.

P394
Treated Grave’s disease? think again!
Aswapthia Sirnath, Ben Whittelow & Sharaf Ibrahim
Queen Mary Hospital, Sidcup, Kent, UK.

Forty-seven years old lady presented to another hospital with symptoms of diarrhea and sleep disturbance. She was found to have abnormal thyroid function tests (TFTs) with FT4, 44 FT3, 12 and TSH 3.4. She had no goiter or thyroid eye signs. Thyroid ultrasound showed a multinodular goiter and an uptake scan was consistent with thyrotoxicosis. She was commenced on carbimazole and in 2001 radio-iodine was administered for a presumed diagnosis of Grave’s disease. She was subsequently diagnosed with hypothyroidism and commenced on levothyroxine. The dose was gradually increased to 350 g, however THM remained elevated. In 2006 she was referred to our hospital. TFTs showed elevated TSH 8.5 and FT3 38. Heterophile antibodies were negative. Hypothyroid with poor treatment compliance was suspected. Over the next 2 years adjustments were made to the levothyroxine dose and repeated advice on compliance given. Copies of clinic letters from the first hospital were then obtained with details of initial biochemistry and the diagnosis reviewed. In January 2006 serum calcium was high 3.03 mmol/l with a high parathyroid hormone at 113. Ultrasound and nuclear medicine imaging suggested a left lower pole parathyroid adenoma. MRI scan of the pituitary demonstrated a 4 cm pituitary adenoma with suprasellar extension, compressing the optic chiasm. Biochemical assessment of pituitary function was normal. Neck exploration was performed and a single left sided parathyroid nodule was removed. Histology demonstrated parathyroid hyperplasia. Two weeks later trans-sphenoidal pituitary surgery was performed. The surgery was complicated by post-operative meningitis. She remains on levothyroxine treatment. Initial genetic testing for MEN1 did not demonstrate a mutation. TSH-secreting pituitary adenomas are rare and diagnosis could be easily missed. Middiagnosis and inadvertent thyroid ablation is known to result in the enlargement of TSHomas. Regular and retrospective review of diagnoses can be vital in reaching the correct diagnosis.

P395
An evidence based protocol for the early identification and management of hypocalcaemia following total thyroidectomy
Neil Sharma, David Howe, Neil Gittines & John Watkinson
University Hospital Birmingham NHS Foundation Trust, Birmingham, UK.

Temporary hypocalcaemia following total thyroidectomy occurs in around 30% of patients and is usually due to inadvertent damage to the parathyroid glands. Whilst mild cases are easily managed with oral calcium supplementation, there is the potential for severe calcium deficit with the ensuing risks of cardiac dysrhythmias, tetany and in extreme cases death. It is common practice to have a post-surgical protocol to allow the early identification of these patients. Whilst many protocols rely solely on post-operative serum calcium levels, more are now utilising parathyroid hormone (PTH) assays to predict those at risk of developing hypocalcaemia. In most of these centres, it is possible to have a PTH result within hours of the request. Unfortunately, in many UK hospitals, laboratories will not process PTH assays overnight or indeed daily. For this reason there is no widely accepted protocol, which incorporates the test, despite the growing body of evidence supporting its use. Our unit is a tertiary referral centre performing around 100 thyroid operations per year. Within our multidisciplinary clinic we have an ongoing prospective database of 1483 patients who have undergone thyroid surgery over the last 15 years. Of the 505 patients who underwent total thyroidectomy, 22.5% developed temporary hypocalcaemia. Based on our experience and the latest evidence, we have produced a protocol to allow the prompt identification and management of this complication. It has the advantage over other protocols in that it allows for a lack of overnight laboratory availability without compromising patient safety. We also propose that it is possible to accurately identify patients who will not suffer hypoparathyroidism allowing their safe and early discharge. To date we have tested the protocol on 31 consecutive patients undergoing total or completion thyroidectomy (eight of whom developed temporary hypocalcaemia), demonstrating its efficacy and reliability in a clinical setting.

P396
A case of thyroid hormone resistance in a family with three generations of thyroid disease
Doros Polydorou, Daniel Kannapan, Sam Kenz, Angela Paisley & Tara Kearney
Salford Royal Foundation Trust, Manchester, UK.

