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# SOCIETY FOR ENDOCRINOLOGY BES 2015

2–4 November 2015

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Edinburgh, UK

## Abstract Book

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# Plenary Lecturers' Biographical Notes

# Society for Endocrinology Starling Medal Lecture

Robert Semple, University of Cambridge,
Cambridge, UK

Robert Semple is a Reader in Endocrinology and Metabolism and Honorary Consultant Endocrinologist at the University of Cambridge, UK. He read Biochemistry and then Medicine at the University of Cambridge before internal medical posts in London. He returned to Cambridge for specialist training in Diabetes and Endocrinology, interrupted by doctoral studies with Prof. Sir Stephen O'Rahilly, focussing on transcriptional regulation of adipose tissue metabolism.

For the past 12 years he has focussed on rare human disorders of insulin action and growth. His research aims to identify novel acquired or genetic defects underlying insulin resistance and related conditions, both to accelerate diagnosis and to enhance treatment of affected patients, and to draw inferences, through physiological study of affected patients, about the pathobiology of common forms of metabolic disease. This work has played a key part in the establishment of a National Severe Insulin Resistance Service in Cambridge.

Dr Semple is also a fellow of Clare College, Cambridge, and Director of the Cambridge MB PhD programme.

# Society for Endocrinology Medal Lecture

Waljit S Dhillo, Department of Endocrinology and Metabolism,
Imperial College London, London, UK

Professor Waljit Dhillo is a Professor in Endocrinology and Metabolism and Consultant Endocrinologist, Imperial College London. He completed his medical training at St Bartholomew's Hospital Medical School, University of London in 1994. During this time he also completed an Intercalated BSc in Biochemistry (awarded First Class Honours) funded by the Medical Research Council. He then completed his general medical training in London Hospitals. In 1997 he joined the North West Thames Rotation in Diabetes and Endocrinology as a Specialist Registrar. During this time he completed a PhD on the area of novel neuropeptides regulating appetite as a Wellcome Trust Clinical Training Fellow at Imperial College with Professor Sir Steve Bloom. In 2004 he was awarded a National Institute for Health Research (NIHR) Clinician Scientist Fellowship and appointed Clinical Senior Lecturer & Consultant in Diabetes & Endocrinology at Imperial College London. Following this he was awarded an NIHR Career Development Fellowship and promoted to Reader in 2009. In 2011 he was promoted to Professor in Endocrinology & Metabolism. In 2015 Professor Dhillo was awarded a prestigious NIHR Research Professorship.

Professor Dhillo's research investigates novel aspects of endocrine control of obesity and reproductive function. His research has focused on understanding the neuroendocrine mechanisms which are important in the regulation of food intake. Professor Dhillo's research investigates the mechanisms by which gut hormones mediate their effect. He was awarded the Royal College of Physicians Linacre Medal for this work. These findings have identified CNS pathways which have potential as novel targets for the development of anti-obesity drugs.

Professor Dhillo's recent translational research has identified the novel hormone kisspeptin as a potential novel therapy for infertility. Professor Dhillo has carried out the 'first time into human' studies of kisspeptin.This work was awarded the American Endocrine Society Award for Excellence in Clinical Research and the British Society for Neuroscience Investigator Prize.

# Society for Endocrinology Transatlantic Medal Lecture

Gordon Hager, Bethesda, Maryland, USA

Dr Hager received his PhD in Genetics at the University of Washington, and pursued postdoctoral studies with Dick Epstein at the Institut de Biologie Moleculaire in Geneva and with Dr William Rutter at the University of California-San Francisco. After moving to the NIH, he developed the mouse mammary tumor virus (MMTV) system as a model to study hormonal regulation of gene expression, and utilized this system to describe the first known hormone responsive sequences. He reported the first evidence that nucleosomes were positioned at specific sites across a regulatory region, and showed that nucleosome reorganization was central to the mechanism of glucocorticoid receptor (GR) action, the first formal argument that nucleoprotein transitions were involved in gene regulation by nuclear receptors.

In 2000, Dr Hager and colleagues reported the first direct observation of a transcription factor binding to an authentic regulatory sequence in living cells. Using this system, he discovered that the GR undergoes rapid exchange with regulatory elements in the continued presence of ligand, developing the 'hit-and-run' hypothesis for transcription factor action. This highly unexpected development challenged the classic view of transcription factors as statically bound to regulatory elements, and opened a new paradigm in the study of gene regulation.

Dr Hager has been extensively involved in genome wide characterization of epigenetic states, and the dynamics of chromatin transitions. He demonstrated that several transcription factors fail to produce footprints incellular chromatin because of their brief residence times, and developed the concept of 'dynamic assisted loading,' a mechanism central to regulatory element function. This concept combines data from genome wide factor localization studies, investigation of chromatin remodeling factors, and real time dynamic studies in living cells.

Dr Hager is currently Chief of the Laboratory of Receptor Biology and Gene Expression, and Chair of the Center of Excellence in Chromosome Biology at the NCI.

# Society for Endocrinology Dale Medal Lecture

Rajesh V Thakker, Radcliffe Department of Medicine, University of Oxford, Oxford, UK

Rajesh Thakker is the May Professor of Medicine at the University of Oxford, UK. He received his medical degree from the University of Cambridge in 1980, and from 1981 to 1988 he undertook postgraduate clinical and research training at The Middlesex Hospital, The Hammersmith (Royal Postgraduate Medical School, RPMS), Hospital, and Northwick Park (MRC Clinical Research Centre) Hospital (London). In 1988, he was appointed MRC Clinician-Scientist, Consultant Physician/Endocrinologist and Senior Lecturer at the MRC Clinical Research Centre, Northwick Park Hospital, and RPMS, Hammersmith Hospital, where he established his laboratory and team. In 1995, he was appointed Professor of Medicine at the RPMS, and in 1999 as the May professor of Medicine at the University of Oxford.

Professor Thakker's research has been focused in elucidating the molecular basis of the neuroendocrine tumours that are associated with multiple endocrine neoplasia type 1 (MEN1) and of disorders of calcium metabolism, and in translating these advances to clinical practice. His research has received continuous support for 30 years from the Medical Research Council (MRC), and more recently he has received a Wellcome Trust Investigator Award and a National Institute for Health Research (NIHR) Senior Investigator Award.

His research contributions have received many prizes and awards which include: the Society for Endocrinology Medal (1995); European Journal of Endocrinology (European Federation of Endocrine Societies; EFES) Prize (1989), Graham Bull Prize for Clinical Science, Royal College of Physicians, UK (1999); Louis V. Avioli Founder's Award (American Society for Bone and Mineral Research) (2009); and Jack W. Coburn Endowed Lecturership (American Society of Nephrology) (2012). He was elected Fellow of the Academy of Medical Sciences (FMedSci) in 1999 and Fellow of the Royal Society (FRS) in 2014. He served as a member of the Council of the Society for Endocrinology (2003–2006) and currently serves as the Chairman of the NIHR/MRC Efficacy and Mechanisms Evaluations (EME) Board (2008–present).

# British Thyroid Association Pitt-Rivers Lecture

Anthony Hollenberg, Chief, Division of Endocrinology, Diabetes and Metabolism, Beth Israel Deaconess Medical Center, Professor of Medicine Harvard Medical School, USA

Anthony Hollenberg, MD is Chief of the Division of Endocrinology, Diabetes and Metabolism, at Beth Israel Deaconess Medical Center, USA. He is a Professor of Medicine at Harvard Medical School. Dr Hollenberg received his MD from the University of Calgary in Canada in 1986. He completed his Internal Medicine residency in 1989 and was chief resident in Medicine in 1990–1991, both at the Beth Israel Hospital. Dr Hollenberg then completed a fellowship in Endocrinology, Diabetes, and Metabolism at Massachusetts General Hospital in 1993 and was recruited back to Beth Israel to start his laboratory. Dr Hollenberg's own research focuses on the hormonal regulation of metabolism, with a particular emphasis on the role of thyroid hormone. His work has important ramifications for the regulation of body weight and metabolism.

# Clinical Endocrinology Trust Lecture

Wiebke Arlt, Institute of Metabolism and Systems Research, University of Birmingham, and Centre for Endocrinology, Diabetes and Metabolism, Birmingham Health Partners, Birmingham, UK

Wiebke Arlt is the William Withering Chair of Medicine and Director of the Institute of Metabolism and Systems Research at the University of Birmingham, Birmingham, UK.

Wiebke Arlt trained in General Internal Medicine and Endocrinology at the University of Würzburg, Germany, under the auspices of Bruno Allolio, followed by postdoctoral research at the University of California at San Francisco, CA, USA, hosted by Walter Miller. She came to Birmingham in 2002 following an invitation by Paul Stewart, funded by a German Research Council (DFG) Heisenberg Senior Fellowship. She was awarded a Medical Research Council UK Senior Clinical Fellowship in 2004. In Birmingham, she has served as the Head of the Centre for Endocrinology, Diabetes and Metabolism from 2008 until her recent appointment as the Director of the newly founded Institute for Metabolism and Systems Research (IMSR). Her research focusses on steroid metabolism and action in human disease, with a clinical focus on adrenal and gonadal disorders.

Prof. Wiebke Arlt has served on the Executive Committee of the European Society of Endocrinology and the Steering Committee of the European Network for the Study of Adrenal Tumours (ENSAT) and currently is the Chair of the Clinical Committee of the Society for Endocrinology.

She has received several national and international awards, including the EJE Prize of the European Society of Endocrinology, the Society for Endocrinology Medal, the Ernst Oppenheimer Award of the Endocrine Society USA and the Graham Bull Prize in Clinical Science of the Royal College of Physicians. She has been elected a Fellow of the Academy of Medical Sciences in 2010.

# Clinical Endocrinology Trust Visiting Professor Lecture

Richard J Auchus, Division of Metabolism, Endocrinology, and Diabetes, University of Michigan, Ann Arbor, Michigan, USA

Dr Richard J Auchus is Professor of Pharmacology and Internal Medicine in the Division of Metabolism, Endocrinology, and Diabetes at the University of Michigan and Director of the Diabetes, Endocrinology, & Metabolism Fellowship Program at Michigan. He did postdoctoral work and training at the University of California, San Francisco prior to joining the faculty at UT Southwestern in Dallas. He served as Acting Chief of the Divisions of Endocrinology and Translational Research in Dallas before his relocation to Michigan in 2011.

Dr Auchus and his group are active in research projects ranging from basic chemical principles of steroid biosynthetic enzymes and steroid mass spectrometry to clinical and translational investigation in disorders of the pituitary, adrenal ovaries, and testes that cause hypertension, infertility, and obesity. The common theme of all his work is steroid and sterol biosynthesis and action with an emphasis on the chemistry of human diseases. He collaborates with a range of investigators spanning a broad range of science from clinical neurobiology to molecular mechanisms regulating hormone production and action from nematodes to human beings. His clinical interests also focus on pituitary, adrenal, and reproductive diseases that involve disorders of steroid production, and he is particularly interested in the care of adults with genetic disorders of steroid biosynthesis and action. He has been the recipient of several awards and honors such as Burroughs Wellcome Clinical Scientist Award in Translational Research and the Jean D. Wilson, M.D. Award for Excellence in Scientific Mentoring at UT Southwestern. He has authored over 180 journal articles and 30 book chapters, and he has presented at a diverse range of national and international conferences.

# Society for Endocrinology European Medal Lecture

Frédéric Jaisser, Cordeliers Research Centre, INSERM U1138,
Paris, France

Frederic Jaisser, MD, PhD has a permanent position as Director of Research at the National Institute of Health and Medical Research (INSERM) from 1996. Dr Jaisser received his medical training and degrees from the Reims Medical School and qualified as Nephrologist in 1990. In 2003, he joined the Collège de France in Paris as part of an independent INSERM team and is currently the director of a team of the INSERM Unit U1138, at the Cordeliers Research Centre, Paris. He is the head of the 'Integrative Physiology and Pathophysiology' Department of the Cordeliers Research Centre. Since 2010, he is the Scientific Delegate of the Pathophysiology Committee of the French National Research Agency. He has been recently appointed as French coordinator for Life Sciences in the ECOS French-South America exchange program.

The aim of his current studies is to improve the understanding of the pathophysiological roles and signaling pathways whereby the hormone aldosterone promotes pathologies in various organs including the kidney and the cardiovascular system. His work combines cellular and molecular approaches, animal physiology, pharmacological studies and has implications in human diseases. His interest includes translational research aimed at identifying and validating biomarkers for Mineralocorticoid Receptor activation in cardiovascular and kidney diseases and novel therapeutic use of MR antagonists. He has published 100 papers (H index 30, 2500 citations).

Dr Jaisser belongs to several research networks, including EU-granted programs. More recently he was appointed as Coordinator of a European Network on Aldosterone with more than 45 laboratories from 15 EU countries and two associated countries (Chile and Mexico). The network is dedicated to the Aldosterone field, covering the continuum from experimental to clinical studies.

# Society for Endocrinology International Medal Lecture

Geoffrey Hammond, Professor and Head, Cellular and Physiological Sciences,
University of British Columbia, Canada

Dr Hammond obtained his BSc from the University College of North Wales. After obtaining an MSc in Steroid Endocrinology from the University of Leeds in 1974, he continued postgraduate work in Biochemistry at the University of Oulu, Finland, and received his PhD in 1978. After postdoctoral training at the University of California San Francisco, Dr Hammond was appointed as an MRC (UK) Research Fellow at the University of Manchester in 1981.

From 1984 to 2002, Dr Hammond held appointments in the Departments of Obstetrics and Gynecology, Biochemistry, Pharmacology and Toxicology, and Oncology at the University of Western Ontario (UWO). Between 1986 and 1997, he held an Ontario Cancer Research and Treatment Foundation Research Scholarship. In the 1990s he served as the first Director of the Cancer Research Laboratories at the London Regional Cancer Centre, and received an endowed chair in Molecular Toxicology in 2000. In 2002, Dr Hammond was recruited by The University of British Columbia (UBC) as a Professor in Obstetrics and Gynaecology and Scientific Director of the Child and Family Research Institute. He is currently Professor and Head of Cellular and Physiological Sciences at UBC and holds a Tier I Canada Research Chair in Reproductive Health.

Dr Hammond is recognized for his work on steroid hormone action and extracellular steroid-binding proteins in particular. He has published $>200$ scientific articles, and has served on numerous editorial boards and organizing committees of international meetings. Dr Hammond has held several patents, and has collaborated with the diagnostic and pharmaceutical industries.

# Plenary Lectures

## Society for Endocrinology Starling Medal Lecture

### PL1

**Hyperactive PI-3-kinase signalling without hormone excess: between cancer and endocrinology**

Robert Semple
University of Cambridge, Cambridge, UK.

Peptide hormones stimulate responses in target tissues by triggering enzyme activation inside cells and thus the generation of 'second messenger' molecules. Two of the most important second messengers in endocrinology are cAMP, whose production is stimulated by activation of $G\alpha_s$ G proteins in response to hormones such as TSH and ACTH, and phosphatidylinositol-3,4,5-trisphosphate ($PIP_3$), generated by phosphatidylinositol-3-kinase (PI3K) in response to hormones such as insulin and IGF1. Nearly 25 years ago postzygotic activating mutations in $G\alpha_s$, encoded by GNAS, were discovered to underlie the classical endocrinopathy McCune-Albright syndrome, which features hormone-independent activation of a variety of $Ga_s$-coupled peptide hormone receptors. We and others have recently added to this by discovery that somatic mosaic activating mutations in components of the PI3K pathway underlie a wide variety of overgrowth disorders, ranging from mild generalised overgrowth to severe overgrowth of only some parts of the body. A subset of patients also have severe insulin-independent hypoglycaemia. Elucidating the genetic defect underlying this group of disorders immediately affords the prospect of targeted therapy with small molecule inhibitors as well as yielding insights into the role of the PI3K pathway in humans in health and disease. An overview of the newly defined 'PIK3CA-Related Overgrowth Spectrum' (PROS) will be given, with discussion of the underlying mutational spectrum, cell biology and prospects for therapy.

DOI: 10.1530/endoabs.38.PL1

## Society for Endocrinology Medal Lecture

### PL2

**Kisspeptin – a vital trigger of puberty with therapeutic potential**

Waljit Dhillo
Imperial College London, London, UK.

Infertility affects one in six couples in the UK. Identification of novel factors which are critical to reproductive function could lead to improved therapies for infertility.

Kisspeptin has been identified as a key regulator of the reproductive system. Defective kisspeptin signalling causes a failure of reproductive hormone release (hypogonadotrophic hypogonadism) in rodents and man leading to a failure to go through puberty.

I have determined the effects of kisspeptin on stimulation of reproductive hormone release in humans. Kisspeptin infusion to male volunteers significantly increased plasma LH, FSH and testosterone. Administration of kisspeptin to female volunteers increased plasma LH in all phases of the menstrual cycle with the greatest effect in the preovulatory phase.

I have recently determined the therapeutic potential of kisspeptin in patients with infertility:

i) Hypothalamic amenorrhea is defined as the cessation of menstruation due to abnormal signalling between the hypothalamus and the pituitary gland. It accounts for over 30% of cases of amenorrhea in women of reproductive age. I have shown that kisspeptin administration stimulates reproductive hormone release and can restore LH pulsatility in with hypothalamic amenorrhea.

ii) IVF treatment is now widely and successfully used to enable infertile couples to conceive. However, IVF treatment can result in the potentially life threatening condition, ovarian hyperstimulation syndrome (OHSS) due to the pharmacological use of human chorionic gonadotrophin (hCG) to stimulate oocyte maturation in current IVF protocols. A more physiological stimulus for oocyte maturation, such as kisspeptin, may avoid this dangerous side effect and improve the safety and efficacy of IVF treatment. I have shown that kisspeptin can effectively and safely trigger oocyte maturation resulting in the birth of healthy children without OHSS.

My future work aims to deliver novel kisspeptin based therapies into the clinic to improve the outcomes of patients with reproductive disorders.

DOI: 10.1530/endoabs.38.PL2

## Society for Endocrinology Transatlantic Medal Lecture

### PL3

**An integrated view of nuclear receptor/chromatin interactions: From genome wide to real time molecular dynamics**

Gordon Hager, Ville Paakinaho, Sohyoung Kim, Stephanie Morris, Songjoon Baek, Thomas Johnson, R Louis Schiltz, David Ball, Tatiana Karpova, Erin Swinstead & Diego Presman
National Cancer Institute, Bethesda MD, USA.

Transcription factors (TFs) regulate gene expression by interacting with chromatinized DNA response elements (REs). Access to these elements is dramatically restricted by chromatin organization, and modification of the nucleoprotein structure to allow factor binding is a key feature of cell selective gene regulation (Molecular Cell 29:611, 2008; Molecular Cell 43:145, 2011). Local transitions in chromatin access (often characterized as DNaseI hypersensitive sites (DHSs)) are often associated with the action of ATP-dependent chromatin remodeling proteins (Nature Struct. Mol. Biol. 21:73, 2014; Molecular Cell 14:163, 2004); ATP-dependent remodeling may be a universal feature of these transitions. Methodologies to characterize these processes are crucially limited on two fronts. i) ChIP-seq, Dnase-Seq, FAIRE, etc. all collect signals averaged across large cell populations, and cells in these populations are highly asynchronous with respect to enhancer and promoter modification states. ii) These processes are highly dynamic, often with factor/template interactions persisting only for a few seconds (Science 287:1262, 2000; Nature Commun. 5:4456, 2014; Molecular Cell 56:275, 2014). We have integrated genome wide ChIP-seq and Dnase-Seq datasets with data from live cell imaging. We will discuss a model (Dynamic Assisted Loading) for regulatory element function that integrates critical observations from live cell imaging, genome wide characterization of binding factors, and the biochemistry of remodeling complexes.

DOI: 10.1530/endoabs.38.PL3

## Society for Endocrinology Dale medal lecture

### PL4

**Calcium regulation: from rhinos to molecules**

Rajesh Thakker
University of Oxford, Oxford, UK.

G-protein coupled receptors (GPCRs) comprise the largest superfamily within the human proteome, and are frequent targets for hormones and drugs. Important insights about the roles of GPCRs in endocrinology have been provided by studies of clinical disorders, as illustrated by those of calcium regulation, which involves the parathyroids, first discovered in an Indian Rhinoceros by Sir Richard Owen in 1849. The parathyroids and kidneys express the extracellular calcium-sensing receptor (CaSR), a family C GPCR, that regulates calcium homeostasis by detecting alterations in plasma calcium concentrations and activating G-protein mediated signalling cascades, which modulate parathyroid hormone secretion and urinary calcium excretion. The gene encoding the CaSR is located on chromosome 3q21.1, and CaSR mutations resulting in loss-of-function or gain-of-function lead to familial hypocalciuric hypercalcemia (FHH) and autosomal dominant hypocalcaemia (ADH), respectively. Such CaSR mutations are detected in $\sim$65% of FHH patients, referred to as FHH type 1 (FHH1), and $\sim$70% of ADH patients, referred to as ADH1. Genetic linkage studies in other FHH kindreds had revealed additional loci on chromosomes 19p and 19q13.3, designated FHH2 and FHH3, respectively, indicating genetic heterogeneity for FHH. A hypothesis-driven study established that FHH2 and ADH2 are due to loss-of-function and gain-of-function mutations of G-protein subunit $\alpha_{11}$ ($G\alpha_{11}$), encoded by *GNA11*. A hypothesis-generating study established that FHH3 is due to loss-of function mutations affecting adaptor protein-2 sigma subunit ($AP2\sigma$). AP2, a hetrotetrameric complex, is involved in clathrin-mediated endocytosis and FHH3-associated $AP2\sigma$ mutations, which all affect the Arg15 residue that interacts with the dileucine motif of cargo proteins and comprise Arg15Cys, Arg15His and Arg15Leu, result in increased CaSR cell surface expression likely due to decreased CaSR internalisation. Such $AP2\sigma$ mutations are found in $>$20% of FHH patients who do not have CaSR or $G\alpha_{11}$ mutations. These studies have provided new insights into GPCR signalling and trafficking.

DOI: 10.1530/endoabs.38.PL4

## British Thyroid Association Pitt-Rivers Lecture
### PL5

Anthony Hollenberg
Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, Massachusetts, USA.

Thyroid Hormone exerts key effects on metabolism via its actions on diverse cell types. Central to interpreting its actions is the ability to understand how different tissues integrate and respond to circulating thyroid hormone levels. The use of novel mouse models and metabolite profiling has allowed for a new understanding of how different tissues respond to thyroid hormone to regulate metabolic function.

Partially supported by *Clinical Endocrinology Trust*.
DOI: 10.1530/endoabs.38.PL5

## Clinical Endocrinology Trust Lecture
### PL6

**Mining the steroid metabolome in adrenal hormone excess**

Wiebke Arlt[1,2]
[1]Institute of Metabolism and Systems Research, University of Birmingham, Birmingham, UK and [2]Centre for Endocrinology, Diabetes and Metabolism, Birmingham Health Partners, Birmingham, UK.

The adrenals are at the centre of the steroid universe, with the adrenal cortex producing aldosterone, cortisol and the adrenal androgen precursors DHEA and androstenedione. Hormone excess (or deficiency) is a characteristic feature of the majority of adrenal diseases and recent years have seen major progress in dissecting adrenal steroid excess through novel approaches. This includes steroid metabolomics, the mass spectrometry-based analysis of steroid production in conjunction with computational data analysis to yield new insights into steroid flux and mechanisms underlying steroid excess and steroid-related disorders. In this lecture I will present novel insights into the diagnosis, prognosis and also the pathophysiology of adrenal steroid excess including adrenal tumours, Cushing syndrome and primary aldosteronism, derived from the application of steroid metabolomics.

Generously supported by *Clinical Endocrinology Trust*.
DOI: 10.1530/endoabs.38.PL6

## Clinical Endocrinology Trust Visiting Professor Lecture
### PL7

**Confronting the last frontiers of endocrine hypertension**

Richard J Auchus
Division of Metabolism, Endocrinology, and Diabetes, University of Michigan, Ann Arbor, Michigan, USA.

In the 60 years since primary aldosteronism (PA) was described, our understanding of its pathophysiology and approaches to diagnosis and treatment has improved remarkably. Despite this progress, $<1\%$ of patients with PA are ever screened for this condition, which accounts for 5–8% of hypertension (HTN). The resistance to screening for PA probably derives primarily from the complexities and uncertainties inherent in its evaluation and management. How many types of PA exist, and how do these various forms develop? What is idiopathic hyperaldosteronism? Is our approach to PA the best we can do? How can we encourage broader screening? I will explore some recent developments in PA that challenge some long-held concepts, raise new questions, and suggest alternate strategies.

Besides PA, other more rare forms of mineralocorticoid-mediated HTN exist, in which cortisol, corticosterone, or 11-deoxycorticosterone act on the mineralocorticoid receptor (MR) to cause HTN. Generously assuming that 10% of HTN is due to a well-defined state of mineralocorticoid excess, then why do 60–80% of unselected patients with HTN demonstrate good blood pressure responses to MR antagonists? In chronically salt-loaded societies, how much MR activation is enough to cause hypertension? How do we know if MR activation is responsible for HTN in a given individual? I will review some of these rare genetic and acquired forms of HTN, new disease mechanisms, and additional forms of HTN that might be related to MR and its ligands.

Finally, it is important to consider the mild phenotypes. As our ability to dissect and to characterize abnormalities in steroid biosynthesis improve, we now understand that some diseases are more common than previously assumed and that patients with milder defects can have different clinical manifestations than those that characterize the 'classic' syndromes. Because MR-mediated HTN exerts greater end-organ damage than other forms of HTN, the identification and characterization of patients with these milder phenotypes looms as a strategic frontier for endocrinology to conquer.

Generously supported by *Clinical Endocrinology Trust*.
DOI: 10.1530/endoabs.38.PL7

## Society for Endocrinology European Medal Lecture
### PL8

**Repositioning mineralocorticoid receptor antagonists in renal diseases: pathophysiological basis and therapeutic implications**

Frederic Jaisser
Cordeliers Research Centre, INSERM U1138, Paris, France.

The mineralocorticoid receptor is a ligand-activated transcription factor involved in renal ion homeostasis and blood pressure control. The MR is also expressed in non-renal targets such as heart, vessels, adipose tissue and immune cells where its role remains to be defined. Pharmacological MR antagonists like spironolactone, canrenoate and eplerenone are used for decades in patients with hypertension or, more recently, with heart failure. Therefore pharmacokinetic and toxicology profiles have been extensively studied. Contra-indications in the nephrology field in particular context like acute renal ischemia or chronic kidney diseases are mainly related to the absence of specific safety trials.

Accumulating data, both at the preclinical (rodent and large animal models) and clinical (safety and interventional studies) levels, will allow repositioning of these drugs and their use in novel clinical indications where, for example, microvascular function and hemodynamics are central.

I will describe the role of aldosterone and mineralocorticoid receptor activation in vascular function and will focus on the microvasculature underlying the physiological and pathophysiological role of vascular MR in retinal and renal injuries where alteration of local hemodynamics is important (eye diseases, acute kidney injury, cyclosporine nephrotoxicity) as well as future plan for repositioning MR antagonists in ischemic kidney diseases and renal transplantation.

DOI: 10.1530/endoabs.38.PL8

## Society for Endocrinology International Medal Lecture
### PL9

**Controlling the freedom of steroids in health and disease**

Geoffrey Hammond
University of British Columbia, British Columbia, Canada.

Upon their release from the steroidogenic cells of the adrenals, gonads and placenta, biologically active steroids are sequestered and transported in the blood bound by several proteins, including albumin, sex hormone-binding globulin (SHBG) and corticosteroid-binding globulin (CBG). Together they also regulate the non-protein bound or 'free' fractions of steroid hormones in the blood and therefore control their ability to exit the blood circulation and enter target tissues and specific cell types. As such, these plasma proteins can be viewed as the primary gatekeepers of steroid action.

Albumin binds steroids with limited specificity and low affinity but its enormous capacity allows it to effectively buffer major fluctuations in the plasma levels of steroids. By contrast, SHBG and CBG play more dynamic roles in controlling the freedom of steroids to act in health and disease. They bind biologically active steroids with very high affinity and specificity, with SHBG binding androgens and estrogens and CBG binding glucocorticoids and progesterone. They are both glycoproteins but structurally unrelated and function in very different ways that extend well beyond a simple transport or buffering function in the blood. The liver is the major site of production for plasma SHBG and CBG, but their genes are also expressed in several other tissues, in which these proteins appear to function differently in extravascular compartments than in the blood. Programmed fluctuations in the tissue specific expression of SHBG and CBG occur throughout development, and abnormal plasma levels have been linked to disease risk or occur in response to pathologies. Understanding how the unique structures of SHBG and CBG determine their specialized functions, how changes in their plasma levels are controlled, and how they function outside the blood circulation provides insight into how they control the freedom of steroids to act in health and disease.

DOI: 10.1530/endoabs.38.PL9

# Society For Endocinology Journal Awards

## Society for Endocrinology Journal Award – *Journal of Endocrinology*

### JA1

**Potential biomarker of metformin action**

Ling He, Shumei Meng, Emily L Germain-Lee, Sally Radovick & Fredric E Wondisford

*Journal of Endocrinology* 2014 **221** 363–369. DOI: 10.1530/JOE-14-0084

DOI: 10.1530/endoabs.38.JA1

## Society for Endocrinology Journal Award – *Journal of Molecular Endocrinology*

### JA2

**1,25-vitamin D3 promotes cardiac differentiation through modulation of the WNT signaling pathway**

Su M Hlaing, Leah A Garcia, Jaime R Contreras, Keith C Norris, Monica G Ferrini & Jorge N Artaza

*Journal of Endocrinology* 2014 **53** 303–317. DOI: 10.1530/JME-14-0168

DOI: 10.1530/endoabs.38.JA2

## Society for Endocrinology Journal Award – *Endocrine-Related Cancer*

### JA3

**Aberrant DNA hypermethylation of SDHC: a novel mechanism of tumor development in Carney triad**

Florian Haller, Evgeny A Moskalev, Fabio R Faucz, Sarah Barthelmeß, Stefan Wiemann, Matthias Bieg, Guillaume Assie, Jerome Bertherat, Inga-Marie Schaefer, Claudia Otto, Eleanor Rattenberry, Eamonn R Maher, Philipp Ströbel, Martin Werner, J Aidan Carney, Arndt Hartmann, Constantine A Stratakis & Abbas Agaimyg

*Endocrine-Related Cancer* 2014 **21** 567–577. DOI: 10.1530/ERC-14-0254

DOI: 10.1530/endoabs.38.JA3

## Society for Endocrinology Journal Award – *Clinical Endocrinology*

### JA4

**Drugs that interact with levothyroxine: an observational study from the Thyroid Epidemiology, Audit and Research Study (TEARS)**

Savannah A Irving, Thenmalar Vadiveloo & Graham P Leese

*Clinical Endocrinology* 2015 **82** 136–141. DOI: 10.1111/cen.12559

DOI: 10.1530/endoabs.38.JA4

## Society for Endocrinology Journal Award – *Endocrine Connections*

### JA5

**Role of endogenous ACTH on circadian aldosterone rhythm in patients with primary aldosteronism**

Takuhiro Sonoyama, Masakatsu Sone, Naohisa Tamura, Kyoko Honda, Daisuke Taura, Katsutoshi Kojima, Yorihide Fukuda, Naotetsu Kanamoto, Masako Miura, Akihiro Yasoda, Hiroshi Arai, Hiroshi Itoh & Kazuwa Nakao

*Endocrine Connections* 2014 **3** 173–179. DOI: 10.1530/EC-14-0086

DOI: 10.1530/endoabs.38.JA5

# Symposia

## Endocrinology meets the environment

### S1.1

**Later life consequences of maternal vitamin D deficiency – MAVIDOS study**

Nicholas Harvey
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Low concentrations of 25-hydroxyvitamin D, the major circulating storage form, are common in the general population. Over recent decades, there has been increasing evidence for a role of vitamin D in disease pathogenesis far beyond the musculoskeletal system. Thus, many studies have investigated whether low levels of circulating 25-hydroxyvitamin D have a detrimental effect on pregnancy outcomes, for both mother and offspring, and whether supplementation with vitamin D might ameliorate such effects. We comprehensively surveyed this literature in a recent systematic review, funded by NIHR HTA. Suggestive positive associations were observed between maternal 25-hydroxyvitamin D concentration/vitamin D supplementation during pregnancy, and offspring birthweight, serum calcium concentrations and bone mass, with some evidence for a protective effect of maternal 25-hydroxyvitamin D concentrations on pre-eclampsia. Overall, though, there was insufficient evidence to recommend vitamin D supplementation in pregnancy for any single health outcome. Such findings reinforce the need for high quality randomised control trials, such as the UK MAVIDOS Maternal Vitamin D Osteoporosis study, a multicentre, randomised, placebo-controlled, double-blind trial of 1000 IU/day vitamin $D_3$ (cholecalciferol) vs placebo from 14 weeks gestation till delivery of the offspring, in which the primary outcome is offspring DXA-measured bone mass, with pregnancy outcomes assessed as secondary endpoints. This study tests, in an interventional setting, earlier observations linking low maternal 25-hydroxy-vitamin D concentration to reduced offspring bone mass, and gain valuable information regarding the role of vitamin D in pregnancy for other health outcomes. Such a rigorous interventional approach is essential to enable research questions to be adequately answered, such that alterations to public health policy maybe confidently based on robust evidence.

DOI: 10.1530/endoabs.38.S1.1

### S1.2

**Iodine deficiency**

Mark Vanderpump
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Iodine is an essential component of the thyroid hormones thyroxine ($T_4$) and triiodothyronine ($T_3$) which play a crucial role in brain and neurological development. The ideal dietary allowance of iodine recommended by World Health Organisation (WHO) is 150 μg of iodine per day which increases to 200–250 μg/day in pregnancy. Severe iodine deficiency may be associated with impairment in the psycho-neurological outcome in the progeny because both mother and offspring are exposed to iodine deficiency during gestation (and the postnatal period). Controlled studies performed in iodine-deficient regions have confirmed that iodine supplementation eliminated new cases of cretinism, reduced infant mortality and improved cognitive function in the general population. Even mild iodine deficiency is thought to lead to reductions of 10–15 in the intelligence quotient (IQ) points. Many people are still deficient in iodine, despite major national and international efforts to increase iodine intake, primarily through the voluntary or mandatory iodisation of salt. The WHO estimates that two billion people, including 285 million school-age children, still have iodine deficiency, defined as a urinary iodine (UI) excretion of less than 100 μg/l. Recent epidemiological data suggest that iodine deficiency may also now be an emerging issue in industrialised countries such as the UK, previously thought of as iodine-sufficient. International efforts to control iodine deficiency are slowing, and reaching the third of the worldwide population that remains deficient poses major challenges.

DOI: 10.1530/endoabs.38.S1.2

### S1.3

**Environmental influences on autoimmunity**

Wilmar Wiersinga
Academic Medical Center, Amsterdam, The Netherlands.

Autoimmune endocrine diseases are seen as multifactorial 'complex' diseases in which immune reactions against self-antigens from endocrine glands develop against a particular genetic background facilitated by exposure to environmental factors. A number of these factors have been identified, and most interestingly they can be protective for one disease but a risk for another disease. Much has still to be learned about gene-environment interactions. As an example we will discuss autoimmune thyroid disease (AITD), in which environmental factors contribute for about 30% to the development of AITD. Smoking is a well-known risk factor for Graves' disease, but protects against Hashimoto's disease. Moderate alcohol intake decreases the risk on both Graves' and Hashimoto's disease. Stress exposure is a risk for Graves' but not Hashimoto's disease. There is insufficient evidence that Yersinia enterocolitica infection is linked to AITD, despite its biological plausibility. Low selenium and low vitamin D levels might increase the risk of developing AITD, but data are still inconclusive. Current options for preventive interventions in subjects at risk to develop AITD (like family members of AITD patients) are very limited.

DOI: 10.1530/endoabs.38.S1.3

## Zoning in on adrenal tumours

### S2.1

**Somatic mutations and adrenal remodelling in hyperaldosteronism**

Morris Brown
University of Cambridge, Cambridge, UK.

Primary Aldosteronism (PA), due to a unilateral aldosterone-producing adenoma of the adrenal (APA), is the commonest curable cause of Hypertension, but the prospects for cure fall with age. APAs rarely increase in size, suggesting an origin much earlier than the development of resistant hypertension. Most APAs have gain-of-function somatic mutations which result in increased $Ca^{2+}$ entry, and constitutive activation of aldosterone production. Women with larger APAs, and cells resembling zona fasciculata (ZF) cells, are likely to have *KCNJ5* mutation, whilst smaller APAs in men, with resemblance to zona glomerulosa (ZG) cells, are more likely to have mutations of *ATP1A1, ATP2B3 or CACNA1D*.[1,2] The number of different gain-of-function mutations within one gene (19 in CACNA1D), and overall frequency of APAs, suggest a common driver, which we believe may, paradoxically, be salt. A striking difference between ZG of human adrenals and other species is the sparseness of aldosterone synthase expression, and an irregular, even atrophic, ZG. Caspase-3 and Tunel stains for apoptosis are positive. A microarray of ZG cells found several genes which are many-fold upregulated in human ZG (vs ZF) that do not feature in similar analysis of rat adrenals.[3] Functional analysis of some of these genes (e.g. LGR5, DACH1) showed that they inhibit aldosterone production, and may cause cell loss. Since the CYP11B2$^{-/-}$ mouse (no aldosterone synthase) has apoptotic ZG cells, we hypothesize that aldosterone protects against apoptosis, and that the prevailing salt-induced suppression of aldosterone in human ZG selects for ZG cells with mutations causing constitutive aldosterone production. This hypothesis would explain why ZG-like APAs tend to be small. The selective advantage comes not from proliferation, but from synthesis of aldosterone. Indeed, on $^{11}$C-metomidate PET CT, ZG-like APAs are often detected as small, bright hot spots within adrenals previously reported as 'normal'.

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## S2.2

**Advances in genetic causes of adrenal Cushing's**

Felix Beuschlein
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The advent of new genetic techniques that allow for high-throughput sequencing in surgical tumour tissues and germline DNA has boosted progress in many fields of biomedical research. The technique has been proven to be particularly fruitful in the area of endocrine tumours with many new driver genes being identified over the last few years that are involved in cell growth but more importantly in hormonal autonomy. Examples account for aldosterone producing adrenal adenomas, insulin producing neuroendocrine tumours, growth hormone producing pituitary adenomas and pheochromocytomas. Also, the field of Cushing's syndrome has been moved forward on several fronts by identifying new genetic and molecular mechanisms that ultimately result in the clinical phenotype of hypercortisolism.

Following a whole genome sequencing approach in patients with familial cases of bilateral macronodular adrenal hyperplasia the group of Jérôme Bertherat identified germline mutations in the ARMC5 gene together with second hits in adrenal nodules as the cause of the disorder. This finding has not only opened the possibility to explore new pathophysiological mechanisms in adrenal steroidogenesis and cellular growth but also has led to the clinical application of genetic testing for case finding and prospective clinical follow-up. Based on exome sequencing from tumour tissue an European consortium was able to pinpoint mutations in the catalytic subunit of PKA (PRKACA) as the underlying genetic event in around one third of cases of cortisol producing adrenal adenomas. In addition, genetic duplications of the same gene were identified in a subgroup of patients from the NIH with bilateral micronodular hyperplasia. Interestingly, in the adenoma patients, there was a clear genotype/phenotype correlation with the most severe disease course in mutation carriers.

In summary, the last two years have witnessed significant progress in the elucidation of molecular mechanisms driving the clinical phenotype of endogenous hypercortisolism with all its metabolic and cardiovascular sequelae. The future will show how these findings will translate into clinically tangible progress.

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## S2.3

**The new genetics of phaeochromocytoma**

Anne-Paule Gimenez-Roqueplo[1,2,3]
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Paragangliomas and pheochromocytomas (PPGL) are neuroendocrine tumors with a very strong genetic component. A germline mutation in one of the 13 different susceptibility genes identified so far explains about 40% of all cases. Genetic testing, which is indicated in every patient, can be guided by the clinical presentation as well as by the secretory phenotype and by the immunohistochemical analysis of the tumors. The diagnosis of an inherited form drives clinical management and tumor surveillance[1].

While whole-exome sequencing studies showed that PPGL is characterized by a low mutation rate of 0.3 mutations per megabase similar to other neural crest-derived tumors, the first integrative genomic analysis of PPGL, carried out by the French COMETE network, demonstrated that mutation status in PPGL susceptibility genes is strongly correlated with multi-omics data and revealed the crucial role of predisposing mutations as being the main drivers of PPGL[2]. PPGL subtypes can be defined by a set of unique genomic alterations that represent different molecular entities. Transcriptomic studies identified two main molecular pathways, activating either the hypoxic pathway or the MAPkinase/mTOR signalling. This comprehensive analysis further illustrated the functional interdependence between genomic and epigenomic dysregulations. Indeed, DNA methylation profiling uncovered a hypermethylator phenotype in SDH-related tumors and revealed that succinate is acting as an oncometabolite, inhibiting 2-oxoglutarate-dependent dioxygenases, such as HIF prolyl-hydroxylases and histone/DNA demethylases[3]. miRNA sequencing identified miRNA expression clusters strongly associated with mRNA expression profiling. 'Omics' data suggested new therapeutic targets for patients with a metastatic PPGL and new diagnosis and prognostic biomarkers. The knowledge of specific genomic alterations should provide a real help for individual patient management and should guide the choice of targeted therapy for malignant cases. New 'omics'-based tests are likely to be transferred from research laboratories to clinical practice to offer the access to a precise molecular classification of PPGL to practicing clinicians with the goal of establishing a personalized medical management of affected patients.

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# Fanning the flames of mitochondrial function

## S3.1

**Novel regulation of fat metabolism in cancer**

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The nutrient demands of cancer cannot be met by normal cell metabolism. Cancer cells undergo dramatic alteration of metabolic pathways in a process called reprogramming, characterized by increased nutrient uptake and re-purposing of these fuels for biosynthetic, bioenergetic or signaling pathways. Partitioning carbon sources toward growth and away from ATP production necessitates other means of generating energy for biosynthetic reactions. While much research has examined the use of glucose and glutamine by tumor cells, a subset of cancers have a high capacity and preference for fat oxidation. Our knowledge of pathways that drive dependency on fatty acid oxidation in cancer is limited. Prolyl hydroxylase domain proteins (PHDs, also called EGLN1–3) are a family of oxygen and $\alpha$-ketoglutarate dependent enzymes that have been linked to fuel switching in cancer. Through their unique ability to integrate cellular stress and nutrient status with coordination of metabolic outputs, PHDs are well poised to play pivotal roles in tumor progression and survival. PHDs are well known to regulate glycolytic metabolism through prolyl hydroxylation of the master transcriptional regulator hypoxia-inducible factor. Here, we reveal a novel mechanism through which the PHD family of proteins regulates the use of an alternative fuel in cancer: fatty acids. We find that PHD status serves as an indicator of fatty acid metabolic status and informs the susceptibility of a particular cancer to pharmacological inhibitors of fatty acid oxidation.

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## S3.2

Abstract unavailable.

## S3.3

**Mitochondrial disease: problems and solutions**

Mitochondrial diseases are a common but challenging group of genetic conditions. The involvement of both the nuclear and mitochondrial genome means that the inheritance pattern is complicated. Mitochondrial diseases affect many different organs and may present to a variety of different physicians including endocrinologists. The treatment of mitochondrial diseases is challenging with no curative treatment available for the majority of patients. It is important to give symptomatic treatment such as optimum management of diabetes and cardiac problems. In view of the absence of curative therapies for patients with mitochondrial disease, prevention of disease transmission is important for families. For those families with nuclear inherited mitochondrial

disease the reproductive options are similar to those of other nuclear genetic conditions. For those families with mitochondrial DNA mutations it is much more challenging. Mitochondrial DNA is maternally inherited and is present in multiple copies. Whilst techniques such as chorionic villus biopsy and preimplantation genetic diagnosis are valuable to some families, others will not benefit from this technique. Mitochondrial donation is a new reproductive option for these families which involves the transfer of the nuclear genes from an oocyte or zygote of a women that carried the mitochondrial DNA mutation into an oocyte or zygote from a donor woman. Thus any child born would have the nuclear genes of both parents but the mitochondrial DNA from a donor.

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## It's all in the timing: rhythms underlying endocrine systems

### S4.1

**Sleep: what endocrinologists should know about the body clock**

Jonathan Johnston
University of Surrey, Surrey, UK.

Endogenous biological rhythms are commonplace throughout the natural world and can be broadly categorised as being ultradian (period $<$24-h), circadian (period of $\sim$24-h), or infradian (period $>$24-h). Circadian rhythms have been studied in detail in mammalian species, including humans. These rhythms are driven by cell autonomous clocks found in the suprachiasmatic nuclei (SCN) of the hypothalamus and also throughout the rest of the body. Circadian clocks influence the body's physiology via numerous output pathways. Within any given tissue, it is estimated that around 10–20% of the transcriptome, proteome, and metabolome is under circadian control. Moreover, nearly half of all murine genes are circadian in at least one tissue. Circadian time is also communicated via regulation of signalling molecules (e.g. hormones) and behaviour. One key behaviour that exhibits circadian control is sleep. The regulation of sleep is described by a two-process model in which circadian regulation of sleep-arousal (process C) combines with a homeostatic measure of sleep pressure (process S) that increases during wakefulness and dissipates during sleep. Although clocks and sleep are sometimes studied separately, there are clear reciprocal links between these two aspects of physiology; sleep propensity exhibits a circadian rhythm and disruption of sleep duration or timing alters circadian rhythmicity. Some hormones (e.g. melatonin and cortisol) are well-known to exhibit robust daily rhythms with well-defined relationships to the sleep–wake cycle. More recent research has clearly demonstrated widespread interactions between clocks, sleep, endocrinology, and physiology. One such example is the control of glucose homeostatis, which is controlled at multiple levels by circadian rhythms and is adversely effected by altered quantity and quality of sleep. This talk will explain why both circadian time and sleep history are important considerations in the study of endocrine function and broader physiology.

DOI: 10.1530/endoabs.38.S4.1

### S4.2

**Rhythms of adrenal glucocorticoid secretion**

Francesca Spiga
University of Bristol, Bristol, UK.

The activity of the hypothalamic–pituitary–adrenal (HPA) axis is characterized by an ultradian (pulsatile) pattern of glucocorticoid secretion that is critical for optimal transcriptional, neuroendocrine and behavioural responses to glucocorticoids. We have investigated the molecular mechanisms underlying the origin of glucocorticoid ultradian rhythm within the rat adrenal gland. We show that this rhythm of glucocorticoids depends on highly dynamic processes within adrenocortical steroidogenic cells, that includes rapid phosphorylation of proteins involved in the acute corticosterone response to a pulse of ACTH, and rapid transcription of steroidogenic genes, including *StAR* and *MRAP*. We also show that ultradian corticosterone secretion is further associated with rapid and transient transcription of nuclear receptors that regulate steroidogenic genes expression, including *SF-1*, *Nur77*, and *Dax-1*. By using a model of immunological stress we show that disruption of these dynamics leads to abnormal glucocorticoid secretion, as observed in disease and critical illness in both human and the rat. Finally, by using mathematical modelling we show that intra-adrenal negative-feedback mechanisms involving the activity of the glucocorticoid receptor appears to be an important factor in the dynamic regulation of these processes.

DOI: 10.1530/endoabs.38.S4.2

### S4.3

**Dynamics in hypothalamic–pituitary function over multiple time-scales**

Paul Le Tissier
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Changes in hormone output to regulate physiological processes is a feature of all endocrine systems, with the added complication that for many systems, the pattern as well as level of output, determines the response of target tissues. Periodic patterns of hormone secretion can occur over multiple time scales, from ultradian to circadian, infradian, and circannual and may be dependent on physiological status. The activity and output of lactotroph and somatotroph cells of the pituitary and their regulation by the hypothalamus is highly dynamic and dependent on physiological status. Using transgenic mice allowing identification and manipulation of pituitary cells and their regulatory hypothalamic neurones, we are dissecting the processes underlying the plasticity of pituitary output and its patterning. Studies to date have overturned accepted dogma that variation in pituitary cell number and altered hypothalamic regulation accounts for altered output. At the pituitary level, we have showing that homotypic cells are organized into distinct, characteristic motifs, which are modified by physiological status. These allow cell–cell communication, resulting in coordination and modification of responses to hypothalamic regulation. Remarkably, the reversibility of altered cell organization in response to physiological demand is also dependent on cell type: reorganization of lactotrophs during lactation is maintained following weaning and is associated with an enhanced output following the repeated demand of a further lactation. These pituitary-level changes do not dominate hypothalamic regulation: changes in the output of, for example tuberinfundibular dopamine neurones, must also occur to allow maintenance of high circulating prolactin concentrations required for lactation. To further study hypothalamic–pituitary functional relationships, novel tools and methodologies are required. The development and use of some of these will be described.

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## Premature ovarian insufficiency

### S5.1

Abstract unavailable.

### S5.2

**Neurological and psychological effects of premature ovarian insufficiency**

Eva Hogervorst
Loughborough University, Loughborough, Leicestershire, UK.

We recently reviewed the evidence for neurological effects of premature ovarian insufficiency (Hogervorst 2014, ESHRE in press) to provide guidelines for ESHRE.

Several observational studies reported that an early age at menopause increased risk for dementia and was associated with worse cognitive function and dementia pathology.

However, sex hormone treatment up to the natural age at menopause could off-set this risk. It is currently not clear how long hormone treatment can be maintained. Cell culture studies suggest that estrogen treatment can accelerate pathology but maintains healthy neurons.

Large treatment trials such as WHIMS suggested that adverse hormone effects, such as breast cancer risk and cardiovascular disease do not occur until up to 5–7 years after initiation of hormone treatment. Negative effects of hormones on brain function have not been shown in recently menopausal women. Our Cochrane reviews have shown that these negative effects of combination hormone therapy on cognition have mainly been found in women over the age of 65 years.

Our other reviews (Clifford 2009) suggest that midlife lifestyle changes such as exercise, cessation of smoking, and healthy diets to reduce metabolic syndrome and consequent risk for cardiovascular disease and dementia in later life are particularly important for women (Hogervorst 2012).

In sum, limited evidence suggests that hormone treatment for POI up to the natural age at menopause combined with healthy lifestyle changes may reduce

risk for dementia in later life. In addition, treatment should probably not be continued if cardiovascular risk increases (diabetes mellitus and atherosclerosis) to reduce risk for dementia. Women at risk for both cardiovascular disease and dementia (who carry the APOE epsilon 4 genotype) do not seem to benefit from hormone treatment but we did not find that other polymorphisms associated with estrogen synthesis or metabolism affected risk (Thornton 2010).

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## S5.3

Abstract unavailable.

## Clinical implications of thyroid genomics (Supported by *Journal of Molecular Endocrinology*)

## S6.1

**Thyroid genomics: relevance to thyroid hormone therapy**

Colin Dayan
Cardiff University, Cardiff, UK.

Many processes are involved between ingesting thyroid hormone and the hormone having an effect within cells. These include hormone absorption, transport into cells, deiodination (for activation and inactivation), export from cells, receptor binding, and activation. Rare, major single gene defects have been reported at many though not all of these steps. Genome wide analyses have also identified multiple common loci associated with TSH and to lesser extent $FT_4$ and $FT_3$ levels. Since variation in thyroid hormone levels between individuals even within the reference range has effects on disease processes such as hypertension, cardiovascular disease, metabolic syndrome, and bone density, the effect of these variants on an individual's set point for thyroid function may be relevant to considering thresholds for thyroid hormone replacement. Rare variants with major effect in a small number of individuals may not yet have been identified. The effects of genetic variation in factors beyond the serum level of thyroid hormone are likely to also affect disease processes. Common, functional variants are difficult to define, and attention has focussed on the Thr92Ala variant in deiodinase 2, in which a homozygous polymorphism in the coding region is present in around 13% of Caucasians. The functional significance of this variant is debated and recent studies suggest that it may have effects on neuronal cellular function independent of its deiodinase activity. Associations with osteo-arthritis and bipolar disorder have been reported, as well as psychological well-being on thyroid hormone and response to therapy. 'Thyroid hormone bioavailability' – the combination of low thyroid hormone levels and Thr92Ala homozygosity has also been associated with reduced IQ in children. Not all studies have replicated these effects and further well powered studies are required. Defining these effects more precisely should allow us to determine individually adjusted thresholds for initiating thyroid hormone therapy in order to derive net benefit as well as determining if there is any biological basis for the need for combination $T_4$ and $T_3$ therapy in selected individuals.

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## S6.2

Abstract unavailable.

## S6.3

**TCGA genomic characterization of papillary thyroid carcinoma**

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Objective
Recent advances in next-generation sequencing (NGS) technology have permitted comprehensive genomic characterization of the most common types of cancer. The Cancer Genome Atlas (TCGA), a program of the U.S. National Institutes of Health (a joint NCI and NHGRI effort), was created to systematically analyze the genome of the most common types of cancer, including the most common type of thyroid cancer, papillary thyroid carcinoma (PTC). TCGA has performed the first of several large-scale attempts to define the genomic landscape of thyroid cancer by identifying the somatic genetic alterations of a significant cohort of PTC.
Methods
The TCGA Research Network completed an integrated genomic analysis of 496 PTCs using NGS and other pan-genomic technologies, together with detailed pathologic and clinical data.
Results
Significant knowledge was known about the genetics of PTC. However, the comprehensive nature of the TCGA project revealed many novel aspects of the genetics and epigenetics of PTC. Mutually exclusive novel single nucleotide variants and gene fusions, thought to be driving events, were identified. Based on gene expression profiles, PTCs were divided into BRAF-V600E-like and RAS-like groups, which displayed distinct differentiation and signaling properties. Differentially expressed miRs were identified and several were hypothesized to play a prognostic role in PTC.
Discussion
This study revealed many novel genetics alterations in the PTC genome. However, the primary conclusion of this study is PTC represents a genetically diverse group of neoplasms, with BRAF-driven tumours being most diverse and also fundamentally different from RAS-driven tumors.

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## The rise and rise of the FGFs in endocrinology

## S7.1

Abstract unavailable.

## S7.2

**FGF21: starvation hormone to a clinical drug?**

Alexei Kharitonenkov[1], Archita Agrawal[1], Pengyun Li[1], Richard DiMarchi[1] & Diego Perez-Tlive[2]
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Fibroblast growth factor 21 (FGF21) is an emerging regulator of energy homeostasis and a novel target for the development of therapies to treat diabetes, cardiovascular disease, and obesity. FGF21 was discovered in an *in vitro* high throughput screen. It was later shown to have impressive metabolic pharmacology including glucose, lipid, and body weight lowering effects in a variety of animal models, including non-human primates. When tested clinically an FGF21-based analog demonstrated dramatic efficacy in management of circulating lipids but failed to produce a robust glycemic lowering. The basis of this discrepancy in human study relative to pre-clinical investigations remains a conundrum and an area of active investigation. More recently, FGF1 and related structural analogs have emerged as capable of providing impressive metabolic benefits when administered to mice, and as such proposed as an alternative to FGF21-based therapy. Our work is focused on enhancing the inherent properties of FGF21 and further interrogating the mechanism for FGF1 signaling relative to FGF21.

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## S7.3

**The increasing understanding of FGF23 in pathogenesis and treatment of diverse endocrine disease**

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Phosphate is an essential ion for mineralization of bone. Hypophosphatemia leads to rickets and osteomalacia whereas hyperphosphatemia promotes ectopic calcification in soft tissues. FGF23 is a polypeptide hormone secreted by bone that regulates serum phosphate concentration. It acts on kidney to decrease phosphate reabsorption, and 1,25-dihydroxy vitamin D secretion which in turn reduces phosphorus absorption by the gut. Its action on the renal tubule appears to require PTH activity. Secretion of FGF23 in humans is regulated by several factors including dietary phosphorus, 1,25-dihydroxy vitamin D, and iron status. However, the phosphate-sensing pathway through which dietary phosphorus regulation occurs remains unknown. The hormone contains a proteolytic cleavage site and both intact bioactive FGF23 and inactive N- and C-terminal fragments are present in serum. Clinical assays are available for intact FGF23 and for C-terminal FGF23 which measures intact hormone plus C-terminal fragments. The C-terminal fragment may act as an antagonist to the FGF23 receptor. The FG23 receptor is a complex comprising FGFR1 and Klotho a co-receptor produced and secreted by the kidney. Diseases of the FGF23/1,25-dihydroxy vitamin D/PTH endocrine axis are caused by both rare monogenetic and common acquired diseases. The commonest genetic disease with increased secretion of FGF23 is X-linked hypophosphatemia (XLH) causing rickets and osteomalacia whereas hyperphosphatemic familial tumoral calcinosis is due to decreased FGF23 activity. The commonest acquired disease of over secretion of FGF23 is chronic renal failure. Treatment with monoclonal antibody to FGF23 in XLH adult patients aimed at decreasing FGF23 activity, increases serum phosphate and 1,25-dihydroxy vitamin D without adversely affecting other aspects of mineral homeostasis. Such treatment may prove useful in other genetic and acquired diseases of excess FGF23 secretion.

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# Fine-tuning of metabolic state for optimal pregnancy outcome

## S8.1

**Revolutionising type 1 diabetes metabolic control in pregnancy**

Helen Murphy[1,2], Zoe Stewart[3] & Roman Hovorka[3]
[1]University of East Anglia, Norwich, UK; [2]Cambridge University NHS Foundation Trust, Cambridge, UK; [3]University of Cambridge, Cambridge, UK.

Continuous glucose monitoring (CGM) has highlighted the gap that exists between our expectations of tight metabolic control and the realities of actually achieving this, particularly during type 1 diabetes pregnancy. Longitudinal measurements indicate that despite overall 'good' HbA1c levels, pregnant women with type 1 diabetes spend 8 h/day with blood glucose levels above the recommended targets. New closed-loop (CL) or artificial pancreas approaches integrate insulin pump delivery (CSII) with CGM via computerized algorithms.
The vital component of a CL system for use during pregnancy is a control algorithm which can function safely despite the physiological changes in glucose turnover, endogenous glucose production, and insulin kinetics. We have defined these changes in type 1 diabetes pregnancy and completed proof-of-concept phase I feasibility studies evaluating overnight CL, in early (12–16 weeks) and late (28–32 weeks) gestation. Pilot feasibility studies demonstrated near-normal overnight glucose control (85–100% time within the target range of 3.5–7.8 mmol/l), during early and late gestation. In a subsequent 24-h crossover study comparing CL vs conventional CSII, CL achieved excellent overnight control (95–100% time in target), with 80% overall time in target, during meals, snacks and physical activity. Phase II unsupervised home studies evaluating the feasibility, safety and efficacy of CL in real-life home settings are currently underway with encouraging preliminary data. The next challenge is to translate promising preliminary results into real-life benefits for mothers with type 1 diabetes and their offspring.

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## S8.2

**Pregnancy and obesity**

Rebecca Reynolds
University of Edinburgh, Edinburgh, UK.

One in five women in the UK is obese (BMI $>30$ kg/m$^2$) at antenatal booking. Maternal obesity is associated with complications for the mother including increased risk of developing gestational diabetes, pre-eclampsia and need for caesarean section. For the offspring short term complications include risk of macrosomia and need for admission to the neonatal unit. It is now apparent that the effects of maternal obesity for the offspring extend beyond the neonatal period with increased risk of obesity in childhood, adolescence and adult life. In a recent record-linkage study we demonstrated that maternal obesity is associated with increased risk of premature mortality and hospital admissions for cardiovascular events in her adult offspring. Animal models suggest the adverse effects of maternal obesity on offspring outcomes are 'programmed' *in utero*. To investigate underlying mechanisms we have been carrying out a case–control study of very severely obese pregnant women (BMI $>40$ kg/m$^2$) vs. normal weight controls. We characterise maternal weight, body composition, and metabolic profiles through pregnancy and study infant growth and development at birth and 3 and 6 months. Placenta and cord blood are collected at birth. Our findings suggest early interventions to improve weight and diet in obese pregnant women are urgently needed. As lifestyle interventions in obese pregnancy are challenging, we have conducted a randomised controlled trial (EMPOWaR) in obese pregnant women using the insulin sensitiser metformin vs placebo. Outcomes for mother and child will be discussed.

DOI: 10.1530/endoabs.38.S8.2

## S8.3

**Autoimmune thyroid disease before and during pregnancy**

Bijay Vaidya
Royal Devon and Exeter Hospital, University of Exeter Medical School, Exeter, UK.

Autoimmune thyroid diseases, including Graves' disease and autoimmune thyroiditis, are common in women of childbearing age. Poorly controlled Graves' disease is associated with an increased risk of fetal loss, premature birth, pre-eclampsia, intra-uterine growth retardation, and thyroid storm. Antithyroid drugs, propylthiouracil and carbimazole (or its metabolite methimazole), cross placenta and may cause hypothyroidism and goitre in the fetus. Carbimazole is associated with characteristic embryopathy, whilst propylthiouracil has also been shown to be associated with liver failure and congenital malformations. Women presenting with Graves' disease before and during pregnancy must be counselled about the risks of uncontrolled thyrotoxicosis and adverse effects of antithyroid drugs. Autoimmune thyroiditis can manifest as overt or subclinical hypothyroidism. Both overt and subclinical hypothyroidism in pregnancy is associated with impaired neurological development of the offspring and other adverse pregnancy outcomes, including miscarriage, premature birth, pre-eclampsia, low birth weight and gestational diabetes. Women with hypothyroidism need increased dose of levothyroxine in pregnancy to remain euthyroid. Recent studies have highlighted suboptimal management of hypothyroidism in pregnancy, with associated adverse pregnancy outcomes. Whether all pregnant women should be screened for thyroid function remains controversial. About one in ten euthyroid women have thyroid peroxidase or thyroglobulin antibodies. These women carry an increased risk of subfertility, miscarriage, and premature birth. One randomised controlled trial has shown that levothyroxine in such women may reduce the risk of miscarriage and premature birth. This presentation will discuss impact of autoimmune thyroid diseases in pregnancy, and evidence base for their management.