An 18-year-old male referred to endocrine department 3 years ago with symptoms of tremor in both hands and query thyrotoxicosis. Presenting TFTs showed raised T4 and T3 levels with normal TSH (FT4 4.5 pmol/l, FT3 – 3.3 pmol/l and TSH of 1.4 mU/l). There was family history of thyroid disease with both the patient’s father and grandmother diagnosed with overactive thyroid. Patient’s TFTs were reproduced with different assays confirming their validity. Differential diagnosis was between TSHoma and thyroid hormone resistance syndrome. Anterior pituitary endocrine profile was unremarkable.

Patient had MRI of pituitary, which was unremarkable. Was initially treated with carbimazole but this failed to suppress his TSH. A blood sample was subsequently sent for DNA testing which confirmed a heterozygous mutation of the thyroid hormone receptor gene at exon 9 (p.Met313Thr) which is associated with thyroid hormone resistance. DNA screening was also offered to the father who was negative for the mutation indicating that the disorder may have arisen de novo in the son which can occur in about 15% of cases. Resistance to thyroid hormone syndrome (RTH) is a rare disorder, usually inherited as an autosomal dominant trait. Patients with RTH are usually euthyroid but can occasionally present with signs and symptoms of thyrotoxicosis or rarely with hypothyroidism. The syndrome is characterized biochemically by elevated
serum thyroid hormone levels, non-suppressed TSH and reduced tissue responsiveness to thyroid hormones.

Discussion
Learning point from this case is that assessment of clinical thyroid status is vital in order to make the correct diagnosis. The case highlights the differential diagnosis of elevated free thyroid hormone levels in conjunction with a non-suppressed TSH, which can occur due to assay interference with heterophile antibodies, TSH/Thy or thyroid hormone resistance syndrome. A brief summary and overview of RTH syndrome will be given.

P397
The use of district-wide laboratory database to identify new patients with persistent hyperthyroidism
Yasmeen Khalid, B M Singh, Varadarajan Baskar & Harit N Buch
New Cross Hospital, Wolverhampton, UK.

Aim
The aim of our study was to explore the possibility of using a district-wide laboratory database as a governance tool to ensure the optimum management of patients with biochemical hyperthyroidism.

Patients and methods
A complete list of patients on whom TFT were requested over a 3-month period was obtained and patients with unequivocal hyperthyroidism were identified. General practitioners (GP) of patients not referred to the specialist endocrine team during the preceding 6 months were sent a standard letter. The letter provided information on the test result and made a recommendation to seek specialist opinion. For patients in whom referral could not be made, the reason for non-referral was sought and appropriate advice was provided to achieve euthyroid state.

Results
Three hundred and forty-seven TFT were requested over a 3-month period of which 88 patients were hyperthyroid. Of 59 (67%) patients had either attended or were waiting to attend endocrine appointment while 29 (33%) patients had not been referred to endocrine team and were sent the standard letter. No response was received for 11 patients and responses received for the remaining 18 patients were as follows – five were referred to endocrine team, seven were on thionine with readjustment of its dose after receiving the letter, three were attending another local clinic and three were being managed in the primary care due to comorbidity. Of 3/29 patients had persistent hyperthyroidism at the end of 3 months after the initial TFT and for two of these patients no response had been received to the standard letter.

Conclusions
A regular audit of the laboratory database provides an excellent opportunity for specialist endocrine team to oversee optimal management of patients with hyperthyroid biochemistry and can form an excellent tool for district-wide endocrine governance process.

P398
Dilated cardiomyopathy and atrial fibrillation secondary to resistance to thyroid hormone
Arsl Ullah, Atif Munir & Sath Nages
James Cook University Hospital, Middlesbrough, UK.

Background
Resistance to thyroid hormone (RTH) is a rare autosomal dominant condition of altered tissue responsiveness to thyroid hormone (TH) characterised by elevated serum FT₃ and FT₄, and non-suppressed TSH levels caused by mutation in the thyroid receptor (TR) β gene. Different isoforms of TR are expressed in the heart and regulate genes that encode structural and regulatory proteins. The syndrome is characterised by a variable clinical phenotype and clinical manifestations may be non-specific or absent. We report a case of RTH due to TR β receptor mutation presenting with atrial fibrillation and dilated cardiomyopathy.

Case report
A 43-year-old man presented with palpitations and congestive cardiac failure due to atrial fibrillation. He had no other symptoms of thyrototoxicosis. Clinical examination revealed a small goitre but no other features of hyperthyroidism. Echocardiography showed severe global systolic impairment. Thyroid function showed TSH 4.47 mIU/l (Ref 0.27–4.2), FT₃ 27.5 pmol/l (Ref 10–21)), FT₄ 6.7 pmol/l (Ref 3.5–6.5). TBI and TPO antibodies were negative. Alcohol intake was 30 Units/week. Thyroid function was suggestive of RTH. Assay interference due to heterophil antibodies was excluded by checking TFT’s on a different analytic platform. Pituitary MRI excluded a TSH secreting tumour. The patient was managed medically with bisoprolol, digoxin, ramipril and warfarin and repeat echocardiography showed significant improvement in ventricular function. Genetic studies confirmed a heterozygous point mutation in the ligand binding domain of the TR β gene confirming RTH.

Discussion
Clinical manifestations of RTH are variable due to heterogeneity of factors that modulate action of TH. Animal models of RTH have shown reduced myocardial contractility. We postulate that hyperthyroxinaemia due to RTH resulted in dilated cardiomyopathy that was exacerbated by alcohol excess. The effects of RTH on circulatory parameters and alterations in ventricular characteristics need further exploration.

P399
Iopanoic acid: a bridge to surgery when all else fails in complicated hyperthyroidism
Victoria Parker, Alistair Green, Piyush Jami, David Halsall, Krishna Chatterjee & Helen Simpson
Addenbrookes Hospital, Cambridgeshire, UK.

We report five cases of hyperthyroidism with complex management issues, which were successfully treated with iopanoic acid prior to thyroidec-
momy. Three patients (cases 1–3) had Graves’ disease; two developed agranulocytosis on carbimazole and one had uncontrolled severe thyrotoxicosis and poor compliance with treatment. Radioiodine was either declined or contraindicated due to radiation protection issues. All patients were rendered euthyroid with iopanoic acid 500 mg BD in combination with propranolol and underwent uncomplicated thyroidec-
tomies.

A further two patients (cases 4 and 5) had Eisenmenger’s secondary to congenital cyanotic heart disease and persistent atrial fibrillation. They developed amiodarone induced thyroiditis (AIT) with no uptake on thyroid scintigraphy suggestive of type 2 AIT and precluding radioactive iodine therapy. They were resistant or intolerant to treatment with anti-thyroid drugs and steroids and subsequently underwent thyroidec-
tomies after pre-treatment with iopanoic acid 500 mg bd. In all cases, uncontrolled thyrotoxicosis was prevented and euthyroidism was achieved within an average of 9.8 days (range 2-20) after iopanoic acid treatment. Iopanoic acid, an iodine-containing oral contrast agent, is a competitive inhibitor of type I sodium/hydrogen dehydrogenase, reducing conversion of T₄ to T₃ in peripheral tissues. Consequently, patients treated with iopanoic acid prior to surgery exhibit a modest reduction in FT₃ levels with a disproportionately larger reduction in its metabolic FT₃, leading to rapid restoration of clinical euthyroidism.

<table>
<thead>
<tr>
<th>Case</th>
<th>Pre-iopanoic acid FT₃ (pmol/l)</th>
<th>FT₃ (pmol/l)</th>
<th>Pre-surgery FT₃ (pmol/l)</th>
<th>Pre-surgery FT₃ (pmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>98.2</td>
<td>46.3</td>
<td>45.4</td>
<td>4.3</td>
</tr>
<tr>
<td>2</td>
<td>63.6</td>
<td>N/A</td>
<td>16.0</td>
<td>4.8</td>
</tr>
<tr>
<td>3</td>
<td>72.7</td>
<td>&lt;30</td>
<td>34.8</td>
<td>4.2</td>
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<tr>
<td>4</td>
<td>95.0</td>
<td>12.5</td>
<td>87.9</td>
<td>7.2</td>
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<tr>
<td>5</td>
<td>133.0</td>
<td>28.2</td>
<td>55.4</td>
<td>7.4</td>
</tr>
</tbody>
</table>

FT₃ (NR 11.5–22.7 pmol/l) FT₄ (NR 3.5–6.5 pmol/l).

These cases highlight the utility of iopanoic acid in preparing a thyrotoxic patient for thyroidec-
tomy when all other treatments have failed or are unsuitable.