DOI: 10.1530/endoabs.38.S8.3

# The endocrinology of the thin (Supported by *Endocrine Connections*)

## S9.1

**Endocrine dysfunction in anorexia nervosa**

Karen Miller[1,2]
[1]Massachusetts General Hospital, Boston, Massachusetts, USA; [2]Harvard Medical School, Boston, Massachusetts, USA.

Anorexia nervosa is a common psychiatric disease, with a prevalence of 1–2% of college-aged women, characterized by chronic starvation. Nutritional deprivation is complicated by serious and multi-axis endocrine dysregulation. This includes abnormalities in GnRH secretion resulting in hypothalamic amenorrhea, with resultant estrogen and androgen deficiency, which is usually but not always reversible with weight and psychiatric recovery. GH resistance at the level of the

liver, resulting in low serum IGF1 levels and, through feedback, elevated GH production is also characteristic of anorexia nervosa. It has long been known that anorexia nervosa is also characterized by hypercortisolemia in a subset of patients, and more recently, abnormalities in appetite-regulating and enteric peptide levels have been demonstrated. Consequences of endocrine dysfunction in anorexia nervosa include severe bone loss, which is observed in the majority of such women, despite young age.

DOI: 10.1530/endoabs.38.S9.1

## S9.2

Abstract unavailable.

## S9.3

Abstract unavailable.

## Blood and guts: how the intestine transduces nutritional cues to endocrine signals (Supported by *Journal of Endocrinology*)

## S10.1

Abstract unavailable.

## S10.2

**The glucoregulatory role of small intestinal nutrient- and pharmacological-sensing**

Frank Duca
University Health Network - Toronto General Research Institute, Toronto, Ontario, Canada.

The gastrointestinal tract is anatomically positioned to play a crucial role in the regulation of metabolic homeostasis, providing critical postingestive negative feedback to regulate both exogenous energy intake, as well as endogenous glucose production. This talk will highlight the ability of small intestinal lipid sensing to lower glucose production via a gut–brain–liver axis that is dependent on local gut peptide signaling. Furthermore, recent evidence indicates that the antidiabetic agents, metformin and resveratrol, activate intestinal energy sensory mechanisms to remotely lower hepatic glucose production via a neuronal network, which are critical for their overall glucose-lowering effectiveness. A better understanding of these pathways lays the groundwork for intestinally targeted drug therapy for the treatment of diabetes.

DOI: 10.1530/endoabs.38.S10.2

## S10.3

**Short chain fatty acids in the regulation of energy homeostasis**

Gary Frost
Imperial College London, London, UK.

In recent years, there has been a renewed interest in the role of dietary fibre in obesity management. Much of this interest stems from animal and human studies that suggest increased intake of fermentable fibre can improve body composition. A growing number of reports have demonstrated that the principal products of colonic fermentation of dietary fibre, short chain fatty acids (SCFAs), contribute to energy homeostasis via effects on cellular metabolic pathways and receptor mediated mechanisms. In particular, over the past decade it has been identified that a widespread receptor system exists for SCFAs. These G protein-coupled receptors, free fatty acid receptor 2 (FFAR2) and 3 (FFAR3) are expressed in numerous tissues, including the gut epithelium, adipose tissue and liver. Investigations using FFAR2- or FFAR3-deficient mice suggest that SCFA-mediated stimulation of these receptors at different tissue sites modulates metabolic processes that control energy intake, utilization and expenditure.
The importance of SCFAs to metabolism has been further emphasised in studies where germ-free mice have received gut microbiota transplants. These investigations highlight that the transfer of gut microbiota compositions that produce different levels of SCFAs in the colon influence body weight gain and adiposity. Increasing colonic SCFAs is therefore an attractive target to improve metabolic health in humans. However, translating the positive outcomes observed in animal studies into humans remains a major challenge due to the difficulty in reliably increasing SCFA production in the human colon. Our group in partnership with the University of Glasgow have developed a method to deliver SCFA to the colon orally. We have demonstrated the potential of SCFA to effect body weight over a 6 month period using this technology. However, the mechanism which underlie this observation remains unclear, potential possibilities will be discussed in this lecture.

DOI: 10.1530/endoabs.38.S10.3

## Epigenetics in endocrine-related cancers (Supported by *Endocrine-Related Cancer*)

## S11.1

**Oestrogen receptors and epigenetics in breast cancer aetiology and outcome**

Simak Ali
Imperial College London, London, UK.

Oestrogens promote breast cancer development and progression by binding to the oestrogen receptors. Oestrogen receptor-alpha (ER) is a transcription regulatory protein that is activated upon binding oestrogen and acts by controlling gene expression in breast cancer cells and is the target for endocrine therapies that inhibit its activity by competing with oestrogen for binding to ER (anti-oestrogens) or by inhibiting oestrogen biosynthesis (aromatase inhibitors). These therapies have contributed to the decline in breast cancer mortality in recent years. However, many patients either do not respond, or eventually relapse, necessitating a better understanding of the mechanisms of gene regulation by ER, towards improved patient stratification and the development of drugs for the treatment of advanced breast cancer.
Gene regulation by ER requires the concerted action of diverse chromatin remodelling and histone modification enzymes, acting at the regulatory regions of ER target genes. Thus epigenetic drivers play essential roles in oestrogen action in breast cancer. Interestingly, we have also demonstrated the importance of DNA repair enzymes in the regulation of ER target gene expression. In particular, we have shown that DNA strand break generation is integral to chromatin remodelling/modification in gene regulation by ER. These findings and their implications for the treatment of ER-positive breast cancer will be presented.

DOI: 10.1530/endoabs.38.S11.1

## S11.2

**Epigenetic regulation of androgen receptor function in prostate cancer**

Moray Campbell
Roswell Park Cancer Institute, Buffalo, New York, USA.

The androgen receptor (AR) is a major therapeutic target once prostate cancer has progressed. Even invasive cells remain initially responsive to androgen

deprivation therapy (ADT) but ultimately become ADT-recurrent, which is lethal. Aggressive ADT-recurrent cells nonetheless retain active AR signaling, but in a distorted form as a result of the actions of transcription factor co-regulators, including the corepressors, for example as revealed by the TCGA consortium. We have examined how corepressors distort AR signaling in prostate cancer progression by determining the choice and capacity of AR binding. Combining ChIP approaches with TCGA data revealed that NCOR1 and NCOR2/SMRT have altered interactions with the AR in cell models of different stages of disease, and led to altered DNA CpG methylation in tumors. Genome-wide ChIP-Seq approaches established the NCOR1 and NCOR2/SMRT cistrome in AR sensitive and ADT-recurrent models. An integrative genomic pipeline revealed that the choice of binding site and the effect of gene expression was extremely dynamic. Notably, distal NCOR2/SMRT binding significantly associated with gene repression. In ADT-sensitive models, activation of the AR receptor induced the NCOR2/SMRT to proximal binding where it was more frequently associated with elevated gene expression. Interestingly, basal NCOR2/SMRT distribution in ADT-recurrent models reflected the patterns in ADT-sensitive models following AR stimulation. These findings suggest that the NCOR2/SMRT function is distorted in ADT models to phenocopy the effect of AR stimulation. Supporting a pro-tumorgenic role, NCOR2/SMRT staining on a TMA of 600 men following radical prostatectomy revealed that elevated co-repressor staining was associated with several poor outcome measures including time to treatment failure. Thus NCOR2/SMRT elevation may play a role to enhance AR actions in aggressive cancers and associates with worse disease outcome.

DOI: 10.1530/endoabs.38.S11.2

## S11.3

**Epigenetic signalling and acquired drug resistance in ovarian cancer**

Robert Brown
Imperial College London, London, UK.

Epigenetic events, such as gene promoter DNA hypermethylation linked to changes in gene expression, are causative of resistance to cytotoxic chemotherapy drugs such as cisplatin in ovarian cancer cell line models, and associated with response to chemotherapy and survival of high grade serous ovarian cancer patients. We are studying the role of epigenetic change throughout the patient journey and how this associates with gene expression changes which can be selected for during treatment to give rise to drug resistance. Based on these studies we propose that epigenetic change in tumours, potentially caused by DNA damage and repair, generates diversity and gene expression states that can rapidly evolve through drug treatment in a multi-step process, with the consequence in patients of acquired resistance and treatment failure. As an example, inactivation of the DNA mismatch repair gene *MLH1* by DNA methylation leads to replication bypass and platinum damage tolerance, which can be reversed by DNA demethylating agents. These studies have led to clinical trials of epigenetic therapies as potential tumour chemosensitisers, with biomarkers inherent to the trial design. Current trials have demonstrated the importance of identifying less toxic agents, rational approaches to drug combinations and the need for appropriate stratification biomarkers. To address this we are evaluating novel histone deacetylase (HDAC) and histone methyltransferase (HKMT) inhibitors with associated biomarkers and initiated ChIP-Seq studies of histone marks in patient derived tumours.

DOI: 10.1530/endoabs.38.S11.3

# Corticosteroids - getting to the heart of the matter (Supported by *Journal of Molecular Endocrinology*)

## S12.1

Abstract unavailable.

## S12.2

**Selective targeting of the mineralocorticoid receptor in cardiovascular disease**

Morag Young
The Hudson Institute of Medical Research, Melbourne, Victoria, Australia.

Sustained mineralocorticoid receptor (MR) signalling promotes cardiac inflammation and fibrosis, which ultimately leads to cardiac failure. While the clinical use of MR antagonists are protective in cardiac disease, hyperkaelemia, and other off target effects have limited their use. In order to identify key MR-dependent mechanisms of heart disease progression that are distinct to normal renal electrolyte control, we have developed a series of tissue selective MR null animal models and determined their responses to the DOC/salt model and other models of cardiac remodelling. We have shown that the MR plays a critical and selective role in the macrophage, cardiomyocyte and in endothelial cells in the progression of cardiac inflammation and fibrosis. Macrophage MR signalling drives the proinflammatory macrophage responses to tissue injury and is central to the onset of fibrosis. The MR in cardiomyocytes has important roles in early chemoattractant signals and determines the hearts response to ischemia reperfusion. Regulation of endothelial cell function by the MR is dependent upon the vascular bed and, but the MR has a central role in macrophage recruitment in these cells. Together, recent data from our lab and elsewhere support a broad but carefully orchestrated role for MR signalling in the cardiovascular system in cardiac pathology and also in the physiological setting. Defining the key mechanistic whereby MR signalling pathways in macrophages and other non-epithelial cells in the cardiovascular system is an essential first step towards identification of therapeutic targets that may preserve potassium homeostasis, especially those cell types in which the MR is most likely acting as a cortisol receptor.

DOI: 10.1530/endoabs.38.S12.2

## S12.3

**Mineralocorticoid receptor antagonists: lessons from clinical trials**

Faiez Zannad[1,2,3]
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Mineralocorticoid receptor antagonists (MRAs) improve survival and reduce morbidity in patients with heart failure (HF), reduced ejection fraction (REF) with severe symptoms (RALES, spironolactone) or with mild symptoms (EMPHASIS-HF, eplerenone), and in patients with left ventricular systolic dysfunction and heart failure after acute myocardial infarction (MI) (EPHESUS, eplerenone). These clinical benefits are observed in addition to those of angiotensin-converting enzyme (ACE) inhibitors or angiotensin receptor blockers (ARBs) and β-blockers. The morbidity and mortality benefits of MRAs may be mediated by several mechanisms of actions, including antifibrotic mechanisms that slow HF progression, prevent or reverse cardiac remodelling, or reduce arrhythmogenesis. Both eplerenone and spironolactone have demonstrated survival benefits in individual clinical trials. Pharmacologic differences exist between the two drugs, which may be relevant for therapeutic decision-making in individual patients. Although hyperkalaemia events were reported in the major MRA clinical trials, these risks can be mitigated through appropriate patient selection, dose selection, patient education, monitoring, and follow-up. When used appropriately, MRAs significantly improve outcomes across the spectrum of patients with HF–REF. Consequently, in the latest international guidelines, an MRA is strongly recommended (grade I, level of evidence A) for all patients with persisting symptoms (New York Heart Association classes II–IV) and an ejection fraction $\leq 35\%$ despite treatment with an ACE inhibitor (or ARB) to reduce the risk of HF hospitalisation and the risk of premature death. As the totality of evidence grows, new strategies are needed to ensure the uptake of clinical trial evidence into clinical practice, from appropriate patient selection to optimal monitoring practices. The expansion of guideline recommendations for MRAs to include less sick patients may serve as a stimulus to develop such strategies or processes of care. MRAs are being evaluated in several new patient populations, including HF with preserved systolic function, ST segment elevation MI without HF, chronic kidney disease, end-stage renal disease on haemodialysis, resistant hypertension, atrial fibrillation, diabetic nephropathy, and other conditions in which aldosterone contributes to disease pathophysiology.

DOI: 10.1530/endoabs.38.S12.3

# Clinical Management Workshops

## Workshop 1: How do I do it? (Supported by *Clinical Endocrinology and Endocrinology, Diabetes & Metabolism Case Reports*)

### CMW1.1

**How do I report and interpret a dual-energy X-ray absorptiometry scan?**

Nicola Peel
Sheffield Teaching Hospitals, Sheffield, UK.

Dual-energy X-ray absorptiometry (DXA) forms an important component of fracture risk assessment. The objective of the report is to enable confident management by the non-expert recipient. A good report summarises the relevant information, provides a clear clinical interpretation and may also provide detailed management recommendations or refer to agreed management protocols. It needs to achieve a balance between over-simplification and inclusion of unnecessary detail.

Most referrers will have limited knowledge of the DXA technique and may not be aware of factors influencing the validity of the results such as osteoarthritic change in the spine causing artefactual overestimation of bone mineral density (BMD), which, if severe, may render the measurement uninterpretable. Artefacts may have a more limited impact and can be minimised by altering the scan analysis, for example to exclude a fractured vertebra from the region of interest. Decisions about the optimal scan analysis will be made by the DXA operator but the reporting clinician makes the final decision whether an unreliable result should be reported. It is important to highlight any unreliability and hence uncertainty in the report.

BMD measurements are interpreted in light of the clinical context, taking account of factors influencing bone health other than BMD including fracture history and independent risk factors for fracture. The history may highlight indications and contra-indications to specific therapeutic approaches. For example, evidence of poor visual acuity may suggest a need for assessment of falls risk and a history of oesophageal surgery or renal impairment would preclude bisphosphonate therapy.

DXA reporting should take place within a robust healthcare governance framework. All staff involved in scan acquisition, analysis and interpretation should undertake appropriate training and continuing professional development. The reporting process should be routinely monitored to ensure quality and consistency and ensure that the output is fit for purpose.

DOI: 10.1530/endoabs.38.CMW1.1

### CMW1.2

Abstract unavailable.

### CMW1.3

Abstract unavailable.

### CMW1.4

**How do I manage adrenal suppression?**

Jeremy Tomlinson
University of Oxford, Oxford, UK.

Two to three percent of the UK population are prescribed glucocorticoid (GC) therapy and their adverse effects are associated with significant morbidity and mortality. Suppression of the hypothalamo-pituitary–adrenal (HPA) axis with the potential risk of adrenal crisis is a recognised complication of therapy. There are significant clinical challenges, not only recognition and diagnosis of the condition, but also in terms of management. There is no doubt that the prevalence of adrenal suppression is under recognised and our own data has suggested that it may be present in over 30% of patients taking prescribed GC therapy. Oral or parental GC use is most frequently associated with adrenal suppression, but it can occur across all routes of administration. In addition to the differential potency of individual GCs, we have identified a dose-dependency of effect with inhaled GCs. The 250 μg short Synacthen stimulation test (SST) is the most commonly used dynamic assessment to diagnose adrenal suppression, but recent studies investigating the use of a random morning cortisol to determine adrenal reserve and limit the use of the SST will be discussed.

The principles of management are reliant upon GC replacement in those individuals no longer exposed to therapeutic GCs who have inadequate adrenal reserve with repeat dynamic assessments of HPA axis function to determine when the HPA-axis has recovered, accepting that the time to recovery can be highly variable. In those individuals continuing to take prescribed suppressive doses of GCs, there is a fine balance between ensuring adequate GC cover at times of intercurrent illness or stress whilst not contributing to the adverse effects of exogenous GC excess.

DOI: 10.1530/endoabs.38.CMW1.4

### CMW1.5

**How do I prevent thyroid eye disease after radioiodine?**

Kristien Boelaert
University of Birmingham, Birmingham, UK.

Thyrotoxicosis is a common disorder affecting up to 3% of the UK population and Graves' disease is the most common aetiology. Clinically relevant thyroid eye disease is present in 25–50% of patients with Graves' disease causing significant disfigurement and morbidity in 5–10% of patients. At the onset of ophthalmopathy, 80–90% of patients have hyperthyroidism, with the rest having euthyroidism or hypothyroidism. Risk factors for development of eye complications include severe biochemical hyperthyroidism and cigarette smoking.

The treatment options for Graves' disease include a prolonged course of antithyroid drugs, the administration of radioiodine and total thyroidectomy. Radioiodine therapy has been associated with development or worsening of Graves' ophthalmopathy and steroid prophylaxis is effective in prevention of progression of pre-existing ophthalmopathy. Development of hypothyroidism is another risk factor for development or worsening of thyroid eye disease and should be avoided through prompt $T_4$ replacement therapy.

Radioiodine treatment is not recommended for patients with active eye disease, whereas steroid prophylaxis is recommended for patients with clinically apparent but stable or quiescent eye disease. Typical steroid regimens involve a high dose of oral glucocorticoids for 2 months, but lower doses given for 6 weeks could be equally effective. Prophylactic steroid treatment is not recommended for patients without evidence of ophthalmopathy because of the low absolute risk of developing severe eye disease after radioiodine.

Patients who smoke are at a higher risk of worsening of Graves' ophthalmopathy than non-smokers, regardless of the type of treatment given. A consensus statement recommends routine steroid prophylaxis in smokers given radioiodine treatment, even if signs of eye disease are absent. This symposium will review the current evidence and international guidance regarding the prevention of thyroid eye disease following radioiodine administration.

DOI: 10.1530/endoabs.38.CMW1.5

### CMW1.6

**How do I manage suspected non compliance for thyroxine replacement?**

Jacqueline Gilbert
King's College Hospital NHS Foundation Trust, London, UK.

Optimal treatment of hypothyroidism is usually anticipated to require a daily dose of 1.6–1.8 μg/kg (body weight)/day of levothyroxine (L-$T_4$) in order to restore the TSH within the normal range. Patients who require significantly larger doses of L-$T_4$ than anticipated, e.g., $>2$ μg/kg body weight of L-$T_4$/day with a persistently elevated TSH warrant further investigation. Biological causes should be excluded, however sub-optimal compliance with medication remains the main cause of treatment failure.

Absorption of oral L-$T_4$ takes place within the small intestine (60–80% of ingested dose) and is maximal when the stomach is empty, peaking within the first 3 hours of absorption. Food, dietary fibre, and express coffee may interfere with $T_4$

absorption as may commonly used drugs, e.g., bile acid sequestering agents, ferrous sulphate, aluminum containing antacids, calcium carbonate, raloxofene, and proton pump inhibitors. Drugs that may increase the excretion or turnover of $T_4$ include phenytoin, rifampicin, and carbamazepine. Malabsorptive disorders, e.g., coeliac disease and inflammatory bowel disease reduce the fraction of the ingested L-$T_4$ dose that is absorbed. Other causes include atrophic gastritis, *Helicobacter pylori*, liver disease, and previous gastrointestinal surgery.
Non-compliance with medication is a challenging situation that must be explored with a sensitive and non-judgemental approach. Patients' health beliefs, understanding of their condition and fears of adverse side effects of medication may need to be addressed. Directly observed therapy in a supportive environment over an agreed timeframe may be suitable for some patients. If non- compliance is suspected by the clinician but denied by the patient, a L-$T_4$ absorption test helps to demonstrate that L-$T_4$ can be absorbed into the systemic circulation. Options may include using a supervised 1 mg bolus of oral L-$T_4$ or a week's supply of a weight related bolus dose of oral L-$T_4$ with measurement of TSH and $fT_4$ samples at baseline and post dose as per protocol.
This weekly administration was conducted for 4 consecutive weeks. TSH is rechecked 1 week after the final dose. Measurement of $fT_4$ 120 min after the ingestion of a weight related dose can be used to show maximal $T_4$ absorption.
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DOI: 10.1530/endoabs.38.CMW1.6

## Workshop 2: Management of pituitary disease: beyond the adenoma

### CMW2.1

Abstract unavailable.

### CMW2.2

Abstract unavailable.

### CMW2.3

Abstract unavailable.

## Workshop 3: Biological therapies - cause and cure of endocrine diseases

### CMW3.1

**The thyroid and rituximab**

Mario Salvi
Endocrinology, Fondaziobe Cà Granda IRCCS, University of Milan, Milan, Italy.

Since our first report on successful treatment of one patient with moderate-severe GO in 2006, several non-controlled studies have suggested that RTX can be more effective in active GO than intravenous methylprednisolone (ivMP). One randomised controlled trial comparing RTX to placebo and one comparing RTX to steroids in moderate-severe GO were recently published.
We have randomised 32 patients with active moderate-severe GO to be treated with RTX or ivMP and studied the decrease of the CAS as a primary end point. At 24 weeks, the CAS decreased more significantly after RTX and 100% of patients improved compared to 69% after ivMP ($P<0.001$). Dose finding analysis has shown that a single dose of 500 mg RTX is as effective as two doses of 1000 mg, two weeks apart. Disease reactivation was never observed in patients treated with RTX, but in 5 (31%) after ivMP. Either treatment was not effective on proptosis, palpebral aperture, and the total eye score, but RTX proved to be more effective than ivMP on motility and quality of life, thus suggesting that, compared to steroids, it may act as a disease modifying therapy. Stan *et al*. did not find RTX effective in treating active GO, when compared to placebo. The study was conducted on 21 patients, of whom two, after RTX, developed optic neuropathy. Major differences that may have influenced the outcome of their study are a much longer disease duration (11.2 months vs 4.5 months), a greater number of patients previously treated with steroids (40% vs 19%) and a lesser degree of motility involvement (mean diplopia score 2 vs 3.5). RTX may be a good alternative to steroids in non-responders, but its administration should be limited only to experienced centres, until the results of multicentre RCT trials on a greater number of patients will be available.

DOI: 10.1530/endoabs.38.CMW3.1

### CMW3.2

**Endocrine sequelae of biological therapies (Campath, other MABs, etc.)**

Carla Moran
University of Cambridge, Cambridge, UK.

Biological therapies include interleukins, interferons, and MABs. Over the past few years, the use of MAB to treat cancer and other diseases such as multiple sclerosis (MS) have increased; some of these frequently cause endocrine side effects.
Alemtuzumab, a MAB directed at CD52 on T and B lymphocytes, is very effective in reducing relapse rates and improving disability in relapsing remitting MS, however is frequently (16–35%) associated with the onset of thyroid disease, most commonly Graves' disease (GD), which can occur many years after it's use. Family history of thyroid disease and smoking are risk factors. A subset of affected patients follow a fluctuating course, which can mean control of disease is difficult. Eye disease seems less common than in non-Alemtuzumab induced GD, however it is not entirely clear whether these patients run a more indolent course and experience similar relapse rates.
Agents that modulate immune checkpoint proteins such as CTLA4 and programmed death 1 (PD1) have been shown recently to be effective in advanced melanoma, NSCLC and other cancers. The anti-CTLA4 MAB ipilimumab results in hypophysitis in up to 17% of patients, with a predilection to TSH and ACTH deficiency. Many patients have diffuse pituitary enlargement on MRI. Recovery is variable, with the hypothalamus–pituitary–gonadal axis recovering most frequently (57%), but the hypothalamus–pituitary–adrenal axis only recovering in 1–2%. High dose steroids are often given but may not significantly alter the course. The PD1 inhibitors Nivolumab and Pembrolizumab are frequently associated with thyroid dysfunction (hypothyroidism in up to 10%, hyperthyroidism up to 6.5%). Thyroiditis has also been described but the frequency is not yet known.
Given their efficacy, it is likely that we will encounter these agents and their side effects more frequently in clinical practice, however the optimal surveillance and management strategy is not yet clear.

DOI: 10.1530/endoabs.38.CMW3.2

### CMW3.3

Abstract unavailable.

## Workshop 4: How do I do it? (II) (Supported by *Clinical Endocrinology and Endocrinology, Diabetes & Metabolism Case Reports*)

### CMW4.1

Abstract unavailable.

### CMW4.2

Abstract unavailable.

### CMW4.3

**How far do I investigate chronic fatigue?**

Annice Mukherjee
Salford Royal NHS Foundation Trust, Manchester, UK.

Chronic fatigue presenting in the endocrine clinic is often intangible and difficult to assess. From the patients perspective fatigue is very disruptive to quality of life, exacerbates health related anxiety and can trigger functional symptoms. Patients with symptoms of chronic fatigue frequently have multiple contributory components to their symptoms, including endocrine, behavioural, and other medical factors. Investigation and management approaches will depend on whether the patient has existing hormone deficiency or excess, or whether they have been referred for a possible endocrine cause of fatigue without a clear endocrine diagnosis. Optimising endocrine replacement therapy in patients with known endocrine disease is important and can significantly improve quality of life. Validating symptoms, appropriate focussed diagnostics, dovetailed with practical symptom management is essential whether or not and endocrine cause of ongoing fatigue is suspected or identified. These strategies can help direct the fatigued patient towards appropriate management and sustainable quality of life improvement.

DOI: 10.1530/endoabs.38.CMW4.3

### CMW4.4

**How do I manage hirsutism?**

Thomas M Barber
University of Warwick and UHCW NHS Trust, Coventry, UK.

Hirsutism is one of the most common clinical features encountered in women attending endocrine clinics. It can also be extraordinarily difficult and challenging to manage well. In this brief presentation I will discuss management of hirsutism, using three major challenges as a scaffold:

Challenge 1: diagnosing the underlying cause

Hirsutism, the presence of terminal hairs distributed in a male-like pattern in women, affects between 5 and 10% of women. Polycystic ovary syndrome (PCOS) is the commonest cause of hirsutism, but many endocrine conditions manifest clinically with hirsutism (including Cushing's syndrome, CAH, and androgen-secreting ovarian and adrenal tumors). Diagnostically therefore, hirsutism is inherently challenging and it is important for the healthcare professional to invest time and effort at the onset of hirsutism, to diagnose correctly the underlying cause, particularly to exclude a neoplastic origin.

Challenge 2: being sensitive to the patient's needs

Unfortunately, hirsurtism is considered as a 'cosmetic' issue by NHS funders, and as such many treatments such as laser therapy are not widely available currently under the NHS. Hirsutism however has been termed as the 'thief of womanhood' and as such can be associated with substantial psychological distress which should not be under-estimated. Such distress can have far-reaching deleterious consequences. Dealing sympathetically with such distress in an empathic way forms an essential component of effective management.

Challenge 3: choosing an effective treatment (with a limited evidence-base)

One of the biggest challenges to management of hirsutism is choosing an effective therapy. Whilst there are plenty to choose from, including laser therapy, electrolysis, anti-androgens and eflornithine cream, there are substantial hurdles which include the need for self-funding for some of these, and general lack of clear evidence of efficacy from the literature. There is a clear need for a robust evidence-base for hirsutism management, preferably a long-term prospective study, to inform future guidelines.

DOI: 10.1530/endoabs.38.CMW4.4

### CMW4.5

**Managing hormone replacement therapy in the learning disabled young adult: a personal practice**

Helen Spoudeas
University College Hospitals, London, UK.

Teenagers and young adult survivors of congenital and acquired brain injury caused by developmental defects (e.g. septooptic dysplasias), brain tumours and their treatment, or intracerebral and systemic disease (e.g. histiocytoses and meningitis), are often both learning disabled and requiring lifesaving (cortisol and desmopressin) and life-enhancing (sex steroid, thyroxine, and growth) hormone replacement due to panhypopituitarism.

Managing the parental expectation and obtaining the understanding of the young person on the context of their level of understanding often requires a team approach and several sessions. Achieving adherence for life saving therapy whilst respecting choice, require formal assessment of intellectual capapcity and psychological/psychiatric insight into individual expectations (family and patient), perception, fear and careful discussion of risk-benefits of therapy at their level of understanding.

This workshop presentation will showcase particular examples and contextualise the legal and practical frameworks in which we aim to achieve adherence.

DOI: 10.1530/endoabs.38.CMW4.5

### CMW4.6

**How do I manage men who have used anabolic steroids?**

Andrew Toogood
Queen Elizabeth Hospital Birmingham, Birmingham, UK.

Anabolic agents are used to enhance performance through their effects on muscle mass, strength, and stamina. The prevalence of anabolic steroid use is difficult to quantify amongst the general population, but estimates derived from anonymous questionnaire studies in various populations suggest use may be common. Up to 4% of 18 years old American males have reported use at least once. Reported use of these agents rises significantly amongst army recruits and further still in elite athletes. Anabolic steroids are freely available from the internet and are used particularly amongst the gym population. Chronic use is associated with suppression of the hypothalamic–pituitary–adrenal axis, sexual dysfunction, infertility, testicular atrophy, and gynaecomastia. Other adverse effects are focused on the cardiovascular system, are hepatotoxic and can psychological disturbance.

The patient presenting to the endocrine clinic is most likely to complain about infertility or sexual dysfunction. A full history with detailed drug history should be recorded and a full physical examination undertaken. Measurement of gonadotrophins, testosterone, and SHBG will confirm suppressed gonadotrophins in the context of a low testosterone. A full blood count and liver function and enzymes should also be taken as part of the assessment for complications. The mainstay of management is abstinence from anabolic steroids. It may take 12 months, but recovery of the hypothalamic–pituitary–gonadal axis should be complete. Occasional measurement of gonadotrophins and testosterone levels confirm recovery and abstinence. In some cases referral to addiction services may be required to provide support during withdrawal from anabolic steroids.

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# Applied Physiology Workshop

## Evolving model systems for complex tissues

### APW1.1

**Modelling human diseases with stem cell derived hypothalamic neurones**

Florian Merkle[1,2], Alexander Schier[1,3] & Kevin Eggan[1,3]
[1]Harvard University, Cambridge, Massachusetts, USA; [2]University of Cambridge, Cambridge, UK; [3]Broad Institute of Harvard and MIT, Cambridge, Massachusetts, USA.

Neurons in the hypothalamus play central roles in physiology and behavior, and their loss or dysfunction cause common human diseases such as obesity and the sleep disorder narcolepsy. Efforts to study these diseases have been hindered by the inaccessibility of human hypothalamic neurons. I developed a robust method to generate hypothalamic neurons from human embryonic and induced pluripotent stem cells. These stem cell-derived hypothalamic neurons include the AGRP and POMC neurons that regulate feeding and the hypocretin/orexin neurons that regulate sleep and are lost in narcolepsy. Stem cell-derived neurons express the expected marker genes, have the characteristic morphology of their *in vivo* counterparts, and survive transplantation into the mouse brain. Using the CRISPR/Cas9 system I have generated knock-in reporters for multiple hypothalamic neuron types and introduced disease-associated genetic variants. I will use these cellular models to study the phenotypic consequences of common and rare genetic variants in disease-relevant cell types.

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### APW1.2

Abstract unavailable.

### APW1.3

**Cell replacement and regeneration of β cells as novel therapy for diabetes**

Neil Hanley[1,2]
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The last couple of years have seen an advance in generating human β-cells from pluripotent stem cells. It is now clear that cells of greatly increased maturity can be obtained from robust protocols. In addition to improving the prospects of cell replacement therapy, these developments allied to greater understanding of how human β-cells are first generated in the body are allowing development of more complex model systems. These model systems are likely to be important in gaining mechanistic understanding of developmental monogenic causes of diabetes (e.g. maturity onset diabetes of the young, and permanent or transient neonatal diabetes) and deciphering the relevance of genome-wide association studies in type 2 diabetes. Recent advances and data will be discussed.

DOI: 10.1530/endoabs.38.APW1.3

# Early Career Prize Lectures

## ECP1.1

### The physiology and pharmacology of the fasting-induced hormone, FGF21

Bryn Owen
Imperial College, London, UK.

The ability to adapt to changing environmental stimuli, such as the availability of food, is essential for the survival of species. As such, complex mechanisms have evolved to maintain metabolic homeostasis during periods of nutritional challenge. In 2007, the Fibroblast Growth Factor 21 (FGF21) was identified as a nuclear receptor-regulated hepatokine that is induced during starvation. Since then, considerable effort has been devoted to elucidate the breadth of actions of FGF21, its target-tissues, mechanisms of action, and unexpected pharmacological benefits. Consistent with a proposed role in the adaptive starvation response, it was found to induce gluconeogenesis, ketogenesis and insulin sensitivity. It can also promote longevity and food-seeking behaviour, inhibit growth, disrupt the circadian rhythm, and cause female infertility. We showed that many of these effects are mediated via FGF21-signalling in the hypothalamus. This defined a liver–neuroendocrine axis for the systemic adaptation to fasting. The work also enabled us to shed light on the apparently counterintuitive observation that pharmacological administration of FGF21 causes weight-loss in obese animals. We showed that this is also due to central FGF21-signalling. Specifically, it stimulates sympathetic nerve activity and this increases energy expenditure via the brown adipose tissue. We are currently investigating the fascinating effects of FGF21 on mood and reward-behaviour. In summary, significant and rapid progress has been made in understanding the physiological actions of this recently-identified hormone. On-going clinical trials will soon determine the potential of FGF21-based therapy for the treatment of obesity, and its complications, in humans

DOI: 10.1530/endoabs.38.ECP1.1

## ECP1.2

### Kisspeptin- A 'key regulator' of reproductive physiology, integrating limbic circuits with the regulation of reproductive hormones

Alexander Comninos[1], Jelena Anastasovska[1], Amar Shah[1], Matthew Wall[3], Channa Jayasena[1], Xiaofeng Li[2], Ali Abbara[1], Shaku Narayanaswamy[1], Gurjinder Nijher[1], Chioma Izzi-Engbeaya[1], Lysia Demetriou[3], Sophie Clarke[1], Mohammad Ghatei[1], Jimmy Bell[1], Paul Matthews[1], Stephen Bloom[1], Kevin O'Byrne[2] & Waljit Dhillo[1]
[1]Imperial College, London, UK; [2]King's College, London, UK; [3]Imanova Imaging, London, UK.

Kisspeptin is a recently identified reproductive hormone which serves as a crucial activator of the Hypothalamic–Pituitary–Gonadal (HPG) axis via stimulation of GnRH neurones. We have previously observed that a single kisspeptin injection increases LH pulsatility while twice daily kisspeptin injections can even advance the menstrual cycle. Furthermore, we have demonstrated that kisspeptin restores LH pulsatility in women with hypothalamic amenorrhoea and can potently stimulate egg maturation in women undergoing IVF, thereby unveiling novel therapeutic actions.
However, the effects of kisspeptin are not limited to the HPG axis, yet there is paucity of data in this regard. Kisspeptin and its cognate receptor are also expressed within the amygdala, a key limbic brain structure with important roles in social and reproductive behaviours. In addition, the amygdala exerts an 'inhibitory brake' on reproduction via inhibitory neuronal projections to hypothalamic centres involved in reproductive hormone release. By mapping brain neuronal activity (using Manganese-Enhanced MRI) in adult rodents we demonstrated a marked decrease in neuronal activity within the amygdala following kisspeptin administration. Subsequently, we investigated functional relevance by assessing the LH response to direct intra-amygdala administration of kisspeptin or kisspeptin antagonist in adult rodents. This revealed that direct intra-amygdala administration of kisspeptin elicited a dose-dependent increase in LH secretion. In addition, blocking endogenous kisspeptin signalling specifically within the amygdala by administering intra-amygdala kisspeptin antagonist decreased LH secretion as well as LH pulse frequency. This provides the first evidence for a novel pathway in which kisspeptin inhibits neuronal activity in the amygdala, thereby releasing the amygdala's 'inhibitory brake' on reproduction, resulting in stimulation of gonadotrophin secretion and pulsatility. Furthermore, these studies suggest that kisspeptin can integrate limbic circuits with the regulation of reproductive hormones. We now translate this work into humans, employing functional MRI to interrogate human brain activity in response to kisspeptin.

DOI: 10.1530/endoabs.38.ECP1.2

# Meet the Expert Sessions

## Cutting edge imaging of whole endocrine organs (Supported by *Clinical Endocrinology*)

### MTE1

**Cutting edge imaging of whole endocrine organs**

Helen Christian
University of Oxford, Oxford, UK.

Recent advances in tridimensional (3D) tissue imaging and development of reporter proteins have provided new insight into the dynamic relationship between tissue structure, function and dysfunction in a number of fields of research. In developmental biology 3D microscopy has enabled building of quantitative 3D atlases of embryo morphology and gene expression at cellular resolution and in neuroscience 'connectomics', the building of 3D brain connectivity maps is a rapidly expanding area of research. Recently, in endocrinology, *in vivo* whole tissue 3D imaging by the use of two photon excitation microscopy has revealed the importance of cellular networks in the pituitary and endocrine pancreas which direct development and function. However, optical microscopy methods are limited in resolution compared to electron microscopy. Advances in electron microscopy include the development of serial block face scanning electron microscopy which enables the 3D visualisation of fixed tissues with fine ultrastructural detail. Furthermore, correlative light and electron microscopy (CLEM) integrates the advantages of 3D light and electron microscopy on the same sample to enable the subcellular localisation of dynamic processes *in vivo* with the resolution power of the electron microscope. Most recently the emerging technology of light-sheet microscopy allows live imaging of organs with high spatiotemporal resolution over long periods of time. The current state of the art in 3D biological imaging techniques will be explored with a focus on recent applications to the study of endocrine systems.

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## T4 vs T4/T3 – evidence vs expectations? (Supported by *Clinical Endocrinology*)

### MTE2

Abstract unavailable.

## Optimising fertility in teenage cancer survivors (Supported by *Clinical Endocrinology*)

### MTE3

**Optimising fertility in teenage cancer survivors**

Richard Anderson
University of Edinburgh, Edinburgh, UK.

Recent advances in the treatment of teenage cancers have led to an increasing focus on the late effects of treatment, amongst which fertility is prominent with surveys ranking potential loss of fertility as amongst the most important concerns of teenage and young adult patients. The various treatments for teenage cancer including chemotherapy, radiotherapy and surgery can all potentially compromise fertility with the most toxic therapies being alkylating agent based chemotherapy and pelvic irradiation. Assessment at the time of diagnosis and prior to starting treatment is therefore critical in providing patient information and suggesting potential fertility preservation strategies, where relevant. Sperm cryopreservation is well established but prediction of spermarche is difficult. Cryopreservation of testicular tissue is being developed but remains experimental. For girls, ovarian tissue cryopreservation may be an option and for older teenage girls ovarian stimulation for oocyte vitrification may also be possible, although there is limited experience in developing appropriate protocols for such patients. It is important to recognise that most teenagers treated for cancer will retain their fertility and thus for many, assessment and reassurance are appropriate. Following treatment, conventional semen analysis provides the best predictor of male fertility but some young men may well be reluctant to have this investigated. In young women, premature ovarian insufficiency (POI) provides a clear indicator of loss of fertility, and in those with ongoing ovarian activity the key question may be prediction of the fertile lifespan. There is also evidence for a significant prevalence of infertility in women even with maintained ovarian activity following cancer therapy. Radiotherapy to the uterus carries a substantial risk of miscarriage or obstetric complications and pregnancy in such women should be managed in a high risk obstetric unit. Pregnancy following egg donation should also be regarded as high risk. There is increasing interest in the use of serum anti-Müllerian hormone to predict reproductive lifespan, but its ability to predict fertility in young women is probably very limited.

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## Investigation and management of TSHoma (Supported by *Clinical Endocrinology*)

### MTE4

**Investigation and management of TSHoma**

Mark Gurnell[1,2]
[1]Wellcome Trust-MRC Institute of Metabolic Science, University of Cambridge, Cambridge, UK; [2]Cambridge University Hospitals Foundation Trust, Cambridge, UK.

Background
Thyrotropinomas (TSHomas) have traditionally been considered a rare, albeit important cause of thyrotoxicosis. However, a recent report suggests their prevalence may be 2–3 times higher than previously suspected. In addition, although early case series described a predominance of invasive macroadenomas, recent findings (including in our own cohort of 40 patients) confirm that microadenomas are being increasingly diagnosed, and the clinical/biochemical phenotype appears to be more variable than previously reported.
Investigation
When faced with a patient with raised thyroid hormones (TH) and apparent inappropriate (non-suppressed) TSH, the first step is to exclude potential confounding medications (e.g. heparin, amiodarone) or intercurrent illness (including acute psychiatric disorders). Thereafter, it is important to screen for assay interference (e.g. due to heterophilic antibodies or familial dysalbuminaemic hyperthyroxinaemia (FDH)) to avoid unnecessary further investigation. Once a diagnosis of genuine hyperthyroxinaemia with inappropriate TSH secretion has been confirmed, a complex pathway of investigation is often required to reliably differentiate between TSHoma, resistance to thyroid hormone due to loss-of-function mutations in the beta isoform of the TH receptor (*THRB* RTH), and rare disorders of TH transport or metabolism. Undue reliance on pituitary MRI findings may result in inappropriate surgery for a pituitary incidentaloma in RTH, or failure to recognise a microTSHoma that is not readily visualised on standard MRI (equivalent to the microcorticotroph adenomas found in patients with Cushing's disease).
Management
Transsphenoidal surgery is the mainstay of treatment for TSHoma. However, somatostatin analogue (SSA) therapy is finding increasing use, both in the preparation of patients for surgery, but also as an adjunct following incomplete tumour resection, or even as primary medical therapy. Radiotherapy remains an important option in more aggressive/invasive tumours.

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## What every endocrine researcher should know about genome editing

### MTE5

Abstract unavailable.

## Frontiers in the management of hypoparathyroidism (Supported by *Clinical Endocrinology* and *Hypopara UK*)

### MTE6

**Frontiers in the management of hypoparathyroidism**

Karen Winer
NIH, Bethesda, USA.

Treatment of hypoparathyroidism with vitamin D analogs and calcium does not restore normal physiologic regulation of calcium homeostasis in the bone and kidney and may lead to renal insufficiency due to progressive nephrocalcinosis. Replacement with PTH potentially addresses this problem but until recently, hypoparathyroidism was the only classic hormonal insufficiency state not treated with its missing hormone.

Over the past two decades, we have evaluated various PTH 1-34 regimens including once-daily and twice-daily PTH 1-34 injections without the use of calcitriol or Ca supplements in adults and children of all etiologies for up to 10 years. With increased frequency of injections, the total daily dose of PTH can be reduced, in most cases by at least 50% and serum calcium can be maintained in the normal range throughout the day with reduced fluctuation. Lower doses produce less stimulation to the bone and reduced the risk of transient episodes of hypercalciuria. For each regimen, the PTH 1-34 dosage was individualized throughout treatment to maintain optimal calcium homeostasis.

To further refine replacement therapy, we recently studied PTH delivery by insulin pump compared with twice daily injections. Pump delivery produced normal, steadystate calcium levels with minimal fluctuation and avoided the rise in serum and urine calcium levels just after a PTH injection. Pump delivery of PTH allowed for simultaneous normalization of bone markers, serum calcium, and urine calcium excretion levels. This represents a significant therapeutic breakthrough, which has not been achieved in any other treatment study of hypoparathyroidism.

The recent approval of PTH 1-84 in the treatment of hypoparathyroidism represents an important milestone in the treatment of this rare disease. Although the two peptides have not been directly compared, PTH 1-84 and PTH 1-34 have similar PK and PD profiles. Thus, one can assume many of the principles learned from studies of PTH 1-34 also apply to PTH 1-84. Therefore, a key area of future study is to determine individualized titrated doses of PTH 1-84 without the simultaneous use of calcitriol and calcium supplementation. Treatment of hypoparathyroidism with both PTH analogs should potentially restore normal physiologic regulation of Ca homeostasis in the bone and kidney.

The essential therapeutic principles that underlie successful treatment of this rare disorder are: i) Every patient has individual PTH requirements based upon their disease etiology and tendency for hypercalciuria. ii) Smaller, more frequent doses of PTH replacement by subcutaneous injection reduces stimulation to bone and kidney and results in lower calcium excretion and markers of bone turnover; iii) PTH delivered by pump produces the most physiologic biochemical profile. The normalization of serum and urine calcium and markers of bone turnover should be the therapeutic treatment goal in the management of hypoparathyroidism with PTH replacement therapy.

DOI: 10.1530/endoabs.38.MTE6

## Designer Receptors Exclusively Activated by Designer Drugs (DREADD) - Novel pharmacological handles on the endocrine system (Supported by *Clinical Endocrinology*)

### MTE7

**Designer receptors exclusively activated by designer drugs (DREADD) – novel pharmacological handles on the endocrine system**

Alastair Garfield[1,2]
[1]University of Edinburgh, Edinburgh, UK; [2]Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, Massachusetts, USA.

Chemogenetic technologies afford the real-time control of molecularly-defined populations of neurons in a spatially and temporally specific fashion. The ability to remotely activate and silence neurons within the context of genetically-intact freely behaving mice provides unprecedented insight into their physiological function and pathological potential. Used in combination with other genetically-encoded tools this approach facilitates the deconvolution of the neural circuitry governing sensorimotor responses to (patho)physiological state. Here we review the principles and applications of chemogenetics, drawing upon our recent research into the discrete circuits that regulate homeostatic hunger/satiety and counter-regulatory autonomic responses to hypoglycemia, respectively.

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## Updated thyroid cancer guidelines (Supported by *Clinical Endocrinology*)

### MTE8

**Meet the Expert 8: updated thyroid cancer guidelines**

Petros Perros
Department of Endocrinology, Newcastle upon Tyne Hospitals NHS Foundation Trust, Newcastle upon Tyne, UK.

Previous UK guidelines (2002, 2007) focused on reducing regional variations in practice, concentrating expertise (especially surgical), and eliminating clinical, radiological and biochemical evidence of persistent disease. The 2014 guidelines aim to improve the overall survival while enhancing the health-related quality of life of patients. This presentation will discuss areas of the guidelines that are significantly different from the previous edition of the guidelines and are most relevant to practicing endocrinologists: i) entry of patients into the cancer pathway and initial investigation of thyroid nodules, ii) management of micorcarcinomas, iii) managing uncertainty, and iv) patient quality of life.

DOI: 10.1530/endoabs.38.MTE8

## Endocrine and non-endocrine aspects of hypothalamic syndromes (Supported by *Clinical Endocrinology*)

### MTE9

Ashley Grossman
University of Oxford, Oxford, UK.

The term pituitary tumour usually implies an adenoma arising from anterior pituitary tissue, but in fact some 2–5% of 'tumours' in this region are not adenomas, and many of these arise from the para-sellar region or within the hypothalamus. One clue as to the non-adenomatous origin of such tumours is the presence of diabetes insipidus, which is almost never present with a primary pituitary adenoma. Some 1% of sellar masses are metastases, most often from the frequent primary sites (lung, breast and prostate, but including lymphomas which my be primary). Other major pathologies include lymphocytic and granulomatous hypophysitis (now sometimes seen arising from immunomodulatory therapy for cancer), craniopharyngiomas, arachnoid cysts, germinomas, Langerhans cell histiocytosis and neurosarcoidosis, and Rathke's cysts which may be primarily intra-or supra-sellar.

Endocrine assessment and replacement follow the guidelines for pituitary adenomas, but there may be additional problems involving the control of appetite, thirst, sleep-weight patterns, and body temperature regulation. Craniopharyngiomas in particular are associated with such changes, especially obesity, which may be exacerbated by radical surgery. There is some evidence that bariatric surgery can help in this situation, but adipsia can lead to profound problems which may become intractable and lethal. Poikilothermia requires careful attention to regulation of the environmental temperature.

Thus, while anterior pituitary defects associated with adenomas can generally be treated and may lead, according to recent studies, to a normal quality of life, such hypothalamic syndromes can be extremely difficult to manage. It is essential that any therapeutic modalities are considered in this light, and are not allowed to exacerbate such defects.

DOI: 10.1530/endoabs.38.MTE9

## Diagnosis and management of SIADH (Supported by *Clinical Endocrinology*)

### MTE10

**Diagnosis and management of SIADH meet the Expert 10**

Chris Thompson
Beaumont Hospital, Dublin, Ireland.

Hyponatraemia is the commonest electrolyte abnormality in hospitalised patients and every published series has demonstrated that hyponatraemia is associated with increased mortality and substantial morbidity, including longer duration of hospital stay. The debate between the contribution between hyponatraemia and the causative condition to the increased mortality remains to be resolved, but increasing evidence points to an independent association with hyponatraemia. It is not clear whether all causes of hyponatraemia increase mortality; in particular, it

is not certain whether the prognosis is better or worse in hyponatraemia due to SIADH or non-SIADH causes. The diagnosis of SIADH requires the distinction from hypovolaemic or hypervolaemic hyponatraemia, and the exclusion of glucocorticoid deficiency, and is helped by clinical alogorithms. Many patients with SIADH will see rapid resolution of hyponatraemia with treatment of the underlying cause – for instance SIADH due to pneumonia. However, chronic symptomatic hyponatraemia may need specific therapy. First line therapy recommended by most guidelines is fluid restriction, despite the absence of a firm evidence base, the difficulty in adherence, and well known predictors of clinical failure, such as UOsm $>600$ mOsm/kg. Second line treatments include demeclocycline, frusemide plus oral salt, urea and vasopressin receptor antagonists, of which only tolvaptan is licensed in Europe. All, with the exception of tolvaptan, lack prospective randomised trial evidence, and urea has no license or available preparation for clinical use. Tolvaptan is effective and has well documented guidelines to prevent overcorrection, but widespread use is limited by the high unit cost of the drug. Acute hyponatraemia ($<48$ h), particularly when associated with neurological irritation, carries a high mortality, and is associated with permanent neurological damage in survivors. Emergency elevation of plasma sodium is needed, and current recommendations are for the use of bolus hypertonic saline to ensure an elevation in pNa of 4–6 mmol/l in 4 h, with gradual elevation over the first 24 h of 8–12 mmol/l. Higher rates of rise should be corrected with IV dextrose, and/or dDAVP, to prevent osmotic demyelination.

DOI: 10.1530/endoabs.38.MTE10

# Skills

## Skills 1: Working with the media

### SK1.1

**Why should we engage with the media?**

Richard Quinton[1,2]
[1]Institute of Genetic Medicine, Newcastle University, Newcastle-on-Tyne, UK; [2]Newcastle-on-Tyne Hospitals NHS Foundation Trust, Newcastle-on-Tyne, UK.

Lord Reith famously characterised the BBC's purpose as 'to educate, inform and entertain' (EIE). News and current affairs is a multibillion dollar industry that is rapidly expanding beyond traditional print, audio and vision formats, allowing the media to reach out even to the most isolated and disadvantaged populations worldwide. The media have an insatiable appetite for stories about medicine, healthcare and related scientific advances, because these strongly resonate with their audience.

Journalists operate within significant constraints, typically including income-insecurity, intense pressure to deliver features within tight deadlines, Arts-weighted educational background, and little time/space to engage with their audience. Time for background research is limited, so they need trusted sources to rapidly turn to for feedback or corroboration. They cannot devote time and energy to a story that is unlikely to have a concrete outlet, so their first expert point-of-contact may be key to deciding whether or not to pursue it further.

It is dispiriting when features appear to misrepresent reality in an area that is important to us, or where the views of alternative practitioners are given equal weighting to those of undoubted experts in the field – MMR debacle was a low-point in this respect. However, as physicians and scientists with expertise in Endocrinology we can hugely assist journalists in preparing their features more accurately and efficiently and they know it.

For the media, 'hormones are sexy' (and who are we to disagree?), but we can steer them away from sensationalism whilst retaining EIE value. The Society has retained a Media Officer since 1999, because it appreciates the importance of quality information relating to Endocrinology being shared with the public. By giving journalists a single 'go to' point of access to experts in the relevant area of Endocrinology, the Society is in a strong position to positively influence features-in-preparation.

DOI: 10.1530/endoabs.38.SK1.1

### SK1.2

Abstract unavailable.

### SK1.3

Abstract unavailable.

### SK1.4

Abstract unavailable.

## Skills 2: Early Career Symposium: Effective communication: get involved, get engaged!

### SK2.1

**Engaging with the public: conveying the wonders of science**

Matthew Simmonds
University of Oxford, Oxford, UK.

Engaging with the public is becoming a key part of any research career and enables us to share our passion for endocrine research with the world, show how charity and government funding for endocrinology leads to new treatments and encourages the next generation of endocrine researchers. Whilst as researchers we are used to presenting our research at conferences and networking with our peers, engaging with the public about our research can be a daunting prospect. This talk will focus on the numerous different opportunities available to researchers to engage with the public. We will look at opportunities to take part in structured activities run by the Society of Endocrinology and other charities at numerous big science events throughout the year to help convey the wonders of endocrinology. We will also discuss how to create your own interactive stand at public engagement events organised locally within your own institute or department and talk about formal training available to researchers to learn how to engage with the public. For those interested, we will also discuss how to create your own events and provide practical advice on advertising, applying for funding and how to get the most out of these events. Opportunities to engage with the public on a smaller scale will also be discussed, such as presenting your research to local patient groups and using social media to engage with the public and patient groups. This talk will also offer advice on how to pitch your science in a way that makes your research accessible to the public and will give tips on how to tackle potentially tricky questions that can arise, with the aim of providing you with the tools to engage with the public in a way that works for you.

DOI: 10.1530/endoabs.38.SK2.1

### SK2.2

**The battles of writing a lay summary**

Joanna Grey
Association for Multiple Endocrine Neoplasia Disorders (AMEND), Tunbridge Wells, Kent, UK.

Lay summary uses are multitudinous with their overall purpose being to engage the average person's interest and thereafter increase their understanding of a subject unfamiliar to them. In medicine, there is rightly an emphasis on ensuring that a patient fully understands what is happening to them in order that they may become an effective participatory member in their own care. However, literacy levels are surprisingly low, even in developed nations, and it therefore follows that this has a knock-on negative effect on health literacy. Consequently, considerable careful effort is required when communicating complex medical topics to lay people. The Association for Multiple Endocrine Neoplasia Disorders (AMEND) has worked hard since 2002 to help families and other interested people to better understand the complex genetic multiple endocrine neoplasia (MEN) conditions. The evolution of AMEND's patient information resources, together with other work undertaken over the years in a variety of arenas, including research, provides an interesting and thought-provoking snap-shot of issues to consider as well as tools with which to arm yourself in order to win your lay summary battle.

DOI: 10.1530/endoabs.38.SK2.2

### SK2.3

**Making science cool for kids**

James Cannon
Kingston University, Kingston Upon Thames, Surrey, UK.

The last decade has seen an explosion in scientific breakthroughs as technology drives new discoveries. It is an exciting time to be a scientist! So why are the numbers of young people studying science declining?

To arrest this slide there are innumerable initiatives to encourage researchers to engage with young people directly. But, for all that it is exciting to be a scientist,

are scientists exciting? Can social skills learnt by breeding zebrafish truly translate into the classroom?
This session will explore how you can make research as exciting for young people as it is for your colleagues. We will look at good and bad practice and think about how you, as researchers, can make science cool for kids.

DOI: 10.1530/endoabs.38.SK2.3

## SK2.4

**Making your impact statement: pack a punch**

Rajesh Thakker
University of Oxford, Oxford, UK.

Funding and publication in biomedical research has become highly competitive and to succeed, it has become increasingly important to include statements that have broad appeal, i.e., impact, especially in the summaries of grant applications and manuscripts. The key elements in these statements are to keep them short, simple and 'sweet' (i.e. appealing to a wider audience). Consider the three following statements:
i) We've got no money, so we've got to think. (Ernest Rutherford 1871–1937, physicist. Nobel Laureate 1908).
ii) Big questions get big answers (Francis Link 1916–2004, biophysicist and neuroscientist. Nobel Laureate 1962).
iii) Scientific research is one of the most exciting and rewarding of occupations. (Frederick Sanger 1918–2013, biochemist. Nobel Laureate 1958 and 1980).
All three statements are illustrative in being short and simple, and in having applications and implications that are beyond the direct discipline of the person making the statement. In addition, they give important messages in emphasising the necessity of thinking and asking appropriate questions, yet making sure that the fun and excitement of scientific discovery are maintained. Conveying these messages will help to ensure that an impact statement will pack a punch.

DOI: 10.1530/endoabs.38.SK2.4

## SK2.5

**Making social media work for promoting and recruiting to research studies**

Victoria Parker
WT-MRC IMS-MRL, Cambridge University Hospitals NHS Trust, Cambridge, UK.

Recruitment to research studies to meet sample quality, size, and power requirements may be challenging, with underrepresentation of specific gender, age or ethnic groups being a common source of bias within research. Social media are defined as web-based applications that enable creation and exchange of user generated content. Facebook, one of the most renowned social networking interfaces currently has 1.44 billion users worldwide, and cross-sectional studies of user demography suggest a remarkably equal uptake across different ethnic, socioeconomic classes and gender groups. The use of social media in research studies thus provides opportunity to engage and recruit large numbers of eligible participants from diverse populations, at any time, and from potentially anywhere in the world. Furthermore, social networking sites create a valuable conduit for patient and public involvement (PPI) within research and science, but their use should be carefully balanced with the unavoidable loss of confidentiality and anonymity which is inherent in the use of such applications.

DOI: 10.1530/endoabs.38.SK2.5

## SK2.6

Abstract unavailable.

# Futures

## Futures 1: Branching out with endocrinology

### FUT1.1

Abstract unavailable.

### FUT1.2

Abstract unavailable.

### FUT1.3

**The secret life of a core facilities manager**

Natalie Homer
University of Edinburgh, Edinburgh, UK.

With the emergence of core facilities across the academic community has come the need for their good management. Increasingly the role of facilities manager has become a promising career route for graduate and post-doctoral scientists.
A core facility typically offers state of the art instrumentation, fully serviced and maintained with a high level of expertise in their operation and scientific applications. In terms of a mass spectrometry core facility, this can involve receipt and processing of samples, analysis using validated assays, data interpretation, and reporting of results. Servicing, maintenance, upgrades, and good laboratory management are essential if the core facility is to be successful and reliable and maintain a good reputation within the scientific community.
Management of a core facility is quite a unique role in that you are able to fully focus on a technological aspect of your research or studies but also allows you to develop a surprisingly wide range of other skills, e.g., people management, resource timetabling, technical troubleshooting, financial management, and even marketing.
There is no typical day for a core facility manager. It predominantly requires resource and study management but can involve anything from instrument maintenance, managing core staff, and liaising with service engineers and sales representatives. Further to that it can include meeting with potential new investigators to assess scientific viability and financial feasibility of new studies. It is also important to have a strong awareness of current and future technological improvements in your area of expertise in order to future proof the facility. Validating assays for scientific papers and interpreting and reporting results to investigators are also potential requirements, all of which play on different skills. In order to provide an excellent core facility then the first port of call is often the manager, where being approachable and willing to share your knowledge and expertise is a must!

DOI: 10.1530/endoabs.38.FUT1.3

## Futures 2: Overcoming the consultancy hurdle

### FUT2.1

Partha Kar
Portsmouth Hospitals NHS Trust, Portsmouth, UK.

Much has been written and discussed about the role of the specialist within the confines of the modern NHS. There is a growing realisation that having a specialist rigidly based in hospital is becoming difficult to sustain; instead, flexibility is now crucial as primary and specialist care look to work together to improve diabetes care.
With the challenges of the Five Year Forward View (NHS England, 2014) upon us in England, and similar pressures elsewhere, this session looks at nuances within the existing consultant contract that would allow us to develop specialist roles for the future.
The potential abounds for improving the way in which specialists work with primary care. This session will look at exploring the areas of controversy and see what maybe feasible in the future.

DOI: 10.1530/endoabs.38.FUT2.1

### FUT2.2

Abstract unavailable.

### FUT2.3

**Research pathways in endocrinology: a UK and global perspective**

Waljit Dhillo
Imperial College London, London, UK.

Diabetes and endocrinology is a specialty in which we study the fascinating area of the physiology of hormones, which can influence the regulation of every organ in our body. Understanding how hormones are dysregulated in pathological states leads to improved diagnosis and treatment of these conditions. Rapid progress has been made in the field and there are now many classical endocrine systems, which are well established that we have all learnt about as undergraduate trainees. However there is still much to learn about these biological systems, which could lead to an improvement in the lives of patients with endocrine disorders. In addition there are potentially hundreds of hormones that remain undiscovered and the panoply of novel tools now available for scientific discovery means that understanding these novel hormone systems could result in improved treatments for patients. This highlights that our specialty is highly academic and trainees in diabetes and endocrinology should receive training in research in order to meet these needs of our specialty and our patients. In an ideal world with no funding limitations a dedicated period of research time would be built into every diabetes and endocrinology trainee's clinical programme.
In this talk I will highlight the potential research opportunities available to trainees at all stages of their career. Over the last decade health researchers have observed a transformation in the landscape for training and research opportunities through the new training schemes in place for all healthcare professionals. Health researchers can now have access to research training opportunities from the early pre doctoral stages of their career all the way up to Chair level and beyond. There has never been a more exciting and opportune time for diabetes and endocrinology trainees to make a difference to patient outcomes through their research.

DOI: 10.1530/endoabs.38.FUT2.3

## Futures 3: Why endocrinology and diabetes?

### FUT3.1

Abstract unavailable.