P400
Selective malabsorption of thyroid hormone or selective intake?
Rajeev Raghavan, Wolf Woltersdorf & Colin Dayan
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Case 1
Nineteen years old nursing student with primary hypothyroidism, despite thyroxine (T₄) at 200 µg/day and subsequent trial of T₄+T₃, remained significantly symptomatically hypothyroid. Over 4 years worsening hypothyroidism - was evident (TSH 19.1 > 200 and FT₃ 2.1–6.8) despite dose/regime changes. Investigations for malabsorption: normal coeliac screen, B12, ferritin,
FBC, kidney and liver function, autoimmune profile, hydrogen breath test, upper-GI endoscopy/DZ biopsy, and barium meal-follow-through, with folate and vitamin-D deficiency (20 μmol/L). Through this period, she completed her degree. Later full-time work marred by several periods of illness requiring time off. Assay interference with heterophile antibodies was excluded. A ‘thyroid absorption test’ (TAT) – baseline TFTs, supervised administration of oral thyroxine 200 μg, and post-dose TFTs (see Table) all repeated a week later with T4 1000 μg – demonstrated poor absorption and need for high doses to achieve adequate serum levels.

Thyroxine 600-1000 μg daily, then alternate days caused hyperthyroidism (T4 = 27–95). Consequent dose reduction (any dose <500 μg) caused hypothyroidism. She is now having parental thyroxine 200 μg i.m. every 3 days with significantly improved quality of life and TFTs. Of note is a strong family history of hypothyroidism and maternal grandmother also being on parenteral thyroxine.

Case-2
Thirty years old Ex-army chef, primary hypothyroidism diagnosed during pregnancy 9 years prior. Gradually increasing dose of T4 to 1000 μg with suboptimal TFTs. Tests for malabsorption negative. TAT showed good absorption for both 200 and 1000 μg doses. Advised to reduce T4 to 200 μg/day.

<table>
<thead>
<tr>
<th>Assay-timing</th>
<th>Case-1: 200 μg T4 levels</th>
<th>1000 μg</th>
<th>Case-2: 200 μg</th>
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<td>Pre-dose</td>
<td>10.7</td>
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<td>15.2</td>
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<tr>
<td>2-h post dose</td>
<td>10.0</td>
<td>16.4</td>
<td>13.3</td>
<td>34.2</td>
</tr>
<tr>
<td>5-h</td>
<td>–</td>
<td>–</td>
<td>18.5</td>
<td>40.8</td>
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<td>4-h</td>
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<td>11.6</td>
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<tr>
<td>24-h</td>
<td>–</td>
<td>9.6</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

Conclusions
TAT is useful in selected cases of hypothyroidism. We have demonstrated that selective thyroxine malabsorption can occur. The exact underlying defect is currently unknown and treatment can prove challenging.

P401
Management and follow up of post-operative hypocalcaemia after thyroidectomy: a pilot study
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Background
Although hypocalcaemia is common post thyroidectomy, no national guidelines pertain to its management.

Long-term treatment with calcium and vitamin D replacement predispose patients to nephrocalcinosis and should be avoided in the absence of a clear indication.

Aim
To identify the incidence and management of post-operative hypocalcaemia following thyroidectomy in a UK teaching hospital with view to formulating management guidelines.

Method
Patients who underwent thyroid surgery between 2007 and 2008 were identified by theatre lists. Case notes were reviewed to determine demographic details, incidence of hypocalcaemia and the treatment given. All patient review appointments were examined to identify if a trial off supplements had been offered.

Results
One hundred and twenty-seven patients underwent thyroid surgery between 2007 and 2008. Ninety-one patients were included in the audit and 36 excluded due to missing data.

Of 33/91 (36.3%) patients were treated with calcium supplements post-operatively. Of 25/91 (27.5%) had documented hypocalcaemia. Mean pre-operative serum calcium (s.c.) was 2.56 mmol/l (0.13). Nadir serum calcium on the first, second and third post-operative day was 2.00, 1.82 and 1.89 mmol/l respectively. No significant differences in serum calcium between men and women post-operatively (P value = NS).

Mean hospital stay (s.c.) following surgery was 3.9 (1.3) days 16 patients were treated with Sandocalc, 4 with 1-α-calcidol and 13 with both. Three patients required intravenous calcium.

Twenty-nine patients were discharged with calcium and/or α-calcidol. At data analysis 8/29 (27.6%) patients remained on supplements. Replacement therapy was discontinued in seven patients at their post-operative clinic review and in 11 patients at the discretion of their general practitioner.

Conclusion
There is wide variation in management of hypocalcaemia post thyroid surgery. Large proportion of patients remain on long-term replacement without a clear indication and a trial off treatment is needed to ascertain the need for long-term therapy.

P402
Clinical management and outcomes of anti-TSH receptor antibody positive pregnancies
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1The Wolfson Diabetes and Endocrine Clinic, Institute of Metabolic Science, Addenbrooke’s Hospital, Cambridge University Hospital NHS Foundation Trust, Cambridge, UK; 2Cambridge University Hospital NHS Foundation Trust, University of Cambridge, Cambridge, UK; 3Department of Clinical Biochemistry, Addenbrooke’s Hospital, Cambridge University Hospital NHS Foundation Trust, Cambridge, UK; 4Department of Paediatrics, Addenbrooke’s Hospital, Cambridge University Hospital NHS Foundation Trust, Cambridge, UK; 5Department of Obstetrics and Gynaecology, The Rosie Hospital, Cambridge University Hospital NHS Foundation Trust, Cambridge, UK; 6Metabolic Research Laboratories, Institute of Metabolic Science, Addenbrooke’s Hospital, Cambridge University Hospital NHS Foundation Trust, Cambridge, UK.

Introduction
Anti-TSH-receptor binding antibodies can cross the placenta and can stimulate or inhibit the fetal thyroid, causing fetal and neonatal thyroid dysfunction. We test for these antibodies in all pregnant women with a history of thyroid disease using a TSH binding inhibitor immunoglobulin (TBI) assay. Our aims were to audit our management of TBI positive pregnancies and to review pregnancy outcomes.

Methods
Serum TBI concentration was measured using the second-generation BRAHMS TRAK luminescent immunoassay (Henningsdorf, Germany). The biochemistry database was searched to identify pregnant patients with elevated TBI (> 1.0 IU/l) over a 1-year period.

Results
TBI was measured in 109 pregnant women. Of 26/109 had TBI > 1.0 IU/l (range 1.1–29.0 IU/l). Maternal thyroid antibodies were Graves’ disease in 16/26, primary hypothyroidism in 9/26 and hypothyroidism following thyroidectomy for adenoma in 1/26. Third trimester TBI was highest in Graves’ patients either taking antithyroid medication during pregnancy (mean 3.3 IU/L) or euthyroid non-treated Graves’ patients (mean 3.3 IU/L), and was lower in patients with hypothyroidism, either following radioiodine and/or surgery for Graves’ (mean 1.60 IU/L), or with primary hypothyroidism (mean 1.8 IU/L). All pregnancies went successfully to term. Third trimester TBI correlated significantly with first neonatal TSH (r = 0.67, P = 0.006). One fetus had fetal goitre and tachycardia, but had no evidence of clinical or biochemical neonatal thyrotoxicosis. One neonate had transient biochemical thyrotoxicosis, which settled with conservative management. Both were born to mothers with Graves’ requiring antithyroid medication during pregnancy. Cord TSH was elevated in five cases. All had mothers with Graves’ disease, 3/5 mothers had required antithyroid medication during pregnancy, and 2/5 had treated hypothyroidism following previous radioiodine and/or surgery. All settled with conservative management.

Conclusions
Neonatal thyroid dysfunction was most likely with maternal Graves’ disease requiring antithyroid medication during pregnancy, and where 3rd trimester TBI was highest. However neonatal thyroid dysfunction settled spontaneously and overall outcomes were excellent.

P403
131I uptake scanning after radiiodine treatment for thyrotoxicosis prevents delayed diagnosis of radiiodine induced hypothyroidism
Eleanor Murray, Doreen Foullis & F E Murray
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Radioactive iodine is a common and effective treatment for hyperthyroidism. Post radio-iodine hypothyroidism is a frequent complication occurring in ~80% of radioidine treated patients. This can be diagnosed clinically by monitoring free T4 and TSH levels but, in our centre, a 131I uptake scan 6 weeks after therapy is
The data suggest that use of a diagnostic 123I uptake scan 6 weeks after radiostimulus treatment may expedite diagnosis of hypothryroidism resulting in earlier prescribing of thyroxine and avoidance of prolonged untreated hypothryroidism. Therefore, in centres where such facilities are available we would continue to advocate routine 123I uptake scan as well as thyroid biochemistry at the first clinic visit after radiostimulus therapy.

P404
Sunitinitib induced hypothryroidism: a retrospective analysis
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1 Diabetes and Endocrinology, Guy’s and St Thomas’ NHS Foundation Trust, London, UK; 2 Medical Oncology, Guy’s and St Thomas’ NHS Foundation Trust, London, UK.