## FUT3.2

**Let me tell you the way it is: from a trainee's perspective**

Julia Prague[1,2]
[1]North West London NHS Trusts, London, UK; [2]Imperial College, London, UK.

Rather than be another careers session of pathway diagrams and tales from many years ago, this short talk will cover honest experiences of current training including tips and tricks with hindsight (relevant to any specialty training), and the highs and lows that happen along the way.

DOI: 10.1530/endoabs.38.FUT3.2

## FUT3.3

**Endocrinology club in your medical school: from concept to reality, led by Cambridge example**

Parisut Kimkool & Rakhee Vaja
University of Cambridge, Cambridge, UK.

We are delighted to have the opportunity to share our experience of setting up a society within our university. As medical students, we often attend insightful talks by doctors in different specialties that can inspire us to explore a particular field in more detail. The events are mainly hosted by student societies, and the major ones at our university include paediatrics and neurology, for example.

Last year, a small group of us felt that endocrinology was underrepresented in this respect. We felt that this was something we could improve, and after talking to our peers, we found that many students were definitely interested in finding out more about endocrinology. We therefore decided to set up an endocrinology society in the hope of increasing its recognition amongst the medical students.

We had a daunting task ahead of us but we managed to turn our concept into reality, and hosted many successful well-attended events in the 1st year. In this talk we will guide you through the process we took and some of the difficulties we faced. Overall it was an insightful experience and we would encourage other students to do the same should they have the opportunity.

DOI: 10.1530/endoabs.38.FUT3.3

## FUT3.4

Abstract unavailable.

# Debate

## This house believes that patients with hypothyroidism should be offered combination treatment with T3 and T4 if they do not respond to Levothyroxine

### D1.1

**This house believe that patients with hypothyroidism should be offered combination treatment with $T_3$ and thyroxine if they do not respond to levothyroxine**

Colin Dayan
Cardiff University, Cardiff, UK.

In large community surveys, there is a consistent absolute excess of psychological caseness in subjects on thyroxine ($T_4$) compared to age and sex matched controls of around 6%. Part of this likely to be 'confounding by indication' – i.e. that some individuals failed to respond to $T_4$ as hypothyroidism was not the cause of their psychological morbidity in the first place. However, the observations that normalisation of intracellular thyroid hormone levels is not achievable with $T_4$ alone in animal models, the fall in $T_3$ levels that occurs after beginning $T_4$, and the preliminary observation subjects with a common polymorphism of deiodinase 2 have worse psychological well being on $T_4$ and appear to benefit from $T_3$, suggest that there may be a subsection of individuals who are not returned o health by $T_4$ alone. Although many randomised studies show no benefit from $T_4$ vs combination studies, studies using higher $T_3$:$T_4$ ratios appear to show some benefit. Despite this, thyroid hormone over-replacement is likely to increase the risk of atrial fibrillation and osteoporosis. Taken together, this information suggests that subjects who report a profound effect on their quality of their life on $T_4$ and are aware of the evidence of potential long-term side-effects of over re-placement should be considered for a trial of $T_3$/$T_4$ until further research information becomes available if they request it. Combination therapy should not be offered routinely, and every effort should be made to minimise doses, emphasise, and mitigate the potential risks and to stop therapy if benefit is not apparent.

DOI: 10.1530/endoabs.38.D1.1

### D1.2

Abstract unavailable.

# Nurse Session

## Nurse Session 1: Multiple Endocrine Neoplasia

### N1.1

**Overview of multiple endocrine neoplasia**

John Ayuk
University Hospital Birmingham, Birmingham, UK.

Multiple endocrine neoplasia (MEN) syndromes are rare autosomal-dominant disorders that predispose affected individuals to benign and malignant tumours involving two or more endocrine glands. Four major forms of MEN are recognised, each associated with the occurrence of specific tumours. MEN1 is due to germline-inactivating mutations of the MEN1 tumour-suppressor gene, and is associated with the occurrence of parathyroid, pancreatic islet and anterior pituitary tumours. MEN2 (previously MEN2A) and MEN3 (previously MEN2B) are due to mutations of the RET protoncogene; MEN2 is associated with medullary thyroid carcinoma (MTC), phaeochromocytoma and parathyroid tumours, while MEN3 is characterised by the occurrence of MTC and phaeochromocytoma in association with a marfanoid habitus, mucosal neuromas and intestinal autonomic ganglion dysfunction. MEN4 was only recently described, and is due to heterozygous mutations in the CDKN1B tumour-suppressor gene; patients develop parathyroid and anterior pituitary tumours, along with gastric and bronchial carcinoids or gastrinomas.
Surgery is the treatment of choice for patients with symptomatic tumours, and biochemical and genetic screening of family members enables prophylactic surgery and early detection of tumours to optimise individual MEN patient care.

DOI: 10.1530/endoabs.38.N1.1

### N1.2

**Genetics and multiple endocrine neoplasia**

Louise Izatt
Guy's and St Thomas' NHS Foundation Trust, London, UK.

Genetic testing plays an increasing role in diagnosing and managing patients with Multiple Endocrine Neoplasia (MEN). Advances in genetic testing technology, combined with a fall in the cost of analysis, provides the opportunity to test more patients as early as possible, to try to confirm or refute whether there is a genetic variant contributing to their endocrine neoplasia. If a MEN syndrome is confirmed genetically, then ongoing management and surveillance can be tailored, following consensus guidelines.
The purpose of this session is to explain some of the key concepts of genetics and MEN. This will include patterns of inheritance, the risks, benefits, and limitations of different types of genetic tests, understanding genetic risk, and the implications for individuals and families when a diagnosis of a genetic condition is made.
In the future, we may expect that knowledge of genetic variants in both the patient's constitutional DNA and in their endocrine tumours may be of importance, as it is likely that in some MEN syndromes the disease pattern will be more complex than monogenic disease. Understanding the functional consequences of how these genetic variants interact will help to customise optimal treatment.

DOI: 10.1530/endoabs.38.N1.2

### N1.3

**Psychological support of the MEN patient**

Kym Winter
AMEND Charitable Trust, Kent, UK.

Working with individuals and families with a complex and unpredictable genetic condition like MEN can present particular challenges for health professionals. This session will aim to support you in supporting your patients by focusing upon the psychological aspects of living with MEN.

DOI: 10.1530/endoabs.38.N1.3

## Nurse Session 2: Nurse-led clinics

### N2.1

Abstract unavailable.

### N2.2

Abstract unavailable.

### N2.3

**Medicolegal aspects of nurse-led clinics**

Helen Turner
OCDEM, Oxford, UK.

On a background of an increasing number of Nurse-Led Clinics and Endocrine Specialist nurses taking on many varying extended roles and responsibilities, 2015 has seen several important developments in the medicolegal aspects of practice.
Nurse-led endocrine clinics often develop ad ho, and whilst there is very helpful guidance available (a Royal College of Nursing accredited Competency Framework developed by the Society for Endocrinology, and the new 2015 Nursing and Midwifery Council code to professional practice), many aspects of these clinics continue to run within an unclear legal framework making staff potentially vulnerable to litigation.
The practice of obtaining informed consent in endocrine nurse-led clinics varies between centres. However, the law surrounding 'Consent' has changed this year; the 'Bolam test', whereby practice accepted by a 'reasonable body of medical opinion' was not negligent, has been extended by the Supreme Court. The test of "adequacy of informed consent of material risk" means that the clinician must make the patient aware of any risk to which a 'reasonable person in the patient's position' would be likely to attach significance.
Whistle-blowing in the NHS is an on-going important issue. Following the Francis enquiry, the 'Freedom to Speak up review' has highlighted 15 principles involving a culture change 'whistle-blowing' or 'raising concerns' by NHS employees regarding the workplace and practice.
Between 2008 and 2012, complaints by patients increased by 196% in nursing and midwifery. There is a contractual duty on NHS staff to report all incidents resulting in moderate/severe harm or death. The Nursing and Midwifery Council has provided draft guidance on a nurse's 'duty of candour'.
Other challenges include adequacy of documentation, equality and diversity, email and telephone advice, recording of consultations, and relationships with pharmaceutical companies. Thus, whilst Nurse-led clinics remain popular and very valuable, awareness of these various issues is essential.

DOI: 10.1530/endoabs.38.N2.3

# Senior Endocrinologists Session

## SE1.1

**The new tachykinin, endokinin: its role in emesis, sialorrhea and smoking in pregnancy**

Philip Lowry
University of Reading, Berks, UK.

The human tachykinin, endokinin (EK), is processed into a 41mer in the human placenta. As synthetic EK(10–41) is as potent as substance *P* at the NK1R, it is likely that the primary function of EK is as the principal placental NK1R agonist mediating local beneficial vasodilatation. Although the emetic and sialogogic actions of substance *P* are well known, its complete absence from the placenta probably explains why its role in pregnancy has not been studied. I propose that the potent natural placental NK1R agonist, EK, by spilling in to the mother's circulation, should now be recognised as the likely cause of emesis and sialorrhea in pregnant women. As EK is post-translationally modified specifically in the placenta with a phosphocholine (PC) moiety, it would bind to placental C-reactive protein (CRP) and form a pentameric agonist complex resulting in local NK1R aggregation/activation. Circulating PC-free EK released from peripheral tissues (e.g. from the lungs during smoking), on occupying placental/uterine NK1Rs, could reduce the receptor aggregation induced by placental pentameric PC-EK/CRP, and may account for the poor placentation/vascularisation seen in smokers. The release of EK from lung tissue may also explain the lower incidence of emesis in pregnant smokers by down regulation of the associated NK1Rs in the area postrema of the medulla oblongata.

DOI: 10.1530/endoabs.38.SE1.1

## SE1.2

Abstract unavailable.

## SE1.3

**Steroid mass spectrometry: a 50 year history**

Cedric Shackleton[1,2]
[1]Centre for Endocrinology, Diabetes and Metabolism (CEDAM), University of Birmingham, Birmingham, UK; [2]UCSF Benioff Children's Hospital, Oakland, California, USA.

The characterization and measurement of steroids in health and disease has been a cornerstone of endocrinology since the 1930s. The first example of a diagnostic steroid for an endocrine disorder was the discovery of pregnanetriol in the urine of patients with the adrenogenital syndrome (CAH) by Guy Marrian in 1937. He went on to head the Clinical Endocrinology Research unit in Edinburgh for many years. For decades, paper and TLC dominated metabolomic (urinary steroid) approaches to steroid analysis, while from the 1960s RIA of circulating hormones and precursors was pre-eminent and remains useful.

Mass spectrometry now dominates the steroid analytical field but it has taken a long time to reach this position. The breakthrough came in the mid-1960s, when a Swedish company introduced the LKB 9000 GC/MS instrument. Researchers at the Karolinska institute had invented a way of combining two very modes, GC with its high-pressure gas flow and mass spectrometry which is conducted in a vacuum. In 1967 this presenter had the privilege to use the first UK LKB 9000 in Professor Charles Brooks' lab in Glasgow to document the structures of steroids excreted by the human newborn.

In the 50 years of steroid mass spectrometry there have been continuous advances, notably for GC/MS the introduction of capillary columns, chemical derivatisation methods and advanced data systems.

The most important advances have been combining HPLC and MS and the introduction of Tandem MS which provides high specificity and sensitivity. Automated HPLC MS/MS systems now allow quick separations and accurate measurement of vanishingly small amounts of hormonal steroids. The high throughput has rendered HPLC/tandem MS the desired method for routine clinical steroid measurements. In spite of being labour intensive GC/MS still holds its own for metabolomic studies, particularly with the introduction of machine learning techniques for disorder diagnosis. Interestingly, pregnanetriol, the mother of steroid metabolites, remains an essential analyte for our metabolic profile studies almost 80 years after Marrian's painstaking identification.

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## SE1.4

**A crackpot ejection theory about China's unique natural glass**

David C Anderson

An interest in art and antiques and a chance discovery in Hong Kong in 2000 led rapidly to an obsession with carvings from Northern China's 5000 year old Hongshan Culture. The most controversial are in a unique silica glass ('shui jing'), with a very high melting temperature (>1600 °C) and no additives or stabilisers, that could only have originated from a large unique meteorite impact. This paper will present results on the carvings of light microscopy coupled with persistent pestering of friendly and well-equipped materials scientists. The study crosses many boundaries – art, culture, history, meteorology, geology, minerals science and exploration – all of little obvious relevance to medicine or endocrinology. Obviously retirement provides opportunities not open to anyone who still has to work for a living. I shall present evidence from the carvings that points to a unique post-impact lateral ejection of molten glass; and that this was later carved by grinding using impact diamond sand. We undertook three expeditions to Inner Mongolia, in 2010, 2011 and 2015. In the first two we visited a Google Unearthed candidate impact site, and in the third tried to locate the mine in the predicted splash field where large amounts of such glass are still being excavated. This mine should be a strong pointer to the location and age of the impact itself, but is shrouded in secrecy possibly for fear of a conflict between business and scientific interests; locating it is a race against time. Our candidate impact site itself lies in the Abag-qi volcanic field, with postulated secondary 'crackpot' volcanism from enormous pressures upon impact. For ongoing work I am fortunate to have the support of colleagues at the Institute of Geological Sciences in Beijing.

DOI: 10.1530/endoabs.38.SE1.4

## SE1.5

**Understanding the biochemistry underpinning hypoglycaemia of the insulin autoimmune syndrome (IAS) is paramount for diagnosis and management**

Adel Ismail
Pinderfields and Leeds Hospitals, Wakefield, West Yorkshire, UK.

IAS is common in Japanese but rare in Caucasians. Highlighting its aetiology is paramount in understanding its clinical and analytical variabilities and can help planning investigations and avoid mistakes in the interpretation of false pancreatic endocrine data commonly associated with this syndrome.

The underlying aetiology of IAS is the presence of high affinity/avidity endogenous insulin autoantibodies in significant amounts in non-diabetics. Clinically these autoantibodies could trigger pathology, namely hypoglycaemia and analytically it could interfere in immunoassays analyses causing erroneous pancreatic hormones data. Two types of insulin autoantibodies (different classes and subclasses) may develop separately, sequentially or in combination namely autoantibodies which bind insulin/proinsulin(s) and receptor autoantibodies (insulinmimetic). Binding of insulin/proinsulin(s) to autoantibodies increase considerably their $t_{1/2}$ and concentrations. The clinical manifestations are highly variable, ranging from mild and transient, to spontaneous, severe and protracted hypoglycaemia necessitating in extreme cases plasmapheresis for glycaemic control, dependent on the intrinsic nature of circulating autoantibodies (i.e. affinities/avidities, titres/concentrations and capacities/valency).

IAS appears to develop in genetically predisposed individuals with history of other autoimmune disorders and/or autoimmune polyendocrine syndromes. Major triggers are infection and drugs containing sulphydryl groups including the cyclic disulphide alpha-lipoic acid (ALA), sold over-the-counter and the internet as a universal antioxidant drug/free radical scavenger; it is also prescribed for conditions such as peripheral polyneuropathy. Recently, over a period of

22 months, seven cases in Caucasians (Italians) receiving ALA were diagnosed with IAS in single medical center in Sicily. It appears that the incidence of drug-induced IAS may not be after all insignificant in genetically predisposed Caucasians too.
Testing for insulin autoantibodies should be included among first line investigations in patients with indeterminate and/or unexplained hypoglycaemia; if present, pancreatic hormones data must be interpreted in the context of immunoassays cross-reactivities.

DOI: 10.1530/endoabs.38.SE1.5

## SE1.6

Abstract unavailable.

# Oral Communications

## Early Career Oral Communications

### OC1.1

**A novel pharmacological approach to target LH and testosterone hypersecretion in women with polycystic ovary syndrome: a randomised, double-blind, placebo-controlled multi-centre randomised clinical trial of the neurokinin B receptor antagonist AZD4901**

Jyothis George[1], Rahul Kakkar[5], Jayne Marshall[4], Martin Scott[5], Richard Finkelman[6], Tony Ho[5], Stuart McIntosh[6], Johannes Veldhuis[3], Karolina Skorupskaite[2], Richard Anderson[2] & Lorraine Webber[4]
[1]University of Oxford, Oxford, UK; [2]University of Edinburgh, Edinburgh, UK; [3]Mayo Clinic, Rochester, USA; [4]AstraZeneca, Cambridge, UK; [5]AstraZeneca, Waltham, USA; [6]Former AstraZeneca Employee, NA, UK.

LH hyper-secretion, driven by increased GnRH pulsatility, underpins excess testosterone secretion – a key clinical feature of PCOS.
The kisspeptin-neurokinin B (NKB)-GnRH pathway has emerged as the pivotal regulator of reproduction. We hypothesised that pharmacologic blockade of NKB may address the central pathophysiology of LH hyper-secretion and hyperandrogenism in PCOS. We undertook a randomised, double blind, placebo-controlled multi-centre Phase II trial (Clinicaltrials.gov NCT01872078) of the NKB receptor antagonist AZD4901. Sixty-seven women (mean $\pm$ S.D.) age $28 \pm 5$ years, BMI $31.5 \pm 6.0$ kg/m$^2$, were randomised to receive 20, 40 or 80 mg/day of AZD4901 or placebo orally for 28 days.
Inter-group comparisons used mixed effects models for repeated measures with baseline values as a covariate. The primary endpoint was change in LH area-under-curve (AUC) between baseline and day 7, quantified over 8 h using 10-min LH sampling.
In the AZD4901 80 mg/day group, mean (geometric) LH AUC decreased from 67.4 ($\pm$1 S.D. limits 42.2, 108.0) at baseline to 36.0 (15.9, 81.6) IU/L*h at day 7, a baseline-adjusted decrease of 52% (95% CI 30–67%) relative to placebo ($P=0.0003$). Correspondingly, LH pulse frequency in this group decreased from 5.79 to 3.73 pulses/8 h, an adjusted mean reduction of 3.55 (95% CI 2.00–5.10%) pulses/8 h relative to placebo ($P<0.0001$). Total testosterone decreased from 2.16 (1.63, 2.87) at baseline to 1.55 (1.06, 2.27) nmol/l at day 7, an adjusted decrease of 29% (95% CI 14–41%) relative to placebo ($P=0.0006$).
Excluding presumed ovulators (serum progesterone $>6$ ng/ml at any study visit), all these endpoints remained significantly reduced at day 28. No statistically significant LH or *T* changes were observed with lower doses.
In summary, AZD4901 reduced serum LH, LH pulse frequency and serum testosterone in this first RCT to manipulate the kisspeptin-neurokinin B (NKB)-dynorphin system in PCOS. No statistically significant LH or T changes were observed with lower doses (Table S3); no drug-emergent serious adverse events were reported. In summary, in this first study to manipulate the kisspeptin-neurokinin B (NKB)-dynorphin system in PCOS, the NKB receptor antagonist AZD4901 specifically reduced serum LH, LH pulse frequency and serum testosterone. Current therapy of PCOS is mainly symptomatic: these findings present NKB antagonism as a potential therapeutic approach to treat the central neuroendocrine pathophysiology of this common clinical condition.

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### OC1.2

**Mutations in IGSF10 cause self-limited delayed puberty, via disturbance of GnRH neuronal migration**

Sasha Howard[1], Leo Guasti[1], Gerard Ruiz-Babot[1], Alessandra Mancini[1], Alessia David[5], Helen Storr[1], Louise Metherell[1], Michael Sternberg[5], Claudia Cabrera[2,4], Helen Warren[3,4], Michael Barnes[2,4], Karoliina Wehkalampi[6,7], Valentina André[9], Yoav Gothilf[8], Anna Cariboni[9,10] & Leo Dunkel[1]
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Background
Timing of puberty is associated with height, cardiovascular health and cancer risk, with a significant public health impact. Previous studies estimate that 60–80% of variation in the timing of pubertal onset is genetically determined. Self-limited delayed puberty (DP) segregates in an autosomal dominant pattern, but the underlying genetic background is unknown.
Methods
We performed whole exome sequencing in 111 members of 18 families from our patient cohort with self-limited DP, with follow-up targeted re-sequencing of candidate genes in a further 42 families. For one candidate gene we defined tissue expression in human and mouse embryos. The effects of gene knockdown were investigated via neuronal migration assays, and *in vivo* using a transgenic zebrafish model with fluorescently-labelled GnRH neurons. *N*-terminal fragment mutations were interrogated via expression in mammalian cells.
Results
We identified four rare heterozygous variants in *IGSF10* in 29 members of ten unrelated families. All four variants were in evolutionarily conserved positions and were predicted by *in silico* analysis to have a deleterious effect on protein function. Statistical tests showed a significant difference in the prevalence of these mutations within DP cases compared to a general population ($P=4.46\times10^{-3}$), and a significant association between these mutations and the delayed puberty trait within our cohort ($P=3.47\times10^{-4}$). *IGSF10* mRNA shows strong expression in the nasal mesenchyme in mouse and human embryos, during the time-period when GnRH neurons migrate from their nasal origin towards the hypothalamus. *IGSF10* knockdown caused reduced migration of immature GnRH neurons in the *in vitro* analysis, and perturbed migration and extension of GnRH neurons in the transgenic zebrafish model. Reduced secretion of mutant protein was demonstrated by western blotting.
Conclusions
We present our novel finding that *IGSF10* mutations contribute to the phenotype of self-limited delayed puberty in humans, through impairment of migration of GnRH neurons during embryonic development.

DOI: 10.1530/endoabs.38.OC1.2

### OC1.3

**RNA-sequencing of mouse adrenals reveals the pathways perturbed by loss of nicotinamide nucleotide transhydrogenase**

Eirini Meimaridou[1], Michelle Goldsworthy[2], Vasileios Chortis[3], Paul Foster[3], Wiebke Arlt[3], Roger Cox[2] & Lou Metherell[1]
[1]Centre for Endocrinology, William Harvey Research Institute, Queen Mary University of London, London, UK; [2]Medical Research Council (MRC) Harwell, Diabetes Group, Harwell Science and Innovation Campus, Oxforsdshire, UK; [3]University of Birmingham, Birmingham, UK.

Nicotinamide nucleotide transhydrogenase (NNT) is a highly conserved inner mitochondrial membrane protein, which supplies high concentrations of NADPH for detoxification of reactive oxygen species (ROS) by glutathione and thioredoxin pathways. In humans, loss-of-function mutations in *NNT* cause familial glucocorticoid deficiency, a potentially fatal, adrenal specific disorder characterized by increased levels of ACTH and low levels of cortisol. *Nnt*$^{-/-}$ mice (MUT) have a 50% reduction in both basal and ACTH-stimulated corticosterone output when compared to their WT counterparts (WT). The reintroduction of *Nnt* into this mouse strain by a BAC transgene (BAC) results in 1.9-fold overexpression of the protein and partially rescues the phenotype. RNA-seq on adrenals from WT, MUT and BAC mice revealed differential expression (fold change $\geqq 1.5$; *P* value $<0.001$) of 91 genes between WT and MUT mice that was reversed in the BAC suggesting these are directly affected by *Nnt* loss. The 91 genes fell into 12 biological processes by gene ontology analysis with significant enrichment (4.13-fold; $P<0.05$) of genes involved in stress response including the heat shock proteins *Dnajb1*, *Hsph1*, *Hspa1a* and *Hspa1b*. Interestingly alpha- and beta-haemoglobins (*Hba-a1*, *Hba-a2*, *Hbb-b1* and *Hbb-b2*) were highly upregulated in the knockout mouse and their levels returned to normal in the BAC rescue. This data suggest that ROS damage to proteins is activating mito- and cytoprotective proteins (haemoglobins and heat shock proteins respectively) that help maintain cell viability. Surprisingly no alterations in antioxidant genes of the glutathione and thioredoxin pathways (*Prdx3*, *Gpx1*, *Sod2*, *Txnrd2*, *Gr*) or in components of the ACTH signalling pathway (*Mc2r*, *Mrap*) were noted, whereas a 25% down-regulation, at RNA level, in mitochondrial steroidogenic enzymes (*Cyp11a1*, *Cyp11b1*) resulted in a 60% reduction at protein level, perhaps providing a mechanism to explain the reduction in corticosterone seen in the mice.

DOI: 10.1530/endoabs.38.OC1.3

## OC1.4

**Adipose tissue-specific androgen generation fuels an adverse metabolic phenotype in patients with polycystic ovary syndrome**

Michael O'Reilly[1], Punith Kempegowda[1], Laura Gathercole[2], Iwona Bujalska[1], Angela Taylor[1], Beverley Hughes[1], Warwick Dunn[4], Robert Semple[3], Jeremy Tomlinson[2] & Wiebke Arlt[1]
[1]Institute of Metabolism and Systems Research, University of Birmingham, and Centre for Endocrinology, Diabetes and Metabolism Birmingham Health Partners, Birmingham, UK; [2]Oxford Centre for Diabetes, Endocrinology and Metabolism, University of Oxford, Oxford, UK; [3]Institute of Metabolic Science, University of Cambridge, Cambridge, Uganda; [4]Birmingham MRC Regional Phenome Centre, University of Birmingham, Birmingham, UK.

Insulin resistance and androgen excess are the cardinal features of polycystic ovary syndrome (PCOS). Severity of hyperandrogenism and metabolic dysfunction in PCOS are closely correlated, but underlying mechanisms remain poorly understood. Aldoketoreductase type 1C3 (*AKR1C3*) is a key source of adipose androgen generation, activating androstenedione to testosterone (T). We postulated that *AKR1C3* plays a critical role linking androgen metabolism and metabolic phenotype in PCOS.

We undertook *in vivo* deep phenotyping in ten women with PCOS and ten age/BMI-matched controls. Patients underwent oral challenge with 100 mg of the androgen precursor DHEA, with serum sampling every 30 min for 4 h and concomitant adipose microdialysis. Androgens in serum and adipose microdialysate were measured by tandem mass spectrometry. Non-targeted serum metabolomics analysis was performed pre- and post-DHEA. In complementary *ex vivo* and *in vitro* studies, we investigated adipose androgen generation, employing primary human adipocyte culture and the preadipocyte SGBS cell line. The impact of androgens upon adipose lipid metabolism was assessed through measurement of *de novo* lipogenesis, free fatty acid (FFA) uptake and β-oxidation.

At baseline, 5α-dihydrotestosterone (DHT) was detectable in PCOS adipose interstitial fluid but not in controls. After DHEA, adipose tissue DHT increased significantly ($P=0.04$); adipose glycerol levels decreased in PCOS more than in controls ($P=0.04$ for AUC), indicative of suppression of lipolysis. Serum metabolomics indicated significant upregulation of lipid and catabolic metabolism in PCOS. Subcutaneous adipose *AKR1C3* mRNA expression correlated with BMI ($P<0.001$). In human adipocytes, insulin up-regulated *AKR1C3* expression and activity, while DHT increased *de novo* lipogenesis and suppressed β-oxidation ($P=0.03$ for both).

Here we provide integrated *in vivo, ex vivo*, and *in vitro* evidence that insulin drives adipose androgen generation through increased *AKR1C3* expression and activity, fuelling a vicious circle of hyperinsulinaemia, adipose androgen generation and lipid accumulation. This identifies *AKR1C3* as a promising therapeutic target in PCOS.

DOI: 10.1530/endoabs.38.OC1.4

## OC1.5

**Interaction of the MR and GR in the nucleus and at DNA**

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Glucocorticoid actions in the brain are mediated by glucocorticoid receptors (GR) and mineralocorticoid receptors (MR). MR and GR bind endogenously circulating glucocorticoids, share a hormone response element in DNA, and are co-expressed in neurons of the hippocampus and hypothalamus. This arrangement suggests MR and GR functionally cooperate in the regulation of gene expression though hitherto poorly described mechanisms. This possibility was previously exampled by the demonstration of MR-GR interactions *in vitro*, but demonstration of an interacting complex binding DNA *in vivo* has not been accomplished. We have utilized a unique cell line (3617ChMR) to study MR-GR interactions *in vivo*. 3617ChMR incorporates a tandem array of the MMTV long terminal repeat (800–1200 GREs) driving viral Harvey-Ras expression. Accumulation of fluorescent GFP-tagged GR and mCherry-MR at this structure is observable microscopically allowing interactions to be studied at chromatinized DNA. Forster resonance energy transfer (FRET) by lifetime measurement, and fluctuation analysis by cross-correlation number and brightness assay (ccN&B) were used to assess MR-GR interactions in living cells. Paired with standard co-immunoprecipitation, these approaches provide evidence for MR-GR interactions in the nucleoplasm and at DNA and reinforce the expectation that such complexes have a transcriptional role. Computational predictions for MR-GR interactions were obtained using the PRISM algorithm. Unexpectedly, these predictions suggested a more diverse potential for MR-GR complexes beyond the simple heterodimer arrangement. Experimental examination of the stoichiometry of the nucleoplasmic complex (N&B method) did not reveal a heterodimer arrangement. Finally, using the array as an indicator of DNA binding, we show GR is cyclically recruited to DNA by pulses of corticosterone application. Conversely the MR, which does not interfere with GR cyclical behaviour, fails to respond to the ultradian pattern of stimulation remaining loaded at DNA during the washout period; albeit undergoing continuous rapid turnover observable by fluorescence recovery after photobleach (FRAP).

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## OC1.6

**Urine steroid metabolomics as a diagnostic tool in primary aldosteronism**

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Introduction

The regular diagnostic workup for primary aldosteronism (PA) can be very demanding and involves multiple invasive as well as time and cost intensive diagnostic tests. Here we have explored the value of urinary steroid metabolome analysis in the diagnosis and differential diagnosis of PA. Previously, urinary 3α,5β-tetrahydroaldosterone (THAldo) has been suggested as a reliable screening test for PA and serum 18-oxocortisol and 18-hydroxycortisol have been reported as diagnostic markers with the potential to distinguish unilateral aldosterone-producing adenomas (APA) from bilateral adrenal hyperplasia (BAH).

Patients and methods

We studied 180 PA patients (103 APA, 71 BAH) in whom PA had been confirmed by saline infusion test followed by adrenal vein sampling for subtype differentiation. We carried out targeted sequencing for disease-causing somatic mutations in the *KCNJ5*, *CACNA1D*, *ATP1A1* and *ATP2B3* genes in tumour tissue obtained by unilateral adrenalectomy, which was available in 75/103 APA patients. The urine steroid metabolome was analysed by gas chromatography-mass spectrometry comprising the quantification of 38 distinct steroids including metabolites of aldosterone, deoxycorticosterone, corticosterone as well as 18OH-cortisol (18OH-F) and 18-oxo-tetrahydrocortisol (18oxo-THF).

Results

As expected, urinary excretion of mineralocorticoids ($p<0.0001$) and mineralocorticoid precursors ($p=0.002$) was significantly higher in PA patients as compared to 89 sex- and age-matched controls. 65% of PA but none of the controls had a THAldo excretion $>69$ μg/24h, with significantly higher THAldo in APAs compared to BAH ($p=0.0082$). Similarly, APAs had significantly higher excretion of 18OH-F ($p=0.0171$) and 18oxo-THF ($p=0.0005$). Genetic analysis of APA tissue revealed mutations in KCNJ5 ($n=29$), ATPases ($n=12$) and CACNA1D ($n=6$) while in 28 patients no known mutation was identified. Patients with KCNJ5 mutations had significantly increased excretion of both 18OH-F ($p=0.0002$) and 18oxo-THF ($p<0.0001$) as compared to the other mutation groups.

Conclusion

Urine steroid metabolomics is a promising approach for the diagnosis and differential diagnosis of PA.

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## Translational pathophysiology and therapeutics

### OC2.1

**Dual 5-α reductase inhibition promotes hepatic lipid accumulation in man as a result of changes to lipid metabolism in adipose tissue and the liver**

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Background and aims
5α reductase 1 and 2 (SRD5A1 (expressed in liver and adipose), SRD5A2 (expressed in liver) inactivate cortisol to 5α-dihydrocortisol in addition to their role in the generation of dihydrotestosterone and therefore regulate the tissue availability of cortisol. Dutasteride (dual SRD5A1 and SRD5A2 inhibitor) and Finasteride (selective SRD5A2 inhibitor) are commonly prescribed, but their potential metabolic effects have only recently been identified. Our principle objective is to provide a detailed assessment of the metabolic effects of SRD5A inhibition and in particular the impact upon hepatic lipid metabolism and the serum metabolome.
Methods
We conducted a randomised study in 12 healthy male volunteers with detailed metabolic phenotyping performed before and after 3-weeks treatment with Finasteride (5 mg od) or Dutasteride (0.5 mg od). Hepatic magnetic resonance spectroscopy (MRS) to evaluate intrahepatic lipid and 2-step-hyperinsulinaemic euglycaemic clamps incorporating stable isotopes with concomitant adipose tissue microdialysis were used to evaluate tissue-specific carbohydrate and lipid flux. In addition, analysis of the serum metabolome was performed using ultra high performance liquid chromatography-mass spectrometry.
Results
Dutasteride, not Finasteride, increased hepatic insulin resistance. Intrahepatic lipid increased on MRS after Dutasteride treatment and was associated with increased rates of *de novo* lipogenesis. Adipose tissue lipid mobilisation was decreased by Dutasteride. Analysis of the serum metabolome demonstrated that in the fasted state, Dutasteride had a significant effect on the metabolome with significant changes in 123 metabolites (compared to 11 by Finasteride) in particular there were markedly increased glycerophospholipids. Finasteride and Dutasteride blunted the effects of insulin on the serum metabolome.
Conclusions
SRD5A has an important role in lipid regulation and dual SRD5A inhibition with Dutasteride is metabolically disadvantageous by direct effects on the liver and adipose tissue. These changes to the lipid profile with dual SRD5A inhibition promotes hepatic lipid accumulation an established precursor to more serious liver disease.

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### OC2.2

**Adrenal vein catecholamine levels and ratios: reference intervals derived from patients with primary aldosteronism**

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Introduction
Phaeochromocytoma localisation is generally reliably achieved with modern imaging techniques, particularly in sporadic cases. Diagnostic doubt can arise due to the presence of bilateral adrenal abnormalities, particularly in patients with mutations in genes predisposing them to the phaeochromocytoma development. In such cases, surgical intervention is ideally limited to large or functional lesions due to the long-term consequences associated with hypoadrenalism. Adrenal venous sampling (AVS) for catecholamines has been used in this situation to guide surgery, although there is little data available to support diagnostic cut offs.
Methods
172 consecutive patients underwent AVS for localisation of established primary aldosteronism (PA) at two centres (St Bartholomew's Hospital, London and Radboud University Medical Center, Nijmegen) with measurement of adrenal and peripheral venous catecholamines in addition to cortisol and aldosterone. Data from six additional patients with phaeochromocytomas who underwent AVS for diagnostic purposes with subsequent histological confirmation is included.
Results
In the PA cohort, total adrenaline (A; 61.4 + 2.5 nmol/l vs 35.1 + 5.1) and noradrenaline (NA; 16.3 + 2.47 nmol/l vs 2.47 nmol/l) were higher in the right adrenal vein than in the left ($P < 0.05$). This gradient was reversed when the NA:A ratio was considered (right: 0.26 + 0.04; left: 0.40 + 0.03; $P < 0.05$). Reference intervals for adrenal venous NA:A were constructed; the ratio for the 97.5th centile was 1.2 on the left and 1.02 on the right. Six patients with phaeochromocytomas underwent AVS for diagnostic purposes with subsequent histological confirmation. Using the 97.5th centile from the PA population, the false negative rate in the phaeochromocytoma group was 0%.
Discussion
This study describes the largest dataset of adrenal venous catecholamine measurements and provides reference intervals in patients without pheochromocytoma. This strengthens the certainty with which conclusions related to AVS for catecholamines can be drawn, acknowledging the procedure is not part of the routine diagnostic work-up and is an adjunct for use only in difficult clinical cases.

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### OC2.3

**Further advances in diagnosis of adrenal cancer: a high-throughput urinary steroid profiling method using liquid chromatography tandem mass spectrometry (LC-MS/MS)**

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Context
Differentiating adrenocortical adenoma (ACA) from adrenocortical carcinoma (ACC) represents a continuous challenge in patients with (often incidentally discovered) adrenal masses, with unfavorable sensitivities and specificities provided by tumor size, imaging and even histology. We have previously developed urine steroid metabolomics as a tool for the detection of adrenal malignancy employing gas chromatography mass spectrometry (GC-MS) for the detection of 32 distinct steroid metabolites (JCEM2011; 96 (12): 3775–84). Using the most informative nine steroids, as determined by machine learning analysis, this method can diagnose ACC with superior sensitivity and specificity to currently used imaging modalities. However, GC-MS is a labor-intensive, relatively expensive and low throughput method.
Methods
Here we developed a high-throughput liquid chromatography tandem mass spectrometry (LC-MS/MS) method capable of detecting 16 distinct steroid metabolites in a single 5 min run. This method was validated assessing linearity, sensitivity, specificity, reproducibility and matrix effects. We collected 24-h urine samples from 130 healthy controls, 294 ACA and 96 ACC patients and analysed steroid excretion both by GC-MS and the novel LC-MS/MS method.
Results
Comparison of steroid analysis results showed very good correlation between the two methods. LC-MS/MS data revealed significant differences in steroid output between ACC and ACA, with 13 of the 16 measured steroids significantly increased in ACC. Steroid data were subjected to Matrix Relevance Learning Vector Quantization, which identified for both methods the same three steroids as

most informative in detecting ACC. These were THS, 5-PD and 5-PT, the metabolites of 11-deoxycortisol, pregnenolone and 17OH-pregnenolone, respectively. Importantly, we could demonstrate very similar diagnostic performances of GC-MS and LC-MS/MS when using 16 steroids.

Conclusion

This work represents an important step in the implementation of urine steroid metabolomics in the routine work-up of adrenal incidentalomas. We anticipate LC-MS/MS screening in all patients, followed by GC-MS confirmatory analysis of samples with a positive screening result.

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## OC2.4

**Development of a long-acting growth hormone antagonist for the treatment of acromegaly**

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Background

The UK acromegaly register reported that $<60\%$ of acromegalics on medical therapy had controlled disease (1). This is because many patients do not respond to somatostatin therapy. Pegvisomant, a growth hormone antagonist (GHA), controls disease in $>95\%$ cases, but is not cost-effective and requires high dose daily injections (2). There is therefore an unmet need for a cost-effective GHA. We have developed a fusion technology for making a cost-effective long-acting GH molecule (3), and generated a GHA by linking mutated growth hormone to its binding protein (GHBP).

Design of GHA

Growth hormone (GH) contains two binding sites. A mutation (G120R) within site two produces a receptor antagonist and mutations in site 1 enhance binding creating a super antagonist. Linking to GHBP delays clearance but has the problem that site 1 in GH binds to GHBP reducing activity. We hypothesised that the addition of a W104A mutation in GHBP would prevent intramolecular binding and generate a potent antagonist.

Methods

Four target molecules were gene synthesised to include either site 2 mutation (GHA1), site 1 and 2 mutations (GHA2), site 2+W104A mutations (GHA3) and site 1 and 2+W104A mutations (GHA4). Proteins were expressed in CHO cells and purified using antibody based affinity chromatography. Analysis by SDS–PAGE confirmed integrity and purity of protein. GH antagonist potency was tested using a GH-specific *in vitro* dual luciferase reporter assay.

Results

Median IC50s of 45 nM (GHA1); 133 nM (GHA2); 40 nM (GHA3) and 16 nM (GHA4) were obtained. Proteins were judged to be stable over an 8 day period when incubated at 4 °C, room temperature and multiple freeze/thaw cycles at −80 °C.

Conclusions

Site 1 mutations designed to enhance binding (GHA2) decreased bioactivity. However, the inclusion of a W104A mutation (GHA4) increased bioactivity. The results supported our hypothesis that the W104A mutation reduced intra and intermolecular binding. GHA4 has the potential to be a long-acting potent GHA and with no requirement for post-translational modification (e.g. pegylation) is likely to be a cost-effective treatment for acromegaly.

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## OC2.5

**Age at first major osteoporotic fracture in Danes aged 50 and over: influence of diabetes on mean age at fracture and 1 year mortality**

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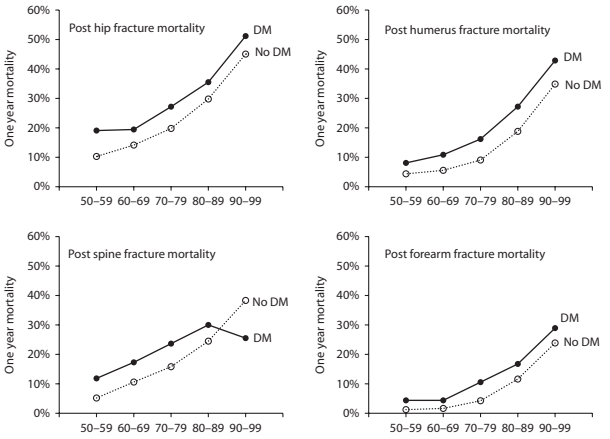
Diabetes mellitus (DM) is associated with an increased risk of fractures in excess of what would be expected from age and BMD. Further, DM is associated with increased risk of mortality in patients with hip fractures and possibly also other osteoporotic fractures.

Study population and methods

Patients aged 50 or over who were treated for fractures of the hip, forearm, humerus and spine over 6 years (2004–2009, $n=146\,256$) not coded as road traffic accidents were tracked in national registers for 1 year post fracture mortality and for a history of DM by ICD-10 code, with a look-back to 1/1/2000.

Results

Patients with DM sustained their first major osteoporotic fracture at a slightly younger age at the hip only (78.1 vs 80.5, $P<0.001$). By contrast, forearm (71.8 vs 69.8, $P<0.001$), and spine fractures (73.2 vs 72.0, $P=0.005$) occurred at a higher age in patients with DM, with no difference in age for humerus fractures ($P=0.34$). Patients with DM had poorer 1-year survival after fracture than fracture patients without DM, with the only exception being spine fractures in the very old (age 90–99).




Conclusions

The increased risk of fractures in patients with DM does not manifest itself in a clinically significant earlier age a first major osteoporotic fracture. Patients with DM have poorer short term post fracture survival than patients w/o DM irrespective of fracture site.

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## OC2.6

**Treatment with the epigenetic modifying compound JQ1+ can significantly reduce the proliferation of pancreatic neuroendocrine tumours in a mouse model of multiple endocrine neoplasia type 1**

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There are currently no curative treatments for metastatic pancreatic neuroendocrine tumours (PNETs), and the 5-year survival is $<50\%$. Such tumours frequently have mutations in chromatin remodelling genes as well as the protein encoded by the multiple endocrine neoplasia type 1 (*MEN1*) gene, menin, which is mutated in up to 40% of sporadic PNETs, and binds the histone methyltransferase MLL1. Histone modifications, and specifically acetylated residues on histone tails, are recognised by members of the bromo and extra terminal (BET) protein family, via their bromodomains, causing alterations in the transcription of growth stimulating genes. We therefore examined the expression of the BET family genes, bromodomain-containing (Brd) 2, Brd3 and Brd4 in PNETs isolated from a conditional MEN1 knockout model, *Men1$^{L/L}$/RIP2-Cre*, whereby menin expression is lost specifically in pancreatic beta cells. We show that Brd2, Brd3 and Brd4 are all expressed in PNETs from this model, in a relative ratio of 3:1:1, making Brd2 the most abundant. Activity of the BET family can be

inhibited by the small molecular probe, JQ1+, through binding to, and inhibiting their bromodomains. We therefore treated female ($n=4$) and male ($n=4$) mice with 50 mg/kg JQ1+, vehicle only, or the JQ1+ negative stereoisomer (JQ1−), by intraperitoneal injection, weekly for 1 month. Bromodeoxyuridine (BrdU) was also administered for the final 3 weeks. At the end of the study, pancreases were harvested and proliferation rates calculated (number of BrdU incorporated cells/mm$^2$ of tissue/days of BrdU administration$\times$100), by immunostaining. We show that after one month, PNETs from JQ1+ treated mice have a significantly lower proliferation rate (3.7%), than both vehicle only (8.1%) and JQ1− treated mice (7.1%), $P<0.005$ and $P<0.05$, respectively; with JQ1+ having an equal effect in both male and female mice. Thus, BET protein inhibitors may represent potential compounds for the treatment of NETs.

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## Steroids and adrenal

### OC3.1

**Molecular diagnosis of patients with adrenal insufficiency using a targeted custom Haloplex next-generation sequencing panel**

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Background
Gaining a correct genetic diagnosis for patients with adrenal insufficiency is important not only to enable genetic counselling within their families, but also for correct treatment and long term management. Adrenal insufficiency is genetically heterogeneous and the long-term sequelae for many of the gene defects, including the progression of the disease and involvement of other tissues, is unknown. Next-generation sequencing (NGS) technologies allow parallel sequence and CNV analysis of multiple genes simultaneously and are therefore ideal to screen genetically heterogeneous disorders.
Methods
We have designed a high-throughput custom Haloplex NGS panel to study 150 known and candidate genes for adrenal insufficiency. As a preliminary study we processed 28 patients without a diagnosis for their adrenal insufficiency. Data analysis was performed using two pipelines, Agilent SureCall Software and Ingenuity Variant Analysis.
Results
A rapid molecular diagnosis was obtained for 11/28 patients including new and previously reported mutations in *HSD3B2* (one patient, homozygous p.R335*), *MC2R* (two patients, homozygous for p.N81fs*3 and p.F235fs*7), *NR0B1* (two patients, hemizygous for p.S431fs*6 and p.L436R), *ABCD1* (two patients hemizygous for p.Q472fs*83 and p.A262T), *STAR* (one patient, compound heterozygous for p.G221S and p.G201D), *CYP11A1* (one patient, compound heterozygous for p.R439* and p.E314K), *NNT* (one patient, compound heterozygous for p.G236V and p.P437L), and *POMC* (compound heterozygous for p.R145C and a regulatory region variant c.-11C>A). Deleterious, single heterozygous changes were discovered in a further 6 patients in *POR, AAAS, STAR, AIRE*, and *TXNRD2* hinting at a genetic diagnosis for these individuals too.
Conclusion
The application of targeted enrichment and NGS can be utilised to aid in the rapid identification of novel and known pathogenic mutations in adrenal insufficiency whilst avoiding the incidental findings associated with whole exome or whole genome sequencing.

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### OC3.2

**Glucocorticoids stabilise the microtubule network to inhibit cell migration**

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Glucocorticoids (GCs) are steroid hormones used to treat inflammatory diseases such as rheumatoid arthritis, but their clinical efficacy is hampered by development of side effects such as impaired wound healing. GCs bind the glucocorticoid receptor (GR) to mediate cellular effects. The inactive GR is held in multi-protein complex in the cytoplasm and upon ligand binding undergoes a conformational change, interacts with cytoplasmic enzymes to mediate non-genomic effects (within minutes) and then translocates to the nucleus to regulate gene expression (over hours). GCs are known to inhibit migration of multiple cell types, although the primary mechanisms underlying this observation remain unclear.
Using a combined strategy of live cell imaging and computational analysis we show that the motion of lung epithelial (A549) cells can be modelled by an alpha stable distribution. We observe that GCs rapidly reduce the average speed of cell migration without changing the nature of the walk statistics. Interestingly, both GR agonists and antagonists ((fluticasone propionate and dexamethasone) and RU486) had a similar effect, suggesting a non-transcriptional mechanism. Using real-time imaging we could not show a rapid GC effect on dynamics of the actin cytoskeleton, but rather demonstrate that the earliest event post-GC treatment is the stabilisation of the microtubule network. Consistent with this, we observed increased acetylation of α-tubulin, a modification required for microtubule stabilisation. This suggests that GR regulates migration following either activation of an acetyltransferase or inhibition of a deacetylase enzyme that targets α-tubulin.
siRNA knockdown of the α-tubulin specific acetyltransferase (α-TAT1) did not affect GC-dependent reduction in migration. In contrast, overexpression of the α-tubulin deacetylase (HDAC6) reversed the effect of dexamethasone, suggesting that treatment with GCs rapidly stabilises the microtubule network to reduce cellular migration by inhibiting HDAC6. This shows for the first time a non-genomic, GC-dependent mechanism involving HDACs which impacts cell function. This is a clear example of how real-time imaging and computational modelling can reveal insight into biological processes.

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### OC3.3

**11β-HSD1-mediated decrease in COX2 expression is abrogated by hypoxia in human dermal fibroblasts**

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Chronic wounds contribute significantly to patient morbidity, mortality and associated healthcare costs. Glucocorticoid (GC) excess and hypoxia are both associated with impaired wound healing (WH) outcomes. The cyclooxygerase 2 (COX2) pathway is an integral component of inflammation and WH. Locally, GC availability is regulated by the enzyme 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) which generates cortisol from inactive cortisone. Although we recently demonstrated that 11β-HSD1 increases during the inflammatory phase of mouse skin WH, a functional role for 11β-HSD1 in human skin remains to be established. Primary human dermal fibroblasts (HDF) were incubated±IL1β, cortisol, and cortisone for 96 h. Ten microgram/ml IL1β increased *COX2* mRNA expression (by qPCR) by 36-fold** (**$P<0.01$), with 50 nM cortisol reducing expression by 64%**. Cortisol decreased IL1β-mediated COX2 expression by 86%**, this was prevented by 5 μM RU486** (GC receptor antagonist). Importantly, 200 nM cortisone also reduced IL1β-mediated COX2 expression by 80%** (time-dependently), this was prevented (dose-dependently) by a selective 11β-HSD1 inhibitor. However, under hypoxic conditions (1% oxygen) cortisone was unable to suppress IL1β-mediated COX2 expression. Moreover, IL1β increased 11β-HSD1 expression by 24- to ninefold** and hypoxia further enhanced this by 86%**. These data are supported by 11β-HSD1 activity assays (using trace amounts of tritium-labelled cortisone substrate), which indicated minimal cortisol generation at baseline (0.1 nM/h). IL1β induced 11β-HSD1 activity by sixfold**, generating 50 nM cortisol in 72 h (cortisol dose–response experiments revealed a minimal concentration of 25–50 nM for GC receptor activation). IL1β-mediated 11β-HSD1 activity further increased by 68%** (1.2 nM cortisol/h) under hypoxic conditions and was undetectable during co-incubation with 11β-HSD1 inhibitor. In summary, we demonstrate that the previously unreported 11β-HSD1-mediated regulation of GC target genes in HDF (e.g. COX2) may be limited in a hypoxic environment. Our findings also suggest a novel synergy between inflammation and hypoxia may drive local GC excess through increased 11β-HSD1, contributing to wound chronicity.

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## OC3.4

### Urine steroid metabolomics as a novel diagnostic tool for early detection of recurrence in adrenocortical carcinoma

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Introduction
Adrenocortical carcinoma (ACC) is an aggressive malignancy with a high rate of recurrence. Regular post-operative follow-up imaging is necessary, but associated with high radiation exposure and frequent diagnostic ambiguity. Urine steroid metabolomics has recently been introduced as a novel diagnostic tool for the detection of adrenocortical malignancy in patients with adrenal incidentalomas. Here we present the first clinical study assessing the performance of this innovative approach in the context of follow-up after complete (R0) ACC resection.
Patients and methods
We included 166 patients from 13 centres registered with the European Network for the Study of Adrenal Tumours (ENSAT). We selected all patients recorded between 2008 and 2015 fulfilling the following criteria: i) recorded on the ENSAT registry as confirmed adrenocortical carcinoma with R0 primary tumour resection and ii) availability of at least two postoperative 24-h urines, one whilst disease-free and the other after recurrence. Twenty-four-hour urines were analysed by gas chromatography–mass spectrometry, with quantification of 38 distinct steroid metabolites. A machine learning-based computational algorithm was employed to detect ACC recurrence.
Results
Twenty-one patients developed 22 ACC recurrences during the study period as documented by serial cross-sectional imaging and biopsy where appropriate. Steroid metabolomics predicted disease recurrence at the time of first abnormal imaging with a sensitivity of 84% and specificity of 95%. Adjuvant mitotane in 12/21 patients did not affect accuracy. In the subgroup of patients for whom a diagnostic pre-operative 24-h urine sample was available ($n=7$), we were able to accurately detect all cases of recurrence (sensitivity and specificity 100%). In seven cases, biochemical evidence of disease recurrence pre-dated the first radiological detection by more than 2 months (range 2–11 months).
Conclusion
Our study provides proof-of-principle evidence suggesting a role for urine steroid metabolomics as a potent diagnostic tool in the follow-up monitoring of ACC.

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## OC3.5

### Glucocorticoid pattern-dependent gene regulation in the rat hippocampus

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Endogenous glucocorticoids are secreted in hourly pulses, establishing a characteristic ultradian rhythm. Accordingly, the glucocorticoid receptor (GR) is activated in distinct pulses, causing target-gene pulsing. In contrast, constant glucocorticoid treatment causes aberrant target-gene overexpression in cell culture models, however this has not yet been described *in vivo*. Therefore, here we assess the effect of altering the glucocorticoid ultradian pattern on transcriptional output in the rat hippocampus.

Male Sprague–Dawley rats were adrenalectomised and replaced with i.v. infusion of pulsatile or constant glucocorticoid at either physiological levels or double concentration to produce large-mass pulses. For the low physiological level infusions, we found differential pattern-dependent regulation of hippocampal glucocorticoid-targets including Per1, KLF15, and SGK1. When the pulse mass was increased, the output of these three targets was not different between constant and pulsatile patterns. Therefore the large-mass pulses caused a similar aberrant transcriptional output of hippocampal target genes to the highly abnormal constant infusion.
Chromatin immunoprecipitation assays for GR, phospho-Ser5 Pol2 and phospho-Ser2 Pol2 during the peak and trough of the pulsatile glucocorticoid infusion indicated that the large-mass pulses resulted in a significant disruption of the normal ultradian rhythm at the DNA template. Notably, we detected a significantly raised nadir-phase binding for GR at regulatory regions and phospho-Ser2 Pol2 within the gene body. Therefore, GR-dependent transcriptional cyclical activity was dysregulated by the large-mass pulses.
Our findings with the larger amplitude pulses may be of clinical relevance as large-mass pulses have been reported in patients with obstructive sleep apnoea. These patients also present with metabolic, cognitive, and affective dysfunction. After treatment, their glucocorticoid profiles normalise, along with an improvement in their clinical profile. Our data suggests that large-mass pulses disrupt the timing of ultradian GR-dependent transcriptional rhythms in the hippocampus, and therefore may contribute to the associated cognitive and affective dysfunction in these patients.

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## OC3.6

### Female 5β-reductase knockout mice are protected from diet induced obesity, insulin resistance, and glucose intolerance

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Steroid hormones and bile acids are potent regulators of metabolic phenotype. The enzyme 5β-reductase (AKR1D1) has a crucial role in bile acid synthesis and also generates 5β-reduced dihydrosteroid metabolites, regulating intra-cellular steroid availability though the clearance of cortisol, testosterone, androstenedione, and progesterone. As AKR1D1 sits at the interface of bile acid synthesis and steroid metabolism, we have hypothesised that it plays a key role in metabolic homeostasis and have generated and characterised an entirely novel, global AKR1D1 knockout (KO) mouse.
As expected AKR1D1KO mice had altered hepatic steroid (*in vitro* cortisone clearance: 100% (WT), 70% (KO); *in vitro* 5α-cortisone/cortisol metabolite generation increased 3.9-fold (KO)), and bile acid metabolism (hepatic bile acid concentration males: $1164\pm626$ pmol/mg (WT), $122\pm42$ pmol/mg (KO), $P<0.05$; females: $310\pm67$ pmol/mg (WT), $113\pm23$ pmol/mg (KO), $P<0.01$). At 10 weeks, KO animals were the same weight as WT littermates with no differences in glucose tolerance. Mice were challenged with a further 20-weeks of high fat diet feeding whereon female, but not male, AKR1D1KO mice were protected from diet induced weight gain (weight gain males: $21.8\pm0.9$ g (WT), $21.4\pm0.7$ g (KO), $P=$NS; females: $27.2\pm0.5$ g (WT), $15.8\pm1.2$ g (KO), $P<0.01$), with reduced adipose tissue mass across all depots (gonadal: $4.0\pm0.2$ g (WT), $2.4\pm0.4$ g (KO), $P<0.005$; subcutaneous: $3.9\pm0.3$ g (WT), $2.4\pm0.5$ g (KO), $P<0.05$; and mesenteric: $1.9\pm0.2$ g (WT), $1.2\pm0.3$ g (KO), $P<0.05$), but with preserved lean mass. Female AKR1D1KO mice were also protected from the metabolic consequences of the high fat diet, with improved glucose tolerance (ipGTT AUC females: 3216 mmol×min (WT), 2601 mmol×min (KO), $P<0.05$) and enhanced insulin sensitivity (ipITT AUC females: 1171 mmol×min (WT), 947 mmol×min (KO)).
AKR1D1KO mice display a sexually dimorphic metabolic phenotype, where female mice are protected from the adverse metabolic effects of a high fat diet. Although the underpinning mechanisms remain to be fully defined, AKR1D1 may represent a future novel therapeutic target for the treatment of metabolic disease.

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## Diabetes and cardiometabolic complications

### OC4.1

**Acute intense exercise restores defective counter-regulation in type 1 diabetes through a process of dis-habituation**

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Hypoglycaemia is an almost unavoidable consequence of iatrogenic insulin therapy in type 1 diabetes. Recurrent hypoglycaemia (RH) results in suppression of normal counter-regulatory hormonal and physiological responses (CRR) to future episodes increasing the risk of severe hypoglycaemia. The mechanisms responsible for this phenomenon remain unclear but may reflect changes in critical hypothalamic glucose sensing neurons (GSN). We set out to indirectly test the hypothesis that hypothalamic GSN 'habituate' to RH and that introduction of a novel stimulus, acute high-intensity (HI) exercise, could restore hypothalamic CRRs through a process of 'dis-habituation'.

Male Sprague–Dawley rats (250–300 g; $n=8$–12/group) were exposed to RH (1 U/kg insulin i.p.; three times per week for 4 weeks or saline control). Subsequently, animals were divided into three groups: i) control, ii) low-intensity exercise (LI: total 25 min: 5 m/min), or iii) high-intensity exercise (HI: total 25 min; 5 min (5 m/min) accelerating (2 m/min) to final 1 min (15 m/min). Twenty-four hours later all rats underwent a hyperinsulinemic–hypoglycaemic clamp with measurement of CRR responses. Glucagon ($240.5\pm27.4$ ng/ml vs $134.6\pm11.1$ ng/ml vs $219.4\pm31.7$ ng/ml) and epinephrine ($8.04\pm0.49$ ng/ml vs $3.12\pm0.28$ ng/ml vs $7.04\pm0.61$ ng/ml) responses to hypoglycaemia were restored in RH animals following HI exercise (control vs LI vs HI exercise respectively). Interestingly, plasma BDNF ($141.1\pm16.8$ pg/ml vs $165.4\pm25.3$ pg/ml vs $333.3\pm57.5$ pg/ml) and β-endorphin ($156.6\pm6.46$ pg/ml vs $186.8\pm11.1$ pg/ml vs $219.6\pm12.6$ pg/ml) levels were significantly elevated in RH animals exposed to HI exercise (control vs LI vs HI exercise respectively). These findings are consistent with the hypothesis that hypothalamic GSN habituate to RH and that introduction of a novel stimulus may re-sensitize them to further hypoglycemia trough a process of 'dis-habitation'. Acute high-intensity exercise may represent a novel therapeutic intervention for people with impaired hypoglycaemia awareness.

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### OC4.2

**Thermal imaging as a novel non-invasive method to measure human brown adipose tissue activity in humans**

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Background
Obesity is a major medical health problem. Human brown adipose tissue (BAT) is potently activated by cold exposure and is a potential novel anti-obesity target. The current gold standard for measuring BAT activity in humans is $^{18}$F-FDG PET/CT. However it has two major limitations – exposure to ionizing radiation and high cost. Since BAT is a thermogenic organ located in the supraclavicular and neck regions, we hypothesised that an increase in temperature in this region detected by thermal imaging as a potential novel method to detect BAT activity in humans.
Objective
To validate thermal imaging of human BAT activation vs the current gold standard $^{18}$F-FDG PET/CT.
Method
PET/CT scans and thermal images were collected from ten healthy young males (mean age $26\pm6$ years) during cold exposure. PET/CT scans were classified as 'BAT positive' ($n=7$) if there was increased signal intensity on the PET/CT scans or 'BAT negative' ($n=3$) if this was absent, during cold exposure. Temperatures within the left supraclavicular region of the thermal images were analysed and overlaid against the PET/CT scans. A further sub-analysis of temperatures of skin directly overlying BAT as seen on PET/CT was carried out.
Results
The hottest 10% of temperatures in thermal images correspond to cold-activated BAT as seen on PET/CT in 'BAT positive' subjects. Thermal imaging of the supraclavicular region detects a significant increase in temperature ($0.31\pm0.08$ °C, $P<0.001$) between baseline and end of cold exposure in 'BAT positive' but not the 'BAT negative' groups. Focussing on skin directly overlying BAT as seen on PET/CT detects a comparable increase in temperature ($0.35\pm0.03$ °C, $P<0.001$) between baseline and end of cold exposure.
Conclusion
Thermal imaging is a novel valid alternative to the current gold standard, $^{18}$F-FDG PET/CT to measure BAT activity in humans which is cost effective and does not expose subjects to ionising radiation.