Sunitinitib, a tyrosine kinase inhibitor, inhibits VEGF-mediated tumour angiogenesis. Following NICE approval, it is increasingly used in the treatment of metastatic renal cell carcinoma. Hypothyroidism in sunitinitib-treated individuals was first described in 2005. The aetiology remains uncertain and possibly reflects a destructive thyroiditis. Incidence rates of hypothryroidism from case series vary between 36 and 85%.

We report a retrospective analysis of thyroid-function in patients treated with sunitinitib for metastatic renal cell carcinoma at a UK tertiary hospital. Twenty-six patients (18 men, 8 women; median age 62.5 years (range 32–80)) were started on sunitinitib between September 2007 and April 2009. All patients received sunitinitib for more than three cycles (25–50 mg/day; 4-weeks-on and 2-weeks-off per cycle). Sixteen patients (61%) had baseline thyroid function tests (mean TSH 2.44 (t.u. ± 2.45) mIU/L, NR 0.27–4.20 mIU/L; mean FT4 16.23 (t.u. ± 4.62) pmol/L, NR 12–22 pmol/L). Pre-treatment TSH was elevated in patients (4.61 and 10.7 mIU/L) increasing to 47.4 and 42.1 mIU/L respectively, after one cycle. At the first follow-up thyroid assessment, which was done between the 1st and 8th cycle (median 3 cycles), the mean TSH was 5.0 (t.u. ± 8.09) mIU/L (∆TSH 2.56, 95%CI 1.76–6.78). Free-T4 was normal with a mean value 15.86 (t.u. ± 1.80) pmol/L. One patient had a thyrotoxic phase before progressing to hypothryroidism. Median time-point for the 2nd follow-up was after 4 cycles (range 3–12) and the third follow-up occurred after 6 cycles (range 3–13). Thirteen patients (50%) had a high TSH (median 11.4 mIU/L (range 5.48–80)) during follow-up and 8 (30%) were started on levothyroxine due to a progressive increase in TSH.

In conclusion, hypothryroidism is a common complication of sunitinitib therapy and may adversely affect tolerance. Local protocols for thyroid surveillance and thyroxine replacement will enhance recognition and enable early treatment of this unusual clinical entity.

P405
Screening and management of thyroid dysfunction in pregnancy
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1 James Cook University Hospital, Middlesborough, UK; 2 Newcastle University, Newcastle-Upon-Tyne, UK.

Introduction
Maternal hypothryroidism is the most common disorder of thyroid function in pregnancy and may influence the outcome of mother and fetus at all stages. Aim
To evaluate screening and management of all high risk pregnant women for thyroid dysfunction.

Method
A retrospective audit as carried out between January 2005 and December 2006. Our local standards were TSH < 3 mIU/L for screening and TSH <2 mIU/L for treatment. Women were identified as high risk if they had known thyroid dysfunction or a family history of diabetes or thyroid disease. Computer based search was performed to extract the data for the study. Women were considered screened if they had thyroid function tests checked any time from date of booking up to delivery.

Results
A total of 8478 women were booked to the antenatal clinic. Of 3673 (43%) high risk pregnant women were identified of whom 57% had a family history of diabetes, 25% thyroid disease, 16% both, and 2% (186) had known thyroid dysfunction. Of 93, 55, 60, and 15% respectively were not screened at all during their entire pregnancy. When screened only 85, 83, 83 and 56% respectively had TSH levels <3 mIU/L. Only 8% (303) were reviewed in combined medical obstetric clinics and required 689 appointments. Of 43% attended in first trimester, 44% in second trimester, and 13% in third trimester. Only 38, 43 and 63% referred in 1st, 2nd, and 3rd trimester had an initial TSH level <2 mIU/L at treatment. Of 77, 80 and 72% successfully achieved TSH levels <2 mIU/L before delivery.

Conclusion
Current screening methods are not adequate with not enough women being tested and not enough achieving target TSH in early pregnancy. Increased awareness of the need for screening and management of thyroid disease in early pregnancy is needed amongst health care professionals in primary and secondary care settings.

P406
Diagnostic and financial benefits of checking TSH receptor antibodies in patients with thyrotoxicosis
Violet Fazal-Sanderson, Theiniing Aung, John A H Wass & Niki Karavitaki Department of Endocrinology, OCDM, Churchill Hospital, Oxford, UK.