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### OC4.3

**5α-tetrahydrocorticosterone exhibits topical anti-inflammatory action with limited adverse effects on angiogenesis**

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Background
The 5α-reduced glucocorticoid, 5α-tetrahydrocorticosterone (5α-THB), displays a dissociated steroid profile exhibiting anti-inflammatory effects in a murine model of thioglycollate-induced peritonitis but failing to induce adverse metabolic effects caused by corticosterone. We assessed the topical anti-inflammatory properties of 5α-THB in a model of irritant dermatitis. Given the adverse effects of steroids on cutaneous wound healing, we also investigated the anti-angiogenic properties of 5α-THB *in vivo*.
Methods
Ears of mice ($n=10$/group) were topically treated with irritant croton oil (CO, 300 μg) alone or with corticosterone (applied at EC50 dose, 5 μg) or 5α-THB (5, 15, and 25 μg) for 24 h. Swelling was assessed by ear weight at cull. To study angiogenesis, polyurethane sponges containing either 5α-THB (3 and 15 mg), corticosterone (3 mg), or vehicle, were implanted subcutaneously in mice (8–12/group) for 21 days. Newly formed vessels were analysed histologically; endothelial and smooth muscle cells and infiltrating macrophages were investigated immunohistochemically (CD31, αSMA, F4/80), and transcript profiles by qPCR. Data are mean±S.E.M., CO/vehicle group set to 100%, $^*P<0.05$.
Results
5α-THB decreased swelling similarly to corticosterone, but required a higher dose (25 μg 5α-THB, swelling reduced to $65\pm8\%^*$ vs 5 μg corticosterone, to $57\pm4\%^*$). Corticosterone decreased the number of new vessels to $15\pm3\%^*$ whereas 5α-THB had less effect even at higher dose (3 mg, $87\pm13\%$ NS; 15 mg, $46\pm7\%^*$). While corticosterone inhibited both endothelial and smooth muscle cell recruitment (to $2\pm0.7$ and $12\pm3\%^*$ respectively) and decreased transcripts of genes involved in angiogenesis and inflammation, 5α-THB inhibited only endothelial cell recruitment at high dose (15 mg, reduced to $20\pm5\%^*$), without affecting the same transcripts. 5α-THB and corticosterone both attenuated the number of macrophages infiltrating the sponges (3 and 15 mg 5α-THB to $48\pm4\%$ NS and $49\pm2^*$; corticosterone to $39\pm3\%^*$). Importantly for repercussion on wound healing and skin homeostasis, 5α-THB unlike corticosterone did not decrease amount of collagen in sponges (3 and 15mg 5α-THB, changed to $106\pm16\%$ NS and $128\pm20\%$ NS vs corticosterone, $30\pm8\%^*$).
Conclusions
5α-THB displays the profile of a safer anti-inflammatory compound for topical application with limited effects on angiogenesis and extracellular matrix indicating it is less likely to impair wound healing or cause skin thinning.

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## OC4.4

**Hyperinsulinaemia due to inhibition of 5α-reductases is ameliorated by liver-selective glucocorticoid receptor antagonism in diet-induced obesity**

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Background
5α-reductase 1 (5αR1) metabolises steroids such as glucocorticoids and androgens, and is highly expressed in murine liver. Genetic disruption of 5αR1 leads to adverse metabolic changes in mice. We hypothesised that dutasteride, a 5αR inhibitor, induces insulin resistance in mice, as in humans, and this effect is underpinned by increased hepatic glucocorticoid action; an experimental paradigm was set up using A-348441, a liver-selective glucocorticoid receptor (GR) antagonist, and then utilised to assessed the contribution of increased hepatic glucocorticoid action to the metabolic consequences of dutasteride.
Methods
C57BL6/J male mice ($n=8$–15/group; age 12 weeks) were given high fat (HF), HF with A-348441 (KaroBio), HF+dutasteride (Dut), or HF+Dut+A-348441 diet for 4 weeks. Glucose tolerance tests (GTT) were performed at week 3, with mice culled at week 4. Plasma insulin and corticosterone were measured by ELISA and plasma glucose spectrophotometrically. Data are mean±S.E.M., $^{\#}P<0.05$ vs HF diet and $^{\$}P<0.05$ vs HF+Dut diet.
Results
Plasma corticosterone concentrations were not changed by A-348441, supporting liver-selective GR antagonism. A-348441 improved metabolic health of mice receiving a HF diet, preventing HF-induced bodyweight gain (34.3±0.5 g vs 31±0.8 g$^{\#}$), and total white adipose depot weight gain (2.46±0.1 g vs 1.58±0.1 g$^{\#}$), and attenuating HF-induced elevations in fasting plasma insulin, fasting glucose and insulin response to GTT (lowered by 52$^{\#}$, 25$^{\#}$, and 44%$^{\#}$ respectively) . Inhibition of 5αRs with dutasteride impaired insulin sensitivity, with increased insulin response to GTT but did not change body weight, total adipose depot weight, fasting insulin, fasting glucose, or glucose response to GTT; A-348441 reduced this hyperinsulinaemia (235.9±17 ng/ml per min vs 329.3±16$^{\#}$ ng/ml per min vs 198.4±25$^{\$}$ ng/ml per min).
Conclusions
Liver-specific GR antagonism ameliorates the metabolic consequences of acute diet-induced obesity. Hyperinsulinaemia caused by inhibition of 5αRs was ameliorated by A-348441, suggesting that hepatic glucocorticoid action plays a substantial role in metabolic dysfunction caused by 5αR inhibition. Moreover, targeting hepatic GR may be beneficial in maintaining metabolic homeostasis in diet-induced obesity.

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## OC4.5

**Glucagon increases energy expenditure independently of brown adipose tissue activation in humans**

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Background
Obesity is a global health concern. Elevating energy expenditure (EE) would be a highly effective treatment approach to treat obesity but no current drugs can safely achieve this. Cold exposure potently increases EE through brown adipose tissue (BAT) thermogenesis in humans. Glucagon elevates EE via BAT in rodents but the mechanism in humans is unknown. We investigated for the first time the mechanism by which glucagon increases EE in humans.
Methods
Eleven volunteers underwent measurement of EE using an indirect calorimeter at the start and end of three interventions: i) cold exposure; ii) control (vehicle) infusion at 23 °C; and iii) glucagon infusion at 23 °C. On each visit thermal images of the neck were taken – an increase in temperature is a non-invasive measure of increased BAT activity. All 11 volunteers also underwent a FDG PET–CT scan with cold exposure. In those in which this confirmed cold-induced BAT activity ($n=8$), they had a second PET–CT scan with either vehicle ($n=4$) or glucagon ($n=4$) infusion (23 °C).
Results
EE rose by 14% with cold exposure and 15% following glucagon infusion ($P<0.05$ vs control). BAT depots identified on the cold scan had significantly (4×) higher metabolic activity than on the vehicle or glucagon infusion scans, which were not significantly different from each other. There was a 0.31 °C rise ($P<0.001$) in neck temperature on thermal images after cold exposure in the BAT positive cohort but not after glucagon or vehicle infusion.
Conclusions
Glucagon and cold exposure have a similar effect in stimulating energy expenditure but glucagon has no effect on the metabolic activity of classical adult supraclavicular BAT compared with cold exposure. This information is of importance to the development of better targeted and safe treatments designed to combat obesity through upregulation of energy expenditure.

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## OC4.6

**Cardiac fibrosis and the balance between glucocorticoid and mineralocorticoid receptors signalling**

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Specific variations in the human glucocorticoid receptor (GR) gene associate with increased cardiovascular disease risk. GR signalling is essential for cardiac maturation *in utero* and adult mice with cardiomyocyte and vascular smooth muscle deletion of GR (SMGRKO mice) have cardiac hypertrophy, fibrosis and impaired function. Intriguingly, levels of left ventricle (LV) mRNA encoding the mineralocorticoid receptor (MR), which is pro-fibrotic in heart, rise postnatally in SMGRKO mice in parallel with the development of cardiac fibrosis. Here, the benefit of MR antagonism in limiting cardiac fibrosis was assessed in SMGRKO mice.
SMGRKO mice (generated via SM22α-Cre mediated deletion of GR) and control (Cre−) littermates were treated from birth with vehicle or 20 mg/kg per day spironolactone, an MR antagonist, administered in the drinking water to lactating dams until weaning then to offspring ($n=10$–13/group). At 8 weeks of age, hearts were collected for histology and mRNA profiling. Data were analysed by two-way ANOVA with Tukey's multiple comparisons test.
Heart weight in male SMGRKO mice was higher than controls irrespective of spironolactone treatment ($P<0.01$). Interestingly, spironolactone modestly reduced heart weight in both genotypes ($P<0.05$).
PicroSirius Red staining showed greater collagen levels in LV of SMGRKO mice ($P<0.001$); spironolactone treatment reduced the magnitude of this genotypic difference. Although spironolactone did not prevent the increase in LV levels of mRNA encoding MR or profibrotic factors (connective tissue growth factor, collagen1α2 and collagen3α1) in SMGRKO mice, it did attenuate collagen1α2 mRNA increases ($P<0.05$).
In conclusion, the modulatory effects of spironolactone on pro-fibrotic signalling suggest that elevated MR contributes to the pro-fibrotic cardiac phenotype discovered in SMGRKO mice. Consequently, MR antagonism may benefit individuals with particular variants of the GR gene. Spironolactone effects on heart weight indicate a role for MR in early life cardiac growth and SMGRKO mice are, potentially, a useful new model to investigate MR-dependent cardiac fibrosis.

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# Thyroid and parathyroid

## OC5.1

**Functional consequences of germline mutations in a novel non-RET medullary thyroid cancer susceptibility gene**

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Whilst the majority of familial medullary thyroid cancer (MTC) is caused by germline mutations of the RET proto-oncogene, there are families and individuals

with predisposition to MTC in whom no RET mutation has been identified (non-RET MTC). Recently, we identified novel mutations in a single gene termed MTC2 in non-RET MTC individuals by whole exome sequencing. The precise role of these MTC2 germline mutations in MTC tumorigenesis is however unclear. Here, we examined the functional consequences of MTC2 mutants V128L and G318Afs*22 to determine their roles in MTC. Luciferase (LUC) reporter assays showed that MTC2-V128L retained transcriptional activity with a significant increase in LUC activity in response to steroid hormone receptor-agonist DPN in HCT116 (3.7-fold; $P<0.01$) and MCF-7 (1.8-fold; $P<0.05$) cells. In contrast, MTC2-G318Afs*22 was incapable of inducing LUC activity in either cell line ($P=$NS). Furthermore, MTC2-G318Afs*22 failed to inhibit ERα-driven luciferase activity in response to either 17β-estradiol ($E_2$) or ERα-agonist PPT, or restrain ERα-driven proliferation of MCF7 cells ($P=$NS compared to ERα alone). In contrast, WT MTC2 and MTC2-V128L inhibited ERα-driven LUC activity (>60%; $P<0.01$) and cell proliferation (>30%; $P<0.05$). As RET expression is known to be stimulated by oestrogen, we then determined the influence of MTC2 mutants on RET in $E_2$- and PPT-treated HCT116 cells. In contrast to WT MTC2, MTC2-G318Afs*22 was unable to oppose ERα-stimulation of the RET proto-oncogene at both the mRNA and protein level ($P=$NS compared to ERα alone). Treatment with anti-oestrogen 4-hydroxytamoxifen was however capable of inhibiting $E_2$-induced RET mRNA expression in cells with MTC2-G318Afs*22. Together these data indicate an emerging role for MTC2 as a novel susceptibility gene in non-RET MTC development, especially as MTC2 mutant G318Afs*22 was associated with higher RET levels. These results also suggest that anti-oestrogens might represent a promising therapeutic strategy for MTC individuals with defective MTC2.

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## OC5.2

### A novel, missense, mutation (P81R) in the TRH receptor gene in congenital central hypothyroidism

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Background

Congenital, isolated, central, hypothyroidism (CCH), is rare and evades diagnosis on TSH-based congenital hypothyroidism screening programmes in the UK. Genetic ascertainment is therefore paramount in enabling prompt diagnosis and treatment of familial cases. Recognised causes include *TSHB* and *IGSF1* gene defects, with only two previous reports of biallelic, highly disruptive (nonsense; R17X, in-frame deletion and missense; p.S115-T117del+T118), mutations in the *TRHR* gene. Here, we describe the first homozygous missense mutation in *TRHR*, associated with a typical phenotype.

Case

A female infant from a consanguineous Pakistani family, presented with prolonged neonatal jaundice and was found to have central hypothyroidism (TSH 2.2 mU/l, NR 0.4–3.5 and free $T_4$ 7.9 pmol/l, NR 10.7–21.8), with otherwise normal pituitary function. With *TSHB* or *IGSF1* mutations being usually associated with profound or X-linked CCH, a *TRHR* mutation was sought.

Results

Sequencing identified a homozygous mutation (P81R) in *TRHR*, substituting arginine for a proline residue in transmembrane helix 2 (TM2) which is highly conserved amongst G-protein coupled receptors (GPCRs). Functional studies showed that although the mutant receptor was expressed and localised to the cell membrane normally, its ability to bind radiolabelled TRH and signal via Gqα was markedly impaired, likely due to disruption of structure of TM2.

Conclusion

We describe the first deleterious, missense *TRHR* defect associated with moderate CCH. Importantly, the location of the mutated amino acid (proline 81) highlights a previously unanticipated functional importance of the TM2 in mediating hormone binding and receptor activation. Future identification of other, naturally-occurring, *TRHR* mutations may map the molecular basis of ligand binding and activation of TRHR which are poorly understood.

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## OC5.3

### Use of $^{11}$C-methionine PET to localise parathyroid adenoma/hyperplasia: a single centre experience

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Introduction

It is established practice to localise parathyroid lesions preoperatively using ultrasound (US) and sestaMIBI (MIBI). Whilst these imaging techniques have good sensitivity/specify, there are patients in which imaging does not localise a parathyroid lesion. $^{11}$C-Methionine PET (MET PET) is an imaging modality where $^{11}$C-methionine, a radioactive tracer, is taken up at sites of protein/peptide synthesis and has been demonstrated to be effective in localising parathyroid lesions. We therefore investigated the clinical utility of this imaging technique at our centre.

Methods

All patients had biochemistry prior to imaging thought to be consistent with primary hyperparathyroidism. Criteria to undergo PET imaging were inability of conventional imaging to identify a parathyroid lesion, potential intrathyroidal parathyroid lesion, and three patients where mediastinal disease was suspected. Twenty patients underwent MET PET over an 18-month period.

Results

MET PET identified a parathyroid lesion in 14/20 patients. Three out of three of these were demonstrated to be mediastinal lesions, leading to a parathyroid adenoma being successfully resected by sternotomy. 11/20 demonstrated disease in the neck. Of these 3/11 parathyroid lesions were very deep in the neck adjacent to vertebrae/oesophagus and not seen with US/sestaMIBI. In 2/11 patients MET PET demonstrated intrathyroidal parathyroid lesions and patients underwent hemithyroidectomy. All parathyroid lesions were confirmed on histology (13 adenoma and one hyperplasia). Of the 6/20 who had negative imaging, one now has a diagnosis of sarcoidosis with elevated 1,25-dihydroxycholecalciferol, one underwent bilateral neck exploration and histology demonstrated parathyroid hyperplasia. The remaining four patients are still being investigated with working diagnoses of FHH in three patients.

Discussion

MET PET is a useful additional functional imaging technique when conventional imaging fails to localise a lesion, where mediastinal disease is suspected or intrathyroidal disease needs confirmation. This can particularly helpful when deciding to refer patients for major surgery.

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## OC5.4

### A novel modulator of cellular invasion and metastasis in endocrine cancer

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Metastasis is a multistep process responsible for the majority of endocrine cancer deaths. Central to the ability of cells to move is the recruitment of actin fibres at the periphery of the cell by key proteins, especially the cortical actin binding protein cortactin. A full understanding of cortactin function is required in order to address metastatic cell activity within endocrine cancer. We used IP-MS to discover protein binding partners, and now identify the proto-oncogene PBF as a new functional binding partner of cortactin, whose expression has recently been correlated with thyroid and breast cancer metastasis, and with colon cancer extra-mural vascular invasion. We show that cortactin and PBF interact and co-localise through immunofluorescence and Proximity Ligation Assays, and that this occurs within or close to the plasma membrane, and preferentially at the leading edge of migrating cells. Oncogenic expression of PBF induced potent cell invasion and migration in thyroid TPC-1 ($P=0.01$), breast MCF-7 ($P<0.001$) and colorectal HCT116 cells ($P<0.001$), which was entirely abrogated by the knockdown of cortactin expression. In $n=43$ matched papillary thyroid cancers, cortactin was significantly upregulated at the mRNA ($P=0.022$) and protein ($P=0.045$) levels, particularly in more aggressive tumours, and significantly correlated with PBF expression. We also demonstrate the interaction between PBF and cortactin through co-immunoprecipitation assays and reveal that artificially targeting PBF to the plasma membrane results in increased cortactin binding, entirely blocking endogenous cellular invasion. Thus, we identify a new modulator of cortactin

function, and show for the first time that cortactin is over-expressed in differentiated thyroid cancer. We propose that modulation of PBF subcellular localisation may present a novel mechanism of addressing *in vivo* tumour cell invasion and migration.

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## OC5.5

### Investigating the genetic architecture of gland-in-situ congenital hypothyroidism by comprehensive screening of eight known causative genes

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Background
Lower cut-offs in TSH screening have doubled the incidence of congenital hypothyroidism (CH), particularly cases with an eutopically-located gland-in-situ (GIS). Although mutations in known dyshormonogenesis genes, or the thyrotropin-stimulating hormone receptor (*TSHR*) may underlie such cases, these genes have not previously been screened comprehensively in a GIS CH cohort.
Study design
We evaluated the relative contribution and molecular spectrum of mutations in eight known causative genes (*TG*, *TPO, DUOX2, DUOXA2, SLC5A5, SLC26A4, IYD* and *TSHR*) in fifty-one CH cases with GIS from 35, ethnically diverse families, using next generation sequencing. Patient genotypes were correlated with biochemical phenotype and pathogenicity of novel mutations analysed *in silico*.
Results
Twenty-nine cases harboured likely, disease-causing, mutations. Twenty cases with single gene defects, most commonly involving *TG* (12 cases), *TPO* (five cases), *DUOX2* (two cases) and *TSHR* (one case), were documented. Novel variants were identified in *TG* (13), *TPO* (6), and *DUOX2* (3). Nine cases harboured pathogenic variants in two different genes: *TG* and *TPO* (1 case); *SLC26A4* and *TPO* (two cases) and *DUOX2* and *TG* (six cases). However, family studies in such digenic cases, showed no clear correlation between genotype and phenotype. Genetic aetiology was not ascertained in 22 patients, generally with milder biochemical CH and including some familial cases.
Conclusions
The aetiology of CH with GIS is complex, with only 57% being due to mutations in *TSHR* or known dyshormonogenesis-associated genes. Combinations of defects in two different genes are common, especially in consanguineous families. Severe CH is most commonly mediated by biallelic *TG* or *TPO* mutations. A high proportion ($\sim$43%) of unsolved cases suggests contribution of hitherto unknown genes or environmental factors to GIS CH.

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## OC5.6

### Safety review of liothyronine use: a 20 year observational follow up study

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Some patients use liothyronine as thyroid replacement therapy as an alternative to L-thyroxine. Trials have examined the potential benefits, but there is little data looking at the relative safety of these two agents. All patients receiving thyroid replacement therapy between 1993 and 2014 in Tayside were included in a cohort study ($n=34\,355$; 319 500 patient years of follow up). Overall 33 955 patients received only L-thyroxine, and 327 received liothyronine in combination with L-thyroxine and 73 on liothyronine alone (total = 400). Using unique patient identification numbers, biochemical, prescribing, hospital admission, radioiodine and general registry office data were linked.
Patients initiating treatment with liothyronine were younger (48 vs 59 years $P<0.001$), but there was no gender difference (85% female vs 82%). They were more likely at baseline to have had thyroid cancer, have a history of previous hyperthyroidism and be treated with anti-psychotic or anti-depressant medication. They were less likely to have cardiovascular disease or be treated with a statin. During a mean follow up of 9.3 years ($\pm$5.6 years) proportional hazards ratios (HR) were reported after adjustment for age, gender, baseline TSH, number of thyroid prescriptions and history of thyroid cancer or hyperthyroidism. For patients taking liothyronine there was no increased risk of death (0.78; 95% confidence interval: 0.54–1.11), fractures (HR 0.79; 0.49–1.27), atrial fibrillation (HR 0.91; 0.47–1.75) or cardiovascular disease (HR 0.90; 0.42–1.92). There was an increased risk of mental health disorders (HR 3.27; 1.02–10.52) for patients taking liothyronine alone, but not for those taking a combination therapy. There was an increased incident use of anti-psychotic medication (HR 2.26; 1.64–3.11). No increased risk of fractures or atrial fibrillation in patients taking liothyronine compared to L-thyroxine was demonstrated. There was an increased risk of mental health disorders if liothyronine was used alone.

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# Advances in reproduction and signalling

## OC6.1

### Neurokinin B receptor antagonist limits kisspeptin-10 induced LH secretion in women

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Background
The hypothalamic neuropeptides kisspeptin and neurokinin B (NKB) are both obligate for normal gonadotrophin secretion. Studies in patients with loss-of-function mutations in NKB signalling suggest that kisspeptin is functionally upstream of NKB, but this hierarchy is unexplored in healthy men and women. We hypothesised that kisspeptin augmentation of estrogen-induced mid-cycle LH secretion will not be abrogated by pharmacological blockade of NKB.
Methods
Ten healthy women were administered NKBR antagonist AZD4901 (Astra Zeneca) 40 mg po bd (or no treatment controls, $n=9$) from cycle day 4–5 for 6 days; transdermal estradiol (200 μg/day) was applied after 5 days and induced LH secretion 48 h later (controls: 0 h: $4.4\pm0.6$; 24 h: $3.1\pm0.3$; 48 h: $8.6\pm1.0$; 72 h $9.1\pm2.8$ IU/l, $P<0.0001$). At 24 h estradiol treatment, women were randomised to 7 h kisspeptin-10 (4 μg/kg per hr) or saline infusion, returning in a subsequent cycle for the alternate infusion. LH was analysed by *t*-test and ANOVA with Bonferroni multiple comparison *post hoc* analysis.
Results
NKBR antagonist did not affect estrogen-induced LH secretion (48 h: $9.7\pm2.2$; 72 h: $6.5\pm1.2$ IU/l vs estrogen only, ns). During estrogen treatment, kisspeptin-10 stimulated LH secretion, from $3.8\pm0.6$ to $17.3\pm4.3$ IU/l ($P<0.0001$) at the end of infusion; LH remained significantly elevated at 48 and 72 h ($15.3\pm4.0$ and $8.1\pm1.3$ IU/l, both $P<0.01$ vs start of infusion). NKBR antagonist did not affect the immediate response to kisspeptin-10 ($21.6\pm5.6$ vs $17.3\pm4.3$ IU/l, ns). However, NKBR antagonist blunted the duration of the response, with LH being significantly lower at 48 h ($7.5\pm1.5$ vs $15.3\pm4.0$, $P<0.05$).
Conclusions
Kisspeptin-10 advanced estrogen-induced LH secretion. Treatment with the NKB antagonist did not affect the positive feedback response to estrogen, and while the immediate LH response to kisspeptin was maintained, the duration was shortened. These data suggest neurokinin B is predominantly proximal to kisspeptin in the regulation of GnRH, but indicate a complex interaction between these neuropeptides.

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## OC6.2

### Kisspeptin-54 safely and effectively triggers oocyte maturation during IVF treatment in women at high risk of developing ovarian hyperstimulation syndrome (OHSS)

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Context
IVF treatment is an effective therapy for infertility, but can result in the potentially life-threatening complication 'ovarian hyperstimulation syndrome' (OHSS).
Objective
To investigate whether kisspeptin-54 can be used to effectively and safely trigger oocyte maturation in women undergoing IVF treatment at high risk of developing OHSS.
Design
Phase 2 multi-dose open label randomised clinical trial.
Setting
Hammersmith Hospital IVF Unit, London, UK.
Patients
Sixty women at high risk of developing OHSS.
Intervention
Following a standard recombinant FSH/GnRH antagonist protocol, patients were randomized to receive a single injection of kisspeptin-54 to trigger oocyte maturation using an adaptive design for dose allocation (3·2 nmol/kg, $n=5$; 6.4 nmol/kg, $n=20$; 9.6 nmol/kg, $n=15$; 12.8 nmol/kg, $n=20$). Oocytes were retrieved 36 h after kisspeptin-54 administration, assessed for maturation, and fertilized by intra-cytoplasmic sperm injection (ICSI) with subsequent transfer of one or two embryos. Women were routinely screened for the development of OHSS.
Main outcome measure
Oocyte maturation was measured by oocyte yield (percentage of mature oocytes retrieved from follicles ≧14 mm on ultrasound). Secondary outcomes include rates of OHSS and pregnancy.
Results
Oocyte maturation occurred in 95% of women. Highest oocyte yield (121%) was observed following 12.8 nmol/kg kisspeptin-54, which was +69% greater than following 3.2 nmol/kg. At all doses of kisspeptin-54, biochemical pregnancy, clinical pregnancy and live birth rates per transfer ($n=51$) were 63, 53 and 45%, respectively. Highest pregnancy rates were observed following 9.6 nmol/kg kisspeptin-54 (85, 77 and 62%, respectively). No woman developed moderate, severe or critical OHSS.
Conclusion
Kisspeptin-54 is a promising approach to effectively and safely trigger oocyte maturation in women undergoing IVF treatment at high risk of developing OHSS.

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## OC6.3

### Mutations in HS6ST1 are causal in self-limited delayed puberty as well as idiopathic hypogonadotropic hypogonadism

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Background
Self-limited delayed puberty (DP) often segregates in an autosomal dominant pattern, suggesting that inheritance is conferred by a small number of genes. However, the underlying genetic background is mostly unknown. By comparison, many genes have been identified where loss-of-function mutations lead to hypogonadotropic hypogonadism (HH). Despite likely overlap between the pathophysiology of delayed puberty and conditions of GnRH deficiency, few studies have examined the contribution of mutations in HH genes to the phenotype of DP.
Methods
We performed whole exome sequencing in 111 members of 18 families from our patient cohort with DP. We filtered the results, seeking potentially pathogenic mutations, with a list of 25 genes identified in the published literature as causal in HH. After follow-up targeted re-sequencing in a further 42 families (288 individuals), one candidate gene was identified. Developmental tissue expression studies and assessment of the enzymatic function of the mutant protein were carried out.
Results
A rare variant in *HS6ST1* (Heparan sulfate 6-O sulphotransferase 1) was identified, present in six affected members of one family and not present in 145 controls. No other pathogenic variants in HH genes were identified. *HS6ST1* codes for an extracellular matrix component critical for normal neural branching. It is thought to be required for the function of FGFR1 and KAL1 *in vivo*, both of which are vital for GnRH neuronal development and normal hypothalamic–pituitary–gonadal axis function. The novel variant was predicted to lie within a highly conserved coiled-coil domain and displayed reduced sulphotransferase activity *in vitro*.
Conclusions
Mutations in *HS6ST1* contribute to the phenotype of DP. However, although mutations in genes controlling GnRH neuronal migration and differentiation may cause both HH and DP, the overlap in the genetic basis for the two conditions appears from our study to date to be limited to a subset of HH genes.

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## OC6.4

### Calcium-sensing receptor internalisation is impaired in cells expressing FHH3-associated AP2σ mutations

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The calcium-sensing receptor (CaSR), a class C G-protein coupled receptor (GPCR) is critical for calcium homeostasis. The presence of CaSR at the plasma membrane (PM) is regulated by a balance between internalisation via clathrin-mediated endocytosis, and agonist-induced PM insertion from intracellular receptor pools, in a mechanism known as agonist-driven insertional signalling. Recently, mutations of the clathrin-mediated endocytic adaptor protein-2 sigma subunit, AP2σ, have been identified in patients with familial hypocalciuric hypercalcaemia type-3 (FHH3). To date, AP2σ mutations have only been identified at one residue, Arg15, and consist of substitutions to Cys, His or Leu. We investigated the effect of AP2σ mutations on CaSR PM expression using total internal reflection fluorescence microscopy. Imaging was performed in HEK293 cells stably expressing AP2σ-WT or FHH3-associated AP2σ-mutants R15C, R15H or R15L ($n=33$–48 cells), and transiently transfected with a CaSR construct with an N-terminal tag containing: a fluorescent bungarotoxin (BTx-594) binding site to monitor endocytosis; and pH-sensitive superecliptic pHluorin to simultaneously measure total PM CaSR. We observed an increase in net PM abundance in all cells on exposure to $Ca^{2+}$, confirming agonist-induced insertion of CaSR following receptor stimulation. Levels of BTx-594 at the cell surface declined rapidly in AP2σ-WT cells consistent with constitutive internalisation of CaSR. Cells expressing AP2σ-mutants R15H and R15L had significantly longer BTx-594 cell surface labelling, indicative of delayed internalisation of the receptor ($P<0.02$). Furthermore, the time to 25% of CaSR internalisation was longer for all cells harbouring AP2σ mutations than AP2σ-WT expressing cells (393.4±63.4s (WT), 1003.3±102.2s (R15H), 688.6±112.1s (R15L), 670.1±99.3s (R15C), $P<0.02$ in all). CaSR activation also induced a second rapid internalisation step in AP2σ-WT expressing cells, that was absent or severely reduced in cells expressing AP2σ-mutant proteins. Thus, FHH3-associated AP2σ-mutations result in delayed CaSR internalisation, indicating an important role for CaSR endocytosis in regulating CaSR cell surface expression and signalling.

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## OC6.5

### Pituitary tumor transforming gene binding factor (PBF): a novel modulator of iodide uptake and target for Src phosphorylation in breast cancer

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Although the sodium iodide symporter (NIS) is expressed in 70–80% of breast cancers, only 20–30% is located at the plasma membrane (PM) and therefore functional. Previous work in thyroid cells leads demonstrated that PBF redistributes NIS from the PM into intracellular vesicles, potently reducing radioiodide uptake. We therefore examined whether increased membranous NIS could facilitate radioiodide therapy for breast cancer. Immunofluorescent microscopy revealed co-localisation between NIS, phosphorlyated (pY174) and total PBF in MCF-7 cells, with PBF transfection leading to increased intracellular NIS staining. Treatment with the Src family kinase (SFK) inhibitor dasatinib inhibited PBF phosphorylation and led to increased NIS PM staining and decreased co-localisation with PBF. PBF significantly repressed radioiodide uptake in MCF-7 and MDA-MB-231 cells expressing exogenous NIS (25 and 30% reduction respectively, both $n=3$ and $P<0.05$), which was restored by dasatanib treatment (1.75- and 2-fold increase, $n=3$, $P<0.05$). Two PBF mutants were next utilised: i) EEN170-172AAA, lacking the predicted Src consensus sequence, and ii) Y174A, disrupting the endocytosis motif. EEN170-172AAA was unphosphorylated, incapable of reducing radioiodide uptake and showed WT subcellular localisation, whereas Y174A did not reduce iodide uptake and was retained in the PM. These data suggest that phosphorylation of PBF, not localisation, is critical for the interaction between NIS and PBF. To investigate Src phosphorylation of PBF, a mutant form of Src (T341I), resistant to dasatinib, was utilised. In the presence of T341I Src, dasatinib no longer rescued PBF's repression of radioiodide uptake in MCF-7 cells, confirming that Src is the kinase responsible for the phosphorylation of PBF. Taken together, these data suggest that PBF alters the subcellular location of NIS and that Src's phosphorylation of PBF modulates the ability of breast carcinoma cell-lines to uptake radioiodide. This has critical implications for adapting NIS as a potential therapy in breast cancer.

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## OC6.6

### Glucocorticoids induce rapid and persistent chromatin remodelling at a glucocorticoid receptor bound locus in macrophages

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Glucocorticoids (GC) are powerful metabolic hormones with anti-inflammatory effects. Exogenous GC are in wide therapeutic use but often cardio-metabolic side effects are limiting and resistance may develop. GC act via a nuclear hormone receptor (GR), which is a ligand activated transcription factor. GR binds DNA at sites distant from target gene promoters such as enhancers. Macrophages are ubiquitous innate immune cells that are major targets of GC and have roles in normal development and homeostasis. GC also antagonise the major macrophage growth factor, Csf1.

We have previously generated genome wide expression and GR-DNA binding data in primary mouse and human macrophages responding to 100 nM dexamethasone. We selected a locus with a highly conserved GC response and GR binding pattern, *Fkbp5*, for further study in mouse bone marrow derived macrophages. Fkbp5 is a GR co-chaperone that is strongly induced by GC 2 h after simulation. Using DNA fluorescence *in-situ* hybridization at the *Fkbp5* locus there was rapid ($<5$ min) and persistent ($>5$ days) chromatin decondensation after treatment with dexamethasone. By contrast a locus with similar expression kinetics, but no local GR binding, *Tmod1*, decondensed in parallel with its expression response. Decondensation at *Tmod1* was abolished by using α-amanitin to block transcription, but the decondensation at *Fkbp5* was not prevented.

In summary in primary macrophages GC induced a rapid, transcription independent, persistent change to higher order chromatin structure, at a locus involved in feedback control of the GC response in both mice and humans. It is not clear how the chromatin decondensation we measured fits with current models of enhancer activity, which require enhancers to come closer to promoters. Further, long range GR-DNA driven chromatin dynamics may be a novel mechanism involved in transcriptional regulation by GC with potential consequent immunological and metabolic effects.

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# Featured Posters

## FP1

### Inhibition of NFAT signalling *in vivo* improves microvascular endothelial function in a mouse model of chronic diabetes

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Vascular disease is a major cause of morbidity and mortality in people who have diabetes. Hyperglycaemia is associated with increased transcriptional activity of nuclear factor of activated T cells (NFAT) in the vasculature. NFAT activation enhances expression of pro-inflammatory mediators including osteopontin, COX2 and IL6, implicated in the development of vascular disease. This study investigated the therapeutic potential of NFAT inhibition on the progression of microvascular dysfunction in Akita diabetic mice.

Male Akita diabetic mice (C57BL6-*Ins* 2; 8–10 weeks old) were randomly assigned to receive NFAT blocker (A-285222) or saline control (0.29 mg/kg i.p., $n=15$–20/group) for 4 weeks. Laser Doppler imaging and iontophoresis of acetylcholine (ACh) and response to localised heating was used to assess microvascular function *in vivo*. The nitric oxide (NO) synthase inhibitor L-NAME (20 mg/kg i.p.) was used to assess the role of endothelium-derived NO. Blood pressure was assessed non-invasively. Plasma cytokines levels were measured by ELISA.

All animals were chronically hyperglycaemic at baseline (blood glucose $>20$ mmol/l). Following 4 weeks' intervention, inhibitor treated animals showed significantly greater vasodilator responses to ACh and heat (ACh: $P<0.05$ vs Control; Heat $P<0.01$ vs Control). This improvement was abolished by pre-treatment with L-NAME. Treatment with A-285222 significantly reduced plasma levels of IL6, and osteopontin when compared with vehicle treated animals. Blood pressure was significantly lower in inhibitor treated animals (Control: $125\pm4.7$ vs Inhibitor: $83.9\pm7.9$ mm/Hg $P<0.05$) and was negatively correlated with ACh response ($r=-0.629$; $P<0.05$).

Inhibition of NFAT attenuated the deterioration of endothelial function and blood pressure associated with diabetes. These effects were abolished by L-NAME suggesting a role for NO. Furthermore, improvements in endothelial function and blood pressure were accompanied by a marked reduction in circulating cytokine levels suggesting a role of inflammation-induced endothelial dysfunction. Inhibition of NFAT activity may therefore provide a novel therapeutic modality for the treatment of microangiopathy associated with chronic diabetes.

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## FP2

### Corticosterone in human saliva is highly abundant and lacks a diurnal rhythm

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Human plasma contains both cortisol (F) and corticosterone (B). B has been largely neglected in humans as it is 10–20 times less abundant however previous studies suggest B and F have differential tissue-specific actions. Compared with F, B has an enhanced response to ACTH, relatively higher concentrations in brain and CSF and differential transmembrane trafficking by ATP-binding cassette (ABC) transporters. Plasma F is bound to corticosteroid-binding globulin (CBG) and albumin with only 5–10% unbound. Our unpublished data suggest F has greater affinity than B for CBG and plasma B:F ratio is higher in the free than total pool. Salivary glucocorticoid measurement reflects unbound steroid in plasma and we hypothesised that salivary B:F ratio is similarly high compared with the total plasma pool.

With ethical approval, we collected salivary samples from 6 healthy male volunteers 5 times over 24 h (mean $\pm$ S.D. age $32.8\pm11.9$ years, BMI $23.8\pm4.2$ kg/m$^2$). B and F concentrations were measured by ELISA, using antibodies without significant cross-reactivity. Multiple pooled morning and evening samples from 1 volunteer were analysed by LCMS/MS for validation of ELISA results.

In healthy male samples, mean $\pm$ S.D. B on waking was $3.94\pm1.24$ nmol/l and F was $8.17\pm3.67$ nmol/l whereas bedtime concentrations were $3.64\pm1.68$ and $0.63\pm0.23$ nmol/l, respectively. The mean B:F ratio was higher at bedtime than on waking ($0.55\pm0.23$ vs $6.28\pm3.49$, $P=0.01$). LCMS/MS analysis confirmed high morning salivary B with lack of diurnal variation compared with F.

B and F are subject to differential peripheral handling. The B:F ratio is higher in saliva than in plasma, consistent with lower protein binding of B than F in plasma and/or selective steroid trafficking by ABC transporters in salivary glands. Diurnal B variation is absent compared with F, so that B concentrations are higher than F in the evening, consistent with differential adrenal control of B and F.

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## FP3

### Maternal genotype is an important determinant of the outcome of antenatal glucocorticoid treatment in GR$^{+/+}$ and GR$^{+/-}$ foetal mice

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Glucocorticoids are routinely administered to pregnant women at risk of pre-term delivery to mature foetal organs and improve neonatal survival. Previous work in glucocorticoid receptor (GR)-deficient mice showed that GR activation is essential for maturation of the foetal heart. Here, we tested the hypotheses that i) antenatal glucocorticoid exposure, prior to the normal increase in glucocorticoid levels, will advance foetal heart maturation and ii) this would depend on maternal GR genotype.

Female GR$^{+/-}$ and GR$^{+/+}$ mice were crossed with male GR$^{+/-}$ mice to generate GR$^{+/+}$, GR$^{+/-}$ and, from GR$^{+/-}$ intercrosses only, GR$^{-/-}$ (glucocorticoid-resistant controls) littermate foetuses. Dexamethasone (100 μg/kg per day) or vehicle was administered in the drinking water of pregnant dams from E12.5 ($n=3$–6/group). *In utero* high frequency ultrasound was performed at E15.5.

Myocardial performance index (MPI), a measure of combined systolic and diastolic function, did not differ between GR$^{+/-}$ and GR$^{+/+}$ littermates in vehicle-treated GR$^{+/+}$ or GR$^{+/-}$ dams. Dexamethasone treatment of GR$^{+/+}$ dams did not affect MPI in either GR$^{+/-}$ or GR$^{+/+}$ foetuses. However, compared to vehicle, dexamethasone treatment of GR$^{+/-}$ dams decreased MPI (indicating improved cardiac function) in their GR$^{+/-}$ foetuses (mean $\pm$ S.E.M.:vehicle $=0.746\pm0.020$, dex $=0.620\pm0.028$; $P<0.01$, $n=13$–16) whilst having no effect on MPI in their GR$^{+/+}$ foetuses (Mean $\pm$ S.E.M.:vehicle $=0.713\pm0.044$, dex $=0.687\pm0.082$, $n=4$–7). Examination of the influence of maternal genotype showed MPI in GR$^{+/+}$ foetuses was higher in GR$^{+/-}$ dams than GR$^{+/+}$ and was unaffected by dexamethasone treatment. Importantly, whilst MPI was elevated in GR$^{+/-}$ foetuses in GR$^{+/-}$ dams compared to GR$^{+/+}$ dams (Mean $\pm$ S.E.M.:GR$^{+/-}$ dams $=0.769\pm0.027$, GR$^{+/+}$ dams $=0.585\pm0.075$, $n=4$), this was reversed by dexamethasone treatment (two-way ANOVA interaction $P\leq0.01$).

Precocious GR activation therefore improves foetal heart function, but only in GR$^{+/-}$ foetuses from GR$^{+/-}$ dams, suggesting foetal heart maturation is dependent on both foetal and maternal factors. Foetal factors could include reduced GR density in GR$^{+/-}$ mice and maternal factors may include the higher circulating plasma levels of glucocorticoid in GR$^{+/-}$ mice.

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## FP4

### GLP-1 reduces cerebrospinal fluid secretion and intracranial pressure: a novel treatment for idiopathic intracranial hypertension?

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Background

Idiopathic intracranial hypertension (IIH) predominantly affects obese women of childbearing age. IIH is characterised by increased intracranial pressure (ICP) which results in visual loss and disabling headaches. ICP dis-regulation results from imbalance of cerebrospinal fluid (CSF) production rate (at the choroid plexus epithelial cells) and drainage. Treatments for IIH are limited but weight loss is established as disease modifying.

Glucagon like peptide-1 (GLP-1), an incretin with weight modifying properties, has been shown to have a natriuretic effect in the kidney through inhibition of the $Na^+H^+$ exchanger in proximal tubule cells. CSF secretion is controlled by ion channels and pumps akin to an inverted renal proximal tubule. The choroid plexus (CP) expresses GLP-1 receptor (GLP-1R). Therefore, we hypothesise that GLP-1 modulates CSF secretion at the choroid plexus and reduces ICP.

Results

GLP-1 receptor mRNA (QPCR) and protein (WB) were detected in the rat CP. Immunohistochemical analysis of CP explants showed that GLP-1R localised to the cytoplasm and apical surface of the epithelial cells. After Exendin-4 (GLP-1R agonist) treatment GLP-1R immunoreactivity was translocated to the apical cell surface. GLP-1R mRNA and protein levels were also increased. Evaluation of the downstream signalling pathway on primary CP epithelial cells identified a twofold increase in cAMP after Exendin-4 treatment ($P<0.01$). Exendin-4 also significantly reduced the activity of $Na^+K^+$ ATPase, a marker of CSF secretion

(39.3±9.4% of control; $P<0.05$). *In vivo* ICP recording in adult rats ($n=6$) demonstrated that Exendin-4 significantly reduced ICP (58.3±5.0%, $P<0.0001$).
Conclusions
We have identified a novel GLP-1 signalling pathway in the CP. We demonstrate that treatment with a GLP-1 agonist significantly reduces CSF secretion *in vitro* and ICP in rats. GLP-1 therapy may represent a novel therapeutic avenue for conditions of raised ICP such as IIH and may have additional long term advantages of weight reduction.

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## FP5

### Generation of GnRH neurons from human embryonic stem cells and induced pluripotent stem cells of healthy individuals and patients with Kallmann's syndrome

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GnRH neurons are vital for reproductive competence. These neurons originate mainly in the nasal epithelium and migrate to the preoptic region of the hypothalamus during foetal development. Defective migration may result in Hypogonadotropic Hypogonadism (HH), a condition in which puberty is never or only partially achieved.
Little is known about the molecular ontogeny and regulation of GnRH neurons. Their anatomical localisation and small numbers (about 1000) makes experimental studies extremely difficult. Immortalised GnRH-releasing cell lines have provided functional insights but they carry several limitations. Human embryonic stem cells (hESCs) and induced pluripotent stem cells (iPSCs) have been differentiated into many neuronal types but not into mature GnRH neurons. We have created GnRH-expressing neurons from hESCs and iPSCs. iPSCs were generated by reprogramming dermal fibroblasts from Kallmann's Syndrome patients and unaffected family members. iPSCs lines were karyotypically normal and displayed pluripotency features, including self-renewal, expression of pluripotency markers, and ability to differentiate to cells of all three germ layers. GnRH-expressing neurons were produced by generating Neural Progenitor Cells (NPCs) through prolonged BMP inhibition over several passages. NPCs express early neuronal markers such as PAX6 and Nestin and can be passaged and expanded. Terminal differentiation was achieved by incubating the cells in basal neuronal media supplemented with FGF8. After 21 days neurons express mature neuronal markers TUJ1 and MAP2. GnRH expression can be detected by immunocytochemistry and RT-PCR. Mature neurons also express CXCR4 but very little to no KISSR1.
We plan to use this system to investigate basal and stimulated GnRH release. We will also investigate developmental changes over time by extending the culture period. Our model of GnRH neurons derived from hESCs/iPSCs could provide a reliable system for studying molecular mechanisms underlying developmental changes and conditions such as HH.

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## FP6

### The role of 11β-hydroxysteroid dehydrogenase type 2 in the central regulation of blood pressure and salt appetite

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Increased intake of sodium is postulated to be controlled by aldosterone-sensitive cells in a select region of the adult mouse brain, the nucleus of the solitary tract (NTS). These cells express the enzyme 11β-hydroxysteroid dehydrogenase type 2 (HSD2) which inactivates glucocorticoids, allowing selective activation of mineralocorticoid receptors by aldosterone. However in the developing brain, HSD2 is widely expressed to protect against adverse glucocorticoid action, which increases susceptibility to affective and cognitive disorders as adults. To determine the role of developmental versus adult expression of brain HSD2, we investigated the phenotype of mice with lifelong deletion of brain HSD2 (HSD2f/f.nestin-cre) and mice with adult deletion of HSD2 in the NTS using lentiviral delivery (HSD2f/f.v-CRE) compared to their respective controls. The phenotypes (salt appetite, BP, baroreceptor response (BRR) and cognition), can be categorized as either due to glucocorticoid fetal programming effects, or unregulated activation of 11β-HSD2 expressing neurons in the NTS.
Salt appetite increased in both HSD2f/f.nestin-cre and HSD2f/f.v-cre cohorts (percentage increase of 65% $n=8$ and 46% $n=6$, respectively), leading to an increased blood pressure in both groups (percentage increase of 12% and +8%, respectively). Similarly, the BRR is impaired in brain HSD2 KO mice, indicating all phenotypes are mediated by NTS neurons. However, spatial recognition memory (Object-in-Place task) is abolished in HSD2f/f.nestin-cre mice. Whereas, HSD2f/f.v-cre mice have a marginally reduced memory, but still retain short-term memory.
Our data suggests that neural HSD2 protects against inappropriate activation of MR by corticosterone to regulate salt appetite and salt-induced rises in blood pressure in the adult mouse. However, spatial recognition memory is not influenced by deletion of 11β-HSD2 in the adult brain, confirming this phenotype is underpinned by developmental programming by glucocorticoids.

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## FP7

### Regulation of adipogenesis by wnt10b signalling in cultured human adipocytes taken from an obese patient with a WNT10B-C256Y mutation

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Background
Adipogenesis is a key process leading to adipose tissue (AT) expansion. However, managing this event is critical to limit excessive fat accumulation and disease risk. Adipogenesis appears controlled in part, through Wnt10b signalling which reduces adipogenesis via inhibiting CCAAT/enhancer-binding protein alpha (CEBPα) although the molecular mechanism remains unclear.
Aims
Abdominal subcutaneous AT (AbdSc AT) was collected from a, non-diabetic metabolically normal obese, female subject with a naturally occurring WNT10B-C256Y mutation, (rendering a non-functional Wnt10b protein (Age: 19yr, BMI: 62Kg/m$^2$)), to examine the impact of this mutation in Wnt10b signalling during *in vitro* adipogenesis.
Methods
AbdSc adipocytes were cultured from a WNT10B-C256Y subject and compared with control adipocyte cells from lean non-diabetic subjects (Age: 30.7 mean± S.E.M. 4.7 years, BMI: 21.9±1.2 kg/m$^2$; $n=3$). Adipogenesis was assessed over time (0–14 days) by genes regulating adipogenesis and Wnt signalling as well as lipid accumulation, glycerol release and insulin-stimulated glucose uptake.
Results
During adipogenesis the WNT10B-C256Y cells accumulated significantly more lipid and insulin stimulated glucose uptake than control cells ($P<0.05$), whilst glycerol release remained similar. In WNT10B-C256Y adipocytes, mRNA CEBPα expression increased throughout adipogenesis still rising at Day 14, whereas in control cells this peaked at day 6. Whilst axis inhibition protein 2 (AXIN2, a key gene in regulating CEBPα) appeared down-regulated in the WNT10B-C256Y adipocytes in contrast to the control cells ($P<0.01$). WNT10B-C256Y adipocytes significantly raised phospho-β-catenin protein ($P<0.05$) and lowered T cell transcription factor 7 (TCF7) expression ($P<0.05$).
Conclusions
These data highlight that Wnt10b plays an inhibitory role in adipogenesis via a negative feedback loop to reduce CEBPα through AXIN2. Taken together this data indicates that the feedback loop is dysregulated in the subject with the WNT10B-C256Y mutation fuelling her continual fat accumulation, and future risk of metabolic disease.

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## FP8

### Reduced circulating levels of kisspeptin, human chorionic gonadotropin, placental-like growth factor, soluble fms-like tyrosine kinase-1 and soluble endoglin but not prokineticin-1 are associated with miscarriage risk

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Background
Miscarriage is the spontaneous loss of pregnancy occurring prior to 24 weeks of gestation, and can be devastating for affected couples. Abnormal placental development is observed in two-thirds of miscarriages. Recently, the circulating placental markers kisspeptin, prokineticin-1 (PK-1), soluble fms-like tyrosine kinase-1 (sFlt1), soluble endoglin (sEng) and placental growth factor (PlGF) have been identified and investigated for potential associations with miscarriage. However, no previous study has evaluated the predictive value of these markers in identifying pregnancies which later result in miscarriage.
Methods
We conducted a prospective cohort study including over 900 asymptomatic women attending their booking antenatal visit at a single obstetric centre. In each patient, a single measurement of plasma PK-1 and kisspeptin, and serum human chorionic gonadotropin (hCG), sEng, sFlt1 and PlGF was made, and pregnancy outcome was monitored prospectively.
Results
During singleton pregnancies, multiples of median (MoM) kisspeptin, hCG, sFlt-1, PlGF and sEng were 63, 43, 36, 30 and 11% lower in women later experiencing miscarriage when compared with unaffected pregnancies, respectively. MoM PK-1 was not significantly different in the miscarriage group when compared with unaffected pregnancies. Associations of kisspeptin, hCG, sFlt-1 and PlGF with miscarriage remained significant despite adjusting for subject age, gestation, smoking, blood pressure, and body mass index during logistic regression modelling.
Conclusions
Our data suggest that circulating levels of kisspeptin, hCG, sFlt-1 and PlGF but neither sEng nor PK-1 may be independently associated with miscarriage risk in asymptomatic women attending their antenatal booking visit. These data further our understanding of placental function, and have important potential implications for utilising novel hormonal markers to detect adverse clinical outcomes during pregnancy.

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## FP9

**How well can we measure oestradiol?**

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Traditionally, oestradiol measurement is performed by immunoassay. The sensitivity of these assays is not sufficiently sensitive for certain patient groups such as males, post-menopausal females and children. There is also a potential for interference from structurally related compounds or heterophilic antibodies. LC–MS/MS is an attractive alternative as it has increased sensitivity and specificity. We reviewed the requests for LC–MS/MS analysis during the first 6 months of its availability. During this period 20% of samples were sent for specific LC–MS/MS analysis due to suspected immunoassay interference. Of these, three samples also gave raised oestradiol concentrations using LC–MS/MS. One sample with undetectable oestradiol was from a breast cancer patient who had undergone an oopherectomy as the oestradiol concentration was detectable by immunoassay. Because it remained detectable after oopherectomy, the sample was referred for LC–MS/MS analysis which identified interference in the immunoassay. Another sample was on a child who was being considered to have an oestradiol secreting tumour based on immunoassay results. Upon analysis using LC–MS/MS, oestradiol were undetectable, thus preventing any further investigation.
The remaining 80% of samples were from breast cancer patients. There are reports in the literature of treatments for breast cancer causing interference in immunoassays. We performed spiking experiments of common treatments for breast cancer: tamoxifen, anastrozole, exemestane and fulvestrant and measured the samples by immunoassay and LC–MS/MS. Fulvestrant was the only drug found to cause interference in the immunoassays, however the potential for metabolite interference for the other drugs remains.
We have shown several cases where the availability of this LC–MS/MS assay for oestradiol has had a direct impact on the quality of patient care and has proven to fill gaps previously not covered by immunoassay. This assay is available in an accredited NHS laboratory to make it accessible for all clinicians and their patients in the UK.

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## FP10

**A dedicated Turners clinic improves adherence to UK recommended best practice and is well liked by patients**

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Background
Prior to 2014 Portsmouth patients with Turners syndrome (TS) were seen in a variety of endocrine clinics. Screening investigations e.g. cardiac echo, necessitated further appointments. The Turners Syndrome Support Society (TSSS) recommend that ladies should be under the care of a specialist multi-disciplinary TS clinic, equipped to manage the specific medical problems associated with the syndrome.
Innovation
Portsmouth Endocrine Department established a dedicated Turners MDT clinic staffed by Consultant Endocrinologist and nurse specialist. Liaison with cardiology and audiology led to provision of same day echocardiography and audiological tests. Patients attend for the whole morning arriving together. 30 minutes are spent discussing a relevant topic, subsequently patients have their screening tests and consultant review.
Audit
We reviewed the notes of all patients with TS registered on our database. Compliance with recommended monitoring pre dedicated clinic. Compliance with recommended monitoring post clinic inception. Patient satisfaction survey sent to all participants. Repeated non attenders were excluded.
Results
A marked improvement was seen in adherence to recommended monitoring as per the TSSS checklist after establishment of the clinic. Satisfaction survey results were overwhelmingly positive. Women appreciated having all investigations on the same day, and enjoyed meeting and talking to others similarly affected.
Future plans
Our cohort size suggests we are not seeing all affected women in our catchment area. The joint clinic has been advertised to local GPs raising awareness of TS and the recommended screening schedule. Additionally, we have recognised the frequency of abnormal liver function tests in this group, thus a Hepatologist will join the team providing same day fibroscans and review.

| Screening test | Before joint clinic (%) | After joint clinic (%) |
|---|---|---|
| Annual blood tests | 65 | 100 |
| Echocardiography | 65 | 100 |
| Audiology test | 50 | 90 |
| DEXA scan | 35 | 80 |
| Thyroid autoantibodies | 20 | 100 |
| Coeliac antibodies | 35 | 100 |

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## FP11

**Potential molecular mechanism of AIP-mediated cellular invasion**

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Background
Heterozygote germline mutations in the aryl-hydrocarbon receptor interacting protein (AIP) gene play a role in the pathogenesis of pituitary adenoma development in familial isolated pituitary adenoma as well as simplex pituitary adenoma cases. AIP mutation positive patients develop often aggressively growing tumours in early teenage years.
Aims
The aim of this study was to perform comparative gene expression analysis of AIP mutation-positive (AIPpos) pituitary adenomas to discover the genes/pathways responsible for the aggressive clinical phenotype of these tumours.
Methods
Gene expression analysis on normal pituitary, AIP mutation positive, familial AIPneg as well as sporadic somatotrophinomas ($n=25$) using the Affymetrix

Gene-Chip array. Differential expression of selected genes was validated by RT-qPCR and immunohistochemistry. *In vitro* stimulation of epithelial-to-mesenchymal transition (EMT) was performed on stable AIP-knockdown cells using forskolin, as well as TGFbeta1 and assessed using the EMT markers by Western blotting. *In vitro* invasion assay was performed on AIP siRNA-knocked down BxPC3 cells using BioCoat-Matrigel invasion chambers.

Results

One of the top altered pathways in AIPpos adenomas was the 'EMT'. Validation by RT-qPCR and immunohistochemistry showed significant decrease for EMT markers E-cadherin, beta-catenin, PERP, ESRP1 and increase for ZEB1 ($P$ ranging $<0.05$ to $<0.0001$). *In vitro* EMT stimulation lead to induction of EMT as indicated by down-regulation of epithelial marker (E-cadherin, $17\pm0.4$ and $24\pm0.7$, respectively, $P=0.001$) and up-regulation of mesenchymal marker (ZEB1, $3\pm0.28$ and $2\pm0.10$, respectively, $P=0.02$) as well as an increase in actin stress fibres formation. Invasion assay revealed that AIP silencing led to $\sim$45% increase in invasion compared to non-targeting siRNA ($627\pm99$ and $294\pm46$, respectively, $P=0.02$).

Conclusions

This study revealed a unique molecular signature related to increased invasiveness in AIPpos pituitary adenomas and therefore provides a potential molecular mechanism to explain how these tumours acquire more aggressive behaviour.

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## FP12

### Characterising the functional significance of the first reported mutations in the pituitary tumor-transforming gene binding factor

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Pituitary tumor-transforming gene binding factor (PBF) is a ubiquitous glycoprotein which is over-expressed in thyroid, breast and other endocrine cancers, and modulates cellular invasion, radioiodine uptake and thyroid hormone efflux. Papillary thyroid cancer patients with high PBF expression show decreased disease-specific survival compared to those with lower expression. PBF expression has recently been correlated with breast cancer metastasis and colon cancer extra-mural vascular invasion. Previously classified as a proto-oncogene, the first ten substitution-missense mutations of PBF have recently been reported via the COSMIC and TCGA databases, suggesting PBF may in fact be an oncogene. We have therefore examined the biological implications of all 10 mutations in thyroid and breast cells. Substitution mutations generally resulted in clear alterations in the 3D structure of PBF. Western blotting revealed that mutations C51R and G106R inhibited PBF dimerisation and glycosylation in vitro. Anisomycin half life studies in SW1736 thyroid and MCF7 breast cancer cells demonstrated that the C51R mutation resulted in increased protein stability compared to wild type, whereas V55I, W59F, R87C and S103L, G106R, G106V, G106W and R146W mutants were less stable. BrdU proliferation assays revealed that C51R and V55I induced a significant proliferative advantage in thyroid and breast cells compared with WT, whilst R140W significantly repressed proliferation. Mutant C51R was mainly confined to the endoplasmic reticulum while R140W was apparent in the Golgi apparatus. Cell invasion assays demonstrated significantly reduced cell invasion in mutant C51R, accompanied by increased binding to the cortical actin binding protein cortactin. Both C51R and R140W showed decreased cellular migration, but retained the ability to repress radioiodide, a functional hallmark of WT PBF. This is the first description of mutations in PBF, a gene implicated in the initiation and progression of thyroid and other cancers. Taken together our data suggest that mutations in PBF might represent rare novel aetiological events in human cancer.

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# Poster Presentations

## Bone

### P1

**FHH3-associated AP2σ mutations impair MAPK signalling pathways**

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Familial hypocalciuric hypercalcaemia type-3 (FHH3) is caused by loss-of-function mutations of the sigma subunit of adaptor protein-2 (AP2), a ubiquitously expressed heterotetrameric protein with a fundamental role in endocytosis of transmembrane proteins. FHH3-associated AP2σ mutations impair internalisation of calcium-sensing receptor (CaSR) giving rise to FHH. CaSR predominantly signals via $G\alpha_{q/11}$ leading to intracellular calcium release, and activation of mitogen-activated protein kinase (MAPK) pathways. We have previously shown AP2σ mutations impair intracellular calcium release and therefore hypothesised that MAPK signalling may also be altered. To determine the effect of AP2σ mutations on MAPK signalling we undertook two approaches: examination of immediate signalling events by measuring phosphorylated ERK1/2 (pERK1/2) using AlphaScreen assays; and examination of late signalling events using a serum-response element (*SRE*) luciferase reporter in HEK293 cells stably expressing AP2σ-WT or AP2σ-mutant (R15C, R15H or R15L) proteins and transiently expressing CaSR. Exposure to increasing (0–10 mM) extracellular calcium concentrations ($[Ca^{2+}]_e$) led to increased AlphaScreen pERK1/2 responses in a dose-dependent manner; however, AP2σ-mutant cells had significantly reduced responses at higher $[Ca^{2+}]_e$ compared to AP2σ-WT ($P<0.05$ $n=4$). Furthermore, activation of the CaSR with 5mM extracellular calcium induced increased *SRE* reporter expression in all cell-lines; however, reporter activity in AP2σ-mutant cells was significantly reduced compared to AP2σ-WT ($P<0.001$, $n=4$). Finally, we investigated the effect on ERK1/2 signalling in lymphoblastoid cell-lines derived from leukocytes of FHH3 patients with AP2σ-R15C mutations, alongside control cells from unaffected family members. Western blot analyses of lymphoblastoid cells exposed to 5mM extracellular calcium demonstrated an increase in pERK1/2, but responses were significantly reduced in AP2σ-R15C expressing cells ($P<0.05$). Furthermore, AlphaScreen analyses confirmed that AP2σ-R15C lymphoblastoid cells had reduced pERK1/2 responses compared to AP2σ-WT cells ($P<0.001$, $n=4$). In conclusion, our studies demonstrate FHH3-associated AP2σ mutations impair MAPK signalling which may have implications for cell proliferation and survival.

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### P2

**Exploring the *N*-ethyl-*N*-nitrosourea mutagenesis DNA archive for mutations in nuclear factor I/X to derive mouse models for Marshall-Smith syndrome**

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Marshall-Smith syndrome (MSS) is a congenital disorder affecting skeletal and neural development due to mutations in the nuclear factor I/X (*NFIX*) gene. Of these mutations, 61% are small insertions/deletions, 12% are splice site mutations and 27% are large exonic deletions clustered in exons 6–10 of the *NFIX* gene. In order to derive a MSS mouse model, the *N*-ethyl-*N*-nitrosourea (ENU) mutagenesis DNA archive was screened for mutations in *NFIX*. Three point mutations were identified. The first mutation caused a T to A transversion 8 nucleotides prior to exon 5 (IVS4-8t>a). The second mutation caused a T to G transversion in exon 4 (Trp214Gly). The third mutation caused a G to T transversion in exon 8 (Ala356Ser). The three mutations were characterised using *in vitro* minigene and expression assays to investigate whether the IVS4-8t>a mutation affected splicing and whether the Trp214Gly and Ala356Ser mutations affected NFIX cellular localisation and function respectively. RT-PCR using RNA from the *NFIX* minigene assay showed that the IVS4-8t>a mutation did not affect splicing. *In vitro* expression assays using NFIX cDNA with the Trp214Gly and Ala356Ser mutations showed that these mutations did not affect NFIX cellular localisation. As the mutations are found within the C-terminal transactivation/repression domain they might affect the expression of downstream target genes. Western blot analysis showed an increase in NFIX protein level in the Trp214Gly mutant and a reduction in NFIX protein level in the Ala356Ser mutant. Reporter assays under the control of NFIX binding sites showed that only the Ala356Ser mutation reduced NFIX transactivation activity at the glial fibrillary acidic protein (*GFAP*) locus while repression activity at the Bobby sox (*BBX*) locus was unaffected, consistent with *in vivo* data from MSS patients cell lines. ENU induced Ala356Ser mutant mice may therefore provide a representative model for MSS.

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### P3

**Reduction in daily hydrocortisone dose in adrenal insufficiency improves significantly bone mineral density – results from a 2-years prospective trial**

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Introduction
Patients with primary adrenal insufficiency (PAI) and patients with congenital adrenal hyperplasia (CAH) receive life-long glucocorticoid (GC) replacement therapy. Today daily GC doses are still higher than the reported adrenal cortisol production rate, and are not able to reproduce the physiological secretion pattern. This might result in long-term morbidities such as osteoporosis. Until now no prospective trial was performed investigating the long-term effect of GC dose changes in PAI and CAH patients.
Subjects and methods
Prospective, longitudinal study including 57 patients with PAI (42 women) and 33 patients with CAH (21 women) over $28.7\pm5.6$ months. Bone mineral density (BMD) was measured by DXA scan. Patients were divided into three groups depending on changes in daily hydrocortisone (HC) equivalent dose (group 1: unchanged $25.2\pm8.2$ mg ($n=50$); group 2: increased $18.7\pm10.3$ to $25.9\pm12.0$ mg ($n=13$); group 3: decreased $30.8\pm8.5$ to $21.4\pm7.2$ mg ($n=27$)).
Results
Patients of group 1 showed unchanged normal *Z*-scores of lumbar and femoral areas. Patients of group 2 showed a significant decrease in *Z*-scores of femoral neck and Ward's triangle ($-0.15\pm1.1$ to $-0.37\pm1.0$ ($P<0.05$); $-0.45\pm1.1$ to $-0.71\pm1.0$ ($P<0.05$)), whereas patients of group 3 showed a significant increase in *Z*-scores at lumbar and femoral sites (L1–L2: $-0.96\pm1.1$ to $-0.76\pm1.2$ ($P<0.05$); L1–L4: $-0.93\pm1.2$ to $-0.65\pm1.5$ ($P<0.05$); total hip: $-0.40\pm1.0$ to $-0.28\pm1.0$ ($P<0.05$)). No changes in BMI over time were seen within the groups. No changes in osteocalcin was documented during the study, however beta-crosslabs increased significantly in group 2 over the study period ($P<0.05$).
Conclusions
For the first time we were able to show that reduction in HC equivalent dose is resulting in an increase in BMD, whereas a dose increase results in a worsening of BMD. This data emphasises to aim at the lowest possible GC replacement dose in AI patients to avoid long-term side effects.

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### P4

**Mutations in G-protein subunit $\alpha_q$ (GNAQ) are not a cause of familial hypocalciuric hypercalcaemia**

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Familial hypocalciuric hypercalcaemia (FHH) is an autosomal dominant disorder characterised by hypercalcaemia and inappropriately low renal calcium excretion. FHH can be classified into three types: FHH1, caused by calcium-sensing receptor (CaSR) loss-of-function mutations, accounting for >65% of cases; FHH2, due to loss-of-function mutations of the G-protein $\alpha_{11}$ subunit ($G\alpha_{11}$); and FHH3, resulting from loss-of-function mutations in the adaptor protein-2 sigma subunit (AP2σ), encoded by AP2S1, identified in 25% cases. The genetic cause in the remaining FHH patients is unknown. Activated CaSR signals predominantly via the $G\alpha_{q/11}$ family of G-proteins leading to activation of mitogen-activated protein kinase, and intracellular calcium release. $G\alpha_q$ and $G\alpha_{11}$ are highly related, have identical tissue expression, and considerable functional overlap. Somatic

mutations have been identified in both genes in uveal melanoma, and a parathyroid-specific mouse double knockout of *Gnaq/Gna11* phenocopies mice deleted for one allele of CaSR, indicating that the protein family is important in CaSR signalling. We therefore hypothesised that *GNAQ* mutations may be a cause of FHH. We examined for mutations within the seven exons, exon-intron boundaries and untranslated regions of *GNAQ* by sequence analysis of leukocyte DNA, isolated from twenty FHH patients who did not have CaSR, *GNA11* and *AP2S1* mutations. No mutations were identified in the coding or non-coding regions of GNAQ. Binomial probability analysis, using the assumption that mutations in *GNAQ* would occur at a prevalence of 20% in FHH patients negative for mutations in CaSR, *GNA11* and AP2S1, indicated that the likelihood of detecting at least one *GNAQ* mutation was >99% in this sample size of twenty patients. Therefore, we conclude that it is unlikely that loss-of-function mutations in *GNAQ* contribute to FHH pathogenesis, and that $G\alpha_{11}$ may have a more critical role in calcaemic tissues than the closely related $G\alpha_q$.

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## P5

**Soy protein with isoflavones reduce bone turnover markers in women during their early menopause – a randomised double blind parallel study**

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Importance
The major factor contributing to the high incidence of osteoporosis in older women is the reduction in estrogen synthesis. Isoflavones have a similar structure to 17-β estradiol and can act as selective estrogen receptor modulators.
Objective
To determine the safety and effect of soy isoflavones on bone during early menopause.
Design
Parallel, double blind, study.
Setting
Secondary care research institution at United Kingdom.
Participants
200 women within 2 years after the onset of their menopause.
Intervention
Participants were randomised to either 30 g soy protein with 66 mg isoflavone (SPI) or 30 g soy protein alone that was isoflavone free (SP), daily for 6 months.
Outcome measures
The primary outcome of this study was assessing any change in plasma bone turnover markers (βCTX and PINP). The secondary outcome for this study was assessing any change in cardiovascular risk markers and thyroid function.
Results
The area under the curve (AUC) was significantly smaller for plasma β*CTX* (bone resorption marker) with SPI compared to SP (0.25 vs 0.36 μg/l; *P* value <0.01). There was no significant difference in AUC of P1NP (46.8 vs 45.4; *P* value = 0.64). However, there was a significant reduction of P1NP concentrations (bone formation marker) between 3 and 6 months with SPI. The AUC for fasting glucose, fasting insulin and hsCRP were significantly lower with SPI compared to SP.
Conclusions
This study suggests that soy may confer a beneficial effect on bone health with a significant decrease in bone turnover markers seen for both resorption and formation after supplementation with 30 g soy protein and isoflavones that was not seen for soy protein alone. The initial reduction of osteoclast function, followed by attenuation of coupled osteoblast function through bone remodelling cycle is analogous to the mode of action of anti-resorptive agents used in post-menopausal osteoporosis. There was also an improvement of cardiovascular risk markers.

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## P6

**Increased circulating sclerostin levels in type 2 diabetic rats are not associated with changes in bone sclerostin production**

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Type 2 Diabetes Mellitus (T2DM) is associated with decreased bone quality and a higher prevalence of fractures. Sclerostin is an inhibitor of bone formation produced by osteocytes and its expression is elevated in serum of diabetic patients. We examined the effect of T2DM on bone architecture and sclerostin levels in a rat model of T2DM and the influence of hyperglycaemia on sclerostin production by bone cells *in vitro*. Bone architecture was measured by microCT in 14 weeks-old Zucker diabetic fatty (ZDF) and control Zucker lean male rats ($n=6$/group). Sclerostin expression was quantified in serum at 9, 11 and 13 weeks using ELISA and in rat femurs using qPCR. The number of osteocytic empty lacunae was measured on tibia sections of ZDF and control rats. Osteoblast-like UMR106 cells were cultured with increasing concentrations of glucose and sclerostin mRNA expression and protein release determined by qPCR and ELISA, respectively. Our results showed that ZDF rats have lower trabecular bone mass and mineral density compared to controls, due to decreases in bone volume and thickness. They also exhibit impaired bone connectivity and cortical bone geometry. Serum sclerostin levels were higher in ZDF compared to lean rats at 9 weeks (+40%, $P<0.01$), but this difference disappeared after 11 weeks. Bone sclerostin mRNA levels were similar in ZDF and lean rats. The number of osteocytic empty lacunae in bones of ZDF and lean rats was comparable. Glucose dose-dependently stimulated sclerostin mRNA levels and protein release in UMR106 cells. Altogether, our data indicate that sclerostin production is increased by hyperglycemia *in vitro* and in serum of ZDF rats at onset of diabetes, but bone sclerostin levels and the number of apoptotic osteocytes are not elevated in diabetic rats. Further studies are required to determine whether sclerostin contributes to the deleterious effect of T2DM on bone.

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## P7

**Differential effects of parathyroid hormone on key regulators of osteoblast mineralisation**

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Intermittent PTH therapy is currently the only anabolic therapy for osteoporosis. As the mineralisation of the extracellular matrix of bone is essential for normal function it is vital that the effects of PTH on key regulators of mineralisation are uncovered. Ablation of Alpl, Phospho1 or Smpd3 results in skeletal hypomineralisation and as such this study examined the effects of bovine (b)PTH 1–34 on their expression. MC3T3 (clone-14) osteoblast-like cells display temporal increases in Phospho1, Alpl and Smpd3 expression (150, 60 and 60-fold respectively by day 10; $P<0.001$). At day 10, Phospho1 mRNA was significantly reduced after a 15 min exposure to bPTH (50 nM; 80% decrease; $P<0.001$) Further reductions were evident after 1 and 6 h bPTH exposures (96 and 93% decrease respectively; $P<0.001$) and persisted to 24 h (48% decrease; $P<0.05$). Smpd3 expression was similarly reduced after 6 and 24 h exposures (97 and 91% respectively; $P<0.001$). PHOSPHO1 and SMPD3 protein was markedly reduced after 24 and 48 h bPTH treatment. In contrast, Alpl mRNA levels increased after 1 (2.8-fold; $P<0.05$) and 6 h (3.6-fold; $P<0.001$) bPTH exposure which was consistent with increased TNAP protein after 24 and 48 h bPTH treatment. Phospho1, Smpd3 and Alpl showed dose dependent (0.05–50 nM) responses to 24 h bPTH treatment. Indeed, a 50% reduction ($P<0.001$) in Phospho1 and Smpd3 expression was achieved with 0.5 nM bPTH with comparable changes at the protein level. Induction of Alpl mRNA and protein was achieved with 5 nM bPTH. The cAMP activator forskolin induced a suppression of Phospho1 comparable to the effects of bPTH (93 and 96% respectively). Forskolin stimulated Alpl expression (2.4-fold; $P<0.05$). The suppression of Phospho1 by bPTH was partially obstructed by the PKA-inhibitor, PKI 5–24 (45% reduction compared to 75% observed in bPTH only cultures). In summary, bPTH shows

potent effects on PHOSPHO1, SMPD3 and TNAP during osteoblast-mineralisation with initial studies implicating the cAMP/PKA signalling pathway.

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## P8

**Type 2 diabetes, bone mineral density and disc height**

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Background
Although it is well established that subjects with type 2 diabetes (T2D) have an increased fracture risk, there have been conflicting reports on the relationship of T2D to bone mineral density (BMD). Such discrepancies could be due to failure to adjust for potential confounding factors which could influence BMD.
Objectives
To assess the relationship between type 2 diabetes and BMD at the femoral neck and spine in diabetic and non-diabetic subjects, after adjusting for multiple covariates which are known, or suspected to, affect BMD. Intervertebral disc height was also investigated in view of its possible relation to fracture risk.
Methods
A hundred patients with type 2 diabetes of at least 5 years duration (mean age 63 years) and 86 non-diabetic subjects (mean age 59 years) were recruited. A cross-sectional study was carried out whereby BMD T-scores and disc heights between the twelfth thoracic (T12) and the third lumbar (L3) were compared between the two study groups.
Results
There was a higher spine BMD T-score on monovariate analysis (mean $\pm$ S.D. $0.08 \pm 1.2$ vs $-0.29 \pm 1.24$; $P = 0.049$ respectively) in diabetic subjects. However, there were no significant differences in T scores in either the spine or femoral neck after adjustment for potential confounding variables between T2D subjects and controls. Diabetic patients had a statistically lower intervertebral disc height between the 2nd and third lumbar vertebrae when compared to controls both on monovariate analysis as well as after adjustment for potential confounders (mean adjusted difference of 0.028 cm, $P = 0.02$).
Conclusion
We found that diabetes exerts no significant independent effect on BMD. However, there was significantly lower disc height in patients with T2D. This may contribute to the increased vertebral fracture risk in subjects with T2D. Further studies need to be carried out in order to try and further assess the relationship between BMD, disc height and T2DM.

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## P9

**The decreased plasma levels of sclerostin but not Dickkopf-1 are associated with increased risk of osteoporotic fracture and lower bone mineral density in Korean postmenopausal women**

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Background
Although sclerostin (SOST) and Dickkopf-related protein 1 (DKK1) are major regulators in bone metabolism, the associations between these soluble Wnt antagonists and osteoporotic fracture (OF) in Asians, who may have distinct biologic characteristics with Caucasians, are inconclusive. Furthermore, there have been no clinical studies separately considering non-vertebral and vertebral fractures in terms of the blood levels of SOST and DKK1.
Methods
This is a case–control study in postmenopausal Korean women. Among 513 consecutive subjects not taking any drug or having any disease that could affect bone metabolism, 105 cases having any kind of OF (i.e., non-vertebral and/or vertebral fractures) and age- and BMI-matched 105 controls were enrolled. Non-vertebral (i.e., wrist, forearm, humerus, hip, and pelvis) and morphological vertebral fractures were identified by an interviewer-assisted questionnaire and lateral thoracolumbar radiographs, respectively. Bone mineral density (BMD) and plasma levels of SOST and DKK1 were also measured.
Results
Plasma SOST was markedly lower in subjects with OF than their controls. Each S.D. decrement of plasma SOST concentration associated with a multivariable-adjusted odds ratio of 1.75 for any kind of prevalent OF. The odds for OF was 2.79-fold higher in subjects in the lowest SOST tertile compared with those in the highest SOST tertile. Importantly, all these associations were still significant when the non-vertebral and vertebral fractures were analyzed separately. However, prevalent OF did not associate with plasma DKK1 levels regardless of the fracture type and the adjustment model that was employed. Consistently, plasma SOST levels, but not DKK1, related positively with BMD values at all measured skeletal sites.
Conclusions
Circulating SOST but not DKK1 could be a potential biomarker for predicting bone health in Asian populations.

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## P10

**A mutation in the calcium sensing receptor (previously known to cause neonatal severe hyperparathyroidism in the homozygote state) causing familial benign hypocalciuric hypercalcaemia in the heterozygote**

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Familial Benign Hypocalciuric Hypercalcaemia (FBHH) is a benign autosomal dominant condition characterised by elevated serum calcium and parathyroid hormone (PTH) and low urine calcium. It is a genetically heterogeneous disorder but the majority of cases (type 1 FBHH) can be shown to be due inactivating mutations in the Calcium Sensing Receptor (CASR). This is a guanine nucleotide-binding-protein (G-protein) coupled receptor that signals through the G-protein subunit $\alpha 11$ (G $\alpha 11$). Patients who are homozygotes for inactivating CASR mutations present with Neonatal Severe Hyperparathyroidism (NHPT) which, in contrast to FBHH, is characterised by severe hypercalcaemia and early death. Three sub types of FBHH are recognized. Type 1 is the most common and is due to inactivating mutations in the CASR. Type 2 is due to mutations effecting G$\alpha 11$ which result in loss of function, while type 3 is due to adaptor-related protein complex 2, sigma 1 subunit (AP2S1) mutations which result in altered calcium-sensing receptor endocytocis. In FBHH, mutations result in the CaSR being less sensitive to serum calcium so that the 'set point' for serum calcium is reset at a higher value, leading to hypercalcaemia and increased PTH secretion. Although FBHH is a benign condition it can be confused with primary hyperparathyroidism because the two are similar in terms of biochemistry. It is important therefore to confirm the diagnosis of FBHH in patients with hypercalcemia and raised PTH in order to avoid unnecessary parathyroidectomy.
We report a kindred with FBHH type1 due to a CASR mutation that changes Cysteine to Tyrosine at amino acid 582 in the CASR. This mutation is listed in the 'Professional' version of the Human Genome Mutation Database as causing NHPT in the homozygote but there are no instances listed of this mutation being causative for FBHH type 1 in the heterozygote.

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## P11

**The effect of oestradiol circadian rhythm on the bone mineral density of adult males**

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Background
Circulating levels of total oestradiol ($E_2$) decrease with age in adult males; the effect of altered $E_2$ circadian rhythm is uncertain. We hypothesised that age-related changes in the circadian rhythm contribute to decreased BMD in older males and investigated this.

Methods
Nineteen subjects were studied: six young-healthy (YH) males (mean age (years) 27.3 ± 4.6) with normal BMD, eight older-healthy (OH) males (mean age (years) 70.5 ± 2.1) with normal BMD, and five older males with osteoporosis (OO) (mean age (years) 75 ± 2.9). Groups were matched for body weight and BMI; subjects excluded had histories of bisphosphonate, corticosteroid, calcium, and/or vitamin D use and skeletal disorders. Volunteers were hospitalised with blood samples obtained every 30 min over a 24-h period. Levels of total $E_2$ and bioavailable $E_2$ were calculated. Circadian rhythm was analysed.
Results
Total 24-h mean bioavailable $E_2$ was less in the OH cohort compared to the YH group (16.7 ± 2.2 pmol/l vs 26.3 ± 3.6 pmol/l; $P < 0.0001$); 24-h mean total $E_2$ concentrations reflected this pattern ($P < 0.0001$). The OO group had significantly less 24-h mean bioavailable $E_2$ than the OH cohort (12.5 ± 1.8 pmol/l vs 16.7 ± 2.2 pmol/l; $P < 0.0001$). IGF1 levels were significantly greater in the OH compared to the OO cohorts (group mean MESOR 112 ± 24 μg/l vs 72 ± 13 μg/l). Total oestrogen demonstrated a concerted circadian rhythm in all three groups, but bioavailable oestrogen did not demonstrate circadian rhythmicity in older men with decreased BMD.
Conclusion
Twenty-four hours mean bioavailable $E_2$ and IGF1 levels are significantly lower in older men with osteoporosis compared to healthy counterparts. Osteoporotic subjects demonstrated a disrupted circadian rhythm with respect to bioavailable oestrogen. Our study demonstrates the role of circadian $E_2$ rhythms in the maintenance of BMD.

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## P12

**Familial hypocalciuric hypercalcaemia due to *AP2S1* mutation in a patient with failed parathyroidectomies: a case report**

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Familial hypocalciuric hypercalcaemia (FHH) is a rare condition and can be mistaken for primary hyperparathyroidism (PHPT). Distinguishing this from the later is vital to avoid un-necessary surgery as this is a benign condition. Ca:Cr excretion ratio > 0.01 in a spot urine is widely used to rule out FHH. However this was calculated from 24 h urine samples on the original studies.
We present a case of 46-year-old lady who presented with symptomatic hypercalcaemia, initially diagnosed as PHPT. Peak C.Ca was 2.78 with low normal $PO_4$, and normal PTH, 5.7 pmol/l. USG and SestaMIBI were suggestive of possible left inferior parathyroid adenoma. Cortical bone mineral density was normal on DEXA. Initial urine Ca:Cr excretion ratio was 0.04. Following surgery she had persistent hypercalcaemia (C.Ca 2.93 mmol/l) and histology showed normal parathyroid tissue. Repeat SestaMIBI was negative and MRI neck showed a possible left parathyroid adenoma. Second surgery also failed, histology confirmed normal parathyroid tissue. However patient improved symptomatically after second surgery in spite of persistent hypercalcaemia. A 24 h urine calcium was low at 1.58 and subsequent genetic analysis confirmed FHH type 3 with mutation in *AP2S1* gene (c.43C > T_p.Arg15Cys). Most cases of FHH are due to mutation in calcium-sensing receptor (CaSR) gene mutation (> 65%) and in the rest, *AP2S1* mutation accounts for more than 20%. Disease causing mutation in this gene was first described in two unrelated kindreds by Nebit *et al*. (1), presented in BES 2013. It is not uncommon for these patients to have undergone parathyroid surgery in the literature. With lack of data on true prevalence of FHH, it is likely that some of these patients are misdiagnosed as PHPT. Low 24 h urine calcium, normal cortical density, and hypermagnaesemia are useful clues in addition to positive family history to make a correct diagnosis.
Reference
1. Nesbit MA, Hannan FM, Howles SA, *et al*. Mutations in *AP2S1* cause familial hypocalciuric hypercalcemia type 3. *Nat Genet* 2013 **45** 93–97.

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## P13

**Pre-operative localisation of parathyroid adenomas in patients with primary hyperparathyroidism: can a single modality of imaging be adequate?**

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Aim
Pre-operative localisation of primary hyperparathyroidism (PHPT) has been based on ultrasound (US) of neck and MIBI scans with an expectation that solitary parathyroid adenomas (PA) would be localised with reasonable accuracy and sensitivity, to enable mini-parathyroidectomy. The aim of our audit was to assess the utility of both these imaging in patients with parathyroid adenoma.
Method
Retrospective analysis was performed on all patients who had surgery for PHPT. A list of patients who had PA proven on post-operative histology was collated. Pre-op localisation modalities on these patients were analysed.
Results
*Baseline*: Data on 220 surgeries were analysed. Mean age 58 years (18–84); 77% were females. Post-op histology showed: Parathyroid adenoma 155 (70.5%), hyperplasia 10 (4.5%), diffuse hyperplasia 20 (9.1%), parathyroid carcinoma 3 (1.4%), normal 17 (7.7%), and others 12(6.8%). US was done in 164 (75%), MIBI in (81%), and both 149 (68%).
*PA overall*: Of the 168/220 who had a PA localized on US or MIBI, 130 of these were confirmed accurate after histology (77.4%); 20 had hyperplasia (11.9%); nine were normal (5.4%); six were others (3.6%); and parathyroid cancer 3 (1.7%). Sensitivity, specificity, and positive predictive value (PPV) to identify adenoma for US was 80, 36, and 78% and for MIBI 83, 40, and 80% respectively.
*US and MIBI preformed*: 149 patients had both US + MIBI (112 of these were PA on histology). 78/112 (70%) were concordantly identified. US identified a further 14 (12.5%) and MIBI a further 13 (11.6%). So the sensitivity of US or MIBI on their own was 82%. The sensitivity and PPV of US and/or MIBI localising an PA were 94 and 78% respectively. On 16/149 (11%) patients both US + MIBI showed false localisation when histology was not consistent with PA (five were normal).
*US and MIBI negative*: 14/149 (9.4%) had both scans negative: Histology showed: PA 7(50%), normal 4 (29%), and hyperplasia in three.
Conclusion
i) Sensitivity of US or MIBI is around 80%; combining the two scans enhances the chance of localisation. ii) Pre-op localisation may not be possible in 5% of patients despite the pathology being an adenoma. iii) False localisation can be an issue in about 4% of patients, even allowing for hyperplasia as an accepted confounder.

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## P14

**A rare presentation of primary hyperparathyroidism**

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A 25-year-old woman with hypertension was referred with 12 months history of watery right eye and slowly growing swelling in the lateral wall of the right orbit. On examination there was proptosis with superior and medial displacement of the right globe. CT scan of facial bones revealed a 3.5 cm swelling with internal cortical scalloping and calcification arising from the zygoma within the right lateral orbital wall. Further lucent lesions were identified in the frontal bone, pterygoid process, alveolar process, and mandible. Detailed fundal examination revealed choroidal folds that were suggestive of significant indentation of her right globe by the lytic bone lesion. She underwent orbital decompressive surgery. Histology of the curetting was consistent with brown tumour. Subsequent investigations showed serum calcium 3.24 (RR 2.10–2.60) mmol/l, parathyroid hormone 62.3 (RR 1.6–6.9) pmol/l, and 25-hydroxyvitamin D 27 nmol/l with normal renal function. She underwent parathyroidectomy for primary hyperparathyroidism (PHPT) and a plum sized left lower gland was removed. Other glands were unremarkable. Histology showed parathyroid adenoma.
Brown tumours represent the late stage of bone remodelling in hyperparathyroidism. Whilst any part of the skeleton can be affected, the mandible is the most commonly affected facial bone. Multifocal maxillofacial brown tumours especially of the zygoma of the orbit are very rare, certainly as an initial presentation of PHPT. Differentiating brown tumours from other giant cell tumours such as fibrous dysplasia and reparative granulomas is difficult histologically and radiologically. A definitive biochemical diagnosis of PHPT alongside the radiological findings and bone histology confirmed brown tumours

due to PHPT. Following successful parathyroidectomy, calcium and PTH were normal and the bony swelling resolved over 18 months.

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## P15

**Lessons learnt after 'failed' parathyroidectomy**

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Aims

To define and identify patients who have 'failed' parathyroidectomy by a single surgeon. To explore common features of these cases to refine our pathways.

Methods

This case note review began with a retrospective audit of 123 patients having neck exploration from January 2009 to May 2014. The dataset of clinicopathological information obtained from the electronic Trust records, was interrogated to identify patients fulfilling the following: Operations resulting in normal or no tissue removed; operations in which parathyroid adenoma was identified but with calcium on day 1 then close to 6 months remaining $>2.6$ mmol/l. Statistical differences between 'successful' and 'failed' distributions were examined with a Mann–Whitney *U* test and themes were explored using case notes.

Results

Only 13 of the 123 patients demonstrated normal histology and despite significantly higher post-operative calcium in this group, only seven remained hypercalcaemic at 6 months. In the complete cohort, day 1 calcium in those with positive histology was slow to correct in 15 patients, but only two patients had lasting hypercalcaemia. The biochemical diagnosis of primary hyperparathyroidism was secure in six out of the seven patients with normal histology, with one other altering course post-operatively. Although they didn't all have urine CCCR, there was no statistical difference in this or pre-operative PTH for this group. Although only two were symptomatic of hypercalcaemia this was not unusual for the whole dataset, and all fulfilled at least one indication for parathyroidectomy as per the 2008 Third International Workshop. Most striking although not statistically significant was that four of this group didn't have any positive imaging.

Conclusions

Post-operatively the diagnoses of the 'failed' parathyroidectomy patients were reviewed to be sound. Management revolved around a sestamibi scan, which when showing new findings led to repeat surgery. Those without repeat positive imaging are still a clinical dilemma.

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## P16

**Melorrheostosis: a rare cause of bone pain and limb deformity**

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We report a case of a rare genetic condition of disordered bone turnover requiring complex multidisciplinary management.

A 42-year-old lady presents with a 2-year history of worsening pain in both her legs and feet. She has a long standing history of structural equinus deformities worse on the right ankle and permanently walking on her toes. She also has fixed flexion deformities at both her knees. She normally is not able to weight bear and uses crutches on both sides to mobilise. These problems had started at the age of 7 and have progressively gotten worse. She had recently migrated to the UK and she was managed by her GP with simple analgesia. Her serum bone profile was essentially normal. Imaging of her affected limbs showed extensive radiological appearances in her lower limbs that are classical of melorrheostosis, the linear hyperostosis and 'dripping candle wax' appearance of her long bones. Bone scintigraphy scan confirmed the disease being confined to both her lower limbs. Treatment trial with bisphosphonates did not help in alleviating her symptoms of pain. There were no surgical options in terms of improving her symptoms and a conservative approach was adopted with involvement of physiotherapy team. Melorrheostosis is a rare disease with an incidence of $<1$ in a million. It is believed to be as a result of a disordered bone formation with hyperossification of bones as well as surrounding soft tissue including muscle, tendons, and joints resulting in progressive limb deformities. The exact aetiology has not been completely delineated however *LEMD3* gene mutation has been implicated. Melorrheostosis is a progressive condition resulting in significant decline in function and chronic pain.

Currently there is no known effective treatment and management involves symptom control with the aim of maintaining as much function as possible in the affected limbs.

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## P17

**Denosumab improves bone density in a female patient with severe anorexia nervosa**

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Case report

A 29-year-old female with a 17-year history of severe enduring anorexia nervosa attended our unit. Osteoporosis was diagnosed aged 24 and she had developed a left calcaneal fracture after minimal trauma 3 weeks prior to presentation. Her bone mineral density at this time confirmed the presence of osteoporosis at the lumbar spine and total hip (*T*-score $-3.3$ and $-2.9$ respectively) and her body mass index was low at 15.1 kg/m$^2$. She declined therapy previously with oestrogen and bisphosphonate therapy and was not keen to undertake daily injections. A decision was made to commence therapy with Denosumab 60 mg by s.c. injection every 6 months with monitoring of serum calcium and co-administration of calcium and vitamin D. A further measurement of bone mineral density was made 2 months after completing 3 years of therapy with Denosumab. During the period of treatment the patient did not experience any adverse effects related to the treatment. There was no evidence of hypocalcaemia nor were there further fractures. Bone mineral density increased substantially at the lumbar spine The measurement at the left femoral neck showed a reduction of $-5.7\%$ from its pre-treatment value (Table 1).

**Table 1**

| Scan date | Age | BMD (g/cm$^2$) | *T*-score | BMD change | |
|---|---|---|---|---|---|
| | | | | Vs baseline | Vs previous |
| Lumbar spine: | | | | | |
| L1–L4 | | | | | |
| 2015 | 32 | 0.789 | −2.3 | 4.0% | 14.8% |
| 2012 | 29 | 0.687 | −3.3 | −9.4% | −8.3% |
| 2011 | 28 | 0.750 | −2.7 | −1.1% | −1.1% |
| Left total hip | | | | | |
| 2015 | | 0.610 | −2.7 | −2.9% | 1.4% |
| 2012 | | 0.601 | −2.8 | −4.2% | −3.9% |
| 2011 | | 0.626 | −2.6 | −0.3% | −0.3% |

Conclusion

Denosumab is a potentially efficacious treatment for osteoporosis in anorexia nervosa.

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## P18

**Improved glycaemia following parathyroidectomy for primary hyperparathyroidism**

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Primary hyperparathyroidism (PHPT) is not uncommon. It has varied presentations ranging from asymptomatic disease to the classical 'stones, groans, and moans'. The incidence and prevalence of frank diabetes mellitus (DM) is significantly increased in patients with hypercalcaemia. It may be difficult to differentiate symptoms of hypercalcaemia from DM. We present a case of improved glycaemia in a patient with DM following removal of parathyroid adenoma.

A 45-year-old female within a week of diagnosis with T2DM was referred to us for being symptomatic with extreme lethargy, polyuria, polydipsia, muscle aches and pains, iron deficiency anaemia, and dysphagia. She had coexisting uncontrolled hypertension, PCOS, obesity, osteoarthritis, depression past history of DVT, and bladder tumor removal. She was vitamin D deficient with a level $<10$ nmol/l. Biochemistry was suggestive of PHPT with raised corrected calcium

of 3.04 mmol/l and an elevated PTH of 10.7 pmol/l. Glycaemic control was poor with a HbA1c of 74 mmol/mol. She had low haemoglobin of 11.4 gm% and a low MCV 75.7. Ultrasound of parathyroids showed a large 3.25×2.1 cm hypoechoic soft tissue mass inferior to lower pole of left lobe of thyroid suggestive of parathyroid adenoma. She refused to undergo upper GI endoscopy. Hypercalcaemia was managed with i.v. fluids and pamidronate infusion. Attempt was made to improve her glycaemic control on Metformin, Gliclazide, and Novomix 30 insulin. It was rather difficult to engage her with our services. 17 months into the diagnosis of PHPT, she finally agreed to have parathyroidectomy. Histology revealed a left lower lobe parathyroid adenoma. One and 6 months post-operatively, HbA1c improved to 58 and 51 mmol/mol. Her insulin needs dropped considerably.

This case highlights that primary hyperparathyroidism might contribute to hyperglycaemia in patients with DM. Parathyroidectomy may result in improvement in glycaemia and may reduce the need for use of anti-diabetic drugs or their dosage. Review of hypoglycaemic s is warranted post-parathyroidectomy.

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## Clinical biochemistry

### P19

**A comparison of plasma copeptin and AVP responses during saline infusion studies**

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Introduction

Copeptin is the C-terminal fragment of proAVP and secreted in equimolar amounts with AVP. While AVP is unstable *in vitro* and has proved difficult to measure in clinical practice, copeptin is relatively stable and can be measured using an automated immunoassay. Therefore copeptin measurement offers potential as a more practical alternative to the direct measurement of AVP in the investigation of polyuria/polydipsia.

Methods

AVP, copeptin, and plasma osmolality were measured in parallel using plasma samples from 15 patients undergoing a hypertonic saline stress test. AVP was measured using in-house radioimmunoassay and copeptin using the Brahms Kryptor immunoassay. AVP and copeptin values were correlated with plasma osmolality using an in-house normogram allowing comparison of AVP response to a reference population. AVP and copeptin responses to hypertonic stress were compared.

Results

In 12 cases the AVP response was considered normal. In three cases the AVP response was considered sub-normal, with AVP failing to demonstrate sufficient response to an osmotic stimulus, consistent with cranial diabetes insipidus. In all 15 cases the response of copeptin was equivalent to that of AVP. In all 12 cases with a normal AVP response, the peak copeptin was at least 12.7 pmol/l (range 12.7–73.4) and copeptin concentration was at least 4.6 pmol/l in samples with osmolality $\geq$300 mOsm/l. In the three cases with a sub-normal AVP response consistent with diabetes insipidus, two were associated with very poor copeptin response (maximum copeptin $\leq$2.6 pmol/l). In the remaining case both AVP and copeptin responses were below the expected normal range.

Conclusions

The results of this small study suggest that copeptin provides equivalent information to AVP when measured during hypertonic saline infusion tests. Further study is required to determine the diagnostic performance of copeptin measurement during hypertonic saline infusion in the investigation of polyuria/polydipsia.

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### P20

**Use of ADH antagonists results in lower hospital resource usage: a retrospective cohort study**

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This retrospective cohort study examined Hospital Episode Statistics (HES) and Hospital Pharmacy Audit (HPA) data from 41 hospital trusts to understand how hyponatraemia secondary to syndrome of inappropriate antidiuretic hormone secretion (SIADH) is currently treated in England, and to understand the associated resource use. Hyponatraemia is the electrolyte disturbance that is most commonly encountered in clinical practice (affecting 10–30% of hospitalised patients), with SIADH a common cause; responsible for about 30% of patients with hyponatraemia. After application of inclusion/exclusion criteria, 3,060 patients had a robust diagnosis of SIADH. Of these patients, 927 were treated with demeclocycline, 120 were treated with tolvaptan and 2013 had no pharmacological treatment for SIADH recorded, which may mean they had fluid restriction, saline administration or received no treatment. Treatment with tolvaptan was associated with a shorter length of hospital stay, lower inpatient costs and fewer subsequent A&E attendances than treatment with demeclocycline. Demeclocycline treatment was associated with fewer outpatient attendances than tolvaptan treatment. Patients who had no recorded pharmacological treatment had lower inpatient costs, but more outpatient appointments and A&E attendances than those given either demeclocycline or tolvaptan. This study showed a significant discrepancy between reported incidence of hyponatraemia/SIADH and the number of patients given appropriate clinical codes for these conditions. A code for hyponatraemia was present in 59 661 out of 3 508 638 patients (1.7%), and 8.9% of these had a code for SIADH. This level of under-reporting has potentially serious implications for hospital remuneration.

This study shows that hyponatraemia secondary to SIADH represents a substantial and under-reported healthcare burden in England. Management of SIADH is variable, but treatment with tolvaptan could potentially result in lower resource usage for hospitals and the wider health economy than treatment with demeclocycline.

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### P21

**Genetic hypocalcaemia: a case of 22q deletion syndrome**

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Introduction

A 22q11.2 deletion syndrome (velocardiofacial syndrome) is an autosomal dominant disorder affects various organs including the parathyroid gland. Because of its incomplete penetrance, multi-system affectation, and variable clinical presentation, the diagnosis is often delayed or never made. Delayed diagnosis may have significant impact on morbidity and mortality. We present a patient with a long history of clinical features of a syndrome which was diagnosed after a delay of many years.

Case

A 46-year-old man presented to the Emergency Department following a seizure. He had a low serum calcium level of 1.94 mmol/l. After treatment he was lost to follow-up and re-presented four years later with a further seizure. His serum calcium level was 1.98 mmol/l. He was prescribed calcium-vitamin D preparation and referred to the Endocrinology team. History taking elicited occasional seizures in childhood with no formal diagnosis of epilepsy. He had undergone a cleft palate repair as a child and had mild learning difficulties. On examination he had low-set ears and a scar indicating previous cleft palate repair. Further investigations revealed an inappropriately low serum parathyroid hormone level of 1.4 pmol/l with normal vitamin D levels in the presence of hypocalcaemia. His clinical presentation led us to suspect a genetic mutation so he was referred to the medical geneticist for further evaluation. Micro-array studies confirmed a micro-deletion on the long arm of chromosome 22. Subsequent echocardiogram demonstrated a dilated aortic root await cardiac MRI. An audiometry revealed sensory neuronal deafness.

Conclusion

Hypocalcaemia in the young especially with phenotypic dimorphism should be addressed with increased diligence. We should have a low threshold for referral to medical genetics as they will require multidisciplinary team input and regular follow-up once a genetic diagnosis is established.

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## P22

### Effect of cortisol assay bias on the overnight dexamethasone suppression test: implications for the investigation of Cushing's syndrome

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Background
NEQAS data demonstrate a divergence in bias of cortisol immunoassays over the last 10 years. Despite this, a serum cortisol of 50 nmol/l has been universally applied as the cut-off for the overnight dexamethasone suppression test (ONDST), the commonest screening test for Cushing's syndrome.
Aims
To assess the effect of assay bias on interpretation of the ONDST and determine the necessity for a method-specific cut-off.
Methods
120 serum samples sent for cortisol analysis as part of an ONDST were collected prior to disposal. Samples were anonymised and aliquots prepared for cortisol analysis by four different immunoassays (Abbott Architect, Roche E170, Beckman Access, and Siemens Centaur), and both cortisol and dexamethasone analysis by LC–MS/MS. Precision at three different cortisol concentrations (~30, 75, and 100 nmol/l), using sample pools prepared from patients not on interfering steroids, demonstrated inter-assay CV of <10% for all cortisol assays at concentrations above the lower reporting limit (LRL) of the assay. Case notes were reviewed to ascertain clinical indications (49% adrenal incidentalomas) and final diagnosis (13% confirmed Cushing's).
Results
Cortisol concentrations for each patient sample above the LRL for each assay were compared to the corresponding LC–MS/MS cortisol. Mean biases were −19.5 nmol/l (Architect), 15.2 nmol/l (E170), 0.3 nmol/l (Access), and −3.9 nmol/l (Centaur). Dexamethasone was detected in 111 samples, with optimal concentrations (>5.6 nmol/l) in 88 samples; samples without dexamethasone ($n=6$) were removed from further analysis. Using the 50 nmol/l cortisol cut-off, 29/108 samples were screen positive by all methods, 60/108 screen negative, and 19/108 samples were discrepant, of which one subsequently had confirmed Cushing's syndrome. Sensitivities and specificities at a 50 nmol/l cut-off varied from 87.5 to 92.5% for the Abbott, to 93.8 and 91.2%, respectively, for LC–MS/MS.
Conclusions
Assay bias affects performance of the ONDST and dexamethasone measurements are required for accurate interpretation.

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## P23

### Using SDHB immunostaining in characterising pheochromocytoma and paraganglioma

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Germline mutations account for hereditary phaeochromocytoma (PCC) and paraganglioma (PGL) syndromes. SDHB immunostaining can be used to functionally characterise SDH status on PCC and PGL tumours. Genetic testing of multiple candidate genes is increasingly performed in patients presenting with PCC/PGL tumours. We investigated the effectiveness of SDHB immunostaining as an initial screening tool in identifying *SDH* mutations.
This was a retrospective analysis of 23 randomly selected patients with PCC (10) and PGL (13); all benign lesions who were referred for genetic testing. Age (41 years, mean, range 15–69), 12 M and 12 F. Ten patients had PCC of which six were left sided and one bilateral. Abdominal and head and neck PGLs were present in seven patients each and thoracic PGLs in two. SDHB immunostaining was performed on all tumour samples using Sigma prestige antibodies (HPA 002868 100 U/l – anti-SDHB rabbit MAB) at a dilution of 1:1000. Staining was done using Leica BOND-III IHC stainer at ERZ (high pH) for 30 min. In PCC, 70% of the tumours were sporadic and RET, VHL, and SDHC–variants of unknown significance (VUS) contributed the rest (10% each). In the PGL group, 15% were sporadic, *SDH* mutations accounted for 38% and the rest, VUS. Immunostaining was found to be negative in the tumour sample of all patients with *SDHB* mutations and one SDHD, weakly positive with diffuse cytoplasmic blush in two SDHD and strongly positive in all sporadic, *RET*, *VHL*, and *VUS* mutations.
Our pilot data from a large cohort (~150 patients) indicate that SDHB immunostaining can reliably predict SDH mutational status and adds value in characterising patients with VUS.

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## P24

### Serum cortisol: what is your laboratory measuring?

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Background
Accurate measurement of serum cortisol is essential in the investigation of the HPA axis. It has been documented that routine immunoassays are liable to both under- and over-recovery leading to inaccurate results and subsequent inappropriate investigations. This study seeks to provide an up-to-date assessment of the accuracy of the major immunoassay platforms and compares results to a liquid chromatography–tandem mass spectrometry (LC–MS/MS) candidate reference measurement procedure (cRMP).
Design
Serum remaining from routine analysis was aliquoted and distributed to four different centres for cortisol analysis by their respective immunoassay. In addition, aliquots were analysed by a routine LC–MS/MS assay and a LC–MS/MS cRMP to provide metrologically traceable results for comparison. Cohort groups included: males ($n=42$), postmenopausal females ($n=44$), pregnant patients ($n=68$), patients taking prednisolone ($n=42$), and patients taking the 11β-hydroxylase inhibitor, metyrapone ($n=27$). Cortisol-binding globulin (CBG) was measured in the postmenopausal female and pregnant cohorts.
Results
Considerable bias was observed across the male (−15 to +15%) and postmenopausal female (−13 to +19%) cohorts. In both the prednisolone and metyrapone samples, all immunoassays over-recovered cortisol (prednisolone patients: 142–707 nmol/l and metyrapone patients: 93–118 nmol/l). The pregnancy cohort displayed a bias ranging from −45 to +8%. Higher concentrations of CBG were observed during pregnancy than in the post menopausal state. The routine LC–MS/MS method showed no significant bias relative to the cRMP.
Conclusions
Accurate quantitation of cortisol by current immunoassays is compromised by the non-specificity of the antibody for cortisol and matrix effects. Users should be aware of the limitations of their current assay and consider these when interpreting results. This is especially pertinent in patients taking metyrapone where dosage may be titrated against cortisol concentration.

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## P25

### Vasopressin-2 receptor antagonists: potent but potentially dangerous drugs for the treatment of severe hyponatraemia secondary to syndrome of inappropriate antidiuretic hormone secretion

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Introduction
Vasopression-2 receptor antagonists (VPAs) have been licensed for the treatment of hyponatraemia secondary to syndrome of inappropriate antidiuretic hormone secretion (SIADH). As usage extends to other causes of hyponatraemia, over-rapid correction and hypernatraemia remains as important side-effect. We present a patient with severe SIADH highlighting the need for guidance and vigilance when using these potent drugs.
Case
A 82-year-old lady was admitted for a total thyroidectomy for papillary carcinoma in December 2014 and commenced on tri-iodothyronine while

awaiting radioiodine ablation. She developed severe hyponatraemia, drowsiness and confusion. On review, she had mild hyponatraemia prior to admission and developed severe hyponatraemia secondary to SIADH post-operatively. She was well hydrated, did not have excessive intravenous fluids and was not on any medication that could cause hyponatraemia or SIADH. Despite seven days treatment with fluid restriction and oral Demeclocycline 300 mg three times a day her serum sodium remained below 110 mmol/l. We then gave her a single dose of Tolvaptan 15 mg and checked her sodium levels every 6 h for 48 h and maintained fluid intake 1.5–2 l/day. Despite the rapid rise in her serum sodium levels (17 mmol/l in 24 h) her neurological symptoms improved and her mental test scores were normal.

Investigations

The patient's biochemical test results are shown in the Table 1. A computerised tomography of her head, chest, abdomen, and pelvis did not demonstrate any pathology. Table 2 shows the response to a single 15 mg dose of Tolvaptan.

**Table 1**

| Biochemical test | Results | Reference range |
|---|---|---|
| Serum sodium | 107 | 133–146 mmol/l |
| Serum potassium | 3.2 | 3.5–5.3 mmol/l |
| Serum urea | 3.5 | 2.5–7.8 mmol/l |
| Serum creatinine | 39 | 50–120 μmol/l |
| TSH | 13.3 | 0.3–4.2 mU/l |
| Free $T_3$ | 2.3 | 3.1–6.8 pmol/l |
| Serum osmolaity | 259 | 275–295 mOsm/kg |
| Urine osmolaity | 559 | 300–1000 mOsm/kg |
| Urine sodium | 32 | – |
| Early morning cortisol | 1113 | – |

**Table 2**

| **Time** (h) | **Serum sodium** (mmol/l) |
|---|---|
| 0 | 108 |
| Tolvaptan 15 mg given | |
| 6 | 111 |
| 12 | 117 |
| 18 | 122 |
| 24 | 125 |
| 30 | 126 |
| 36 | 127 |
| 42 | 128 |
| 48 | 128 |

Conclusion

VPAs are potent drugs available for the treatment of hyponatraemia secondary to SIADH. Patients must be well hydrated (not on fluid restriction) and closely monitored to prevent over-rapid correction and hypernatraemia. We recommend 6–8 h serum sodium monitoring for the first 24–48 h after a single dose.

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## P26

### Lactic dehydrogenase, a biochemical marker to predict foetal outcome in pregnancies complicated by intrauterine growth restriction

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Background

The intrauterine foetal environment is crucial for its survival and long term health. Intrauterine growth retardation of the foetus is a pregnancy specific disorder which involves the restriction in the physical and or mental growth of the foetus during the course of the pregnancy. Lactate dehydrogenase is an intracellular enzyme that converts lactic acid to pyruvic acid. It has been proposed as an important marker for liver diseases, myocardial, renal, and pulmonary infarction. Very few studies have been carried out to establish its significance in various disorders of pregnancy and placentation. Intrauterine growth retardation with its complications carry a high maternal morbidity and foetal morbidity and mortality. Predictors for the prognosis of the disease are desperately needed for the effective management. Total serum lactic acid dehydrogenase activity has been found to be elevated in severe pre-eclampsia. Studies have showed increased levels of foetal lactate dehydrogenase in IUGR, but there are very few studies in which maternal serum levels are studied.

Objectives

i) To analyse lactic acid dehydrogenase as a predictor of IUGR and ii) to study the diagnostic efficiency of LDH levels in prediction foetal outcome in IUGR infants.

Methods

This prospective case control study was done between a period from July 2007 to December 2014 at QMH KGMU, and Department of M&RH, SGPGIMS, Lucknow. 490 women attending the antenatal clinics were recruited between 32 and 36 weeks for the analysis for serum lactic dehydrogenase levels in triplicate. Maternal and foetal outcome was noted. The statistical analysis included ANOVA, $\chi^2$ test, and Student's *t*-test. ROC curve was used to attain the sensitivity and specificity of the test. Keeping CI at 95%, significance was determined if $P$ value was $<0.005$.

Results

Out of the patients recruited, 226 cases and 180 controls could be followed for outcome, rest of them defaulted for various reasons. The mean serum LDH in mothers was 296.334 IU/ml in the control arm whereas the mean was 456.36 IU/ml in the study group ($F$ test $=0.000002$; $P=0.0024$). At the cut off of 315 IU/m, the sensitivity was 72.5% and specificity was 60.5%.

Conclusions

Maternal serum LDH is a good prognostic marker to predict for maternal and foetal outcome. It can be used in regular risk scoring systems for methodological analysis of the prognosis of outcome at delivery.

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## P27

### Turn off the taps

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A 85-year-old man presented with a 2-week history of malaise, confusion and agitation and 4 weeks of polydipsia and polyuria, with water intake of 6 l/day. His past medical history included hypertension, congestive cardiac failure, and chronic kidney disease. On examination he was hypervolaemic. Initial investigations were as follows: serum sodium 115 mmol/l, potassium 4.6 mmol/l, urea 8.1 mmol/l (2.5–7.8), creatinine 138 μmol/l (66–112), serum osmolality 245 mOsmol/kg (275–295), paired urine osmolality 169 mOsmol/kg (80–1200), and sodium 21 mmol/l. Fasting glucose, calcium, thyroid function, and 0900 h cortisol were all normal, excluding other causes of polydipsia and hyponatraemia. A presumptive diagnosis of primary polydipsia and dilutional hyponatraemia was made; the patient was unable to tolerate a formal water deprivation test. He was managed with fluid restriction. On this regime his serum sodium levels normalised but the patient remained incessantly thirsty and distressed. Liaison psychiatry assessment revealed a strong preoccupation with thirst, in the absence of obsessive thoughts, psychotic, mood, or anxiety disorder. He demonstrated severe cognitive impairment (blind Montreal Cognitive Assessment 4/22 and Frontal Assessment Battery 4/18). A collateral history suggested a long history of cognitive decline, and a shorter history of compulsion to drink consistently associated with worsening confusion. Mirtazapine was commenced to ease the compulsion and provide night sedation and we utilised sponges and ice cubes to alleviate thirst, with good effect. The patient's sodium on discharge was 135 mmol/l. Our case is an unusual case of primary polydipsia in the absence of mental illness that was successfully managed with the above behavioural and pharmacological measures. Although primary polydipsia is common in patients with psychosis, there is little documentation of cases in the absence of psychopathology. Patients with dementia frequently present with reduced water intake but rarely with excess thirst.

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## P28

**Where are the endocrinologists?**

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Introduction
Hyponatraemia is defined as serum sodium concentration < 135 mmol/l. It is the most common electrolyte disorder encountered in clinical practise. It is associated with an increase in mortality and length of stay, independent of diagnosis and clinical variables. Despite this it is often inadequately investigated and poorly managed. As a number of endocrine conditions can cause hyponatraemia, endocrinologists often have the necessary clinical skills and expertise to manage these patients.
Methods
A retrospective audit was performed of patients admitted to The Great Western Hospital (GWH) serum sodium of 127 mmol/l or less on admission, over a 3-month period. The aims were to evaluate how hyponatraemia is investigated and whether specialist input from the endocrinology team improves the management of these patients.
Results
Seventy-five patients were included in the audit; 27 males (36%) and 48 females (64%). The mean age was 75 years (range 37–94 years). All patients had a serum sodium of 127 mmol/l or less on admission: mean 122 mmol/l (range 108–127 mmol/l). Only 65% of patients had hyponatraemia documented as either a diagnosis or problem. Only 28% of patients had their fluid status documented. Eight investigations were identified as essential when investigating patients with hyponatraemia. Only 4% of patients had all eight investigations completed during admission. Less than 27% of patients had a urine sodium, urine osmolality and serum osmolality requested during admission. Only seven patients (9%) admitted with hyponatraemia were either referred to the endocrinology team or were reviewed by the endocrinology team during admission. These patients had an average of 6.9 out of eight of the essential investigations, whereas those patients not reviewed by endocrinology had an average of 3.4 out of the eight.
Discussion
This audit confirms that hyponatraemia is often not recognised, inadequately investigated and poorly managed. Results suggest that an endocrine opinion is rarely requested and that patients who are reviewed by a specialist are more likely to have appropriate investigations requested, thereby increasing the chance of a being correctly diagnosed and managed.

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## P29

**The epidemiology of hyperprolactinaemia**

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The epidemiology of hyperprolactinaemia is not well characterised in the literature. Using unique patient identifier we were able to link data from biochemistry, prescribing, hospital admissions, radiology, general registry office and maternity data. Observational data was collected for Tayside Scotland between 1993 and 2013. Any patient with a serum prolactin measurement > 1000 mU/l or at least three prescriptions for a dopamine agonist were included. Patients who were pregnant at the time of assay were excluded, unless they had raised prolactin at other times out-with pregnancy. Patients were then categorised into four groups. Patients with a serum prolactin > 5000 mU/l or use of dopamine agonists were classed as having a probable pituitary tumour (group 1). Patients who had a record of being prescribed antipsychotics, tricyclics, SSRIs, dopamine antagonists, opioids, H2 antagonists, verapamil, and methyldopa 6 months before or one after the raised prolactin who did not fit into group 1 were classed as drug induced hyper-prolactinaemia (group 2). Patients who had macroprolactin identified without any other explanation, or who had high concentrations of macroprolactin were classed as having macroprolactin (group 3). The remainder were unclassified (group 4).
Overall 32 289 patients had a serum prolactin assay undertaken, of which 1366 were > 1000 mU/l, of which 65 were during pregnancy. 334 patients were identified as having a pituitary tumour, 415 were drug related, and 174 were due to macroprolactin, thus leaving 378 idiopathic cases. The average prevalence of hyper-prolactinaemia was 107/100 000 of the population, and the incidence rate over the last 20 years was 14 cases/100 000 person-years. Drug induced hyper-prolactinaemia was the commonest cause of raised serum prolactin.

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## P30

**Audit of management of patients with hypomagnesaemia in district general hospital**

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Background
Hypomagnesaemia is one of the most commonly encountered electrolyte disorders in clinical practice. The reported prevalence of hypomagnesaemia varies from 2.5 to 15% in the general population to as high as 65% among patients admitted to intensive care units. There is a general lack of awareness among physicians regarding the prevalence, clinical significance, and management of hypomagnesaemia.
Aim and method
To review current practice of the management of hypomagnesaemia in an inpatient population by conducting retrospective observational study. Fifty-eight patients with moderate to severe hypomagnesaemia were identified, Data was collected from clinical and computer records.
Results
The mean age was 68 years, 18 patients (31%) were males, mean magnesium level on admission was 0.28 mmol/l, 43 patients (74%) had hypocalcaemia with mean calcium level of 1.89 mmol/l. Neuromuscular signs and symptoms were observed in 22 patients (38%). Thirty-seven patients (64%) had two or more risk factors, the PPI was the most common precipitant factor.53 patients (91%) received IV magnesium. PTH, vitamin D, and ECG were checked only in 24, 33, and 59% respectively. Just above 50% have normal magnesium and calcium level on discharge, 46 patients (79%) have modifiable risk factors which have been addressed only in 23 cases (40%). Follow up arranged only for 8 patients (14%). Only 18 patients (31%) have GP notification .13 patients (22%) were readmitted with hypomagnesaemia. Mortality rate was 15% with variable causes of death.
Conclusion and recommendation
In practice, documentation and interpretation of clinical and biochemical findings were poor, due to a combination of the complex, multifactorial aetiology encountered frequently in high risk group, and the lack of a clinically applicable diagnostic algorithm to define the exact cause. An easy to use protocol, to appropriately investigate and manage hypomagnesaemia will need to be introduced and awareness of its existence should be highlighted. This would help in optimising patient's care.

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## P31

**Single-centre audit of the diagnostic performance of plasma metanephrines with seated sampling for the diagnosis of phaeochromocytoma/paraganglioma**

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Introduction
Measurement of plasma metanephrines (PMETS) is widely regarded as one of the best screening tests for phaeochromocytoma/paraganglioma (P/PGL). Current Endocrine Society guidelines recommend that samples for PMETS are ideally collected in the supine position after 30 min rest and interpreted using supine reference ranges, in order to optimise the diagnostic performance of the test. Current practice in our centre is to collect samples for PMETS from seated patients.
Aim
To determine if seated sampling for PMETS provides acceptable diagnostic performance in our centre.
Methods
Clinical and laboratory data of 113 patients were reviewed, gathered over a 4-year period 2010–2014. All had undergone preoperative PMETS measurement (LC-MS/MS) and all had post-operative pathology confirmation or exclusion of P/PGL.
Results
Of the113 patients included in the study, 40 had a histological diagnosis of P/PGL. Of these 40, three were considered pre and peri-operatively to be

non-secretory. The remaining 73 patients had an alternative adrenal pathology. The diagnostic sensitivity of PMETS (either normetanephrine or metanephrine) above the upper limit of our in-house seated reference range was 93%. However, excluding three cases of PGL determined clinically and biochemically to be non-secretory raised the sensitivity to 100%. Diagnostic specificity was 91%. Applying published supine reference ranges made no difference to diagnostic sensitivity in this group of patients, but decreased diagnostic specificity to 77%.
Conclusions
While these data are derived from a relatively small study population, they demonstrate acceptable diagnostic performance for seated PMETS as a screening test for P/PGL. The data highlight a high diagnostic sensitivity for PMETS with seated sampling in our centre.

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## P32

**An unusual presentation of osmotic demyelination syndrome**

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Introduction
Osmotic demyelination syndrome (ODS) or commonly known as central pontine myelinolysis is commonly associated with rapid correction of hyponatraemia resulting in neurological deficits that manifests within days. The population commonly affected include alcohol dependence patients, the malnourished and liver failure patients. We describe a case of ODS developing in an alcoholic patient with symptoms developing 4 weeks after correcting hyponatraemia.
Case
A 44 year old gentleman with heavy alcohol use of up to 40 units a week presents with 1 week history of progressive left hemiplegia, dysarthria and dysphagia. Initial investigations showed he had normal serum biochemistry including serum sodium. Initially thought clinically to be stroke, MRI was done given the atypical history, which showed pontine and extrapontine myelinolysis. On further review it was noted he was admitted 4 weeks ago following alcohol intoxication during which he was hyponatraemic (125 mmol/l) and was treated with 0.9%sodium chloride with rapid correction of serum sodium to 133 mmol/l. He however did not develop any neurological symptoms at the time. Management involved multidisciplinary care with alcohol dependence, dietitian and neuro-rehabilitation team input and after a 6 month of rehabilitation he made a full recovery and returned to normal independent level of function.
Discussion
ODS is a non-inflammatory demyelination disorder in high neuron density areas of the brain resulting in the neurological sequale. This occurs as a result of rapid correction of a chronic osmolar abnormality with rapid fluid shift and osmotic stress related nerve damage. High risk patients include those that do not have adequate compensatory mechanisms for rapid osmotic changes. The neurological consequences of ODS can be significant including permanent neurological damage and even death. Management is aimed at prevention with slow correction in serum sodium of ~8 mmol/l every 24 h especially in high risk groups which should be done proactively.

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## P33

**Management of inpatient hypokalaemia: a District General Hospital (DGH) experience**

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Background
Hypokalaemia (potassium below 3.5 mmol/l) is a common electrolyte abnormality associated with cardiac instability and myopathies. Untreated hypokalaemia can lead to inpatient morbidity and mortality.[1]
Aim
To review the management of hypokalaemia, in terms of potassium replacement therapy, potassium-level monitoring and cardiac monitoring, in Maidstone hospital.
Methods
A cross-sectional study of inpatients with hypokalaemia over 3 weeks. Clinical notes were used to compare management to trust guidelines.
Results
Of the 51 patients (female: male ratio 2:1, mean age 71 years) identified, 63% received no potassium replacement and only 26% were managed according to trust guidelines. The majority (74%) of mild hypokalaemia (potassium 3.0–3.4 mmol/l, $n=39$) was not actively corrected, whilst 10% of moderate hypokalaemia (potassium 2.5–2.9 mmol/l, $n=10$) and 50% of severe hypokalaemia (potassium below 2.5 mmol/l, $n=2$) were also untreated. Potassium replacement therapy was poorly standardised; 40% of initial replacement for mild hypokalaemia was i.v. instead of oral, and replacement regimes for moderate-severe hypokalaemia were variable. Daily potassium-level monitoring until normokalaemia occurred in 66% of patients. During intravenous potassium therapy, 46% had repeat potassium levels after every 40 mmol administered. All cases of severe hypokalaemia had magnesium levels checked. Few patients (17%) with moderate-severe hypokalaemia received a repeat ECG. No patients had cardiac monitoring during intravenous potassium administration.
Conclusion and discussion
This audit demonstrates inadequate hypokalaemia management in our DGH – a malpractice we believe is shared across other DGHs. Lack of education and consensus on hypokalaemia management amongst doctors was a main contributing factor to the poor practice. This highlights the need for society-led guidelines on the management of inpatient hypokalaemia at a national level.
Reference
1. Alfonzo et al. Potassium disorders-clinical spectrum and emergency treatment. Resuscitation 2006 70 10–25

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## P34

**Alkaline phosphatase may predict tumour volume in patients with parathyroid adenoma**

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Background
There is much debate about the best imaging modality for parathyroid adenoma. Parathyroid ultrasound is operator dependent and in skilled hands leads to localisation of tumour. Small adenomas can be difficult to detect and radiologists are helped by clinical and biochemical data to predict ease of adenoma detection. We investigated whether any factors could predict adenoma size.
Methodology
This was a retrospective CWS analysis of patients who had undergone parathyroidectomy between 2010 and 2013. Data collected included serum calcium, PTH, ALP, vitamin D, tumour volume on scan, tumour volume on histology. Linear regression analysis was performed and reported as odds ratio (=OR, 95% CI).
Results
There was a very close correlation between tumour volume on scan and histology (Pearson correlation 0.803, $P<0.001$). ALP and PTH was associated with tumour volume, ALP being more closely associated (0.016 (0.007, 0.025) and 0.019 (0.003, 0.034) respectively). There was no association between pre-op hypercalcaemia and tumour volume (OR 1.7(−.094, 4.36)). Vitamin D was checked in <50% of patients. Regression analysis with three blood tests (ALP, Ca, PTH), showed that ALP was the only independent predictor of tumour size (OR=0.012 (0.003, 0.022)).
Conclusion and discussion
There seems to be a correlation between adenoma size and histological volume suggesting excellent radiological expertise. The correlation between ALP and tumour volume may suggest that the presence of metabolic bone disease is a marker of adenoma size. PTH was also linked to tumour volume, indicating that this might also be a predictor of adenoma size.