Background
TSH-receptor stimulating antibodies are implicated in the pathophysiology of Graves’ disease (GD). The detection of TSH-receptor antibodies (TSHR-AbS) is routinely performed by assays measuring thyrotopin-binding inhibitor immuno-noglobulin and new generation assays are reported to have high sensitivity and specificity in GD. The differentiation of hyperthyroidism (GD or toxic multinodular goiter (TMG) or toxic adenoma (TA)) is important for planning the therapy and an approach combining cost-effectiveness and patient convenience are essential in common practice.

Objectives
To assess the benefits (diagnostic and financial) of checking the TSHR-AbS (TSHR Autoantibody Coated-Tube, RIA, Kronus, ID, USA) alone in a series of patients presenting with thyrotoxicosis.

Patients and methods
Sixty-seven consecutive patients (58 females, median age 38 years (range 23–89) who presented with newly diagnosed thyrotoxicosis were studied. Of these, 54 had GD, 6 TMG or TN and 7 subacute thyrotoxidities (ST). All diagnoses were confirmed with a thyroid uptake scan. Anti-TPO and TSHR-AbS were measured at the time of presentation.

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P407

Should the cytological finding of Thy 3 mandate surgical excision?
Indrani Chakrabarti, Simon Alywin, Alan McGregor, Klaus Martin Schulte, Salvador Diaz-Cano & Jackie Gilbert
Kings College Hospital, London, UK.

Introduction
Current guidelines recommend that thyroid nodules classified as Thy 3 following fine needle aspiration (FNA) should be managed surgically. This results from over-treatment of benign disease. The purpose of this study was to review multi-disciplinary team (MDT) management of Thy 3 FNAs at our institution.

Patients and methods
A total of 109 FNAs were performed between April 2008 and October 2009; 31 were reported as Thy 3. The management of all cases was discussed by the multi-disciplinary team (MDT).

Results
Seventeen patients underwent a diagnostic lobectomy. Four demonstrated malignancy (two papillary carcinomas and two follicular thyroid carcinomas). Two patients underwent repeat FNA prompting surgical excision in one patient (further Thy 3 result) and conservative management in the other (Thy 2).

Of the twelve patients who did not proceed to surgical intervention:
– Six patients were offered surveillance by ultrasound and/or radioactive imaging following MDT consideration that the aspirate had been classified as Thy 3 based solely on the paucity of colloid.
– One patient demonstrated nodule resolution following initiation of levotyroxine therapy for hypothyroidism.

Conclusions
In 23.5% of patients with a Thy 3 FNA, who underwent a diagnostic lobectomy, histology confirmed thyroid carcinoma. Hence the majority of Thy 3 lesions were benign, in keeping with recent published data from other institutions in the UK. Patients with FNAs classified as Thy 3 based solely on the paucity of colloid (19%) with no additional suspicious features were offered conservative management. Short-term follow-up surveillance investigations have revealed no additional supportive evidence of malignancy in these patients but remains under close review.

P409

Screening of six novel candidate genes for association with Graves’ disease
Ruth Tisdall, Matthew Simmonds, Paul Newby, Jayne Franklin, Steephe Gough & Oliver Brash
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Genome-wide association screening (GWAS) has proved invaluable in determining otherwise undetected genetic effects for several common endocrine diseases. The largest GWAS performed in Graves’ disease (GD), to date, has not only confirmed association of several known gene regions, including the HLA region, TSHR and FCRL3, but has also identified several other possible regions of association with GD. As GD shares several susceptibility loci with other endocrine autoimmune diseases, including type 1 diabetes (T1D), several of the novel gene regions detected through GWAS in T1D, including PTPN2, CD226 and IFIH1, have also been associated with GD. A recent meta-analysis conducted on three of the largest T1D GWAS performed, investigating over 820 T1D subjects and controls, identified six novel T1D susceptibility regions including, the intergenic rs165738, gene desert located e6887079, PRKCC rs947474A, CTHX rs3829532, TNP2 rs416903 and C4/PTB6 rs229541 SNPs. To determine whether these six newly detected T1D susceptibility loci could also be associated with GD, these SNPs were screened in a large UK Caucasian GD cohort consisting of 2504 GD patients and 2688 controls subjects. All subjects gave informed written consent and the project was approved by the local ethics committee. None of the six SNPs studied showed any association with GD (P=0.98-0.05). Clinical correlations were performed to determine if these SNPs were associated with a specific clinical GD sub-phenotype including age of onset, severity of Graves’ ophthalmopathy (NOSPECS 2–6), presence of goitre and autoantibody status, however no associations were detected (P=0.99-0.07). This study can confidently rule out the possibility of any major genetic contributions of these six novel T1D regions to GD (OR≥2.15). However we cannot rule out the possibility of these SNPs contributing smaller effects to GD susceptibility (OR<1.15) without screening these SNPs in additional GD datasets of equal/greater size and the use of meta-analysis.