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## P35

**Biochemical evaluation of adrenal incidentalomas referred to endocrine surgery in a large teaching hospital**

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Background
Adrenal incidentaloma (AI) increasingly pose a diagnostic challenge. This retrospective observational study evaluated biochemical investigations performed

in patients referred to Endocrine Surgery with AI and assessed adherence to Guidelines. Biochemical, histological and radiological characteristics of AI were also reviewed.
Methods
Data were collected from Hospital and Laboratory records for referrals between January 2012–April 2014.
Results
125 patients were referred (21 excluded; 19 'bulky adrenals' and two metastases). 104 patients were included, 70 were female, median age 63 years (range 17–87). Review of biochemical investigations showed 90 and 96% of patients had ACTH and cortisol measured following an overnight dexamethasone suppression test (DXMST). Renin, aldosterone and metadrenalines were measured in 80, 88 and 89% of patients, respectively.
Urinary and or plasma metadrenalines were $>3$ times upper reference range in all patients with phaeochromocytoma. Aldosterone was increased in one patient ($n=91$). 32 patients ($n=97$) failed to suppress cortisol following an overnight DXMST and, 23 ($n=24$) also failed to supress cortisol after a low dose DXMST. AI dimensions were available in 87 patients (22 $\geqq$4 cm). Twenty AI were removed: six phaeochromocytoma; 11 adrenal cortical adenoma (six cortisol secreting); one adrenal myelolipoma; one adrenal ganglioneuroma and one adrenal angiomyolipoma.
Conclusions
The majority of patients referred to our Unit had appropriate biochemical investigations performed. This study reports a similar incidence of phaeochromocytoma (5%) and subclinical Cushing's syndrome (7.7%) compared to current literature. In the absence of current UK Guidelines for the investigation of AI, laboratories must work closely with Endocrine Specialists to ensure appropriate biochemical investigations are performed.

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## P36

**Inhibiting more than the proton pump**

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Introduction
Hyponatraemia is defined as serum sodium concentration $<135$ mmol/l. It is the most common electrolyte disorder encountered in clinical practise (1). Proton pump inhibitors (PPI's) are commonly prescribed in the UK, and the indication and duration of treatment is often not reviewed.
Methods
A retrospective audit was performed of patients admitted to The Great Western Hospital (GWH) with a serum sodium of 127 mmol/l or less on admission, over a three month period. The aim was to identify prescribed medications that may be contributing to the hyponatraemia.
Results
75 patients were included in the audit; 27 male (36%) and 48 female (64%). The mean age was 75 years (range 37–94 years). All patients had a serum sodium of 127 mmol/l or less on admission: mean 122 mmol/l and range 108 to 127 mmol/l. 57% of patients had hyponatraemia on more than one occasion in the 12 months prior to admission. 77% of patients were found to be taking prescribed medications which can result in hyponatraemia. These medications belonged to four main groups: diuretics, anti-depressants, anti-epileptics and proton pump inhibitors. Some patients were taking medications from more than one group. The most commonly prescribed medication was PPI's which 48% of patients were taking on admission. 29% of patients were taking diuretics, 25% anti-depressants and 11% anti-epileptics.
Discussion
This audit clearly demonstrates that drugs may be a contributory factor in the development of hyponatraemia. There are often alternatives, for example H2 receptor antagonists rather than PPIs, and clinicians should consider these in patients at risk of hyponatraemia. 57% of patients audited had a previous diagnosis of hyponatraemia suggesting we may be missing opportunities to review prescribed medications.

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## P37

**Asymptomatic hypokalaemia in an identical twin**

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It is estimated that one episode of severe hypokalaemia will occur each week, in a hospital serving a population of 150 000. Proper management of hypokalaemia is important as severe forms may cause fatal cardiac dysrhythmias. However, recognition of hypokalaemia may be difficult as it is mild in the majority and also asymptomatic. We present a patient who was had asymptomatic hypokalaemia, with an identical twin with a similar problem.
A 45-year-old previously well woman presented with asymptomatic hypokalaemia. She had a twin sister who had type 1 diabetes and was on potassium supplements for significant hypokalaemia (she refused further investigations and died of unknown causes elsewhere). She was on Sando K and Factor 50 (no incriminating chemicals) prescribed for chloasma, and denied the use of other prescription or proprietary medication. She was normotensive, her systems examination was normal, and there were no signs of endocrinopathy. Investigations confirmed significant hypokalaemia while on supplements – 2.5–2.7 mmol/l; inappropriate urinary potassium loss (113 mmol/24 h); low magnesium of 0.53 (0.7–1 mmol/l); normal suppression of cortisol after overnight dexamethasone; normal aldosterone and renin levels (while hypokalaemic); normal renal, bone and liver profiles. Arterial pH was 7.476, bicarbonate 30.4 and base excess was 6.8. Molecular analysis demonstrated a SLCA123 mutation.
Hereditary 'channelopathies' should be considered in subjects with hypokalaemic alkalosis after excluding vomiting, upper gastrointestinal loss, Conn's syndrome and diuretic use. Our patient had the common mutation associated with Gitleman's syndrome – incidence about 25/million population, and presents usually in asymptomatic adults. Aldosterone and renin were relatively normal in our patient as she was profoundly hypokalaemic. Her electrolytes normalized on magnesium supplements and spironolactone. The cause for her twin sister's hypokalaemia is speculative as she refused investigations.

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## P38

**Severe hyponatraemia in an inpatient setting – a role for the Endocrinologist?**

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Introduction
Severe hyponatraemia is a medical emergency and can be life-threatening. It requires prompt assessment, investigation and treatment which can be a challenge as it presents to multiple departments. We therefore looked to undertake a review of severe hyponatraemia cases in our 1000-bedded acute trust with the aim of determining most appropriate care.
Aims and methods
Retrospective notes review of all patients with Na $\leq$110 mmol/l between 1/1/14 and 30/4/14 were reviewed to determine treatments and outcomes.
Results
13 patients were identified (from $\sim$18 200 admissions). 11/13 had symptomatic hyponatraemia; in 12/13 the hyponatraemia was a presenting feature. Assessment – 8/13 had a complete drug history, 5/13 had fluid status documented. 3/13 had a complete set of hyponatraemia investigations. 4/13 were diagnosed as SIADH, with a range of diagnoses for others. Management – 0/13 patients were referred to Endocrinology at diagnosis, 5/13 being reviewed later in admission. 6/13 had ITU review, with 3 admissions. Treatments – 3/13 patients had a Na rise of $>$ 12 mmol/l in first 24 h. Outcomes – 4/13 died during admission, rising to 6/13 at 12 months. 6/9 patients had a recurrence of hyponatraemia and 3/9 were readmitted within 3 months of discharge.
Discussion
Results confirm severe hyponatraemia carries a high risk of mortality (46% at 12 months). Those patients surviving to discharge had a high risk of recurrence and readmission. Low rates of effective investigations/assessment/escalation across diverse departments highlight the importance of dissemination of recently published national and international guidelines to all hospital specialties and not just Endocrinology where we know that assessment and management processes are much more robust. The expertise and educational role of Endocrinologists in the management process needs to be encouraged and the investigational pathway highlighted through Biochemistry flagging of the index Na result.

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## P39

**Are we doing to many short synecthan test**

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Background
The short Synacthen test (SST) is frequently used to diagnose adrenal insufficiency; however the role of baseline cortisol. With the recent European shortage of synacthen, there was a pressing need to identify the best possible way to use the resource and rationalize the test. Our aim is to identify whether baseline cortisol can be safely used to rule out adrenal insufficiency in clinical practice.
Methods
All SSTs performed at the OPD, Royal Gwent Hospital over a 3 year period were identified. Cortisol was measured at baseline and 30 minutes following administration of 250 μgm of synecthan. The test was defined as a pass or fail based on a 30 min cortisol $>$ or $\leq$450 nmol/l, respectively. All analyses were performed on the Abbot Architect i2000. Receiver operating Characteristic (ROC) curve was generated to determine the predictive value of the basal cortisol for a failed SST.
Results
257 SST were performed, among them 203(79%) was declared pass and 54 (21%) were failed. Roc curve showed that despite good predictive value (area under the Curve: 0.92, 95% CI 0.88–0.96) no single value of baseline cortisol was equally sensitive and at the same time specific for failed SST. A basal cortisol $<$299 had 100% sensitivity but had a specificity of 55%. It also showed that, by performing SST to only those with a basal value of $<$299 nmol/l, 103 (40%)of the tests could be avoided. This in turn would save a total £4635 for the health service in the cost of tests alone.
Conclusion
Our findings suggest that a baseline cortisol $\geqq$299 nmol/l (for the Abbott Architect assay) could be used to identify whether an SST is clinically necessary. Using a combination of baseline cortisol and the clinical indication, the number of tests performed can be significantly reduced without any cases of adrenal insufficiency being missed.

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## P40

**A case of B cell lymphoma of brain presenting as syndrome of inappropriate antidiuretic hormone secretion**

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Introduction
B cell lymphoma of brain is a rare form of lymphoma of brain and it presetting as SIADH is very rare. In this case it was very difficult to find the cause for SIADH with all routine tests. Ultimate diagnosis was by histopathology.
Case report
68 year old gentlemen admitted with vomiting found to be hyponatraemic with serum sodium of 122 mmol/litre. He was known to have chronic lymphocytic lymphoma treated with chemotherapy with good remission, brochiectasis and hypercholesterolaemia. His admission investigation confirmed SIADH. Than he was investigated for underlying cause for SIADH. There was no evidence of hypothyroidism, adrenal insufficiency, drugs causing SIADH. He had radiological investigation, which did not show any evidence of recurrence of chronic lymphocytic lymphoma or any other malignancy. He was treated with fluid restriction and demeclocycline initially. He had CT scan of head following fall, did not show any acute changes. Subsequently MRI scan of brain was done as he developed weakness of his right lower limb. At this point, he was complaining of headache. He had second MRI of brain which showed suspicious lesions suggestive of degenerative disease or lymphoma of brain. He developed hydrocephalous within a week and gradually deteriorated and died within 6 weeks of admission. Brain biopsy confirmed a rare form of aggressive primary B cell lymphoma of brain.
Discussion
The diagnosis of cerebral lymphoma is often delayed due to the variable presentation and rarity. It is very important to look for secondary causes SIADH, as illustrated here. It could be late effect of chemotherapy or radiotherapy of previous malignancy.
Conclusion
Although SIADH is a diagnosis of exclusion for patients presenting with hyponatraemia, it is important to establish under lying cause. SIADH is a tip of iceberg.

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## P41

**Audit of the diagnosis and management of primary hyperparathyroidism**

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Introduction
Primary hyperparathyroidism (PHPT) is a clinical condition often recognized as a result of biochemical screening. While surgery is indicated in symptomatic hypercalcaemic states, the need and timing of surgery in asymptomatic patients is not always clearcut. We conducted a retrospective audit on the diagnosis and management of primary hyperparathyroidism between the years 2010 and 2014.
Method
Patients were identified through our histopathology, surgery, and endocrine database.
Results
Ninety-six patients diagnosed with primary hyperparathyroidism were identified. (81% females and 19% males). Mean age of the cohort is 68, with 13% under the age of 50. 58% have 24 h urinary calcium measurement, 81% have vitamin D level measurement, and 57% have a baseline DEXA assessment. 68 patients (71%) were referred for parathyroidectomy. 51% of these referred patients were symptomatic with calcium level above 2.85 mmol/l, 26% of the patients were asymptomatic with calcium above 2.85 mmol/l and 21% of the patients were asymptomatic with calcium level $<$2.85 mmol/l. In patients who had localization studies, ultrasound and sestamibi were not concordant in 53%. 31% of the adenomas were localized via other imaging modalities. Surgery was successful in 75% of the cases. 17/68 (25%) patients were not cured by surgery and five patients needed further revision of surgery. Histology confirmed adenoma in 74% of the cases. 8/68 (11%) had further complications post surgery (four had transient vocal cord palsy, one with hypothyroidism, two with hypocalcaemia, and one developed wound infection).
Conclusion
Majority of the patients were referred for surgery as per guidelines. 21% of asymptomatic individuals who do not meet surgical criteria were offered surgery. There is local variance in investigations for vitamin D, urinary calcium, and bone mineral density. Concordance rate of ultrasound and sestamibi imaging is poor and further consensus needs to be reached with regards to optimal imaging modalities.

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## P42

**Novel treatment of refractory hypercalcaemia: a serendipitous discovery**

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Hypercalcemia is uncommon in lymphoma, it is generally a feature of histologically high grade disease with other aggressive clinical features, such as the presence of B symptoms and an elevated lactate dehydrogenase (LDH). The mechanism of hypercalcaemia is unknown but there is strong evidence for humoral factors that may or may not be related to parathyroid hormone (PTH).
Case
A 92-year-old lady who was found to have incidental hypercalcaemia (calcium 2.60–3.50 mmol/l) on routine testing and was relatively asymptomatic at presentation. Imaging revealed the presence of widespread lymphadenopathy and histology confirmed a low grade B-cell non-Hodgkin's lymphoma. She had no B symptoms and her LDH was not elevated. Calcium levels failed to come down on initial hydration and repeated bisphosphanate therapy. Further investigations were carried out to exclude other causes and these came as negative except raised PTH levels of 34 pl/l (9–18 pl/l). This was further investigated with sestamibi and parathyroid USS showed no evidence of parathyroid pathology. Chemotherapy was not immediately initiated, as this was a histologically low-grade disease with no B-symptoms, but was later initiated due to the refractory hypercalcaemia. The patient was treated with ritxuimab and oral chlorambucil. She was also given calcimimetics (cinacalcet) until further evaluation with PTH-related protein (PTHrP) levels. Cinacalcet was discontinued soon due to lack of response on maximum dose. Afterwards patient was commenced on steroids and chemotherapy was continued to complete 6 full

cycles. Subsequently, calcium came down to normal levels and repeat imaging with PET/CT confirmed a complete metabolic remission.
This is a very rare case of refractory hypercalcaemia secondary to malignancy, which failed to respond to initial and more advanced treatment with calcimimetics but responded to steroids/chemotherapy. PTHrP levels are provisionally reported as undetectable but concomitant administration of cinacalcet affects the PTHrP levels that can give false negative results.

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## Clinical practice/governance and case reports

### P43

**What is the prevalence of severe post-operative hypocalcaemia in patients who have undergone parathyroid surgery or a total thyroidectomy at the RVI, Newcastle? Does vitamin D play a role?**

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Background
Hypocalcaemia is a common electrolyte disorder and it can occur following parathyroidectomy or thyroidectomy. It has been suggested that some post-operative hypocalcaemic crises may reflect severe vitamin D deficiency and vitamin D supplementation may have a protective effect.
Objectives
To establish the prevalence of severe post-operative hypocalcaemia (as defined by the requirement for i.v. calcium gluconate) following parathyroid surgery and total thyroidectomy, and to explore its relationship with vitamin D status.
Methods
The sample ($n=466$) comprised of patients admitted to ward 44 at the RVI, Newcastle for parathyroid surgery (selective adenomectomy or full neck exploration) or a total thyroidectomy 7/12/2010–7/01/2014. The list of patients who required i.v. calcium was obtained from e-prescribing records. The data was collected from paper notes and electronic records using a proforma.
Results
The prevalence of severe post-operative hypocalcaemia was 3%. It was the highest in the total thyroidectomy group (6.71%) and the lowest in the minimally invasive parathyroidectomy group (0%). 50% of patients who required i.v. calcium were found to have vitamin D levels $<50$ in the perioperative period, whereas 36% had vitamin D levels $>50$.
Conclusions
Severe post-operative hypocalcaemia is a rare event that is almost never encountered these days in the context of selective parathyroid adenomectomy and only infrequently in the context of full neck exploration and/or excision of more than one parathyroid gland. The relative risk seems to be higher in the total thyroidectomy group, which suggests that these patients should be monitored more closely. Hungry bone disease (defined as prolonged, resistant hypocalcaemia following surgery for primary hyperparathyroidism) no longer appears to exist as a clinical entity at the RVI. Vitamin D insufficiency was common. Correcting vitamin D levels did not provide 100% protection against severe post-operative hypocalcaemia. Further work in that area is needed.

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### P44

**Urgent requirement for better patient selection for short Synacthen tests: results from a clinical audit**

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Background
Short Synacthen tests (SST) are both inconvenient and expensive, especially since the cost of tetracosactide recently increased 15-fold to over £45/ampule. A retrospective review was performed to see whether the number of SST's could be reduced in our institution.
Methods
All 76 adult inpatient (mean age 67) and 106 adult outpatient SST's (mean age 50) from a 12-month period were reviewed for indication and whether a preceding 0900 h cortisol was performed. The 30 min SST response and clinical interpretation were evaluated accordingly.
Results
Of all SST's, only 12% of tests had a 30 min cortisol beneath 500 nmol/l, though 41% of these were interpreted as borderline. Only 32% of inpatient and 52% of outpatient SST's had a preceding 0900 h cortisol measurement; the median of these was as high as 297 nmol/l, meaning a normal SST could be predicted with high certainty in many of these patients. 9% of 0900 h cortisol measurements were $<70$ nmol/l, thereby making an impaired SST a near certainty. 78% of inpatient SSTs were performed while investigating hyponatraemia, hypotension, falls or apparent hypoglycaemia, and all but one of these SST's were interpreted as normal. 46% of outpatient SST's were performed to 'exclude' Addison's in the elective setting, and all of these were negative. 34% of outpatient SST's were requested to complete endocrine workup in absence of any noted signs or symptoms of hypoadrenalism.
Discussion
Significant cost savings could be made by always requiring a 0900 h cortisol before performing SST's. The 0900 h cortisol result should be interpreted in view of low pre-test probability of hypoadrenalism in most patients currently selected for SST's. In addition, an SST is rarely needed in cases where a very low 0900 h cortisol is diagnostic. Strict protocols are required to ensure compliance to facilitate practice improvement.

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### P45

**Using Skype follow-up consultations for patients with thyroid disease**

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Patients with thyroid dysfunction require frequent clinic follow-up, but are often young and have difficulty taking time off work or college, leading to non-attendance and disruption to their treatment. The did not attend (DNA) rate of our endocrine clinic was 21.3%, of whom 44% comprised patients with thyroid disorders. Skype has already been used very successfully for follow-up in the Newham diabetes clinics for our young and ethnically diverse population. We wished to explore whether Skype consultations could be used safely for managing patients with thyroid dysfunction.
Aim
To undertake a pilot study to assess the feasibility of using Skype consultations for follow-up of patients with thyroid dysfunction.
Methods
A baseline assessment was made asking successive patients with thyroid dysfunction attending the endocrine clinic if they were familiar with Skype and would consider having Skype consultations. Patients were asked to complete questionnaires enquiring about 'patient's cost' of attending clinic (travel expenses, loss of earnings, etc.). Two patients were followed from entering to leaving the hospital for their appointment to obtain their 'journey time'. Patients who agreed to Skype consultations completed a feedback questionnaire on the experience.
Results
A typical 'patient's journey time' attending the clinic was 1 h and 44 min, of which only 15 min maximum was spent with the physician. The average cost to the patient attending clinic was £45.80. 85% of patients surveyed were familiar with Skype and expressed interest in having Skype consultations. Thirteen patients had Skype follow-up appointments. Feedback has been very positive, with comments including 'easy to use', 'time and money saving', and 'better than telephone consultations'. The average duration of a Skype appointment was 8.5 min compared to 13 min for a traditional face to face consultation.
Conclusion
Skype consultations for patients with thyroid disease are feasible, acceptable to patients, and potentially cost- saving.

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### P46

**Review in specialist Turner clinic improves management**

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Background
Turner syndrome (TS) is associated with significant morbidity and a reduction in life expectancy. National guidelines have been developed to support the

management of adult patients with TS but little is known about their implementation. We therefore sought to compare the management of patients with TS seen within a single tertiary referral centre by endocrinology, either general-purpose or Turner-specific clinics (TSC), or by other specialties.
Methods
Authorisation was provided by University Hospitals Birmingham (UHB) Clinical Governance Department. All patients coded with TS and seen on an outpatient basis within the last two calendar years were identified via informatics or through a manual search of clinic letters. Data relating to specific management standards covering annual and 5-yearly investigations were extracted from electronic records, including routine observations and laboratory investigations, use of imaging modalities and autoimmune screening.
Results
Data was extracted for 85 patients, 38 from TSC, five from general endocrine clinics, 11 from cardiology, and one from ENT. All patients within the TSC had BMI and BP measured, compared to 97% in general clinics and 58% cardiology/ENT. Excluding unsuitable patients, 92% of TSC patients were on HRT vs 82 and 66% in general endocrinology and other specialities respectively. 97.4% of patients in the TSC had thyroid function checked in the last year (85.7% in general and 8.3% in other), with 68% of patients also having their 5-yearly TPO antibodies. Echocardiograms were used extensively by all clinics, but 92% of TSC patients had received a cardiac MRI in the past 5 years, vs 63.6% seen by cardiology.
Conclusion
Patients attending specialist TS clinics were more likely to receive management in line with national guidance than those seen outside these clinics. Hormone profiling and cardiovascular imaging more frequently met guidance in Turner-specific clinics than cardiology clinics.

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## P47

**A rare incidental cause of Cushing's syndrome**

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A 19-year-old Cushingoid lady was referred with significant hirsutism requiring daily facial shaving despite previous laser epilation and regular waxing. She had a background history of PCOS, polycythaemia, hypertension and had been labelled as having a 'Cushingoid appearance'. Extensive investigations elsewhere (UFCs, MR pituitary, and CT adrenals) had failed to diagnose the syndrome. She was taking metformin to regulate menses and lercanidipine and bisprolol. Spironolactone was commenced with good effect. Owing to her obvious Cushingoid features a decision was made to rescreen. Paired samples revealed an elevated random serum cortisol level (742 nmol/l) with a suppressed ACTH (<5 ng/l). Cushing's syndrome was confirmed following a LDDST (cortisol 512 nmol/l). Repeated CT adrenal revealed a 5 mm left adrenal nodule, not apparent on previous scans. At that time she underwent breast reduction surgery. Unpredictably the histology result suggested Carney complex (CNC), subsequently confirmed with genetic testing (*PRKAR1A* gene mutation). Other features of CNC were observed including facial lentigines, blue nevi and a family history was suggestive with paternal great-grandmother and father (who had been diagnosed with hypertension and obesity at age 19) dying suddenly at a young age.
CNC is an autosomal dominantly inherited MEN syndrome characterized by skin pigmentation, endocrine and non-endocrine tumours (including atrial myxomas). Primary pigmented nodular adrenocortical disease (PPNAD) is the commonest endocrine finding and can cause Cushing's syndrome. Signs and symptoms of hypercortisolism are subtle; develop slowly over years and may be irregular or cyclic. It is likely her relatives were affected with atrial myxomas contributing to their death. This patient's ECHO was normal. She is currently being worked up for surgery and lifelong screening will be required.
This case presents a rare cause of Cushing's and highlights the importance of ongoing clinical and biochemical monitoring if high suspicions of the diagnosis.

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## P48

**Partial response to sunitinib therapy in a metastatic dopamine-secreting paraganglioma**

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Background
Malignant paragangliomas are rare. Predictors of malignant potential include high levels of normetadrenaline and/or dopamine, and the presence of a germline mutation in the succinate dehydrogenase B gene (*SDHB*). Prognosis is poor and treatment options are limited, with only short-term responses observed after $^{131}$I-MIBG therapy or chemotherapy.
Aim
To report response to the oral tyrosine kinase inhibitor sunitinib.
Case report
A 29-year-old gentleman presented with abdominal pain. Ultrasound showed a peri-adrenal mass, later confirmed as a paraganglioma of the organ of Zuckerkandl based on raised 24 h urinary normetadrenaline and CT confirmation. The tumour was successfully excised and post-operative metanephrines and MRI were normal. Genetic testing demonstrated a heterozygous frame shift mutation in exon 4 of the *SDHB* gene. Normal whole body MRI and metanephrines were reported at 1 year, but 16 months after surgery he re-presented with back pain. MRI revealed a 10×6 cm sacral mass. Repeat urine collection showed pure dopamine elevation, and repeat $^{123}$I-MIBG confirmed poor avidity. Surgical review offered no potential for resection, and chemotherapy was not pursued in view of relatively poor response rates to treatment. Ultrasound showed high vascularity, hence treatment with sunitinib was started. Repeat CT at 6- and 12-months confirmed partial radiological and complete biochemical response to therapy; surgical resection is now being reconsidered.
Conclusions
To our knowledge, this is one of the first cases to confirm a partial response to sunitinib in a metastatic *SDHB*-related paraganglioma.It illustrates i) that the presence of a germline *SDHB* mutation, low avidity on $^{123}$I-MIBG scintigraphy and dopamine secretion are all features of malignant disease, ii) that tumour surveillance in asymptomatic carriers should occur at least annually, and iii) that sunitinib may offer hope for the treatment of metastatic phaeochromocytoma and paraganglioma, including a possible neoadjuvant role in preparation for surgery.

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## P49

**Outcome of patients with adrenal incidentalomas: an analysis of 145 patients from a single centre**

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Adrenal incidentalomas are a common clinical dilemma with increasing utilisation of cross-sectional imaging modalities. The aims of management include: i) exclusion of possible malignancy and ii) identification of hormonally active lesions. Our unit has adopted AACE guidelines, including a screen for adrenal androgen hypersecretion. This audit aimed to review the utility of such an approach.
We identified case notes of 145 consecutive adrenal incidentalomas referred to Endocrinology from January 2013 to January 2015. All were subject to radiological scrutiny by a dedicated radiologist, whilst Endocrine investigations, including two 24 h urinary metanephrines, 1 mg overnight dexamethasone suppression test, renin:aldosterone ratio and DHEAS were arranged, together with electrolytes and blood pressure.
Radiologically, 75% percent were considered to be benign adrenal adenomas on the basis of either CT density (<10 HU), CT contrast washout at 10 min (>60%) or in and out of phase MRI. 0.02% were myolipomas and 3.5% phaeochromocytomas. 14% were indeterminate and referred for further investigation or surgery. Of those which were radiologically indeterminate, 25% were resected (four benign adenomas and one ganglioneuroma), 40% underwent follow-up imaging and investigations and were later shown to be non-functional benign adenomas, one was biopsied and found to be an ACC. The remainder either did not attend follow up or were not further investigated due to patient comorbidity.
From a functional perspective, we identified five phaeochromocytomas, all of whom had imaging studies inconsistent with a benign adenoma and raised urinary metanephrines. Six had a 0900 h cortisol of >100 following 1 mg dexamethasone, none of whom had symptoms/signs consistent with cortisol excess. Four were deemed to be normal after further investigation. The remaining two were on medication which could have led to the excess. Two males had elevated DHEAS levels, both of whom had abnormal imaging. One proved to have a phaeochromocytoma. There were two abnormal renin:aldosterone ratios. Both were subsequently confirmed to have Conn's adenomas. 91% of the cohort was hormonally inactive.
On the basis of this audit, we are concerned that adrenal incidentalomas are currently over-investigated. In patients with no symptoms/signs of an underlying endocrinopathy, normal blood pressure, normokalaemia, and imaging consistent with a benign adenoma, universal endocrine investigation is largely unrewarding.

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## P50

**Early post-operative aldosterone concentration can be used to assess outcome from adrenalectomy in aldosterone producing adenoma**

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Introduction
Primary hyperaldosteronism (PHA) accounts for 5–13% of all hypertension and up to 20% of resistant hypertension. Aldosterone producing adenomas (APA) account for 60% of PHA and surgical resection in these patients can be curative. There is no consensus on the need for and duration of follow-up after adrenalectomy. This study assessed the immediate effect of unilateral adrenalectomy for APA on the serum potassium, renin and aldosterone levels. Acute effects of resection of APA on the renin–aldosterone axis have not been reported.
Methods
We prospectively measured renin, aldosterone, and potassium levels between 24 and 48 h post-laparoscopic unilateral adrenalectomy in 23 adult patients with confirmed APA ($49 \pm 10$ years, mean $\pm$ S.D., ten males). We compared pre-operative and post-operative biochemical values.
Results
All 23 patients had hypertension and hypokalaemia at presentation. Mean pre-operative ARR was 1851 ($>200$ suggestive of PHA) and on average patients were on three anti-hypertensive medications. Immediately post surgery, 19 patients were normokalaemic and three patients had mild hypokalaemia and one patient was hyperkalaemic. Mean ARR was 67 and the post-operative aldosterone fell significantly ($930 \pm 120$ pmol/l vs $160 \pm 50$ pmol/l, pre vs post-op). After 3 months, 21 patients were normokalaemic and two had hyperkalaemia. All patients had normal ARR. In the sub-group cured of hypertension, pre-operative ARR was significantly high compared to those who remained hypertensive (mean ARR 2638 vs 1159).
Conclusion
Adrenalectomy is effective in immediately normalising ARR. In our study, 35% (8) patients post surgery had sustained long-term remission with normalisation of BP and potassium. The remaining 65% had normalisation of the ARR and potassium but remained hypertensive. On average these patients needed less than one anti-hypertensive medication and blood pressure was better controlled. Hence we conclude that early post-operative assessment of aldosterone can be used to assess successful APA resection and predict longer-term outcome.

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## P51

**Saccular internal carotid artery aneurysm masquerading as pituitary macroadenoma**

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An 84-year-old lady with hypertension, bladder cancer, and chronic kidney disease, presented with a 3-day history of diplopia on looking to the right, abnormal eye movements, right-sided peri-orbital headache, and diarrhoea. Clinical examination revealed right-sided sixth cranial nerve palsy with normal pupillary light reaction, visual acuity, and visual fields. There were no other neurological deficits. The patient did not have features of hypercortisolism, GH excess, or adrenal insufficiency.
CT brain demonstrated $39 \times 18 \times 15$ mm lesion involving the pituitary fossa, with bony erosion of the right cavernous sinus and to a lesser degree the left cavernous sinus, sphenoid sinus and posterior sella turcica. MRI pituitary without contrast (due to impaired renal function), revealed a heterogeneous signal in the pituitary fossa adjacent to the right internal carotid artery (ICA). Pituitary function tests were normal (LH 18.5 (NR 3.0–8.0 IU/l), FSH (NR 4.0–7.0 IU/l), 48.5, and prolactin 219 (NR $<400$ mIU/l)). The patient had acute on chronic kidney injury (Cr 178 µmol/l). The working diagnosis was non-functioning pituitary macroadenoma causing sixth cranial nerve palsy. After renal function improved with i.v. fluids, contrast MRI pituitary was done. This revealed a homogenously enhancing lesion of the pituitary fossa extending into the sella and suprasellar cistern. CT angiography confirmed a large right ICA aneurysm compressing and displacing the pituitary gland to the left. Owing to operative mortality risk of 50%, and annual aneurysm rupture risk of 8%, the patient was managed conservatively.
Although aneurysms of the ICA involving the pituitary fossa are rare, they need to be excluded before pituitary surgical referral. This case also highlights the importance of performing a contrast pituitary MRI to reliably establish the diagnosis of a pituitary lesion.

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## P52

**Impact of a multidisciplinary diabetic foot clinic on patient outcomes**

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Introduction
Foot disease in diabetes is associated with significant morbidity and mortality. Diabetic foot disease requires complex care with input from a range of healthcare professionals. Scottish Intercollegiate Guidelines Network guideline 116 recommends that all patients with diabetes and foot ulceration are seen at a multidisciplinary foot clinic. Our aim was to assess the impact on patient outcomes of introducing a multidisciplinary foot clinic within NHS Fife.
Methods
The patient group was patients with diabetes with at least one foot ulcer which had not healed within 28 days of presentation. Retrospective data was collected regarding patient demographics, wound healing, amputation rates, cardiovascular risk profile, and mortality, for patients attending the multidisciplinary foot clinic between September 2012 and September 2013. This was compared with data collected from patients receiving specialist podiatrist care only between September 2011 and March 2012.
Results
We collected data from 31 patients from the multidisciplinary clinic, and 137 patients from the specialist podiatry clinics. Our results demonstrate a trend towards a higher rate of wound healing amongst patients attending the multidisciplinary clinic (72.5% vs 62.1%, $P=0.2$). There was a significant reduction in the need for major amputations amongst multidisciplinary attendees (2.5% vs 11%, $P=0.04$). Patients attending the multidisciplinary clinic also demonstrated an improvement in cardiovascular risk profile (HbA1c reduced from 79.5 to 65.5 mmol/mol, $P=0.00002$, mean systolic blood pressure reduced from 136 to 127 mmHg, $P=0.026$, mean diastolic blood pressure reduced from 74 to 69 mmHg, $P=0.006$, and cholesterol reduced from 4.28 to 3.67 mmol/l, $P=0.0009$). No significant improvement in cardiovascular risk profile was seen amongst podiatry clinic attendees. Mortality at 12 months was significantly reduced amongst multidisciplinary clinic attendees compared with podiatry clinic attendees (0% vs 19.0%, $P=0.01$).
Conclusion
Our study demonstrates that the introduction of a multidisciplinary foot clinic has had a positive impact on patient outcomes. We aim to secure resources to establish an ongoing multidisciplinary foot clinic in NHS Fife.

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## P53

**Out-of-hours cover for diabetes and endocrinology: a single UK tertiary centre experience**

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Introduction
The provision of out-of-hours (OOH) care for hospital inpatients is currently a hot political topic with aspirations for 'seven day working' and earlier specialist involvement. Care for patients with diabetes or endocrine problems OOH (which accounts for 75% of each week) is variable and often falls as a default to the general medical team. Our centre (over 1000 beds, all major specialities) has, for many years, provided a 24/7 service for GPs and hospital inpatients in our trust and beyond. This non-resident rota is manned by 'out of programme' specialist registrars with consultant support. Patients can be reviewed in person if required and routine ward rounds are conducted on weekends and bank holidays. To the best of our knowledge there is no published data defining the extent to which diabetes or endocrine problems are encountered OOH.
Methods
Prospective data collection regarding all OOH activity (defined as 1700–0900 h weekdays and all day on weekends and bank holidays) for 1 month (May 2015).

Results
224 OOH clinical interactions occurred during the study period of which 87% occurred on weekends or bank holidays and 81% resulted in a physical review of the patient. There were forty-eight new referrals. The majority were about inpatients within the trust (77%); the remainder related to calls from GPs (13%), patients at home (6%) and other hospitals (4%). Referrals were evenly split overall between diabetes (48%) and endocrinology (52%) with hyperglycaemia and hyponatraemia being the most common reasons for referral respectively. The largest referrer was acute medicine (40%) followed by GP (13%), oncology (10%), and ED (8%).
Discussion
Diabetes and endocrine problems frequently arise OOH and the availability of round the clock specialist advice is well utilised across a range of referring specialities. We would hope, although cannot prove, that our earlier involvement in patient care improves outcome, facilitates earlier discharge and enables admission avoidance if appropriate – all key performance metrics.

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## P54

**TB or not TB: that is the question**

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A 37-year-old man presented with persistent right hip pain and night sweats. CT of chest, abdomen, and pelvis revealed a right iliopsoas abscess from which mycobacterium species tuberculosis isolated. He commenced quadruple agent anti tuberculous chemotherapy. Repeat CT 1 year later showed complete resolution of the abscess but a 3.3 × 1.7 cm mass replacing the right adrenal gland. Considered likely to represent healed tuberculous lesion, treatment completed, and patient discharged. He was referred back to the infectious diseases service eight months later with left loin pain. There was concern over recrudescence of TB. CT showed enlargement of the right adrenal mass (3 × 3.7 cm) with adjacent liver hypodensity. Given the full remission of tuberculous infection he was referred to endocrinology for further investigation. Clinical examination, including blood pressure, was entirely normal. Blood and urine testing was in keeping with a non-functioning adrenal mass. There was concern however about the possibility of malignancy given the complete remission of infection. He underwent laparoscopic right adrenalectomy. The Histopathological diagnosis was tuberculous periadrenal inflammation. No bacilli were present and the ZN stain was negative, as was the TB culture. It was concluded that the adrenal mass represented a sterile lesion of inflammatory change consequent to dead tubercle bacilli.
Lessons
In many countries, tuberculosis remains a major cause of adrenal failure. Most cases of adrenal tuberculosis are found 10–15 years after the initial infection. Spread of tuberculosis to adrenals is usually bilateral, with both adrenals markedly enlarged, while adrenal carcinoma usually affects only one gland. Approximately two thirds of adrenal cortical carcinomas cause symptoms by producing high levels of the adrenal cortex hormones. Adult adrenocortical carcinomas are aggressive tumours with a very poor prognosis. The prognosis depends on the stage, early identification, and intervention is required.

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## P55

**A case of polyglandular autoimmune disease associated with common variable immunodeficiency**

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Introduction
Polyendocrine autoimmune syndrome is a group of disorder involving endocrine glands as a consequence of autoimmunity. Non-endocrine organs also may be affected. An association between autoimmunity and immunodeficiency has also been recognized. We report a case of polyglandular autoimmune disease associated with common variable immunodeficiency.
Case report
A 26-year-old was diagnosed with Addison disease at the age of 4 following recurrent admission for nausea, vomiting and hypoglycaemia. At the age of 5, she developed recurring episodes of ITP triggered by infections. She was further diagnosed to have hypothyroidism at the age of 6 years requiring thyroxine replacement. Grouping of the three diseases, i.e., Addison's, ITP and hypothyroidism suggested a combined diagnosis of polyglandular autoimmune disease. At the age of 17 years she developed alopecia totalis and vitiligo. She subsequently developed auto-immune hemolytic anemia and a persistent hypomagnesaemia thought to be secondary to renal tubular dysfunction. A recent prolonged admission was associated with recurrent sepsis of unknown source, responsive on each occasion to broad spectrum antibiotic therapy. A multitude of investigations were performed during this period, including labeled WBC scans, infection screens (bacterial, parasitic, and fungal) and an immunoglobulin profile. This demonstrated low T-helper (low CD4), low lymphocyte count, and low IgG and IgA (IgG 2.87 and IgA 0.35). She now has confirmation of common variable immunodeficiency and remains under follow up by the department of immunology at the University Hospital of Wales, requiring long term immunoglobulin transfusions.
Conclusion
Systemic autoimmunity and immunodeficiency are not different entities and may be interconnected. Immunodeficiency, although usually caused by a defect in the immune system, can occasionally be secondary to an autoimmune process. The over lap of symptoms and presentations of both suggest that immunodeficiency should be considered in autoimmune diseases and *vice versa*.

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## P56

**Phaeochromocytoma in pregnancy: good luck and judgement lead to a successful outcome**

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Phaeochromocytoma during pregnancy is extremely rare with a frequency of 0.002% pregnancies. However, the risks for the pregnant patient with this tumour are extremely high: with maternal and foetal mortality up to 50% if undiagnosed. In contrast, early diagnosis and treatment during pregnancy decrease the maternal and foetal mortality to $<5$ and 15% respectively.
A 37-year-old female underwent abdominal imaging to investigate iron deficiency anaemia. The anaemia settled with supplements, but a 19 mm, 40 Hounsfield Units incidental adrenal mass was found on non-contrast CT abdomen. She was routinely referred for endocrine evaluation at which new symptoms of breathlessness at rest, palpitations, panic attacks, and anxiety were elicited. This highly suggestive history, despite a very small and atypical lesion, prompted her to be started on α-blockade that day, pending confirmatory investigations. Subsequently, 24 h urinary metanephrines were confirmed as 2× ULN, with serum metanephrines 4× ULN, and with increased focal uptake of the right adrenal on meta-iodobenzylguianidine (MIBG) scan. Standard medical blockade was therefore continued and uptitrated with a view to early surgery.
The patient then reported that she was unexpectedly pregnant with her LMP 2 days prior to her MIBG scan. She was therefore counselled carefully, continued on alpha blockade in close collaboration with the medical obstetric, anaesthetic and surgical teams, and surgery deferred. She then underwent an elective uneventful laproscopic right adrenalectomy at 13 weeks gestation. Both mother and foetus remained well and her baby girl was delivered successfully via elective LSCS 6 weeks ago.
This case illustrates that, where the diagnosis has already been made, multidisciplinary management of the pregnant patient with a phaeochromocytoma is relatively straightforward. However, this patient was extremely lucky that a chance scan led her to be referred and diagnosed much earlier than is typical, that her symptoms were appreciated and taken seriously despite rather unimpressive imaging, and that her unplanned pregnancy arose just after rather than just before the MIBG scan.

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## P57

**Audit on continuous subcutaneous insulin infusion (insulin pump) therapy**

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Objectives
Insulin pumps were introduced in the late 1970s. Their use has continued to grow and the technology has continued to improve along with the development of insulin substrates. They are however a costly therapy, thus NICE recommend pump therapy in the following circumstances; when patients are undergoing regular episodes of severe and sometimes unpredictable episodes of hypoglycaemia, have HbA1c levels over 8.5% despite attempts to lower it, difficult control during pregnancy, wide readings of blood glucose overnight, e.g., dawns phenomenon and nocturnal hypogylcaemia and lastly patients' choice. Our audit's focus was to see if patients on insulin pump therapy complied with NICE guidance and to see whether insulin pump therapies improved their overall diabetic control.
Method
In our audit, 40 patients on insulin pump therapy at The Queens Medical Centre, Nottingham were chosen at random. The patient parameters gathered comprised: type of diabetes, duration of previous therapies and indication for commencing the pump. We also looked at their HbA1cs over intervals across 5 years, weight and BMI. The rates of cannula site infection, diabetic ketoacidosis and admissions for disabling hypoglycaemic episodes were also documented.
Results
Of the patient population studied, there was no improvement in their HbA1c at 5 years, compared to their initial HbA1c. This conclusion was reached by comparing the average and S.D. of the HbA1c at the various time intervals (the average initially being 62.07 and 64.45 at 5 years. The S.D. being: 12.69).
Conclusion
Although, one of the many aims of insulin pump therapies is for tighter control of the HbA1c measurement, this was not seen in our patient cohort. However, patients appeared to benefit from fewer admissions for diabetic ketoacidosis, disabling hypoglycaemic attacks and an overall improvement in quality of life with less need for continuous glucose monitoring and injections.

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## P58

**Vitamin D deficiency in haemodialysis patients; treatment with colecalciferol**

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End stage renal disease (ESRD) is characterised by decreased renal synthesis of 1,25-dihydroxyvitamin D (1,25D). Therapeutic use of 1,25D analogues for the management of renal bone disease is routine. However ESRD patients are also deficient in 25D (the immediate precursor of 1,25D). Since 2014 UK guidelines recommend diagnosis and treatment of 25D deficiency/insufficiency in people with chronic kidney disease, but make no recommendations for dosage or monitoring. This, together with i) a lack of appreciation for the physiological roles of vitamin D beyond mineral homeostasis, ii) a poor understanding of extra-renal 1,25D synthesis, and iii) a misconception that 1,25D therapy alone is sufficient, has meant hypovitaminosis D in ESRD remains prevalent. Vitamin D insufficiency/deficiency in the haemodialysis population of Coventry and Warwickshire was assessed and a clinical guideline for colecalciferol supplementation developed. Preliminary data relating to deficiency, safety, and efficacy are reported. A search of Web of Science, Cinahl, Embase, Medline, Cochrane, and Proquest (February–June 2014) identified 2847 citations. 17 full papers were appraised. The guideline recommends 40 000 IU colecalciferol weekly (for 3 months) if serum 25D $<$50 nmol/l and 20 000 IU weekly if serum 25D 50–75 nmol/l; to be continued long term unless levels increase to $\geq$150 nmol/l. To date, we have preliminary repletion data for 72 of our 350 haemodialysis patients. At baseline, virtually all patients (95.8%) had serum 25D $<$75 nmol/l (58.3% deficient, $<$30 nmol/l and 37.5% insufficient, 30–75 nmol/l). Only three patients had optimal levels ($\geq$75 nmol/l). After 3 months supplementation 92% of patients had 25D $\geq$75 nmol/l (median 118.5; range 80–175). No patients remained deficient and only six patients remained insufficient (median 66.5 nmol/l; range 38–73). No hypercalcaemia was associated with colecalciferol supplementation. These data indicate hypovitaminosis D is prevalent in our haemodialysis population and this is likely to reflect UK haemodialysis patients. Initial data suggest vitamin D repletion following our local guideline is effective and safe.

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## P59

**DIPNECH: under-recognised and a diagnostic challenge**

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A 48-year-old non-smoking female was seen in the chest clinic for cough and breathlessness on a background of asthma type symptoms for 20 years. Her cough was exacerbated by inhalers, productive of yellow sputum and intermittent streaks of blood. On examination, her lungs were clear but coughed continuously with an unusual duration of five minutes. Pulmonary function test showed an obstructive picture. CT scan showed bronchiectasis with multiple small nodules scattered throughout the lungs ranging 4–8 mm. Subsequent scans over 2 years showed these nodules to have slightly increased in size. She remained extremely symptomatic with persistent cough despite steroid inhalers and mucolytics, although found slight benefit on mast cell stabilizer. She underwent video thoracoscopy which proceeded to left thoracotomy to obtain a lung biopsy. The pathological findings were consistent with diffuse idiopathic neuroendocrine neoplasia (DIPNECH). She was referred to Oncology for further management.
DIPNECH is a rare condition which is closely related to carcinoid tumours. Middle aged women represent 92% and most are non-smokers. Symptoms include chronic cough, dyspnea and wheezing. Imaging shows lung nodules and bronchiectasis. The histological appearance takes many forms comprising of generalised proliferation of scattered neuroendocrine cells, small nodules (neuroendocrine bodies) or a linear proliferation of pulmonary neuroendocrine cells superficial to the basement membrane. Extension beyond the basement membrane are termed tumourlets. Nodules $>$5 mm in diameter are classified as carcinoid tumours.
DIPNECH is typically an indolent and non-progressive disorder although it may progress to carcinoid tumour. Respiratory failure may occur. A 'wait and watch' approach can be an option but whenever possible, the patients should undergo surgical excision of the dominant lesion and somatostatin analogues may be considered for symptomatic or tumour control in patients with progressive disease.

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## P60

**Sodium–glucose co-transporter 2 inhibitor associated normoglycaemic ketoacidosis**

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Sodium–glucose co-transporter 2 (SGLT2)-inhibitors represent a new class of drugs that inhibit glucose re-uptake in the proximal renal tubule resulting in glycosuria, with concomitant weight loss and improved glycaemic control in patients with type 2 diabetes mellitus (T2DM). Since becoming available, SGLT2 inhibitors have been implicated in over 20 reported cases of diabetic ketoacidosis or ketosis. In many cases there was an antecedent period of decreased oral intake, and treatment included both i.v. insulin and dextrose.
We report the case of a female in her mid 40's with T2DM on metformin, liraglutide, and canagliflozin, presenting 2 days after elective cosmetic surgery with dyspnea, nausea, and vomiting. She had clinical and laboratory evidence of severe ketoacidosis, associated with severe glycosuria. Labs were notable for pH 7.0, β-hydroxybutyrate level 140.4 mg/dl, urine glucose $>$1500 mg/dl, serum bicarbonate 6 mmol/l, and anion gap was 33, despite serum glucose of only 180 mg/dl and minimally elevated serum lactate.
Based on the observation that most reported cases of ketoacidosis with use of SGLT2 inhibitors share features of antecedent decrease in oral intake, absence of significant hyperglycemia, and presence of significant glycosuria, we posited this ketoacidosis may involve a deficiency of glycogen stores resulting from significant glycosuria, and triggered in susceptible individuals when carbohydrate intake decreases. Thus, we discontinued the initial insulin infusion and continued i.v. dextrose only, which led to complete resolution of clinical and laboratory evidence of ketoacidosis. We believe this is the first report of SGLT2-associated ketoacidosis treated with dextrose alone and therefore supporting a mechanism unrelated to diabetic ketoacidosis.

Until the mechanisms underlying the risk associated with these drugs are identified, such as subclinical genetic defects in hepatic fuel storage and metabolism, clinicians should use caution with any use of these agents during periods of fasting and/or severely carbohydrate-restricted diets.

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## P61

**A novel management of Gitelman's syndrome**

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We present a patient with Gitelman's syndrome who had marked symptoms of myalgia and lethargy secondary to electrolyte imbalances. Initial management with oral potassium and magnesium replacements failed and she had five to six hospital admissions a month via the emergency department for worsening symptoms and needing i.v. electrolyte replacement. Therefore, she had planned i.v. potassium replacement three times a week via the day unit instead. Although this significantly helped her symptoms and reduced acute admissions, it was very disruptive for her to come to the day unit so frequently.

Therefore a novel way of replacement with i.v. potassium in the community was planned. After discussion and planning with the local team, our patient is now managing her i.v. potassium replacement independently at home. This has not only resulted in marked improvement of her symptoms but also, social independence for her to continue with her daily living. Literature surrounding management of pregnancy in Gitelman's syndrome is limited but our patient had a successful pregnancy and delivered a healthy baby boy using this prescribed regime of replacement.

To our knowledge, this is the first case describing successful management of i.v. potassium replacement in a patient with Gitelman's syndrome done by the patient in their home setting. It also demonstrates the benefit of individualising treatment for such patients with a chronic illness to allow some normality of daily living.

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## P62

**Nothing to 'sea' here: turning a blind eye to hyponatraemia**

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Introduction

Hyponatraemia is defined as serum sodium concentration $<135$ mmol/l. It is the most common electrolyte disorder encountered in clinical practise. It is associated with an increase in mortality and length of stay, independent of diagnosis and clinical variables. Despite this it is often inadequately investigated and poorly managed.

Methods

A retrospective audit was performed of patients admitted to The Great Western Hospital (GWH) serum sodium of 127 mmol/l or less on admission, over a 3-month period. The aim was to identify whether a diagnosis of hyponatraemia is made and to evaluate how these patients are investigated.

Results

Seventy-five patients were included in the audit; 27 males (36%) and 48 females (64%). The mean age was 75 years (range 37–94 years). All patients had a serum sodium of 127 mmol/l or less on admission: mean 122 mmol/l and range 108–127 mmol/l. Only 65% of patients had hyponatraemia documented as either a diagnosis or problem. Only 28% of patients had their fluid status documented. Eight investigations were identified as essential when investigating patients with hyponatraemia. Only 4% of patients had all eight investigations completed during admission. Less than 27% of patients had a urine sodium, urine osmolality, and serum osmolality requested during admission.

Discussion

This audit clearly demonstrates that clinicians do not recognise hyponatraemia as a problem, and are therefore not making a diagnosis and requesting the appropriate investigations.

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## P63

**A case to remember**

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Introduction

X-linked adrenoleucodystrophy (XALD) is a progressive disorder affecting adrenal glands, testis, and myelin stability, that normally caused by mutations in *ABCD1* (NM_000033) gene There is a failure of β-oxidation of fatty acids within peroxisomes due to reduced activity of very long chain acyl-CoA synthetase and can be diagnosed by the demonstration of elevated very long chain fatty acid.

Case report

We report a case of a 32-year-old gentleman who was initially presented with low mood, erectile dysfunction, urinary incontinence, spastic paraparesis, and infertility. His brother now deceased had a diagnosis of early onset X-linked adrenomyeloneuropathy and hypogonadism. His mother also had a history of weakness in the leg but not sought a formal neurological diagnosis. He was noted to haveproximal muscle weakness and had a bilateral extensor planter response on examination. Investigations showed a normal LH, FSH, and testosterone. His 0900 h cortisol was 520 nmol/l and an ACTH was 31 ngm/l (normal range). In view of his strong family history a presumptive diagnosis of adrenoleucodystrophy was made. His very long chain fatty acid (VLCFA c26 was 4.18 μmol/l). Genetic test showed an abnormal mutation on *ABCD1* gene. He was treated by the urology team for his erectile dysfunction. Although, his neurological condition remain stable his tiredness was progressively worsen next 6 months. A further short Synacthen test showed a basal cortisol of 273 nmol/l and 30 min post Synacthan the cortisol was recorded 310 nmol/l. He had negative adrenal antibody. A diagnosis of adrenal insufficiency secondary to adrenoleucodystrophy was made. He was started on hydrocortisone and his symptoms improved rapidly.

Conclusion

Adrenal insufficiency can be presented in various forms and young patient with family history and neurological symptoms, adenoleucodystrophy should be considered as a possible diagnosis**.**

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## P64

**Resistant hypocalcaemia secondary to denosumab: two case reports**

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Case 1

A 65-year-old man was admitted with symptomatic hypocalcaemia (corrected calcium of 1.65 mmol/l, PTH 46.6 (1.6–6.8) pmol/l, and vitamin D 51.4 (50–150) nmol/l)) 5 days following denosumab initiation. He had a diagnosis of probable urothelial carcinoma with associated osteoblastic metastases, and 10 days prior to this admission had been treated for hypercalcaemia (corrected calcium of 3.04 (2.20–2.60) mmol/l) with i.v. fluids following which denosumab was initiated. His hypocalcaemia improved with i.v. calcium infusions, but he became significantly hypocalcaemic whenever i.v. calcium was stopped. He required a total of 550 ml of 10% calcium gluconate intravenously with concomitant oral calcium supplementation to improve his hypocalcaemia. After discharge he was maintained on 2.7 g of oral calcium for 2 months following which it was possible to wean off treatment.

Case 2

A 73-year-old woman presented with paraesthesia and severe hypocalcaemia (corrected calcium 1.46 mmol/l), which was associated with an elevated PTH (54.8 pmol/l), and vitamin D deficiency (17.6 nmol/l). She had a history of breast cancer with skeletal metastases, which was treated with denosumab for 4 months up until admission. She required a total of 800 ml of 10% calcium gluconate infusions and oral calcium supplementation during a prolonged 27-day admission due to rapid recurrence of hypocalcaemia on cessation of i.v. calcium infusions. She remained eucalcaemic on 2 g of oral calcium daily and 40 000 units of colecalciferol monthly following discharge.

Discussion

Hypocalcaemia is a recognised complication of denosumab therapy, where fatal cases have been reported in the literature. The above cases highlight the need for close serum calcium monitoring to allow prompt correction of hypocalcaemia. Owing to its long half-life, presentation of hypocalcaemia following denosumab therapy can be prolonged, leading to protracted inpatient admissions requiring aggressive i.v. calcium replacement. It is also prudent to identify and correct potential risk factors such as vitamin D deficiency prior to initiation of denosumab.

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## P65

**Multiple endocrine paraneoplastic syndromes in a patient with lung malignancy**

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A 58-year-old lady presented to hospital with abdominal pain, nausea, and tiredness, and was referred to endocrinology with symptomatic hyponatraemia (serum $Na^+$ 112 mmol/l). Six months previously her serum sodium was normal. She was euvolaemic, and adrenal insufficiency and thyroid dysfunction were excluded. Laboratory investigations were suggestive of syndrome of inappropriate antidiuretic hormone (SIADH) and no causative medications were identified. A CT of her thorax, abdomen, and pelvis revealed a right upper lobe lung nodule and cysts in the liver, kidneys, and adnexae. Her hyponatraemia was unresponsive to fluid restriction or demeclocycline. She was treated with Tolvaptan 7.5 mg on alternate days and subsequently her serum sodium stabilised (128–135 mmol/l). The cysts and right lung nodule were investigated extensively with further imaging studies and appropriate multidisciplinary input; all were felt to be benign. She was readmitted to hospital three months later with oral ulcers, lethargy, and dyspnoea. Her sodium had normalised (137–140 mmol/l) but she had developed new-onset hypokalaemia (2.3–3.3 mmol/l) and pancytopenia. A further CT scan showed a left hilar lung mass with left upper lobe collapse and multiple spiculated nodules throughout the right upper lobe. It showed marked enlargement of both adrenal glands. Paired serum cortisol was 3107 nmol/l and ACTH was 561 ng/l. She was diagnosed with metastatic small cell lung cancer with paraneoplastic SIADH and Cushing's syndrome, and unfortunately deteriorated rapidly.

This case demonstrates the need to be aware that multiple paraneoplastic syndromes can co-exist. Contrary to the more common sequence of presentation, Cushing's syndrome presented as a terminal event and SIADH, usually a secondary phenomenon, was the presenting manifestation. This case also illustrates that SIADH may precede a cancer diagnosis highlighting the importance of exhaustive investigations and close follow-up in patients with resistant hyponatraemia, especially when responsive only to vasopressin receptor antagonists.

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## P66

**National Audit of Transition in Endocrinology: joint between Society for Endocrinology and the British Society of Paediatric Endocrinology and Diabetes**

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Background
Transition is an important stage in the care of a young person with a long term endocrine condition.
Objective
To explore current services for young people (YP) with endocrine conditions from the perspective of paediatric and adult endocrinologists, and YP and their parents using their services.
Methods
There were two components: i) service questionnaire for completion by paediatric and adult endocrinologists. ii) 'Mind the Gap' questionnaire for completion by YP and parents.
Results
Forty-nine service questionnaires were completed (25 by adult endocrinologists) representing 35 centres across the UK and the Republic of Ireland. 233 YP and 200 parent questionnaires from 24 centres were also completed. Out of 16 You're Welcome criteria (Quality Criteria for YP Friendly Health Services) the median number achieved by each centre was 7 (range 3–12). The least frequently achieved criteria were giving YP a hand-held summary at the time of transfer, running clinics outside school/college hours, YP involvement in service evaluation and design and publicising other services for YP. Clinic structure to support transition was available in 80% included multiple joint appointments in 21, single joint appointment in six, age banded clinics were used in two services. Three services had a structured transition programme. 50% of paediatric and adult endocrinologists identified lack of psychosocial support and time as the greatest barriers, in comments paediatric endocrinologists raised concerns about adult services. 56% of services were considered less developed than those in diabetes. 69% of services were undergoing development and 58% with plans.
Conclusion
Despite clinic structures to support transition being in place, most services are achieving $<50\%$ of criteria associated with high quality care for YP. One criteria, involving YP in evaluating and designing services, was achieved in 23%. The 'Mind the Gap' questionnaire will be a useful tool to understand YP and parent experience.
Funded by Clinical Endocrinology Trust.
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## P67

**IgG4-related hypophysitis: a novel candidate to the hypophysitis spectrum**

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A 57-year-old gentleman presented with bilateral painless submandibular swelling, and flu like symptoms. A biopsy from the enlarged submandibular gland showed chronic sclerosing sialadenitis with lymphocytic infiltration. Immunohistochemistry showed plasma cells positive for CD79a and IgG4, and a combination of CD4 and CD8 positive lymphocytes. IgG4 levels were significantly raised (3.16 g/l, NR $<1.3$ g/l). A diagnosis of IgG4 syndrome was made. Chest and abdominal CT imaging did not reveal involvement of other organ systems. Treatment with a reducing dose of oral prednisolone over 6 months resulted in a reduction of IgG4 levels to 2.27 g/l but no significant change in the ultrasound appearance of the submandibular glands. A 6-month trial of hydroxychloroquine was also ineffective. IgG4 levels have increased over time (IgG4 $>3.44$ g/l, Sept 2014).

In October 2014 the patient complained of recent inset of severe thirst and frequent urination. Initial investigations showed serum and urine osmolalities of 309 and 116 mOsm/kg respectively. A subsequent water deprivation test confirmed a diagnosis of central diabetes insipidus. Anterior pituitary function was normal apart from hypogonadotrophic hypogonadism (LH 2.7 U/l, FSH 1.8 U/l, and testosterone 4.4 nmol/l). MR imaging showed a normal pituitary gland but with abnormal thickening and enhancement of the infundibulum. A diagnosis of IgG4-related hypophysitis was made and the patient responded well to desmopressin. Testosterone replacement is currently under consideration. Hypophysitis is a rare complication of IgG4 syndrome. A case series from Japan[1] reported a male predominance, with variable involvement of anterior and pituitary hormone systems. Diabetes insipidus is the most common endocrine complication. There is a lack of trial data to inform the treatment of IgG4 syndrome. Glucocorticoid therapy remains the first-line of treatment but has not proved effective in this case. He is now being considered for a trial of low dose methotrexate. His anterior pituitary function will be monitored over time to determine the extent of disease progression.

Reference
1. Shimatsu *et al*. 2009 *Endocr J* **56** 1033–1041.

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## P68

**Management of thyroid disease in pregnancy: experience from an antenatal thyroid clinic**

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Background
Thyroid disease in pregnancy is common with hypothyroidism predominating.
Objectives
To determine the aetiology of thyroid disease in patients attending an antenatal thyroid clinic, their baseline biochemical and therapeutic characteristics as well as subsequent management.
Subjects/setting
114 women with a mean 31 years ($\pm 5$) age were seen by a consultant endocrinologist in the antenatal thyroid clinic, between September 2013 and November 2014.
Methods
A retrospective audit of case notes and electronic records of patients identified from clinic lists.

Results
Median gestation at initial review was 16 weeks (IQR 13–20). Diagnoses included hypothyroidism (84%), gestational thyrotoxicosis (6%), Grave's (6%), and other (4%). 89% of patients had previous episodes/persisting thyroid disease pre-pregnancy with a mean duration of 5 years (IQR 2.4–8.0). The remaining 11% presented with new thyroid disease during pregnancy with the majority having gestational thyrotoxicosis or positive thyroid TPO antibodies but normal thyroid function. No new cases of Grave's disease were diagnosed during pregnancy. For patients with hypothyroidism median dose of levothyroxine (L-$T_4$) at initial consultation was 100 μg/day (IQR 50–144). Based on initial and then subsequent blood tests (between 5 and 7 weeks after each appointment), 44% did not require any dose adjustment (TSH 1.9 mU/l ± 1.9), whilst 37% had one (TSH 5.4 mU/l ± 6.8), 13% had two (8.2 mU/l ± 6.1), 5% had three (4.0 mU/l ± 0.22), and one patient had four dose adjustments (TSH < 0.01 mU/l). Note that the mean ± S.D. for TSH is quoted at the time dose adjustment was deemed necessary. On final follow up $FT_4$ had normalised in all hypothyroid patients and only one patient had mild elevation of TSH (TSH = 7.2).
Conclusions
Current recommendations suggest a 30–50% increase in $T_4$ for all patients at initial consultation. Our data suggest that this is not necessary for almost half of patients with pre-existing hypothyroidism and in those that do require an increase, the average required is 41 μg (± 19).

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## P69

**Endometrial hyperplasia in transmen: to scan or not to scan?**

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Endometrial hyperplasia has been reported in up to 15% of transmen. For this reason the current clinical practice suggest that the uterus should be scanned every 2 years.
This is the single centre retrospective audit study at the largest UK Gender Identity Clinic to examine the incidence of endometrial hyperplasia in transmen. Between 2006 and 2012. 200 patients having been maintained on testosterone therapy for 2 years, of those 108 transmen were requested to undergo ultrasound scanning and 42 scans were available to us at the time of study.
None of them had had endometrial thickness of > 10 mm.
Abnormalities were seen in eight patients the largest thickness being 8 mm but this was known prior to the patient being started on testosterone therapy. Other issues that were apparent were fibroids PCOS and endometriosis.
This raises the question that the current recommendations biannual endometrial scanning should be maintained however further evidence is required before it is removed from the current type of practice guidelines.

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## P70

**Steroid responsive hypoglycaemia in a patient with spindle cell sarcoma**

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Introduction
We present a case of a gentleman with non-islet cell tumor hypoglycemia (NICTH), a rare cause of spontaneous hypoglycemia that presents clinical challenges in maintaining euglycaemia.
Case report
A 76-year-old gentleman not known to be diabetic presented with confusion and agitation. On admission he had a blood glucose reading of 2.1 mmol/l and clinical examination confirmed an abdominal mass. He was initially resuscitated with i.v. dextrose but continued to experience hypoglycemic episodes. Urine sulphonylurea, serum insulin, pro-insulin, and C-peptide levels tested at the time of hypoglycemia were satisfactory. There was no ketonaemia and the short Synacthen test showed adequate response. His IGF2 and IGF2:IGF1 ratio was elevated. An abdominal ultrasound and CT scan were inconclusive as to the nature of the mass, so biopsy and histology were organized which confirmed a spindle cell neoplasm. To maintain euglycaemia, he had dietician input for complex carbohydrate meals and regular scheduled snacks, but as this failed to sustain his euglycaemia, he was then started on steroid therapy. He had a good response to the dexamethasone. Unfortunately, as curative therapy of the spindle cell tumor was not an option for him, he was referred for palliative follow-up.
Discussion
NICTH is a para neoplastic complication of malignancy resulting from overproduction of incompletely processed IGF2 by the tumor, which results in stimulation of the insulin receptors and increased glucose utilization. The IGF2 also suppresses glucagon and GH release. The aims of treatment in patients with NICTH include maintaining euglycaemia and when possible offering curative therapy for the tumor itself. The usual medical management for spontaneous hypoglycemia in the case of insulinomas has some limited use in NICTH. Steroids are the mainstay of treatment as they are believed to decrease the amount of IGF2.

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## P71

**Does the usual time of rising influence the stimulated cortisol response?**

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Aim
To investigate the relationship between baseline and stimulated cortisol responses in relation to the habitual time of rising and time of test.
Method
The self-reported habitual rising time and time of cortisol testing were recorded in 63 consecutive patients (47F, 17M, age 18–94) undergoing standard stimulation testing with tetracosactide (SynActhen) 250 μg i.v. In total, 75 tests were analysed. A normal response was defined as a 30 min peak cortisol > 450 nmol/l. All tests were performed between 0800–1300 h.
Results
Of the 75 tests, 65 were classified as normal responses; ten had impaired peak values. In the group with normal responses, Pearson's analysis showed no correlation between baseline and 30 min cortisol responses with habitual rising time or the interval between this time and the time of the test. A weak negative correlation was noted between the baseline cortisol value and the actual time of test irrespective of the time of rising ($r$: −0.28, $P$: 0.026). The highest incremental rises were seen in subjects with the earliest time of rising and lowest baseline values ($r$: −0.58, $P$: 0.038). In the subgroup with impaired responses, higher peak cortisols were observed in those where the time of test was closest to their usual time of rising ($r$: −0.66, $P$: 0.037).
Conclusion
In patients with normal adrenal reserve, peak cortisol responses are not significantly influenced by the timing of the test in the morning or the usual time of rising. In those with impaired cortisol responses, the test time and its relation to the time of rising may influence the incremental cortisol response, although this needs to be confirmed with larger patient numbers. For the majority of patients, adrenal stimulation tests can be reliably performed any time in the morning.

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## P72

**A woman with thyroid metastases 21 years after renal carcinoma excision, with biphasic thyroid dysfunction on sunitinib**

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Introduction
The commonest metastases to the thyroid are from renal cell carcinomas (RCC) and 23–48% of all thyroid metastases are RCCs. They may occur many years after RCC excision, the longest reported latent period being 20 years. Sunitinib (SUN), a multiple targeted anti-cancer drug, is increasingly used for RCC therapy but may cause thyroid dysfunction in some – usually hypothyroidism. We report a patient who presented with thyroid metastases 21 years after RCC excision, who developed biphasic thyroid dysfunction during SUN therapy.
Case presentation
A 76-year-old woman who had a nephrectomy for RCC 21 years before, and was on no relevant medication, presented with an enlarging right thyroid nodule. She had a thyroid lobectomy following inadequate FNAC of the nodule. Histology revealed metastatic clear cell renal carcinoma. CT scans revealed possible pancreatic and ovarian metastases but no recurrence at the original site. She was started on SUN for disseminated disease, after multidisciplinary team review. Six weeks later she developed fast atrial fibrillation, and was thyrotoxic – $FT_4$ 26.6 pmol/l (9–19) and TSH < 0.01 mU/l (0.03–4.40). She had no pain in the

neck, difficulty in swallowing, influenza like symptoms or tenderness in the thyroid remnant. Thyrotrophin receptor antibodies (TRAb) were negative. Carbimazole was started in primary care, but was stopped when subclinical hypothyroidism developed a few weeks later. She remained persistently hypothyroid several weeks after stopping carbimazole.

Discussion

i) This subject developed thyroid metastases from a RCC excised 21 years previously. This as far as we are aware, is the longest latent period reported. ii) She developed a biphasic thyroid illness, which was most likely SUN induced (TRAb negative, no clinical features suggestive of painful subacute thyroiditis). Although, SUN commonly causes hypothyroidism, a hyperthyroid phase may occasionally precede this, due to a 'destructive thyroiditis' which is usually mild and produces no local symptoms.

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## P73

**Immobilisation hypercalcaemia in two intensive care patients**

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Immobilisation is a recognised but rare cause of hypercalcaemia. Most reported cases are patients with spinal cord injury or trauma.

Case 1

A 26-year-old female experienced a 5½ months admission to the intensive care unit (ITU) with complications following small bowel resection for Crohn's disease. At 16 weeks she developed hypercalcaemia. Investigations confirmed calcium 3.24 mmol/l, parathyroid hormone (PTH) $<0.6$ pmol/l (1.6–6.9), 24 h urinary calcium 17.64 mmol/24 h ($<5$), calcium:creatinine ratio 6.9, serum β-cross laps 1.55 μg/l (0.1–0.5), and normal renal function. Total parenteral nutrition was providing 3.8 mmol calcium/24 h. The calcium levels returned to normal with a reduction in the calcium content of her TPN and bisphosphonate treatment. She was later found to have multiple osteoporotic spinal wedge fractures. Her calcium levels have remained normal after mobilisation.

Case 2

A 60-year-old man with flaccid quadriplegia secondary to Guillain–Barre syndrome developed hypercalcaemia four weeks into an ITU admission. Feeding was via a nasogastric tube with the feed delivering 35 mmol calcium/24 h. Investigations confirmed calcium 2.79 mmol/l, ionised calcium 1.348 mmol/l (1.12–1.32), PTH 1.4 pmol/l, urinary calcium 3.65 mmol/24 h, and β-cross laps 0.97 μg/l. I.v. pamidronate normalised calcium levels.

Immobilisation results in increased bone turnover with an imbalanced preponderance for osteoclastic bone resorption reflected by raised β-cross laps. This results in hypercalcaemia. These cases demonstrate that immobility of different aetiologies can result in the phenomenon at varying degrees of severity and duration of immobility. Also demonstrated is the impact of total parental nutrition on the severity of the hypercalcaemia which was less severe in enteral feeding, as the low PTH levels resulted in decreased calcium absorption. Pamidronate is confirmed as an effective treatment.

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## P74

**Audit on hyponatraemia in a West Kent Hospital experience**

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Hyponatremia, defined as a serum sodium concentration below 135 mmol/l is acknowledged to be the most common electrolyte disturbance within hospital environment. Hyponatremia, specifically severe hyponatremia, is associated with an increase in morbidity and mortality.

Aim

Audit aim was to evaluate the diagnosis, investigation, and management of hyponatremia in our district general hospital. This is a retrospective audit using lab data and clinical notes.

Results

Across a time span of 2 months 31 patients with hyponatraemia were identified (nine men and 22 women). Mean patient age was 71.9 years. Bimodal distribution for both men (40s and 80s) and women (60s and 80s). Majority (66.7%) of patients were symptomatic. Also generally symptoms were related to severity of hyponatraemia except patients in their 60s were symptomatic regardless of severity. The mean initial plasma sodium concentration was 122.5 mmol/l. Among the essential investigations patients had: urine sodium (22.5%), plasma osmolality (22.5%), urine osmolality (22.5%), and paired samples (12.9%), TSH (45.1%), cortisol (22.5%), glucose (12.9%), and creatinine (100%). Only five patients had all investigations. Correct diagnosis was made only in half of the patients (48%). Of assessment a third had fluid status documented. Only 40% had appropriate fluid management. A quarter had drugs stopped appropriately. In severe hyponatraemia no single patient had daily sodium monitored and none of them had any pharmacological intervention for hyponatraemia.

Conclusions

This audit in our hospital highlighted that hyponatraemia is inadequately managed. Also essential investigations were not done and this has the potential for adverse consequences to the patient. We believe this malpractice is widespread across UK. We are planning to improve our practice through education and guideline but there is still scope for further improvement nationally.

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## P75

**Large benign nerve sheath tumour in the adrenal gland: an incidental finding**

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Nerve sheath tumours originate from myelin that surrounds peripheral nerves and include schwannomas and neurofibromas. They can occur sporadically or as part of neurofibromatosis 1 or 2. Very rarely, they have been described to arise from the viscera including the adrenal gland. It is thought that they arise from Schwann cells around nerve fibres that supply the adrenal medulla. These are rather slow growing and non-functioning adrenal tumours that can be found incidentally or present as a result of compressive symptoms.

We describe the case of a 30 year old lady who was noted to have an incidental 11 cm by 9 cm unilateral adrenal lesion on a CT scan performed to stage her lymphoma. An overnight dexamethasone suppression test, a renin:aldosterone ratio and 24 h urine metanephrine were normal. She had no clinical signs to suggest neurofibromatosis and had no family history of note. A core biopsy revealed spindle cells that were strongly positive with S100 immunostaining and show a very low proliferative index with Ki-67 of $<1$ in keeping with a benign nerve sheath tumour. She has been managed conservatively.

Large mixed attenuated lesions arising from the adrenal gland on imaging may raise the suspicion of malignancy. Biopsy in this case is helpful as the patient is asymptomatic and surgery is mostly carried out if there is any evidence of compression of adjacent vital structures.

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## P76

**Aromatase inhibitor: potential therapy for obesity related hypogonadotropic hypogonadism and gynaecomastia**

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Background

Hypogonadotropic hypogonadism (HH) is commonly associated with morbidly obesity. Gynaecomastia is associated feature of hypogonadism and can be deteriorated by testosterone therapy.

History

A 49-year-old gentleman with a BMI of 53.2 kg/m$^2$ was referred for management of obesity. He has two children (age 18 and 22). He had obesity associated comorbidities including obstructive sleep apnoea and impaired fasting glucose. He reported lethargy, reduced sexual drive, libido, hair loss, and long standing enlarge breasts. Examination revealed an evidence of hypogonadism with feminine feature, hair loss and large grade IV gynaecomastia (Rohrich *et al.*); large hypertropic breasts with severe ptosis and redundant skin. Testes size were 25 ml bilaterally.

Investigations

Karyotype 46,XY, total testosterone 4.4 nmol/l, LH 2.4 mU/l, FSH 2.9 mU/l, SHBG 28 nmol/l, and low calculated free testosterone 114 pmol/l confirming HH. Oestradiol ($E_2$) was elevated in female range 256 pmol/l and serum hCG $<1.2$ IU/l. The high $E_2$ with low testosterone indicates increased activity of aromatase enzyme that converts testosterone to $E_2$ resulting in HH and

gynaecomastia. Other pituitary function and Cushing's investigation were normal. Ultrasound breasts confirmed large bilateral gynaecomastia with no sinister feature.
Management
He was advised on calorie restricted diet and gentle physical activity and lost 6 kg of body weight in 13 weeks. Regarding HH, a trial of aromatase inhibitor was likely to provide more physiological reversal of hormones and potential improvement in gynaecomastia than conventional testosterone therapy. After detail discussion with the patient, he was started on aromatase inhibitor anastrazole 1 mg OD. At 6 weeks, his total testosterone normalised at 19.1 nmol/l with decreasing $E_2$ at 91 pmol/l. Therapy was planned to continue for 6 months to review effect of his gynaecomastia with a repeat ultrasound.
Conclusions
HH and gynecomastia add significant medical and emotional morbidities to already high-risk individuals with obesity. We demonstrated that a case of high aromatase activity as a cause and successful initial therapy with anastrazole, reversing the hormonal imbalance. Effect on gynaecomatia needs further follow up. With an increasingly obesogenic environment, this is likely become major health risk. A further randomise control trial is needed for long-term effect, dose and duration of therapy and safety in comparison with testosterone.

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## P77

**Phaeochromocytoma: experience from a single centre in South India**

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Phaeochromocytoma (PCC) and paragangliomas (PGL) are neuroendocrine tumours derived from the embryonic neural crest, located either within the adrenal gland or at extra-adrenal sites.
Objective
To study the clinical, biochemical, imaging characteristics, and mutational profile of patients with PCC/PGL treated at our institution.
Methods
We collected data of patients with PCC/PGLs managed at Christian Medical College, Vellore, India over a period of 21 years from 1994 to 2014 by review of medical records.
Results
This retrospective study comprised of 180 subjects with PCC/PGL (52% males and 48% females); with median age at diagnosis of 36 years (range 16–72 years). The classical triad of headache, palpitations, and sweating was observed in only 22% of patients. Hypertension was the presenting symptom in 140 (78%) and 62% had headache. Atypical presentations included abdominal pain (42%), fever (6%), and seizures (3%).
Prior to 2008, 24 h urinary vannilyl mandelic acid estimation was done, of which 73% had elevated levels. Urinary metanephrine (uMN) and normetanephrine (uNMN) estimation (from 2008 onwards) revealed elevated uMN alone in 24%, uNMN alone in 12% and both in 64%. The mean largest diameter of the tumours was 6.3 cm ($\pm$1.4 cm). $^{131}$I-MIBG scan was done in 137 patients (76%), among which 120 subjects (88%) showed increased uptake. Genetic testing for familial PCC/PGL was done in 50 subjects, of whom 16 (32%) had germline mutations in any one of the susceptibility genes – *RET4*, *VHL6*, *SDHB3*, and *SDHD3*. Malignant pheochromocytoma was present in 25 (14%) patients; two of these had *SDHB* and *SDHD* mutations. Surgical excision of the tumour/s was carried out in 176 (97%) patients. Of the remaining four patients, one patient was treated with $^{131}$I-MIBG therapy in view of metastatic disease, while the other three patients refused surgery.
Conclusion
This is the largest series of PCC/PGL reported till date in Indian patients. It highlights the unique clinical presentations and significant burden of familial cases in an Indian population.

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## P78

**Pituicytoma, not a 'meningioma': late recurrence in a rare pituitary tumour**

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Pituicytomas are rare tumours, originating from modified glial cells called pituicytes. The lineage of these tumours remains a topic of debate. Approximately 65 cases have been reported in the literature to date, since being formally recognised in 2007. We report a case where the final diagnosis was made after second surgery for late recurrence.
Case
A 54-year-old man presented with left sided visual disturbances in 1998 and underwent transcranial resection of a sellar/suprasellar lesion. Histology was atypical but was concluded as suprasellar meningioma following a second opinion. Surveillance MRI scan, 15 years later, revealed the previously stable small residuum to have grown into a sellar/suprasellar lesion. Tumour was removed endoscopically. The histology was compared with the 1998 sample and both were confirmed as pituicytoma, using newer immunohistochemistry methods.
Discussion
Pituicytoma was recognised as a distinct entity in 2000 and subsequently added to WHO-2007 classification of Tumours of Cranial Nervous System. Generally, these are slow-growing tumours. The presenting symptoms are due to mass effects, especially compression of optic chiasm. Clinical and imaging features are indistinguishable from other sellar tumours. This benign tumour is made up of elongated bipolar spindle cells in a rich capillary network. Immunostaining is often strongly positive for S-100, vimentin, and thyroid transcription factor 1 (TTF1), but negative for pituitary hormones and neuroendocrine markers like synaptophysin and chromogranin. TTF1 is helpful in distinguishing pituicytoma (TTF1 positive, as in this case) from meningioma, schwannoma, and pilocytic astrocytoma, which are TTF1 negative. Definitive management is total resection, often hampered by the hypervascularity. Recurrence within a few years after subtotal resection is well-documented. However, our case recurred 15 years after near-total resection. It is important to be aware that this highly vascular tumour has the potential to re-grow even after a decade. Long-term clinical, neuro-ophthalmic and MRI surveillance is essential.

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## P79

**Somatotroph adenoma localised with composite $^{11}$C-methionine PET/MRI: a cost effective solution**

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Background
Although, the diagnostic accuracy of magnetic resonance imaging (MRI) has increased in recent years, it may fail to detect secretory adenoma in a few patients with GH excess. Managing such patients with acromegaly can be challenging. We present a case where $^{11}$C-methionine PET/CT co-registered with SPGR/volume MRI proved to be helpful in identifying GH secreting pituitary adenoma.
Case history
A 42-year-old male was referred by surgeons with clinical features of acromegaly. He was 192 cm tall with large hands, significant prognathism and shoe size of 13. His only complaint was hyperhidrosis. He was normotensive with normal glucose tolerance. Visual field was intact and echocardiogram showed borderline LVH. Biochemical tests confirmed GH excess with random GH values between 20–30 μg/l and IGF1 103 nmol/l (reference range 13–37 nmol/l). Oral GTT confirmed autonomous growth hormone production (nadir GH 27.5 μg/l). Rest of the pituitary function tests were unremarkable. Post-contrast pituitary MRI, undertaken at two centers and reviewed at two others, revealed an enlarged, partially empty sella with no obvious surgical target. Medical management was initiated. Dopamine agonists did not have any significant impact. Prior to commencement of long-term somatostatin analogues, further imaging was arranged at a specialist center. $^{11}$C-methionine PET/CT co-registered with SPGR/volume MRI detected a resectable pituitary adenoma. He underwent endoscopic removal of the tumor. Immunochemistry confirmed somatotroph adenoma. Results of post operative IGF1 and dynamic GH testing are awaited.
Conclusion
This case highlights the utility of complex imaging modalities in localization and treatment of secretory adenomas of the pituitary gland which are not identified routinely by conventional techniques. In addition to possibility of achieving a cure, this approach has significant cost benefit bearing in mind that this gentleman in his 40's would have been committed to long term somatostatin analogue treatment.

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## P80

**Vitamin D resistant idiopathic hypoparathyroidism**

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A 30-year-old lady presented with three episodes of generalised seizures, paraesthesiae, irritability, and intermittent confusion since her first delivery 5 months ago. Over the past 3 weeks, she had developed exertional dyspnoea and easy fatigue. She was previously fit and well and the recent delivery was eventless. On examination, she demonstrated carpopedal spasm and other typical features of hypocalcaemia. Serum calcium was 0.88 mmol/l (2.10–2.55), magnesium 0.63 mmol/l (0.58–1.03), phosphate 2.3 mmol/l (0.8–1.4), and parathyroid hormone (PTH) 4.2 pg/ml (10.0–68.3). Echocardiogram showed severe left ventricular dysfunction. CT brain was normal. Diagnosis of idiopathic hypoparathyroidism was made and severe hypocalcaemia was considered to be the cause of encephalopathy, neuromuscular irritability, and cardiomyopathy. The patient was started on calcium supplements and α-calcidol and the doses were progressively increased to 6 mg and 4 μg respectively. She was supported with calcium gluconate infusion to control acute symptoms of confusion and seizures. Once the dose of α-calcidol was maximised, calcium infusion was slowly tapered off. However this led to prompt recurrence of symptoms requiring recommencement of i.v. calcium. Coeliac disease was excluded and she was commenced on s.c. PTH 20 μg twice a day. This led to clinical improvement and normalisation of serum calcium level with successful withdrawal of calcium infusion within a week. Her cognitive state normalised and a few weeks later left ventricular rejection fraction increased to 55%. Two months later a trial of withdrawal of PTH led to recurrence of hypocalcaemia (1.5 mmol/l), which normalised on recommencement of PTH therapy. She has now been advised to continue this indefinitely and has remained clinically well and eucalcaemic for the past year. This is an unusual case with only a few case reports in the literature on the use of PTH for hypoparathyroidism as most patients demonstrate an excellent response to calcium and vitamin D therapy.

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## P81

**Glycogenic hepatopathy: a tale infrequently told in the diabetes world**

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We present the case of a 27-year-old man who was first diagnosed with type 1 diabetes mellitus when he presented with diabetic ketoacidosis (DKA) and during later admissions was found to have grossly abnormal liver function tests. Following diagnosis, his diabetic control has been a challenge to manage despite the patient's claim he had been taking his insulin 'religiously'. He has had repeated admissions with DKA and has failed to attend clinic follow-ups on several occasions. Optimum support was offered by the diabetes specialist nurse and dietitian. However, the outcome remained unfavourable with poor glycaemic control and repeated hospital admissions with DKA.

Two years after being diagnosed with diabetes, liver function tests were found to be deranged during a hospital admission with DKA. Gross hepatomegaly was found on examination and confirmed on abdominal ultrasound. Screening for infective, metabolic, and autoimmune causes were all normal. A liver biopsy was performed under ultrasound guidance. Histopathology demonstrated features of pale swollen hepatocytes with glycogen deposition consistent with glycogenic hepatopathy. Improvements in glycaemic control correlated well with alteration in liver enzyme levels. Despite the grossly elevated liver enzymes and the substantial hepatomegaly the prognosis is good with progression of liver disease unlikely.

We are reporting this case to highlight an uncommon and often unrecognised cause of abnormal LFTs in individuals with poorly controlled type 1 diabetes. The features of this condition and theories of the pathogenesis are discussed illustrated by this case report.

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## P82

**Should all short Synacthen tests be agreed by an endocrine team?**

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Background

Short Synacthen tests (SSTs) are used to assess adrenal function by injecting tetracosactide and measuring blood cortisol after 30 and 60 min. Many SSTs at Royal Bournemouth Hospital (RBH) are undertaken externally to the Bournemouth Diabetes and Endocrine Centre (BDEC). There is an increasing cost-implication for undertaking SSTs exacerbated by recent problems with Synacthen supply. A 0900 h cortisol or random cortisol on acutely unwell patients can be sufficient for assessing adrenocortical function without the need for SSTs.

Method

We undertook a retrospective audit of 333 patients over 5 years that underwent SSTs at RBH. We assessed whether prior cortisol samples were undertaken (0900 h or random) and if a prior cortisol was >450 mmol/l, whether a SST was still undertaken. We also looked at whether non-BDEC patients were on steroids at time of testing.

Results

55% (182/333) of SSTs were initiated external to the endocrine department. Only 50% of patients (169/333) had a prior cortisol measurement whether it was 0900 h or random. 20% (35/169) of these were >450 mmol/l and therefore could have avoided a SST. Of those without a prior cortisol the baseline cortisol was >450 mmol/l in 45% (75/164) and a SST could have been avoided. 19% (25/130) of non-BDEC patients were taking steroid therapy (steroid inhaler/nasal spray/kenalog injection) during the test and in many cases this was multiple different steroids.

Discussion

At RBH more than half of SSTs are undertaken by non-endocrinologists and many tests could potentially be avoided by undertaking a 0900 h cortisol. One in five of the tests performed outside the endocrine department were not initiated appropriately with patients being on steroids at time of testing. Discussing all future SSTs with BDEC could improve performance, reduce cost, and prevent unnecessary testing.

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## P83

**Artifactual hypoglycaemia: lesson from a patient with unreliable finger-prick glucose due to absent finger tips**

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Pseudohypoglycemia has been reported in patients with Raynaud's phenomenon, peripheral vascular disease and shock and may result from increased glucose extraction by the tissues because of low capillary flow and increased glucose transit time. We present a frail and comorbid 76-year-old lady with a long standing history of Rheumatoid arthritis/systemic sclerosis/systemic lupus erythematosis overlap syndrome complicated by Raynaud's phenomenon. She was on long-term steroids and various disease modifying drugs over this time period (including hydroxychloroquine). She has no personal or family history of diabetes mellitus. Over the last 18 months, during admissions for various intercurrent problems (urinary infection, colitis, and routine monitoring), she was observed to have very low capillary glucose readings (lowest 0.9 mmol/l) not always with suggestive symptoms or signs. Insulin and C-peptide during one of these episodes of significant hypoglycaemia was in keeping with endogenous hyperinsulinism (capillary glucose 2.3 mmol/l, C-peptide 2361 pmol/l, and serum insulin 56.1 mU/l, although, regrettably, a concurrent venous glucose was not sent). Computed tomography of the pancreas was negative for any obvious insulinoma. Hydroxychloroquine was stopped as a potential cause but hypoglycaemia recurred within six months again picked up during monitoring for intercurrent infection of chest (lowest capillary glucose reading 0.6 mmol/l). The patient remained asymptomatic throughout the hypoglycaemic episodes. It was during this time that the junior doctor noted auto-absorbed finger tips and considered pseudohypoglycemia as possible factor. Discrepant capillary blood glucose readings between the two hands and with corresponding venous glucose were confirmed. It is important for clinicians to recognize and understand this condition to avoid unnecessary investigations and treatment of unsuspected hypoglycaemia.

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## P84

**Decreased conscious level and a renal mass**

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A 70-year-old lady with known hypothyroidism and ischaemic heart disease (previous CABG) presented with tonic clonic seizures. Her BP was 245/110. She was admitted to the intensive care unit. MRI brain showed subcortical oedema with subarachnoid bleed in the left temporal parietal region. Investigation for malignant hypertension showed a solid mass measuring 17×17×11 cm completely replacing the left kidney on CT-scanning. She was discharged home on sodium valproate with a plan for a radical left nephrectomy.

One morning, 2 months later, her husband could not rouse her. No seizure activity was witnessed. The paramedic team found her to be hypoglycaemic (near patient blood glucose reading of 1.4 mmol/l). She was taken to the emergency department and discharged home on the same day. Two days later, her husband found her confused, sweating with incomprehensible speech. She also had haematuria on this occasion. Her blood glucose was 1.8 mmol/l. She was admitted for further investigations. She experienced spontaneous hypoglycaemia during the night and in the morning and blood samples were taken for insulin and C-peptide levels. She was provided with a glucometer and given dietary advice on how to avoid hypoglycaemia.

Her blood results came back showing low insulin (<2 mIU/l) and C-peptide (<33 pmol/l) levels. Further investigations showed an IGF2:IGF1 ratio of 16 (<10). This overproduction of IGF2 supports a diagnosis of non-islet cell tumour hypoglycaemia (NICTH).

NICTH is a rare but serious paraneoplastic complication of malignancy. Most commonly there is tumoural overproduction of incompletely processed IGF2, which results in stimulation of the insulin receptors and increased glucose utilisation. Other potential but less common causes include the production of autoantibodies against insulin or the insulin receptor and extensive hepatic or adrenal infiltration. Treating the underlying malignancy is the mainstay of treatment, but other options include glucocorticoids to prevent recurrent hypoglycaemia.

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## P85

**An index case of MEN1 identified with imaging of the right thigh**

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We present a 33-year-old lady who was diagnosed with primary hyperparathyroidism in 2004 (aged 22). Investigations showed parathyroid hyperplasia and she underwent a parathyroidectomy of three glands with one parathyroid gland implanted into each thigh. She then presented in 2013 with symptoms in keeping with hypercalcaemia. Initial investigations included ultrasound of thyroid and parathyroid glands which showed no evidence of parathyroid adenoma and incidental tiny cysts on the thyroid gland were the only positive finding. She was, however, found to have a functioning parathyroid adenoma of the gland in her right thigh identified on SPECT/CT scanning. This was confirmed with MRI. Interestingly, she went on to have the gland successfully removed by an ENT surgeon. Investigations at the time of re-presentation found her to be positive for MEN1 (c-537G>C heterozygous substitution). This was the index case for a family of patients with MEN1 in the south of Glasgow.

Baseline investigations at time of diagnosis identified a hypervascular tumour on MRI in the distal body of the pancreas, suspicious of an insulinoma. Gut profile testing was satisfactory, and fasting glucose levels were normal. MRI of the brain showed a cystic pituitary macroadenoma, which she remains under surveillance of, as her visual fields are normal. Pituitary axis testing remains within normal limits, other than a slightly raised prolactin level (957 m/Ul).

As well as an interesting presentation of recurrent hypercalcaemia with a final diagnosis of MEN1, this case raises an important discussion point. All young people presenting with primary hyperparathyroidism should be screened for MEN (1 and 2). Diagnosis in this lady allowed a family of MEN1 sufferers to be identified through genetic testing.

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## P86

**Four years of tolvaptan: experience from two large teaching hospitals**

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Introduction

Hyponatraemia is a common cause of morbidity and mortality affecting 15–28% of inpatients. Tolvaptan is a competitive vasopressin 2 antagonist licensed to treat hyponatraemia secondary to the syndrome of inappropriate anti-diuretic hormone secretion (SIADH). There has been concern that tolvaptan treatment may lead to rapid overcorrection of sodium in a minority of patients, thus potentially leading to osmotic demyelination syndrome.

Methods

We conducted a retrospective case note audit of tolvapatan use from May 2010 to September 2014.

Results

Seventeen patients were identified as having been prescribed tolvaptan; eight were males, nine were females (mean age 78.6 years ± 8.5 S.D.). One male patient had a diagnosis of heart failure; the remaining 16 patients had a diagnosis of SIADH. The starting dose of tolvaptan was 15 mg in 16 patients; however, one patient was admitted on 60 mg. The mean sodium prior to starting treatment was 118.7 mmol/l. The mean sodium 48 h post prescription was 125.8 mmol/l. 12 out of 17 patients (71%) had appropriate investigations (cortisol, TSH, osmolalities, and urinary sodium) prior to prescription. Two out of 17 patients (12%) had adequate serum sodium monitoring at recommended 6 h intervals, per BNF guidance, between 0 and 48 h after starting tolvaptan. Three out of 17 patients (17%) exceeded the recommended limits of sodium change. There was however no neurological sequelae to suggest osmotic demyelination.

Conclusions

Tolvaptan is efficacious for the treatment of hyponatraemia. There were no documented episodes of harm from rapid sodium overcorrection in our sample size. The potential for over correction can be minimised by vigilant monitoring and anticipatory corrective action. A model for optimisation of serum sodium monitoring on an in-patient basis is suggested involving careful coordination between pharmacy, laboratory medicine, and medical teams.

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## P87

**A single-centre experience of adrenal vein sampling in a District General Hospital serving a remote and rural population**

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Background

Given the technically challenging nature of adrenal venous sampling (AVS) there is a drive to centralise services to improve successful outcomes. This has potential implications for patients living in remote and rural areas.

Methods

We retrospectively reviewed the case notes of 15 patients who underwent AVS in our hospital, for investigation of primary hyperaldosteronism between 2002 and 2015. We assessed the success rate of cannulation of the right adrenal vein (i.e., an adrenal:peripheral vein cortisol ratio >3.0 in basal state and >5.0 following ACTH stimulation) and evaluated whether the care provided is in accordance with the guidelines of the Endocrine Society 2008.

Results

Two radiologists performed a total of 19 AVS in 15 patients, with three individuals undergoing repeat procedures. The mean age at time of AVS was 50 ± 11 years, with 10 (67%) males and 5 (37%) females. 11 (73%) patients had been referred to the Endocrine Clinic from primary care and 4 (27%) via the renal clinic. All patients had a raised aldosterone:renin ratio, but only 7 (47%) underwent confirmatory testing with a saline suppression test. This increased to 75% after 2008. Morphology of the adrenal glands was normal on CT in 10 (67%) patients, whilst lesions were identified in the right adrenal gland in 3 (20%), and the left adrenal gland in 2 (13%) cases (nodule mean size 15.4 mm). Cannulation of the right adrenal vein was successful in 73% of procedures and localised the diagnosis to a unilateral adenoma in 33%, while 67% of patients were diagnosed with bilateral hyperplasia. All patients with unilateral adenomas underwent adrenalectomy. Cannulation of the left adrenal vein was successful in 14 (94%) cases.

Discussion

Despite the relatively low numbers of patients, the rate of successful cannulation of the right adrenal vein was reassuring and compares favourably to national data, with a success rate of 63%. Arguably better success rates could be achieved if

services were centralised and national success rates improved. However, the additional travel time (potentially 3–6 h drive one way) has implications for our clinic population.

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## P88

**Haemofiltration as a treatment option in refractory life-threatening diabetic ketoacidosis**

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Background
Treating life-threatening diabetic ketoacidosis (DKA) with a pH of $<6.9$ is extremely challenging and often refractory to treatment using standard fixed dose insulin DKA management protocols which may not work effectively at this low pH because of increased insulin resistance. I.v. bicarbonate ($HCO_3$) use in this situation can be considered but remains controversial due to the risk of significant side effects as well as limited evidence in literature. Here we attempt to describe a case of fulminant DKA without renal failure, where treatment with haemofiltration (HF) for severe metabolic acidosis was successful.
Case history
A 23-year-old female with history of recurrent episodes of DKA and poor diabetes control secondary to non-compliance, presented to the emergency department via ambulance after being found collapsed and had successful cardiopulmonary resuscitation for pulseless electrical activity and was subsequently treated with standard DKA protocol.
Investigations on admission
pH 6.752, $HCO_3$ 1.3, lactate 3.1, base excess $-30$, blood glucose 45 mmol/l, blood ketones 6 mmol/l, creatinine 133 mmol, urea 10.8 mmol, and eGFR 43.
Treatment
Despite maximal DKA treatment over three hours, including 5 l of i.v. fluid, and maximum fixed rate i.v. insulin at 15 units/h, she continued to be in severe metabolic acidosis with pH 6.772, $HCO_3$ 1.7, ketones 5, and blood glucose 40.1, without any improvement in her Glasgow coma scale of 8. Options were discussed at length with critical care and endocrine teams regarding use of i.v. bicarbonate therapy vs HF. She was then put on HF which resolved the metabolic acidosis completely within 12 h.
Discussion
Our patient responded to HF with resolution of severe metabolic acidosis. There are no guidelines at present that compare either bicarbonate therapy vs HF for such patients, but HF may be considered as a potentially safe and viable option to correct persistent metabolic acidosis in refractory fulminant DKA.

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## P89

**Adrenal insufficiency caused by bilateral primary adrenal lymphoma**

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Primary adrenal lymphoma is rare and accounts for $<1\%$ of extra-nodal non-Hodgkin's lymphoma cases. It presents with B symptoms, bilateral adrenal masses and hypoadrenalism; and thought to have a poor prognosis. We describe a patient that was diagnosed with and successfully treated for primary adrenal diffuse large B cell lymphoma (DLBCL).
Case
A 62-year-old gentleman with longstanding hypertension presented with a 5-month history of weight loss, fatigue, sweats, and a 1-day history of vomiting and abdominal pain. He was tachycardic, normotensive, and had right flank tenderness but no palpable lymphadenopathy or organomegaly. Admission bloods showed acute kidney injury with eGFR 40 ml/min, hyponatremia (127 mmol/l), hyperkalemia (5.6 mmol/l), and CRP 428 mg/dl. I.v. antibiotics were started for presumed pyelonephritis. Subsequent CT abdomen revealed bilateral necrotic adrenal masses measuring 10.8 and 9.4 cm in their maximal diameter with retroperitoneal lymphadenopathy. The largest nodes were a $35\times 20$ mm aortocaval node and $40\times 32$ mm para-aortic node. 24-h urinary catecholamines and 24-h urinary free cortisol were within normal limits but short Synacthen test confirmed adrenal insufficiency with a baseline cortisol of 230 nmol/l rising to 242 nmol/l. He was commenced on hydrocortisone. Biopsy of the right adrenal mass was performed which showed diffuse lymphoid infiltrates, large pleomorphic cells, and prominent nucleoli positive for CD20, BCL2, BCL6, and MUM1. He was diagnosed with stage 2 BCL2-positive DLBCL. He underwent six cycles of R-CHOP chemotherapy with remission of symptoms and resolution of the masses and lymphadenopathy on CT. Four years later, he remains disease-free but still requires glucocorticoid replacement.
Conclusion
Primary adrenal lymphoma is rare but should be considered in patients presenting with bilateral adrenal masses and B-symptoms. Poor outcomes are reported but our patient has shown an excellent medium-term response to R-CHOP chemotherapy.

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## P90

**Congenital adrenal hyperplasia: are we really lost in transition?**

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Background
Congenital adrenal hyperplasia (CAH) is an autosomal recessive disorder characterised by impaired cortisol synthesis. In the CaHASE study, a surprisingly low number of cases were identified as attending adult endocrine clinics. It has been suggested that patients with CAH are lost to follow up around the time of transition to adult services.
In our service, there is a transition clinic that has a lead paediatric and adult Consultant who attend all clinics. The first clinic in the adult hospital is attended by both adult and paediatric teams. There is specialist nurse support and all patients are offered transfer to a single adult centre. We hypothesised that this structure would improve clinic attendance.
Methods
Electronic records of patients with CAH who underwent transition to the adult service between 2010 and 2015 were examined. Patients seen within 1 year of transition were considered as having transferred. Those not seen in the last 18 months were considered to no longer attend the clinic.
Results
Fourteen patients were identified (nine female). Of those, 11 (78%) continue to attend the clinic in the adult sector (range 3 months–3 years post transition). Of those with no ongoing follow up, one has left the area and the status of two is unknown. Of those two, both missed their initial adult clinic appointment.
Conclusion
For patients with CAH, clinic attendance in the early years following transition in Glasgow appears to be reasonably good, and better than reported in some other centres. This may be due to the design of the clinic. Clinic non-attendance around transition may predict later difficulties with follow up. Future studies might attempt to identify factors that determine clinic attendance and service engagement in patients outwith the transition period.

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## P91

**Audit of Plenadren use in selected patients**

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Background
Patients with adrenal insufficiency sometimes complain of excessive weight gain or feeling unwell on standard hydrocortisone despite dose optimisation. Prednisolone can be used in adrenal insufficiency but has a more prolonged duration of action and blood tests to monitor prednisolone levels are not readily available. Conventional glucocorticoid replacement can lead to over-replacement particularly in the evening which is thought to increase the risk of obesity. Plenadren is a modified dual release preparation of hydrocortisone designed to be taken once daily. In a study of 64 patients with primary adrenal insufficiency, it was found to have a more physiological cortisol day profile compared to thrice daily conventional hydrocortisone. In another study, average weight loss at end of 12 weeks therapy was 0.7 kg compared to standard hydrocortisone. There was significant improvement in systolic and diastolic blood pressure readings.
Audit aim
To ensure that Plenadren was used in appropriately selected patients and assess benefit of therapy.

Results
Eight patients were commenced on Plenadren between May 2013 and December 2014. One patient discontinued Plenadren as they felt worse on it. There was an average weight loss of 1.96 kg with Plenadren therapy (6 months data in five patients and three months data in two patients). More impressive weight losses occurred in those prescribed Plenadren in view of excessive weight gain (average loss 5.4 kg excluding a patient with depression/comfort eating). Hypoglycaemia improved significantly in a patient with longstanding type 1 diabetes negating requirement for insulin pump therapy. Four hours post morning hydrocortisone cortisol levels were generally better on Plenadren. One patient with Addison's disease AddiQol score improved from 50 to 90 and another's score worsened slightly from 102 to 92. Overall, there was a drop in systolic blood pressure but slight increase in diastolic.

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## P92

**Clinical practice, governance, and case reports: hypovitaminosis D as a cause of severe hypocalcaemia in a Nigerian female**

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Background
Severe hypocalcaemia is a life threatening metabolic emergency which must be identified and treated properly to prevent mortality.
Objective
To highlight a rare finding of severe hypocalcaemia due to hypovitaminosis D in a Nigerian female on anti-tuberculous therapy.
Case presentation
A 30-year-old Nigerian female who wears purdah presented to the medical emergency on account of perioral numbness, generalised muscle spasms of a week, with generalised malaise, difficulty with breathing, palpitation, and significant weight loss. She had been on first line anti-tuberculous agents for 32 days earlier following diagnosis of pulmonary tuberculosis complicated by massive left sided pleural effusion which has been drained once. Physical examination revealed an asthenic woman, BMI of 17.0 kg/m$^2$, left axillary lymphadenopathy. Chest examination revealed features in keeping with left massive pleural effusion. She had positive chvostek and Trousseau's signs. Investigations revealed haemoglobin of 11 g/dl, hypomagnaesemia (0.34 mmol/l), low total calcium (1.66 mmol/l), low serum albumin (25 g/dl), low serum vitamin D (8.4 ng/ml), and normal alkaline phosphatase (116 μ/l). A diagnosis of severe hypocalcaemia due to severe vitamin D deficiency was made on a background of complicated pulmonary tuberculosis. She was treated with multiple intravenous calcium and magnesium, oral calcium, and calcitriol. She had chest tube drainage for the massive left pleural effusion, continued on anti-tuberculous therapy. Her clinical state improved and was discharged home in good condition.
Discussion
This metabolic medical emergency is not commonly seen. It is sometimes misdiagnosed as a seizure disorder when patients present with generalised muscle spasms as in this index case. The causes of hypovitaminosis D in the index patient are prolonged use of purdah and rifampicin use. Other causes of hypocalcaemia were excluded.
Conclusion
Prolonged use of purdah dressing is a risk factor for hypovitaminosis D in our patient who developed overt clinical hypovitaminosis D resulting from the potentiating effect of rifampicin used for the treatment of her tuberculosis.

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## P93

**Evaluation of use of combination dapagliflozin and GLP1 agonist treatment in type 2 diabetes**

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Background
Dapagliflozin was the first SGLT2 inhibitor licensed in UK to improve glycaemic control in type 2 diabetes. Whilst it has not been studied for use in combination with GLP1 agonists the modes of action of the two therapies suggest they would be a logical therapeutic combination. We aimed to evaluate clinical experience with this regimen.
Methods
Observational retrospective data was collected from electronic patient records (SCI–diabetes) on all patients prescribed dapagliflozin as an addition to GLP1 agonist across two Scottish health board areas. HbA1c, weight, BP, cholesterol, HDL, ALT, AST:ALT ratio, and eGFR change at 6–18 months were compared to baseline. Discontinuation of therapy and adverse events were recorded.
Results
Eighty-five patients were included (47 males, mean age mean diabetes duration 13 years). All eGFRs were $>60$ on initiation. 74 patients were on liraglutide at initiation and 11 on exanitide preparations. Five patients (8.5%) stopped dapagliflozin during study period. Duration of therapy studied was between 3 and 18 months. Median HbA1c (mmol/mol) at initiation was 76 (IQR 67.5–88) and at 12 months was 64 (56–71.5) ($P \leq 0.001$). Baseline Systolic BP was 132 mmHg (IQR 120–143) and at 12 months was 128 mmHg (120–135). Baseline diastolic BP was 79 mmHg (73–83) and at 12 months was 77 mmHg (72–86) ($P$=NS). Weight was 107.8 kg (IQR 90.4–121.4) at baseline reducing to 102.7 kg (IQR 86.4–120) at 12 months ($P$=NS). No significant change in cholesterol, HDL, ALT, AST:ALT ratio, and eGFR were found post treatment. Adverse events documented included genital mycosis in 9/85 (9.4%) and volume depletion 4/85 (4.7%).
Conclusions
The addition of dapagliflozin to GLP1 therapy appears to effectively reduce HbA1c in type 2 diabetes patients. A non-significant reduction in weight and BP was found. The addition of dapagliflozin to GLP1 therapy offers promise for treatment of type 2 diabetes.

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## P94

**Do guidelines improve the diagnosis and investigation of hyponatraemia?**

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Introduction
Hyponatraemia is common and associated with significant morbidity and mortality. However, it's often not recognised as a serious diagnosis and therefore inadequately investigated and poorly managed. An audit of the management of patients admitted to The Great Western Hospital (GWH) with hyponatraemia confirmed these problems. A hyponatraemia guideline was produced in order to improve diagnosis, investigation, and management of patients with hyponatraemia.
Methods
The guideline was piloted in the Acute Medical Unit at GWH. Following a teaching session and introductory period, a re-audit was performed. Data was collected retrospectively in patients with an admission sodium of $\leq 127$ mmol/l, over a 1-month period. The aim was to identify whether introduction of a guideline improved diagnosis, investigation, and management of patients.
Results
Twenty patients were included; eight males and 12 females. The mean age was 64 years (range 25–88 years). The mean sodium was 119 mmol/l; range 108–127 mmol/l. Following the teaching session and introduction of the guideline, 95% patients had a diagnosis of hyponatraemia documented compared to 65% of patients in the original audit. Eight investigations were identified as essential investigations in hyponatraemia. 40% had greater than or equal to seven investigations compared to only 13% in the original audit. 55% of patients had a urine sodium, urine osmolality, and serum osmolality requested compared to 27% in the original audit. 35% of patients were either seen by the endocrine team compared to 4% in the original audit.
Discussion
This audit demonstrates that the use of education and a guideline has improved recognition of hyponatraemia. It has resulted in an increase in the number of relevant investigations requested and in the number of patients reviewed by the endocrine team. Hopefully, this has led to an improvement in the management of patients with subsequent reduction in morbidity, mortality and length of stay.

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## P95

**Diaphoresis: an unusual initial presenting complaint of Cushing's syndrome**

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Introduction
Diaphoresis, or excessive sweating, is well recognised as a presenting complaint for endocrine disorders such as hyperthyroidism, acromegaly, and phaeochromocytoma. However, diaphoresis is an unusual presenting complaint for Cushing's syndrome.
Case
We present the case of a 35-year-old lady who first presented to health services for symptoms of excessive sweating, and feeling hot most of the time. Whilst initial investigations such as thyroid hormone measurement did not reveal a cause, she subsequently developed classical symptoms of Cushing's syndrome including weight gain with increased central adiposity and a noticeable change in her facial appearance. She also developed hirsutism and proximal myopathy in her legs. She was diagnosed with hypertension 2 years after her initial presentation, and developed easy bruising and thinning of the skin. A low dose dexamethasone suppression test was consistent with pituitary dependent Cushing's syndrome, with an ACTH of 46ng/l and cortisol of 780 nmol/l at baseline, which did not suppress with a cortisol level of 136 nmol/l at 48 h. Inferior petrosal sinus sampling confirmed a pituitary source of ACTH secretion and a pituitary MRI scan identified a low signal lesion in the antero-inferior aspect of the gland thus surgical removal was instigated.
Discussion
Whilst diaphoresis is a common symptom of several endocrine disorders, it is an uncommon presenting complaint for Cushing's syndrome. In this case, the patient was not investigated for the Cushing's syndrome until other more classical symptoms arose almost 2 years later. Diaphoresis is a recognised, but uncommon symptom in the presentation of Cushing's syndrome. Patients presenting with diaphoresis should prompt evaluation for more classical features of Cushing's syndrome.

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## P96

**An unusual case of 'adrenal' Cushing's**

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Background
Phaeochromocytoma represents a rare cause of hypercortisolism, accounting for $<5\%$ of ectopic Cushing's syndrome while $<1\%$ of phaeochromocytomas are accompanied by Cushing's syndrome.
Case presentation
We present a case of a 51-year-old lady admitted to the surgical ward for bowel surgery for severe long-standing constipation. She was referred to the endocrine team for recent-onset severe hypokalaemia and the presence of left-sided lipid-rich adrenal adenoma on abdominal CT scan. She had 10-year history of episodic headaches, flushing, sweating, palpitations, and hypertension. Type-2 diabetes diagnosed 6 years ago was sub-optimally controlled on maximal oral therapy. On examination, she did not have typical cushingoid appearance although she did have facial hirsutism, marked proximal muscle weakness, and extensive oral candidiasis that affected her oral intake and nutrition.
Investigations
Initial tests revealed severe hypokalaemia (2.4 mmol/l) and hypercortisolism (2089 nmol/l), which was unsuppressed by 1 and 8 mg dexamethasone. ACTH level was 855 ng/l suggesting ACTH driven Cushing's syndrome. Androstenedinone level was disproportionately raised (126 nmol/l). Urinary catecholamines and fractionated metanephrines were raised 30-fold. $^{131}$I MIBG scintigraphy was negative but $^{68}$Ga-DOTA-PET–CT scan confirmed that the left adrenal mass was the source of excessive catecholamine production with no evidence of metastasis.
Diagnosis
In view of severe and non-suppressible hypercortisolaemia, high ACTH, elevated catecholamines, and an adrenal mass with a positive PET scan a diagnosis of phaeochromocytoma with ectopic ACTH production was made.
Management
Over the next few weeks she was managed with oral potassium, spironolactone, insulin and block-replacement therapy with metyrapone and dexamethasone. She underwent adrenalectomy following adequate preparation with a combination of phenoxybenzamine and propranolol. Histology confirmed the diagnosis of phaeochromocytoma, which stained positive for ACTH on immunochemistry. Post adrenalectomy she remained normotensive without medication, her constipation resolved, and glycaemic control was excellent on single oral agent. 24-h urine for total catecholamines, metanephrines, returned within normal limits. Hypercortisolism resolved although HPA axis remained suppressed 6 weeks after surgery requiring continuation of replacement steroids.

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## P97

**Accuracy of sample timing with short Synacthen tests at Royal Bournemouth Hospital**

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Background
Short Synacthen tests (SSTs) are used to assess adrenal function by injecting tetracosactide and measuring blood cortisol after 30 and 60 min. Accuracy of timing helps enable successful interpretation of results. A SST is an invasive test although it is normally well tolerated. There is an increasing cost-implication for undertaking SSTs exacerbated by recent problems with Synacthen supply.
Method
We undertook a retrospective audit of 333 patients over 5 years that underwent SSTs at Royal Bournemouth Hospital. We assessed for accuracy of sample timing allowing a 10 min 'sample window' for both samples, i.e. between 25 and 35 min after injection for the first sample and between 55 and 65 min after injection for second sample.
Results
Two tests were excluded from analysis as no sample timing was recorded. 57% (190/331) of SSTs were performed incorrectly with poor sample timing. 46% (151/331) of first samples were outside of the 'sample window' with most of the samples being taken late (median time to test 35 min, range 13–197). There was no second sample in 17% (57/331) of tests. If the first sample was taken late the 30 min gap between the two samples was maintained in the majority of cases leading to delayed second sampling. 47% (129/274) were outside of the second 'sample window' again with the majority being taken late (median time to test 65 min, range 30–198).
Discussion
The majority of SSTs done at RBH are performed incorrectly and this has an impact on the interpretation of these tests. Approximately £2000 was spent analysing cortisol levels and injecting Synacthen in these tests. Discussing all future SSTs with the endocrine team could improve performance and reduce cost.

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## P98

**Hyponatremia: an audit of the initial investigation and management**

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Background
Hyponatremia is the commonest electrolyte abnormality encountered in clinical practice. It is associated with increased mortality and prolonged length of stay. Errors in establishing the aetiology of hyponatremia can lead to inappropriate treatment with adverse outcomes. An accurate diagnosis requires a careful clinical and biochemical assessment. An audit was undertaken to determine current practice at University Hospitals Bristol.
Method
A comprehensive retrospective review of case notes and the laboratory database was conducted for all medical patients admitted between 1/1/13 and 31/3/13, presenting with a serum sodium $<130$ mmol/l. The following standards were used to benchmark quality of care: i) laboratory alerts for very severe hyponatremia, ii) the diagnostic approach as compared against the European Society of Endocrinology Guidelines, iii) communication of hyponatremia management on the discharge summary; and iv) the rates of re-admission/mortality.

Results
Thirty-eight cases of moderate (125–130 mmol/l, $n=19$) or severe ($<125$ mmol/l, $n=19$), hyponatremia were identified. Local laboratory standards for alerting very severe hyponatremia were achieved in 83% (10/12). The aetiology of hyponatremia was determined in only 45%; glucose, TFTs, cortisol, and urine/plasma osmolalities were requested in 68, 50, 18, and 24% respectively. Where SIADH was suspected, 78% had an incomplete biochemical assessment. Only 42% of discharge summaries clearly communicated the diagnosis and management of hyponatremia. Coding data demonstrated that of patients coded 'hypo-osmolality and hyponatremia' during this 3-month window, 29% died within 12 months, 25% were readmitted to hospital within 28 days, and 55% within 1 year.
Conclusion
Hyponatremia assessments in the acute setting are often incomplete. Incorrect evaluation and management may be associated with prolonged in-patient stay and increased re-admission rates in an already vulnerable group of patients with a high background mortality risk. Early specialist input, clarity of diagnosis, and robust communication across the healthcare community could improve outcomes, patient experience and be cost effective overall.

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## P99

**A case of haemorrhagic adrenalitis due to streptococcus oralis**

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A 57-year-old female who was previously healthy was admitted to hospital with a 2-week history of abdominal pain. On admission, she was tachycardic, tachypnoeic, hypotensive, and had abdominal tenderness. Her initial investigations revealed a neutrophilia with her biochemistry showing hyponatraemia (sodium of 124 mmol/l (135–145)) and normokalaemia (potassium of 4.7 mmol/l (3.5–5.5)). She had an urgent abdominal CT scan which showed evidence of a tubo-ovarian abscess for which she underwent emergency laparascopic surgery. The CT scan also revealed bilaterally bulky adrenal glands. Following her surgery, she was admitted into the intensive care unit where despite improvement in her inflammatory markers, she had persistent hypotension. She was empirically started on hydrocortisone with an improvement in her blood pressure and sodium. Microbiological analysis of the pus within the ovarian abscess revealed streptococcus oralis as the responsible pathogen. She then had a short Synacthen test (with prior temporary withdrawal of hydrocortisone) which showed evidence of primary adrenal insufficiency with cortisol readings of 195, 208, and 207 nmol/l ($>550$), respectively, at 0, 30, and 60 min (following 250 μg of i.m. tetracosactide) and a concurrent ACTH reading (at time 0) was 56 ng/l (0–46). Her measured anti-adrenal antibodies were negative. A subsequent CT of her adrenal glands showed cystic attenuation of the glands suggesting atrophy following earlier haemorrhage of the gland. She was discharged from hospital on replacement doses of hydrocortisone after having received intensive education. This case demonstrates the development of Waterhouse Friederichsen syndrome (haemorrhagic adrenalitis) due to *Streptococcus oralis* and even though the typical association has historically been with *Neisseria meningitides*, it alerts clinicians to the possibility of other pathogens. It emphasises the need to search for adrenal insufficiency in patients with refractory hypotension even in the context of septicaemia.

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## P100

**Xerostomia: an unseen consequence of statin use**

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Xerostomia can be classified into two different types. One type is associated with severely reduced salivary secretion. The other involves normal salivary function yet reduced viscosity of saliva or reduced mucin concentration within the saliva, a common finding in elderly people. Studies show that xerostomia is the most common oral side-effect of cardiovascular medication. Statins are common cardiovascular drugs used to treat hypercholesterolemia. A 55-year-old Asian lady, non-smoker, and a teetotal with bilateral xanthelasma was commenced on simvastatin in August 2011 due to hypercholesterolemia and elevated lipoprotein a. She was initially started on nicotinic acid as it is the most effective treatment for elevated lipoprotein a however as it caused her adverse side effects it was stopped and she was subsequently commenced on 20 mg simvastatin, increased to 40 mg after 4 weeks. Unfortunately she started to develop oral dryness, within a few weeks. This was present throughout the day, as well as the night. Owing to reduced salivary flow rate as a result of snoring and mouth breathing during sleep, nocturnal oral dryness is a normal entity, yet day-time oral dryness is a finding associated with systemic diseases such as Sjögrens disease, diabetes, vitamins A and E deficiencies for which she was negative or pharmaceutical drug use, one of which are statins. She was temporarily changed from simvastatin to atorvastatin due to persistently high LDL cholesterol and the high risk of both CVD and PVD associated with high lipoprotein A. atorvastatin was also stopped for 4 weeks and it substantially did help to minimize her xerostomia. Unfortunately side effects such as xerostomia remain unreported by patients owing to their perception as a minor side effect. This indicates that studies underestimate its true prevalence in statin treatment therefore more studies are required on the association between statins and xerostomia.

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## P101

**A complex case of MEN1**

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Objective
We report the rare case of a 45-year-old male with multiple endocrine neoplasm 1 (MEN1) with simultaneous occurrence characteristic tumours leading to a delicately balanced metabolic homeostasis.
Methods
Clinical, biochemical, and radiological data of this patient diagnosed to have MEN1 were analysed and the challenges in the management of these neuroendocrine tumours are discussed.
Case illustration
A 45-year-old gentleman presented with hip pain, acral enlargement, erectile dysfunction, and early morning hypoglycaemic symptoms. He was diagnosed to have MEN1 with primary hyperparathyroidism, acromegaly due to GH and prolactin co-secreting Hardy's grade E pituitary macroadenoma, pancreatic neuro-endocrine tumours (PNETs) with biochemical evidence of both hyperinsulinaemic hypoglycaemia and hypergastrinemia. He also had collagenomas, lipomas, and non-functioning thymic and bilateral adrenal adenomas. He had a delicate metabolic homeostasis due to GH and insulin counterbalancing the glucose and; parathormone and GH counterbalancing serum phosphate. In view of limited mobility due to hip pain, proximal myopathy, and secondary osteoporosis he first underwent three and 1/2 gland parathyroidectomy in 2012. Acromegaly, hyperprolactinemia, and the PNETs were initially medically managed with octreotide LAR and cabergoline. Subsequently, in 2013 he underwent subtotal excision of the pituitary macrodadenoma. After pituitary surgery he had worsening of hypoglycemic episodes despite diazoxide therapy. Hence he underwent distal pancreatectomy with enucleation of PNETs in the head and uncinate process of pancreas, excision of duodenal (D2) submucosal tumours and omental lymphnode dissection. Histopathology confirmed multiple NETs in pancreas, duodenum and lymphnodes, with tumour immunopositivity for pancytokeratin, synaptophysin, and chromogranin (MIB1 index 2–3%). Following the resection of NETs, his GH levels declined; however he was continued on cabergoline for persistent hyperprolactinaemia.
Conclusion
The present complex case of MEN1 highlights the challenges in the management; necessitating a multidisciplinary team approach in view of the delicately balanced homeostasis, multicentric tumours, and risk of recurrence.

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## P102

**Hyperalphalipoproteinaemia in epileptic patient: cardiovascular protection from carbamaezipine use?**

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Background
HDL is a plasma lipid-protein complex of lipids and alphalipoproteins (apolipoproteins A-I and A-II). It is involved in reverse transport of cholesterol from peripheral tissue to liver, allowing cholesterol degradation. HDL cholesterol (HDL-C) levels are inversely associated with cardiovascular risk. HDL-C levels can be elevated due to genetic causes or drugs. We present a case where significantly raised HDL levels caused some concern at the outset.
Case
A 66-year-old female was referred to lipid clinic for elevated cholesterol. She had epilepsy, well-controlled on carbamazepine. There were no peripheral stigmata of hyperlipidaemia. She had no family history of premature coronary artery disease; calculated cardiovascular risk was low. Full fasting lipid profile showed total cholesterol 9.7 mmol/l (3.5–5.0), triglycerides 2.1 mmol/l (0.8–1.8), LDL 5.2 mmol/l (1.5–4.0), and HDL 3.5 mmol/l (1.0–2.10). The total cholesterol:HDL ratio was 2.8 mmol/l (0–4.5). Apolipoprotein A-I was raised at 3.65 g/l (1.25–2.15). Separate cardiology review for chest pain resulted in coronary angiogram and transthoracic echocardiogram, confirming normal coronary arteries, and preserved ventricular function. Hepatology review and Fibroscan for abnormal liver function tests ruled out liver pathology. Repeat lipid profile revealed a similar picture and no lipid-lowering treatment was instituted.
Discussion
Hyperalphalipoproteinemia (HALP) is a condition where HDL-C levels are elevated raising total cholesterol values. LDL-cholesterol may be normal or elevated. Peripheral stigmata of hyperlipidaemia are usually absent. HALP can result from primary causes due to familial genetic defects or can be secondary to drugs, alcohol, or primary biliary cirrhosis. Most patients are incidentally diagnosed. Medical therapy is rarely required. Carbamazepine is well documented to cause elevated HDL-C, attributed to its enzyme inducing effect leading to increased hepatic synthesis of alphalipoproteins. Careful clinical and biochemical evaluation is essential in patients presenting with lipid abnormalities. Statin therapy is not a panacea for all hypercholesterolaemia patients.