P408

Characteristics of patients requiring more than one dose of radioactive iodine to induce cure of hyperthyroidism
Barbara Tortolhska, Jayne Franklin & Kristien Boelaert
University of Birmingham, Birmingham, UK.

The administration of radioactive iodine (131I) is widely used in the treatment of patients with hyperthyroidism. We have previously reported better cure rates in patients receiving a single dose of 600 MBq 131I compared with those treated with lower doses. We set out to evaluate if baseline patient characteristics predict which patients require multiple doses of radioiodine to induce cure. We compared 42 subjects requiring ≥3 doses with 290 patients cured with two doses and with 868 subjects cured following a single dose. The size of the first dose administered was lower (P=0.001) in those receiving multiple doses with an initial dose of 185 MBq administered to 62.6% of patients treated with ≥3 doses versus 41.2% (two doses) and 24.2% (one dose) respectively. The rates of hypothyroidism were higher in those treated with two doses (78.6%) versus subjects receiving a single dose (67.2%) but were lower in patients requiring ≥3 doses (47.6%, P<0.001).

There was a significantly higher proportion of males in those treated with ≥3 doses (31 vs 24.5% (two doses) and 19% (one dose), P=0.04). Subjects requiring a higher number of doses were significantly younger (46.9 years (≥3 doses) versus 48.3 years (two doses) versus 51 years (one dose), P=0.001) and had more severe hyperthyroidism at presentation (mean serum TSH, 64.2 vs 51.7 vs 43 pmol/L, P<0.001). The presence of a palpable goitre was significantly higher in patients requiring 2 (84.5%) or more doses (95.3%) when compared with subjects cured following a single dose (72.6%, P<0.001). The underlying disease aetiology, smoking history, reporting of a family history of hyperthyroidism and the presence of ophthalmopathy were similar in the three groups.

Conclusions
Males, younger patients, those with higher serum TSH concentrations and subjects with large goitres should receive a larger initial dose of 131I.

P410

The physiological role of thyroid hormone in the hypothalamic ventromedial nucleus
John R Counsell, Enrol Richardson & James V Gardiner
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The hypothalamo-pituitary-thyroid (HPT) axis serves to maintain appropriate systemic levels of thyroid hormone (TH) through a negative feedback pathway via the hypothalamic arcuate and paraventricular nuclei. However, the effects of TH in other hypothalamic regions are poorly understood. Triiodothyronine (T3) administration to the hypothalamic ventromedial nucleus (VMN) induces a potent hyperphagic response, although it is unclear whether this is part of a novel physiological pathway or simply an additional component of the HPT axis. This research investigates the physiological role of TH in the VMN through the use of a recombinant adenov-associated virus (rAAV) designed to locally inactivate TH. Activation and inactivation of TH is mediated by the iodothyronine deiodinases (D1, D2, and D3), where D2 functions to deiodinate...

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tetraiodothyronine (T4) to produce the active T3 hormone, and D3 is the inactivating enzyme.

A plasmid encoding D3 cDNA under the control of a CMV promoter was packaged into rAAV to generate a viral titre capable of over-expressing D3 (rAAV-D3). Anaesthetised male Wistar rats received bilateral stereotactic injections of either rAAV-D3 (n = 13) or rAAV-GFP (n = 11) into the VMN. Both groups were dissected 78 days after surgery.

Over-expression of D3 in rAAV-D3 treated rats was confirmed by QPCR, which demonstrated a 10-fold increase in hypothalamic D3 mRNA (P<0.001) in comparison to rAAV-GFP controls. Furthermore, the enzymatic activity of D3 was increased in whole hypothalamic samples (P=0.31), whilst D2 activity was unaffected (0.92). This was accompanied by up-regulation of the T3 transporter, MCT8 (P=0.15), suggesting a reduction in neuronal T3 content. Plasma levels of free T3 (P=0.95) and free T4 (P=0.82) were not altered, however plasma insulin (P=0.38) and leptin (P=0.2) were reduced by rAAV-D3 treatment, thus suggesting that local inactivation of T3 in the VMN directly modulates peripheral metabolism without affecting the HPT axis.
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