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## P103

**Splenunculi masquerading as neuroendocrine tumours**

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Splenunculi or accessory spleens are congenital foci of normal splenic tissue that are separate from the main body of the spleen. They are common, with a prevalence of 16% on contrast-enhanced abdominal CT, and present in 10–30% of post mortem examinations. They are benign, and important to recognise to avoid unnecessary investigations and surgery for suspected malignancies.
Splenunculi are usually asymptomatic and found incidentally. Typically on CT they are well demarcated round lesions, $<2$ cm, and enhance homogeneously on contrast enhanced scans. Their radiological features are similar to the rest of the spleen. Location varies but the reporting of intrapancreatic accessory spleens is rare.
We present two cases of suspected neuroendocrine, pancreatic tumours. The first presented with a large, saddle shaped pulmonary embolus requiring thrombolysis, new onset diabetes and weight loss. CT and MRI pancreas revealed a solitary 14 mm lesion in the tail of the pancreas confirmed on endoscopic ultrasound. Fasting gut hormones and octreoscan were negative. Radiological surveillance showed no change over 12 months. Surgical resection was recommended following MDT discussion. Laparoscopic distal pancreatectomy was completed and histology confirmed a splenunculus.
Case two presented with small bowel obstruction requiring resection for carcinoid tumour. Postoperative CT scan showed solitary mesenteric node involvement. Octreoscan suggested a pancreatic lesion as well as the known mesenteric node. Follow up MRI showed progression of mesenteric disease but no suspicious lesion within the pancreas. Review of this scan and previous CT images show the lesion is most likely a splenunculus.
Advances in imaging techniques are expected to detect intrapancreatic splenunculi more frequently increasing the importance of being able to differentiate these lesions from more sinister pathologies.

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## P104

**UK primary hyperparathyroidism clinical practice audit**

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Background
Primary hyperparathyroidism (PHPT) is a common endocrine disorder which affects 3/1000 of the general population and is associated with excess morbidity and mortality. Clinical practice tends to vary in terms of investigations, monitoring, decisions on intervention, and follow up. Audits in several European countries have previously demonstrated marked variation and divergence from best practice recommendations.
Methods
This project used the 2009 Third International Workshop guidance on the management of asymptomatic primary hyperparathyroidism in order to compare how patients were worked up, investigated and treated for their confirmed diagnosis of PHPT. It was a retrospective, multi-centre audit co-ordinated by the YDEF, with a clear audit process, inclusion, and exclusion criteria.
Results
Demographics – 85% of patients with confirmed PHPT were female. Baseline investigations such as renal function, urinary calcium collection, abdominal imaging, three sites DEXA bone scanning varied both between and within individual centres. Indications for surgery – were not always consistent and 14% who would not qualify for parathyroidectomy according to the guidance, did have an operation. Diagnostic imaging – parathyroid imaging was requested as part of the diagnostic work up in 82.5% of patients, despite this not being indicated in the initial diagnosis of the condition. Sestamibi and/or USS correctly identified the location of parathyroid pathology in 63%.
Conclusions
Clinical practice varies throughout the UK and appears to deviate from the 2009 guidance. Criticisms of the guidelines are multiple and have been partially addressed in the latest 2014 version.

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## P105

**Pregnancy and lactation-associated osteoporosis: two case reports**

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Pregnancy and lactation-associated osteoporosis (PAO) is a rare syndrome of spontaneous fragility fractures, most commonly vertebral, occurring in late pregnancy or lactation. The aetiology and pathogenesis of this osteoporosis are unknown, and early diagnosis and management are essential because of the severity of the morbidity associated with these fractures. The management includes cessation of breastfeeding, use of specific osteoporosis drugs, and adequate analgesia.
We present two cases of young women who developed PAO in the background of no known risk factors for secondary osteoporosis or bone disease. The first case is a 29-year-old lady who presented with bilateral hip pain during the last month of her pregnancy. The pain persisted 2 weeks after delivery, and at that point she had a fall and sustained bilateral neck of femur fracture. The second case is a 34-year-old lady who presented with back pain after the birth of her second child and similar pain persisted with her subsequent two pregnancies. In both cases, DEXA scan showed evidence of osteoporosis and biochemical tests have ruled out other risk factors. The first patient was started on and improved with oral bisphosphonate therapy, and the second patient was managed conservatively.
In conclusion, although a rare syndrome, PAO has to be considered in young pregnant or postpartum women presenting with persistent back/hip pain or spontaneous fragility fractures as early diagnosis and management can avoid the potential debilitating morbidity associated with it.

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## P106

**Pseudohypoparathyroidism: a case of delayed diagnosis**

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A 65-year-old Caucasian gentleman was admitted with weight loss and generally feeling unwell. X ray chest done a week before admission as part of routine investigation by the General Practitioner, showed healing rib fractures with

diffuse sclerosis. Malignancy was initially suspected due to the bony changes in the chest X-ray. Hence a full body CT scan was done and was normal. Incidentally he was noted to have low adjusted calcium of 2.16 mmol/l (2.20–2.60), raised parathyroid hormone (PTH) at 1189 ng/l (914–72), raised alkaline phosphatase (ALP) at 1189 μ/l (40–130) and low 25-hydroxy vitamin D levels of 15 mmol/l (24–167) consistent with vitamin D deficiency. He did not have any symptoms of hypocalcaemia such as perioral paraesthesia, numbness/tingling in the fingers, or muscle cramps. He was treated with high dose vitamin D replacement of 20 000 IU capsule once a week and calcium carbonate 1.5 g tablet twice a day for 8 weeks. X-ray of his hands revealed evidence of short third and fourth metacarpals of both hands. Significantly raised PTH levels could not be explained by vitamin D deficiency alone; hence pseudohypoparathyroidism (PHP) was also suspected based on the clinical finding of the third and fourth metacarpals. Once he was vitamin D replete he received maintenance dose of calcium carbonate and colecalciferol one tablet daily. PHP is a very rare condition and often diagnosis is missed or delayed due to absence of typical physical signs and biochemical features mimicking vitamin D deficiency. It remains a diagnostic challenge. Signs and symptoms vary from person to person. Calcium is mobilized from the bone and hence patients remain relatively asymptomatic. Our patient was minimally affected by the biochemical abnormality and was in his mid-sixties when a diagnosis of PHP made. Appropriate interpretation of biochemical results along with good history taking and physical examination is crucial in diagnosing PHP.

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## P107

**Opiate induced multiple pituitary hormone deficits**

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Opiate use is a well-recognised cause of hypogonadotrophic hypogonadism. Adrenocorticotrophic hormone and growth hormone deficiencies are much rarer. A 56-year-old female presented with weight loss, lethargy, and nausea of ∼3 years duration. She had received treatment with morphine sulphate 100 mg twice daily for ∼15 years for back pain due to disc prolapse and osteoarthritis. A 0900 h cortisol of 110 nmol/l requested by her general practitioner had resulted in her referral. She was receiving no hormonal treatment.

Investigations: short Synacthen test – cortisol levels 0 min 109 nmol/l, 30 min 610 nmol/l, and 60 min 713 nmol/l. ACTH 12.5 ng/l, $fT_4$ 12.8 pmol/l, $fT_3$ 5.6 pmol/l, TSH 1.36 mU/l, prolactin 446 μIU/ml, FSH 17.4 U/l, LH 2.9 U/l, and IGF1 10.2 nmol/l all performed at 0900 h. An insulin tolerance test, during which adequate hypoglycaemia was achieved with a blood glucose level of 1.1 mmol/l coincidental with autonomic nervous system and neuroglycopenic symptoms, produced peak cortisol levels of 169 nmol/l and GH 0.93 μg/l. MRI of pituitary was normal.

This case highlights the need to consider pituitary insufficiency in patients receiving opiate therapy who have otherwise unexplained symptoms. The limits of the short Synacthen test in assessing the integrity of the hypothalamo-pituitary–adrenal axis are also demonstrated. She is asymptomatic at present receiving hydrocortisone and GH treatment.

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## P108

**Separate, sequential, endocrine, and glycaemic effects of ipilimumab and pembrolizumab in metastatic melanoma**

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Ipilimumab, pembrolizumab, and nivolumab – novel immune checkpoint blockade drugs – are increasingly used in the treatment of metastatic melanoma and other cancers. Ipilimumab is an anti-cytotoxic T-lymphocyte antigen 4 (CTLA4) MAB and the first drug shown to improve overall survival in metastatic melanoma. Hypophysitis is a widely described side-effect linked with both lymphocytic inflammation and a direct toxic effect on the pituitary in mouse studies, but has been shown to link with survival in some patients. Pembrolizumab is an anti-programmed death 1 (PD1) antibody active in ipilimumab-treated and naïve patients. Hypophysitis is less commonly described with pembrolizumab treatment, but glycaemic variation was noted in early clinical studies.

We describe the case of a 50-year-old female, with type 2 diabetes (initially on metformin monotherapy) who presented with stage 2a malignant melanoma. A wide local excision was performed but, two years later, she developed metastatic disease. The patient received first line immunotherapy with ipilimumab with some clinical response. She developed low cortisol and prolactin levels, i.e. drug-related hypophysitis, and was treated with prednisolone. Thyroid function and an MRI of the pituitary were normal. Disease progression led to treatment with pembrolizumab. Measurements of HbA1c indicated increasingly unstable glycaemia, peaking at 91 mmol/mol. Gliclazide was then added at which point the HbA1c began to fall.

In terms of altered glycaemia, a phase two trial of ipilimumab reports hyperglycaemia; and hypoglycaemia was mentioned in a different study. There have been two reported cases of diabetes mellitus following pembrolizumab treatment. In one, acute onset of insulin-dependent diabetes was attributed to a possible link between PD1 inhibition and autoimmune diabetes. This case is remarkable in that ipilimumab-related endocrine effects were sequentially followed by pembrolizumab-related glycaemic changes. It is not yet known whether pembrolizumab-associated glycaemic changes are linked to oncological benefit, an area that would require further study.

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## P109

**Resistant hyperparathyroidism and hepatitis: is there a link?**

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A 71-year-old male patient with no significant past medical history was admitted to hospital with acute hepatitis in July 2014 with bilirubin 98 μmol/l, ALT 1703 IU/l, and ALP 223 IU/l. Liver ultrasound showed a normal sized liver with normal echotexture. Liver screen including hepatitis A, B, C, E, and autoantibody screen was negative. Liver biopsy revealed acute hepatitis with patchy necrosis and inflammatory infiltrate consistent with inflammation, drugs, or autoimmune hepatitis.

Whilst an inpatient, he was noted to be hypercalcaemic at 3.51 mmol/l with a hypophosphatemia of 0.50 mmol/l. As he was symptomatic, further investigations as an inpatient showed an elevated parathormone of 35.3 pmol/l and elevated 24 h urinary calcium of 18.1 mmol/24 h. Bisphosphonates and i.v. fluids failed to lower his calcium levels. Imaging revealed a left inferior parathyroid adenoma and he was referred for an inpatient parathyroidectomy. Whilst awaiting this, he was commenced on cinacalcet. Post operatively, his parathormone, calcium, and liver functions tests improved.

A literature search showed that simultaneous development of acute primary hyperparathyroidism and hepatitis is very rare and has been described only once before. It has previously been postulated that antibodies to hepatitis B virus may alter the calcium set point allowing uncontrolled synthesis of parathormone. It is also possible that our patient may have had mild primary hyperparathyroidism previously unknown to us which was then triggered by the hepatitis episode. However, what remains unusual is, his hyperparathyroidism was resistant to regular calcium lowering treatment and therefore, needed inpatient cinacalcet and parathyroidectomy.

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## P110

**Hypogonadism in Noonan syndrome**

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We present the case history of a 33-year-old male with Noonan syndrome diagnosed at 2 months of age, as he had typical facial features (hypertelorism, ptosis, low set ears, and small pointed chin), a heart murmur and bilateral cryptorchidism. At 22 months he underwent patent ductus arteriosus closure and at 24 months, the left testis was excised, for testicular torsion. He had learning difficulties and attended a special needs school and was intermittently reviewed in the paediatric clinic. At 13 years he was noted to be pre-pubertal, and there was no pubertal growth spurt. At 16 years, he was thought to be in early puberty. However, by 18 years, he did not have significant development of secondary sexual characteristics and had a bone age of 13–14 years. At that point he was

commenced on i.m. testosterone (sustanon, 50 mg, 4 weekly). Owing to lack of parenteral support, non-compliance with treatment, and non-attendance to clinics, he did not receive testosterone replacement regularly.
By 22 years, he was 165 cm tall and was reviewed in the adult endocrine clinic with absent secondary sexual features, low libido, micropenis, chest wall deformity, and reduced muscle bulk. Investigations revealed low testosterone (1.1 nmol/l; NR 9.0–32). DEXA bone scan revealed osteoporosis ($T$ score $< -3.0$). Associated vitamin D deficiency was treated. The patient was commenced on i.m. testosterone undecanoate (1000 mg, 12 weekly). Testosterone levels normalised (10–22 nmol/l) and this contributed to wellbeing, increased body hair and musculature.
This case report highlights the difficulties associated with recognition of delayed puberty in conditions like Noonan syndrome (complicated by cryptorchidism). Further, timely initiation of testosterone is vital in order to prevent osteoporosis. Patients with Noonan syndrome need regular follow up throughout childhood and puberty and into adulthood, in specialist multidisciplinary clinics.

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## P111

**Hungry bones: a case of severe complications post parathyroidectomy**

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An 18-year-old female presented to acute services with polydipsia and lower back pain. Routine blood tests revealed hypercalcaemia 3.67 mmol/l with significantly raised parathyroid hormone levels 2986 ng/l (10–65), alkaline phosphatase (ALP) 3330 IU/l (30–130), and low vitamin D 10.3 nmol/l (50–220). A CT scan revealed a 3.5 cm parathyroid adenoma and very osteopenic bones with multiple lucencies in keeping with primary hyperparathyoidism. The parathyroid subtraction scan Tc/MIBI reported a large area of activity in the lower left lobe of the thyroid gland corresponding with the CT findings.
The patient subsequently underwent parathyroidectomy for a 3.5 cm parathyroid adenoma weighing 15 g with histology showing chief cell adenoma. The patient's calcium dropped within 24 h post surgery and she became symptomatic with Chvostek's sign positive, carpopedal spasms, perioral numbness, and paraesthesia. Calcium was1.87 mmol/l and despite treatment dropped to 1.63 mmol/l, phosphate 0.6 mmol/l, and magnesium 0.5 mmol/l.
She required prolonged high dose i.v. calcium and magnesium infusions with oral phosphate, high doses of oral calcium and activated vitamin D supplementation for 'hungry bone syndrome' which continued for over a month. This case highlights a particularly severe case of primary hyperparathyroidism with rarely seen radiological features and development of rare post-operative complications. Despite acknowledgement of the risk, development of hungry bone syndrome was not avoided and the patient spent a significant number of days as an inpatient. This case describes in detail the large amounts of calcium and magnesium replacement regimes required (including i.v. regimes for over a month) that have not previously been documented in literature. It also discusses the potential risk factors for development of hungry bone syndrome.

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## P112

**Carbarmazepine and Cushing's: a cautionary tale of assay interference mimicking disease**

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We present two cases, with apparently confirmed Cushing's. Detailed clinical evaluation did not support the diagnosis, and carbamezepine interference was finally confirmed in both patients.
Case 1
A 44-year-old man presented to a nephrologist with malignant hypertension. Routine investigation for secondary causes revealed hyperreninaemia with marked disparity of kidney size suggestive of renal infarction. 24 h urinary free cortisols (UFCs) were also sent and found to be elevated at 2036 nmol/24 h prompting an endocrine referral. Past medical history included bipolar disorder treated with venlafaxine, carbamazepine, and pericyazine. At his first endocrine visit, he was found to be slim with normal skin character, no bruising, striae, facial plethora, or myopathy. Further assessment confirmed normal bone density, normal HbA1c, with ongoing elevated UFC levels and failure of suppression on low dose dexamethasone suppression test: 348–67 nmol/l. However, since this did not fit with the clinical evaluation, the possibility of carbamezepine interference in the assays was suspected. Psychiatric input was sought to supervise carbamezepine withdrawal, at which all parameters returned to normal.
Case 2
A 68-year-old woman was also referred by a nephrologist with hypertension and obesity. Screening UFCs were minimally elevated (501 and 360 nmol/24 h), as was overnight dexamethasone testing to 514 nmol/l. Clinical evaluation was again not convincing of Cushing's and she too was found to be taking carbamazepine. Biochemical evaluation was repeated with the patient still taking carbamezepine, but with serum samples run on the alternative assay. UFCs remained marginally elevatedat 321 and 331 nmol/24 h and low dose dexamethasone suppression testing with serum cortisol analyser on the new assay now suppressed to 37 nmol/l.
These cases highlight that as well as the known enzyme inducting effects of carbamezepine leading to false positive dexamethasone tests, carbamezepine itself cross reacts with some older cortisol assays. Drug withdrawal or alternative platforms should be used in these cases to prevent unnecessary investigation and anxiety.

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## P113

**Foreign bariatric procedures: help or hindrance for the NHS?**

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We describe the case of a 32-year-old lady Polish lady, who presented to the surgical team at Leeds Teaching Hospitals NHS Trust in July 2012 with abdominal pain and vomiting. The previous year she had undergone a private 'banded bypass' operation in Poland resulting in 60 kg weight loss. On admission she was diagnosed with a slipped gastric band, to be confirmed by laparoscopy. Surprisingly the 'band' was in fact a length of surgical drain sutured to the middle third of the stomach and the patient had in fact received a jejunoileal bypass (JIB) with a blind ending jejunal loop, rather than a Fobi pouch gastric bypass as she had been led to believe. The lower portion of the stomach along with part of the omentum had slipped through this homemade 'band', removal of which alleviated her symptoms. Following discharge it was decided to convert the JIB to a conventional Roux-en-Y gastric bypass (RYGB) due to malabsorptive symptoms, once funding was approved. In the interim she was admitted acutely a second time due to an episode of intussusception within the blind end of jejunum and so the JIB was converted to a RYGB to prevent further occurrences. The complications encountered in this case illustrate why JIB operations have largely been outlawed within the bariatric community as a weight loss procedure. Patients undergoing bariatric procedures abroad frequently encounter difficulties due to a lack of long term nutritional follow up along with poor peri-operative support. Although the case presented is a rarity, these patients may require revision surgery for a variety of reasons, placing extra strain on the NHS and can be further complicated by funding difficulties. Whether the current guidelines for funding of bariatric surgery would have prevented the second admission is unclear.

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## P114

**Variation in levels of macroprolactin in the investigation of secondary hypogonadism**

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Introduction
Macroprolactin is a physiologically inactive form of prolactin, usually composed of a prolactin monomer and an IgG or anti-prolactin antibody molecule. Whilst clinically non-reactive, it interferes with immunological assays used for prolactin detection. It is identified by polyethylene glycol (PEG) precipitation, and levels of macroprolactin are generally believed to remain stable over time.
Case
We present the case of a 36-year-old gentleman who was referred for hyperprolactinaemia and investigation of possible secondary hypogonadism. He reported symptoms of fatigue and erectile dysfunction, which prompted his general practitioner to measure testosterone, prolactin, and macroprolactin on three occasions. Macroprolactin estimations are carried out routinely in our

department on samples with a raised prolactin level (>350 mIU/l in men), unless two previous estimations have already been carried out within the last year. As the blood tests were requested via primary care, a macroprolactin estimation was carried out on each sample. An initial prolactin level was mildly elevated at 832 mIU/l, with a negative macroprolactin and a testosterone level at the lower end of the normal range. However, on repeat testing 1 month later, his prolactin was mildly raised at 432 mIU/l, but with ~80% of the prolactin immunoreactivity in the sample being due to the presence of macroprolactin. Seven months later his tests were again repeated and his macroprolactin was again found to be negative.

Discussion

This case highlights that macroprolactin titres can vary over time in some individuals, which could be significant in determining whether a prolactin level is genuinely raised. Furthermore, it is important to consider that macroprolactin can co-exist with genuine hyperprolactinaemia.[2] Thus some authors have suggested that PEG precipitation be considered prior to every prolactin measurement.[3] Nevertheless this case highlights that a repeat macroprolactin estimation should be considered if measured prolactin levels do not correlate well with a patient's clinical presentation.

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## P115

**Audit of adult GH replacement therapy in Nottingham**

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Introduction

Guidelines for the use of GH in GH deficient adults were issued by the UK National Institute for Clinical Excellence (NICE). To assess current practice in relation to these guidelines, a review of patients receiving GH treatment was performed. The aims were to i) assess if adults with GH deficiency met NICE criteria for GH therapy and ii) identify reasons for initiating or continuing GH treatment if NICE criteria were not met.

Methods

Retrospective case note review of adults and young adults in transition receiving growth hormone therapy at the Nottingham Treatment Centre up to January 2015.

Results

Thirty-one patients (16 males and 15 females) were identified, mean age 45 ± 15 years (S.D.). The majority (39%) of patients had previous pituitary surgery. Twenty-five patients were assessed as adults requiring GH replacement. 19 (76%) of these patients fulfilled all criteria for commencing GH therapy. 6 (24%) patients did not have tests to demonstrate severe GH deficiency but had convincing circumstantial clinical evidence of GH deficiency. 20 (80%) of patients were assessed with QOL-AGHDA questionnaire at baseline. 14 (56%) were reassessed within 12 months and met criteria to continue. The remaining 6 adult patients had childhood GH deficiency and continued treatment having fulfilled NICE guidance criteria.

Conclusion

There were patients who did not meet NICE criteria but had convincing evidence that justified GH treatment. The QOL-AGHDA questionnaire may have limitations given the subjective nature of questionnaires and comorbidities influencing quality of life. There may be a need to consider justifiable use of circumstantial clinical evidence based on clinical judgement and patients' wishes when initiating and reassessing patients on GH treatment.

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## P116

**Erectile dysfunction, diabetes mellitus, and sun-tan: the hypogonadism story with a difference**

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Background

Hypogonadism and erectile dysfunction have diverse causes. Hypogonadotrophic hypogonadism results from pituitary dysfunction, often caused by pituitary adenoma, pituitary surgery, radiotherapy, or use of prescription/non-prescription drugs. Rarer causes should be considered during initial investigation of patients with erectile dysfunction. We present a case of secondary hypogonadism and how further investigations eventually led to the underlying diagnosis.

Case

A 53-year-old Caucasian presented with reduced libido and gradually worsening tiredness. He was on insulin for type 2 diabetes mellitus and levothyroxine for hypothyroidism. Pituitary profile was consistent with hypogonadotrophic hypogonadism and he was started on testosterone replacement. During a subsequent visit, magnetic resonance imaging (MRI) of the pituitary was requested as routine. Two years after initial presentation, at a routine clinic review, he complained of arthralgia and feeling increasingly tired. His skin appeared deeply tanned, which he attributed to recent European travel. MRI scan report was reviewed and showed a hypoplastic pituitary gland with haemosiderin deposition. In view of skin tanning and the MRI findings, serum ferritin was requested to rule out haemochromatosis. The ferritin levels were significantly elevated at 18 186 ng/ml. Subsequent iron studies confirmed iron overload and genetic studies showed homozygous *HFE* gene mutation *C282Y*. Fibroscan showed cirrhosis of liver. He is currently managed with regular venesection.

Discussion

Hereditary hemochromatosis is an autosomal recessive iron storage disorder. Unrestricted absorption of iron from upper small intestine leads to widespread iron accumulation and tissue damage. A high index of suspicion is essential to diagnose hemochromatosis early. Early diagnosis and venesection can avoid the long-term complications from prolonged iron deposition. This case had characteristic features of slate-grey skin pigmentation, diabetes, cirrhosis, arthralgia, hypothyroidism, and hypogonadotrophic hypogonadism. It is therefore important to consider the possibility of a unifying underlying diagnosis when seemingly disparate constellation of findings is encountered in clinical practice.

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## P117

**Cardiac manifestations of a phaeochromocytoma**

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Hypertension is the most commonly recognised cardiac manifestation of a phaeochromocytoma. There are however a variety of other cardiac presentations including arrhythmias and the increasingly reported Takotsubo cardiomyopathy. We present the case of a female patient presenting acutely to the general medical take who was found to have cardiac arrhythmias and transient left ventricular dysfunction.

The initial presentation was breathlessness, chest pain, sweating, and clamminess. Investigations performed on admission demonstrated elevated troponin and dynamic ECG changes (sinus tachycardia with frequent ventricular ectopics and later developed profound anterior T wave inversion from V3 to V6). The working diagnosis was acute coronary syndrome and pulmonary oedema. She underwent cardiology work up including an echo and coronary angiography. The echo was technically difficult due to her tachycardia, but the coronary angiogram showed normal coronary arteries. Her clinical condition deteriorated with labile blood pressure (range 239/129–48/34) and arrhythmias, the possible diagnosis of a phaeochromocytoma was suggested.

Further investigations included; plasma metanephrines and normetanephrines which were significantly elevated (25 000 pmol/l (normal range <510), 18 568 pmol/l (120–1180) respectively), and CT scan demonstrated a 13 cm right adrenal mass, confirming the diagnosis of phaeochromocytoma. Owing to the paroxysmal hypertensive/hypotensive instability intravenous phentolamine was initially used to stabilise her blood pressure. This was then converted to doxazosin, with β-blockers being added prior to discharge. Owing to a pulmonary embolism requiring treatment, the need to improve her overall health status, and instability on the operating table requiring cancellation of the first attempted procedure, the surgery to remove the phaeochromocytoma was delayed. At the second attempt surgery was successful and she made an uneventful recovery.

The case identifies various life-threatening cardiac manifestations of phaeochromocytoma and medication that was used during an acute presentation with a successful outcome.

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## P118

**Management of patients with adrenal insufficiency attending Galway University Hospital compared with current best practice**

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Introduction
Patients with adrenal insufficiency (AI) lack endogenous cortisol and require oral hydrocortisone. In primary AI (PAI) endogenous aldosterone synthesis is also lost and patients require oral fludrocortisone. Important long-term issues include wearing MedicAlert jewellery (MAJ) and adequacy of steroid replacement including fludrocortisone, assessed by plasma–renin activity (PRA).
We identified patients with AI attending our unit and aimed to: i) assess patient education and practices regarding MAJ and ii) assess the frequency of PRA measurement and the levels of same compared with current guideline targets.
Methods
The hospital's Patient Correspondence System identified letters including the terms 'Adrenal Insufficiency' and 'Addison's Disease'. Such identified patients were classified as having primary or secondary AI. Recent hydrocortisone and fludrocortisone doses were recorded. Patients were contacted using registered telephone numbers and invited to participate in the study. Electronic laboratory results were reviewed for PRA measurements.
Results
Review of $>200$ letters identified 39 patients with AI, 21 with PAI and 18 with Secondary AI. 21 patients were successfully contacted and consented to a telephone survey. Of these, 20 (95%) were aware of the need for MAJ and 62% owned MAJ. Among patients with PAI 67% had a PRA performed in the past 5 years and 47% in the previous year. Two thirds of patients had PRA above the recommended target range.
Discussion
Patients with AI require complex and long-term follow-up. Establishing a database of AI patients allows for audit of patient care and adherence to current guidelines. Patients attending our unit are well advised on the need for wearing MAJ but less than two thirds of patients own MAJ. PRA, a useful measurement in determining appropriate fludrocortisone replacement was available on less than half the patients for the previous year and even when available was above the recommended target in over two thirds of patients.

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## P119

**An audit in to the investigation of spontaneous hypoglycaemia: experience at a tertiary centre**

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Background
Adult spontaneous hypoglycaemia in non-diabetic patients is not a diagnosis *per se* but a manifestation of a disease and is often a diagnostic challenge. Although rare, it's important to exclude endogenous hyperinsulinemia, because treatment can be curative. The aim of our audit was to assess the usefulness of various investigations done for spontaneous hypoglycaemia in diagnosing Insulinoma.
Methods
Data on 98 non-diabetic patients referred to our endocrine unit for investigation of hypoglycaemia was obtained. Data was gathered for type of biochemical investigations undertaken – 24 /72 h fast, prolonged glucose tolerance test/mixed meal test (pGTT), insulin/C-peptide levels, radiology investigations, endoscopic ultrasound, and the diagnostic outcomes.
Results
Of $n=98$, their age $44.5 \pm 14.1$ (mean $\pm$ S.D.) years, Females 62%. Prolonged GTT was undertaken in 72 (73%), 24-h fast in 77 (78%) and 72-h fast in three patients. 51 patients (52%) had both 24-h fast and GTT. Prolonged GTT: 27 (27%) had hypoglycaemia on the prolonged GTT. 21/27 had 24 h fast (none had hypoglycaemia on 24 h fast). One had 72 h fast (normal). Insulin/C-peptide abnormal in two patients (one had insulinoma and one had normal pancreas on further investigations). Pancreatic imaging: CT, MRI, and EUS undertaken in 13, 2, and 8 patients respectively. Final diagnosis was Reactive hypoglycaemia in 22 (81.5%) and diabetes mellitus/IGT in four patients and insulinoma in one. 24 h fasting: 6 (7%) patients had hypoglycaemia on 24 h fasting. Five had abnormal insulin/C-peptide with the hypoglycaemia. CT/MRI and EUS imaging was undertaken in all these six patients. Insulinoma proven in four patients, one factitious hypoglycaemia, and one NIPHS.
Conclusion
Prolonged GTT did not add to the 24 h fasting test in the diagnosis of insulinoma, though it picked up other diagnoses.

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## P120

**Cyclophosphamide induced seminiferous tubule damage causing raised FSH and LH, and high testosterone levels**

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Introduction
Cyclophosphamide is known to cause gonadal dysfunction. Specifically, it has been reported to cause damage to the seminiferous tubules causing raised FSH$>$ LH with low testosterone levels. We present the case of a gentleman with raised FSH and LH following treatment with R-CHOP therapy. Interestingly, the testosterone level was high, signifying adequate physiological response.
Case history
A 63-year-old gentleman was referred to endocrine services with a raised LH and FSH, and a mildly raised prolactin, with high testosterone levels. He complained of a several month history of lethargy, dizziness, and headaches. Past medical history included non-Hodgkin's lymphoma, for which he had previously received R-CHOP therapy, and benign prostatic hypertrophy. Physical examination was unremarkable. There were no features of acromegaly, visual field tests were normal, and there were no worrying features of headache.
Investigations and management
Testosterone was raised at 23.3 nmol/l, SHBG was 71.6 nmol/l, and prolactin 489 mU/l. FSH level was high at 25 IU/l and the LH level was 23 IU/l. Remaining pituitary function tests including IGF and TSH were normal. He had an adequate response to short synacthen test ruling out secondary adrenal insufficiency. An MRI scan did not show any pituitary adenoma. As the patient has adequate compensatory response to high FSH and LH with total testosterone levels in high normal range, it was agreed with the patient not to initiate testosterone therapy.
Discussion
The case highlights the complication of seminiferous tubule damage induced by cyclophosphamide therapy, causing raised FSH and LH. In this clinical picture, a low testosterone level is expected. However, there was a raised testosterone, representing adequate physiological response. The differential could be a pituitary gonadotrophinoma. However, this was ruled out with an MRI scan.

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## P121

**A case of morning headache: Doege–Potter syndrome**

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Tumour induced hypoglycaemia is a rare disorder occurring in cancer patients caused by variety of tumours including islet and non-islet tumours. Non-islet cell tumour induced hypoglycaemia (NICTH) is rare paraneoplastic disorders normally associated with pleural solitary fibrous tumour but can also rarely occur in extra thoracic site.
We describe an 86-year-old man not known to have diabetes mellitus who presented with 3-month history of early morning headache and dizziness relieved by consuming large amount of fizzy sweet drink. On examination, he was noted to have large palpable mass on the left upper quadrant. Initial inpatient investigations during symptoms showed plasma glucose of 2.4 mmol/l, insulin $<1.0$ pmol/l (17.8–173), C peptide 81 pmol/l (298–2350), 3-hydroxybutyrate $<0.1$ mmol/l, free fatty acid 0.3 mmol/l, negative serum sulphonyurea screen, and normal short Synacthen test. CT scan of the abdomen revealed huge solitary $22 \times 19 \times 16$ cm well defined, rounded solid heterogeneous retroperitoneal mass with internal ill-defined areas of necrosis. There was no evidence of metastasis on further full body scan. Further work up showed raised IGF2 94.5 nmol/l, IGF1 7.1 nmol/l, and raised IGF2:IGF1 ratio 13.3 (normal ratio $<10$). Subsequent biopsy of the retroperitoneal mass demonstrated spindled shape cells showing mild to moderate nuclear atypia appearance consistent with malignant solitary fibrous tumour. Investigations were consistent with diagnosis of NICTH secondary to extra thoracic solitary fibrous tumour. He was managed conservatively with oral dexamethasone 1.5 mg o.d. which resolves the recurrent hypoglycaemic episodes. Surgical resection was not pursued, as he was deemed not fit for surgery.
In summary, we have described a rare phenomenon of Doege–Potter syndrome, a paraneoplastic phenomenon of hypoglycaemia associated with rare extra thoracic solitary fibrous tumour. It is important to investigate the aetiology thoroughly as management can be tailored individually for each patient.

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## P122

**The effects of maggot debridement therapy on length of hospital inpatient stay**

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In recent years, there is increased interest in using maggot debridement therapy (MDT) for ulcer treatment. MDT has shown beneficial effects on ulcers' rate of healing, need for surgical intervention, and number of antibiotic-free days. In this retrospective study, we looked into the effects of MDT on the length of inpatient stay and need for surgical intervention.

Data were collected from 33 randomly selected patients admitted into hospital with primary diagnosis of ulcer, both diabetic and non-diabetic. 18 patients, of whom six were diabetic ulcers, were started on MDT and antibiotics treatment. The remaining patients, all of whom were diabetic ulcers, were treated with antibiotics only (control group). The average inpatient stay for the MDT group was 18 days, with 54% of the length of stay attributed to the MDT. In contrast, the average inpatient stay for the control group was significantly shorter than the MDT group (11.3 days, $P=0.0014$). There was no significant difference in the need for surgical intervention between the MDT and control groups (11% of MDT patients vs 26.7% of control group, $P=0.87$), although the relatively smaller number of patients in this study could explain this. The shorter length of inpatient stay observed could be due to the recent availability of 'hospital-at-home', of which patients can continue i.v. antibiotics treatment at home, and increased outpatient follow up by podiatrists and clinicians during treatment. We excluded multi-resistant microbes as a contributing factor by analysing the microbiology results.

In conclusion, MDT increases the length of hospital inpatient stay. However, it can potentially be overcome by increasing the funding and experience in managing MDT in an outpatient or community setting, a service not widely available at present in UK. This is necessary for cost-effectiveness since MDT is beneficial in other aspects of ulcer management.

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## P123

**A case of adrenal haemorrhage in severe sepsis**

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A 72-year-old man, otherwise fit and well, with treated hypertension presented to our hospital with abdominal pain and vomiting. He was febrile with deranged liver function tests and elevated inflammatory markers. Abdominal ultrasound showed a right suprarenal mass, and a normal gallbladder.

CT confirmed a $37\times31\times24$ mm right adrenal mass with no significant contrast enhancement, and bilateral pneumonia responsible for the septic picture. Thrombus was noted in the left external iliac artery for which he was anticoagulated. Three days later, following appropriate antibiotic therapy, his pain worsened and repeat CT showed inflammatory stranding around the right adrenal mass and new nodularity due to acute adrenal haemorrhage. Further investigations showed no measurable malignancy and his urinary catecholamine collection was normal. He was referred for an adrenalectomy, however as his pre-operative CT showed the mass was no longer evident, adrenalectomy was not performed. The adrenal gland is particularly vulnerable to haemorrhage. Under stress, there in an increase in arterial blood supply to the glands. As it as only one or two veins, this makes it susceptible to venous thrombosis and increased venous pressure, which can then result in haemorrhage.

Adrenal haemorrhage has several aetiologies. It is commonly associated with meningococcal sepsis, classically known as the Waterhouse–Friderichsen syndrome. It occurs less commonly in the context of sepsis from other pathogens which lead to disseminated intravascular coagulation, as a result of trauma, anticoagulant therapy, and may be spontaneous.

Radiologically, the adrenal glands may calcify following haemorrhage, or as in this case may no longer be visible. In our case, blood cultures were not particularly helpful in identifying an organism. However it is likely that he suffered an adrenal haemorrhage in the context of severe sepsis due to pneumonia, with the added risk factor of anticoagulant treatment.

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## P124

**A case of Graves' disease occurring following cessation of the oral combined contraceptive pill**

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Introduction

Graves' disease is an autoimmune disorder which may lead to thyroid overactivity and eye disease. Oestrogen and progesterone are thought to be immunomodulatory and have been postulated to play an important role in the difference in prevalence of autoimmune disorders between men and women. Autoimmune disorders, including autoimmune thyroid disease, are often quiescent during pregnancy with an increased prevalence postpartum. The increase in immune mediated thyroid disease postpartum has been associated with alterations in oestradiol and progesterone levels.

Case

A 27-year-old lady with a 2-month history of fatigue, tremor, sweating, pruritus, and weight loss. She had been taking the oral combined contraceptive pill (Yasmin) for 3 years, which she stopped just prior to the onset of her symptoms. Examination revealed a diffuse, smooth goitre although no evidence of thyroid eye disease. Thyroid function tests revealed a suppressed TSH at 0.01 mIU/l, free $T_4$ 41.9 pmol/l, and free $T_3$ $>$46.1 pmol/l. TSH receptor antibodies were elevated at 2.2 U/l confirming a diagnosis of Graves' disease. She was treated with anti-thyroid medication and B-blockade with full resolution of her symptoms.

Conclusion

There are no previously reported cases of autoimmune thyroid disease occurring following cessation of the combined oral contraceptive pill. Graves' disease often affects young women, who are most likely to be treated with oral contraceptive agents. In a cross sectional study looking at risk factors for autoimmune thyroid disease, Strieder *et al*. have reported that oestrogen use was associated with a lower rate of hyperthyroidism (RR 0·169) and furthermore oestrogen use was negatively correlated with the presence of TPO antibodies. Thus it seems feasible that the withdrawal of oral contraceptive use may have precipitated the onset of autoimmune thyroid disease in this young woman.

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## P125

**Benign or malignant adreno-cortical tumour; the relevance of size and androgen secretory capacity**

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Serum androgen levels are commonly suppressed in patients with benign cortisol-secreting adrenocortical adenomas due to ACTH suppression. Associated androgen co-secretion usually indicates malignancy. Here, we present a case a very large benign androgen and cortisol-secreting adrenocortical neoplasm.

Case

A 24-year-old female was referred with a 1-year history of secondary amenorrhea associated with hirsutism and acne. She reported irregular menstrual cycles prior to the development of amenorrhea. On clinical examination purple striae were observed over flanks with no other Cushingoid features or abnormalities found on examination. Initial blood tests revealed elevated testosterone (11.3 nmol/l), androstenedione (58.0 nmol/l), DHEA-S (55 μmol/l), and free androgen index (56.8%). 17-OH progesterone, plasma renin:aldosterone ratio and urinary metanephrines levels were normal. Further testing revealed failure to suppress

cortisol following an overnight dexamethasone suppression test (562 nmol/l), coupled with low ACTH (<5 ng/l). A cortisol day curve and a low-dose dexamethasone suppression tests confirmed cortisol over-secretion (cortisol 483 nmol/l and nadir levels of 430 nmol/l respectively). Subsequently adrenal MRI revealed a large right-sided 12×9 cm heterogeneous adrenal tumour. Patient underwent an open right adrenalectomy with post-operative hydrocortisone replacement. Histo-pathological analysis revealed findings consistent with a benign adrenal cortical neoplasm; Weiss score 2 with Ki-67 proliferation index of 1–2%.

Conclusion

Case studies have shown that adrenal mass size is strongly linked to malignant potential, with most benign adenomas limited to <4 cm. Most centres use tumour size of 5–6 cm or greater as an absolute indication for resection due to the high risk of malignancy (35–98%). This case is unusual in that despite the substantial size of the adrenal mass and its hormone secreting properties, the lesion was found to be benign. High levels of adrenal androgens can also be seen in benign adreno-cortical tumours.

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## P126

**Three cases highlighting the varied clinical presentations of adrenal haemorrhages**

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Adrenal haemorrhage is a relatively rare phenomenon which can often present non-specifically. In the case of bilateral haemorrhages, if unrecognised the endocrine sequelae may prove to be fatal. These three cases highlight very different clinical presentations of this important condition.

Case 1

A 74-year-old male was admitted following a fall and fractured neck of femur. His post-operative recovery was complicated by acute myocardial infarction, pneumonia, bilateral pulmonary emboli, and heparin induced thrombocytopenia. On ITU he was noted to have labile blood pressures, with systolic readings ranging from <100 to >200 mmHg. A CT aorta had been reported as showing bilateral adrenal masses (possible myelolipomas), whilst a short Synacthen test confirmed hypocortisolaemia. Re-review of his CT aorta highlighted these were not adrenal masses but in fact represented sub-acute bilateral adrenal haemorrhages. His blood pressure and clinical conditioned stabilised with hydrocortisone and fludrocortisone cover.

Case 2

A 49-year-old male was admitted with sudden onset severe right upper quadrant pain radiating to the back. Arterial blood gas analysis revealed a raised lactate of three; Blood tests were only remarkable for a macrocytosis and raised white cell count of $17.3\times10^9$/l. A CT aorta confirmed acute bilateral adrenal haemorrhages with extension into the retroperitoneum. His short Synacthen test was normal. Of note, a previous CT also highlighted lucencies within the pelvis believed to represent fibrous dysplasia and bilaterally enlarged nodular adrenal glands; He awaits screening for *GNAS1* mutation.

Case 3

A 66-year-old female was referred to the endocrine clinic after an ultrasound for abnormal liver function tests detected an incidental right supra-renal mass. MRI confirmed a 3.7×2.8 cm lesion in the right adrenal gland. Endocrine testing was normal. A subsequent CT for further characterisation showed peripheral calcification and lack of enhancement. Multi-disciplinary team review suggested that this most likely represents adrenal haemorrhage.

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## P127

**Asymptomatic Paget's disease of the bone in a 62-year-old Nigerian man: 3 years post alendronate therapy**

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Introduction

Paget's disease of the bone is a rare endocrine disease especially among Africans and Asians. Hence the detection of a case in a middle-aged Nigerian is of interest.

Case presentation

A 62-year-old Nigerian man in apparent good health was found to have markedly elevated alkaline phosphatase (ALP) of 1179 U/l during a routine medical check-up. He had no history suggestive of Paget's disease of the bone and also had no known family history of bone disease. His general and systemic examinations were essentially normal. Repeat ALP in our centre was 902 IU/l. Cranial CT scan showed diffuse cranial vault thickening consistent with Paget's disease which was confirmed by Tc-99m HMDP. He was placed on 40 mg alendronate tablets daily for 6 months.The patient has remained clinically stable during the 3-year follow-up period. His serum ALP results have also been within normal limits.

Conclusion

Paget's disease can occur in Africans though very rare; alendronate is a useful therapeutic option for treatment.

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## P128

**Does thyroid armour cause autoimmune thyroid disease?**

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We present the case of a 50-year-old lady who initially presented to the endocrine department at Royal Bolton Hospital in 2011 with symptoms of tiredness and lethargy following hysterectomy and bilateral salpingo-oophorectomy in 2003. Hormone replacement therapy was not commenced in view of a strong family history of breast cancer. There is a family history of both autoimmune hypothyroidism and hyperthyroidism. Thyroid function tests were normal, with a positive thyroid peroxidase antibody. She felt her symptoms were due to her thyroid status and was requesting thyroid armour. Following lengthy discussions, no replacement was commenced, but she was referred on for a second opinion.

She represented in 2014 with symptoms of thyrotoxicosis. On further questioning, it revealed that she was commenced on levothyroxine following a single blood test that suggests hypothyroidism. However, she did not tolerate this and resorted to buying thyroid armour herself. She became clinically and biochemically thyrotoxic and was advised to stop thyroid armour.

Her symptoms persisted following discontinuation of thyroid armour. Biochemistry done showed; TSH <0.003 mU/l (0.1–4.0), free $T_4$ 25.9 pmol/l (8–20), total $T_3$ 5.6 nmol/l (1.1–2.8), TSH receptor, and TPO antibodies were positive. Thyroid ultrasound showed diffuse thyroid inflammation and increased vascularity. This is in keeping with autoimmune thyroid disease. She was commenced on carbimazole.

As there was no record of previous TSH receptor antibody, we don't know if she has both stimulating and blocking TSH receptor antibody to account for her symptoms. What we speculate is the possibility of porcine antibodies from thyroid armour which contributed to her autoimmune thyrotoxicosis. There are many articles in relation to thyroid armour in treating hypothyroidism and subclinical hypothyroidism, but none to suggest a causative factor in developing autoimmune hyperthyroidism. The American Thyroid Association encourages future research in long-term outcome trials looking into thyroid extracts.

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## Cytokines and growth factors

### P129

**Expression of cytokines in placenta from pregnancies complicated by intrauterine growth restriction with abnormal umbilical artery Doppler indices**

The cause of intrauterine growth restriction (IUGR) with abnormal umbilical artery (UA) Doppler indices is unknown. Previous studies indicate rearrangement of placental villi occur in IUGR pregnancies, disturbing normal blood flow to the developing fetus. Vasculature integrity is regulated by a variety of factors, including inflammatory cytokines. Inflammatory factors interleukin 6 (IL6), IL8, and CCL2 have been implicated in abnormal placental structure and function in pre-eclamptic and hypertensive mothers. We determined expression of cytokines IL8, IL6, TNFα, and CCL2 in placentae with abnormal UA Doppler IUGR. Fetuses diagnosed with IUGR (weight $<10\%$) and abnormal UA Doppler indices (increased systolic:diastolic ratio, absent, or reversed end diastolic flow) were compared to fetuses with normal parameters. Placentae were collected from IUGR and control (normally-grown) subjects from both singleton and multiple pregnancies. For singletons, IUGR ($n=7$) and controls ($n=5$) were unrelated. For multiple pregnancies, IUGR fetuses ($n=4$) were compared to the normally-grown control fetuses ($n=5$) from the same pregnancy. At delivery, one central and four peripheral 3 mm biopsies were collected and stored. RNA was isolated. *IL6*, *IL8*, *CCL2*, and *TNFα* mRNA expression was determined with quantitative PCR. In singleton pregnancies, IL8 was significantly ($P<0.05$) increased in IUGR placenta. IL6, TNFα, and CCL2 were not differentially expressed in singletons. In multiple pregnancies, IL6 was significantly ($P<0.05$) decreased in IUGR placenta, while other genes were not differentially expressed. These data establish a role for interleukins in IUGR placenta. Although a causal relationship is not yet established, the increase in IL8 in IUGR placenta is consistent with inflammation that may contribute to poor placental development. IL6 has both pro and anti-inflammatory functions and the decrease seen in IUGR placenta from multiple gestations proposes non-genetic origins of the IUGR pathology.

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### P130

**Interleukin 27: a predictive marker for diabetic retinopathy progression**

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Background
Inflammatory cytokines were considered to play a determinant role in the development and progression of different microvascular diabetic complications including diabetic retinopathy. interleukin 27 (IL27) is a cytokine with dual proinflammatory/anti-inflammatory effects that mediates pathogenesis of different inflammatory diseases.

Aim
To assess the serum and aqueous levels of (IL27) as proinflammatory/anti-inflammatory cytokine in diabetic patients with or without retinopathy and to assess the correlations between its levels with conventional risk factors for diabetic retinopathy such as serum glucose, cholesterol, triglycerides levels, and HbA1c.
Methods
Sixty diabetic patients with and without retinopathy, along with 20 healthy controls, were enrolled in the study. Serum levels of IL27 were assessed using ELISA. Serum lipid profiles, glucose levels, and whole blood HbA1c were also detected.
Results
Mean aqueous levels of IL27 in patients with proliferative diabetic retinopathy ($6.1\pm2$ ng) and non-proliferative diabetic retinopathy ($6.7\pm2.7$) were significantly elevated as compared to either diabetic patients without retinopathy ($4.6\pm0.5$) or healthy control group ($4.1\pm0.8$). Mean serum levels of IL27 were elevated in diabetic patients with retinopathy compared with those without retinopathy or control group. Aqueous IL27 was positively correlated with conventional risk factors for diabetic retinopathy such as serum glucose, cholesterol, triglycerides levels, and HbA1c.
Conclusion
This study showed significant elevated levels of IL27 in the serum and aqueous humour of patients with diabetic retinopathy and a correlation between its aqueous levels with some of diabetic retinopathy risk factors such as serum glucose, cholesterol, triglycerides levels, and HbA1c.

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## Growth and development

### P140

**Hormonal control of β-cell proliferation in fetal ovine pancreatic islets *in vitro***

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Hormonal control of pancreatic β-cell development in the fetus during late gestation remains unclear. In fetal sheep hypothyroidism causes an increase in pancreatic β-cell mass in association with increased plasma insulin and leptin concentrations. This study investigated the effects of triiodothyronine ($T_3$), insulin and leptin on proliferation rates in fetal ovine pancreatic β-cells *in vitro*. All procedures were approved by the Animal Care and Use Committee at the University of Arizona. Pancreatic islets were isolated from twin sheep foetuses ($n=9$) after euthanasia between 133 and 142 days of gestation (term ~145 days). Islets were cultured for 48 h with increasing concentrations of $T_3$, insulin and leptin. Proliferating cells were identified by the incorporation of nuclear 5-ethynyl-2′-deoxyuride (EdU) added to the media for the final 24 h and expressed as a percentage of the total insulin-positive cells determined by immunocytochemistry. A minimum of 200 EdU-insulin positive cells were counted for each treatment. Data (mean ± S.E.M.) were assessed by one-way ANOVA followed by Tukey's post hoc test.
Pancreatic β-cell proliferation *in vitro* was inhibited by all concentrations of $T_3$ (0 ng/ml: $7\pm0.5\%$; 0.1 ng/ml: $5\pm0.4\%$; 1 ng/ml: $3\pm0.3\%$; 10 ng/ml: $2\pm0.2\%$; $P<0.05$) and increased by insulin at 10 ng/ml only (0 ng/ml: $5\pm0.4\%$; 0.1 ng/ml: $5\pm0.5\%$; 1 ng/ml: $6\pm0.5\%$; 10 ng/ml: $10\pm0.9\%$; $P<0.05$). Leptin induced a biphasic response whereby proliferation was suppressed at the lowest concentration and increased at the highest concentration (0 ng/ml: $7\pm0.5\%$;

0.1 ng/ml: 4±0.5%; 1 ng/ml: 5±0.5%; 10 ng/ml: 10±0.9%; $P<0.05$).
Proliferation of β-cells isolated from the ovine fetal pancreas is sensitive to physiological concentrations of $T_3$, insulin and leptin. Changes in these hormones may be responsible for the increased β-cell mass observed in the hypothyroid sheep fetus and may have consequences for pancreatic function in later life.
This work was supported the Nigel Groome Studentship and the Society for Endocrinology Practical Skills Grant.
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## P141

**Inter-correlations between placental genes regulating foetal glucocorticoid exposure and IGF2 in maternal severe obesity: a mechanism for higher birthweight?**
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Background
Maternal obesity in pregnancy associates with higher birthweight. A key pathway is through placental regulation of hormones controlling foetal growth. As excess foetal glucocorticoid exposure associates with lower birthweight and since placental Insulin-like Growth Factor (IGF2) may be modulated by glucocorticoids, we hypothesised that the expression profiles of placental genes leading to reduced glucocorticoid exposure and increased IGF2 mRNA level correlate with higher birthweight in severely obese pregnancy.
Methods
The mRNA levels of placental genes regulating glucocorticoid synthesis (11β-HSD1), clearance (11β-HSD2), sensitivity (NR3C1-αIGF2 and IGF2R were quantified in term placental samples from lean (BMI $\leq$ 25 kg/m$^2$, n=42) and very severely obese (SO BMI $\geqq$ 40 kg/m$^2$, n=43) pregnancies exclusive of antenatal steroids and gestational diabetes. Neonatal anthropometry was obtained from hospital records. Ethical approval was obtained.
Results
The standardised (SDS) birthweight, SDS birth length, SDS BMI and all mRNA levels were similar in both groups. In SO placentas only, mRNA levels of 11β-HSD1 and NR3C1-α positively correlated with IGF2 and IGF2R (all $\rho<0.30$, $p<0.05$). Higher placental 11β-HSD1 ($\rho=0.29$, $P=0.09$), NR3C1-α ($\rho=0.54$, $P=0.001$), and lower 11β-HSD2 ($\rho=0.42$, $P=0.012$) mRNAs (consistent with increased foetal glucocorticoid exposure) correlated with shorter birth length in lean only. Higher placental 11β-HSD1 ($\rho=0.35$, $P=0.04$) and NR3C1-α? ($\rho=0.35$, $P=0.04$) also correlated with increased SDS BMI in lean only. None of the genes correlated with SDS birthweight in lean. Only in SO group did we observe correlations between increased placental IGF2 mRNA and higher SDS birthweight ($\rho=0.40$, $P=0.005$).
Discussion
The inter-correlations between glucocorticoid-linked genes with IGF2 and IGF2R in SO placentas, but not in controls, support a modulatory role of glucocorticoids on placental foetal growth through excess IGF2 exposure in foetuses of SO pregnancy. Further, the contrasting correlation profiles of placental gene expression and infant's size at birth between lean and SO group suggest a protective-adaptive response in SO placentas that prevent growth restriction and support excess birthweight.
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## P142

**Human foetal adrenal gland development and effects of maternal smoking**
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Introduction
The human foetal adrenal gland (HFA) is a highly active endocrine organ, producing large amounts of DHEA and DHEAS. Our understanding of HFA development is limited, however, and species differences mean that animal models are only of partial use. Maternal cigarette smoking is known to increase the post-natal risk of health complications of the foetus, and the mechanisms involved may include effects on the HFA.
Aim
To examine normal HFA development during the second trimester and determine whether it is affected by maternal smoking.
Methods
HFAs were obtained from elective terminations (REC04/S0802/21) of second trimester foetuses between 11-21 weeks of gestation. Foetuses were grouped according to sex, gestational age and maternal smoking. Key enzymes in the HFA steroidogenic pathway were investigated using real-time PCR, Western blot and immunohistochemistry (IHC).
Results
Maternal smoking was associated with a significant (P=0.004) increase in the growth trajectory of the HFA and a progressive decrease in plasma ACTH concentration ($P<0.001$) in male foetuses (n=53). The most highly expressed transcript was CYP17A1. Maternal smoking was associated with increased variability of *STAR* (P=0.001), *CYP17A1* (P=0.02) and *CYP21A2* (P=0.04) transcript expression in a sex-dependent manner although protein levels (Western blot) were unaffected. Transcripts levels of *CYP11B1, CYP11B2, SULT2A1*, and *HSD3B* were unaffected by maternal smoking. IHC of STAR, CYP11A1, and CYP17A1 showed that enzyme expression was predominantly found in the foetal zone. HSD3B was expressed at low levels in the definitive zone.
Conclusions
The HFA dominates foetal steroid endocrinology and this study provides detailed developmental data for the critical second trimester period. We also found that maternal smoking during pregnancy can dysregulate adrenal growth and transcript levels although it remains to be determined whether post-natal health and adrenal function are significantly compromised.
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## P143

**Vitamin D promotes myogenic differentiation and induces an antifibrotic phenotype in primary cultures of skeletal muscle derived satellite cells and fibroblasts**

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Purpose of study
Skeletal muscle wasting is a serious public health problem associated with aging, chronic kidney disease, and AIDS. Vitamin D (VD) is most widely recognised for its regulation of calcium and phosphate homeostasis in relation to bone development and maintenance and for its synergistic effects on target organs such as PTH glands. Recently, it has been shown to improve muscle performance and reduce falls in VD deficient older adults. However, little is known of the underlying molecular mechanism or the role it plays in association with myogenic differentiation and on muscle fibrosis. We examined the effect of 1,25-$D_3$ -the active form of VD- on myogenic cell differentiation and on the generation of an anti-fibrotic phenotype in skeletal muscle derived cells.
Methods
Primary cultures of skeletal muscle derived satellite cells and fibroblasts were isolated from the tibialis anterior, soleus and gastrocnemius muscles of 2-month-old C57/BL6 male mice and then treated with or without 1,25-$D_3$ in a time course manner. Expression of vitamin D receptor (VDR), collagen I, III, pro and anti-fibrotic factors, muscle lineage and angiogenic markers were assessed by ICC, IF, PCR arrays and confirmed by real time qPCR and western blots.
Summary
The efficiency of satellite cells isolation determined by PAX-$7^+$ cells was 86%. It was confirmed that Satellite cells expressed VDR. Addition of 1,25-$D_3$ (100nM) to satellite cells induces: i) increase expression of Troponin-I and II, ii) increase expression of IGF-I and IGF-II, iii) increase expression of Follistatin (−a Myostatin inhibitor) and iv) a decrease expression of Mstn (Myostatin- a key negative regulator of muscle mass). Fibroblast isolated with a 90% efficiency determined by Vimentin$^+$ and $\alpha$-SMA$^-$ cells showed a decreased expression of collagen I and III after being challenged with TGF-β alone or in combination with 1,25-$D_3$.
Conclusion
Vitamin D posses a clear myogenic effect on satellite cells (adult muscle stem cells) in charge of reconstitute the muscle after muscle injury or muscle waste. It also posses an anti-fibrotic effect on fibroblast of muscle origin. This study provides a mechanistic justification for VD replenishment in muscle waste conditions such as AIDS, cancer, congestive heart failure, renal failure, characterized by lost of muscle mass and excessive collagen deposition (fibrotic process) and also in VD deficient older adults who are known to have age-related loss of muscle mass and strength and an increased rate of falls.

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## P144

**Hypothyroidism, altered leptin and c-reactive protein synthesis characterised pregnancy exposed to genistein**

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This study examined the mechanism by which genistein produced adverse effects in pregnant laboratory rats. Pregnant rats were divided into control (Con) and genistein (Gen) force fed (2 mg/kg) groups. At terminal gestation day (GD) ranging from 0 to 20, the rats were sacrificed, and blood samples and amniotic fluids were collected. Thyroid hormone, C-reactive protein (CRP) and leptin assay was carried using the blood samples. Leptin was also assayed in the placenta and amniotic fluid supernatant. Oral exposure of pregnant rats to genistein significantly altered maternal $T_3$, (GD18; Con 1.65±0.01, Gen 1.03±0.04 nmol/l), $T_4$ (GD6; Con 29.60±0.00, Gen 36.04±1.29 nmol/l), Leptin (Placenta GD20; Con 0.08±0.01, Gen 0.31±0.02 ng/ml, amniotic fluid;GD 20; Con 0.02±0.00, Gen 0.35±0.05 ng/ml) in genistein group. These changes were accompanied with loss of embryonic implants and a decreased foetal and placental weight. Oral exposure of pregnant rats to genistein precipitated hypothyroidism, altered some metabolic hormones with a reduction in foetal and placental growth and increased resorption of embryonic implants.

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# Neoplasia, cancer and late effects

## P145

**Nicotinamide nucleotide transhydrogenase (NNT) as a novel molecular target in adrenocortical carcinoma – impact of NNT knockdown on adrenocortical cell proliferation, redox balance and steroidogenesis**

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Nicotinamide nucleotide transhydrogenase (NNT) is a NADPH-generating mitochondrial proton pump with a central role in mitochondrial antioxidant pathways. Recent studies revealed inactivating NNT mutations in patients with familial glucocorticoid deficiency, indicating a selective susceptibility of the adrenal cortex to NNT deficiency and oxidative stress. Here we explored the potential value of NNT as a therapeutic target in adrenocortical cancer. We delineated the distinct effects of NNT loss on cellular proliferation and steroidogenesis, employing two *in vitro* knockdown models, including transient siRNA-mediated knockdown and shRNA-mediated stable loss of NNT in the adrenocortical cell line NCI-H295R. Transient NNT knockdown impaired cellular redox balance, resulting in a lower ratio of reduced to oxidised glutathione on luminescence-based quantification. Assessment of proliferation using a fluorescent DNA dye revealed a loss in cellular viability and decrease in proliferation with NNT knockdown (64±8% decrease compared to scrambled siRNA-transfected cells, $p<0.01$). NNT-deficient cells also became exceedingly sensitive to mild chemically induced oxidative stress. After long-term culture, H295R cells with stable NNT knockdown appeared to develop compensatory mechanisms, improving their redox balance and proliferative potential. This adaptation was associated with alterations in glycolytic and oxygen consumption rates, as demonstrated by extracellular flux analysis using Seahorse XF technology. Steroid profiling by liquid chromatography-tandem mass spectrometry revealed a distinct profile induced by transient NNT knockdown, comprising lower 17OH-progesterone but, surprisingly, higher androstenedione and cortisol synthesis, with a pronounced increase in 11β-hydroxylase activity (ratio of 11-deoxycortisol/cortisol 93±16 in cells transfected with scrambled siRNA vs. 58±8 with NNT knockdown, $p<0.01$). A similar steroidogenic pattern was observed with stable knockdown. Our study suggests a potential role of NNT inhibition as a novel therapeutic approach in advanced adrenocortical carcinoma. Steroid profiling reveals a surprising increase in glucocorticoid and androgen synthesis with NNT loss, challenging the previous association of impaired redox balance and adrenal insufficiency.

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## P146

**The somatostatin analogue pasireotide decreased proliferation and increased apoptosis in pancreatic and pituitary neuroendocrine tumors in a MEN1 mouse model**

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Improved therapies for pancreatic and pituitary neuroendocrine tumors (NETs), which may occur in Multiple Endocrine Neoplasia type 1 (MEN1), are needed. We assessed the effects of pasireotide, a somatostatin analogue with high affinity for somatostatin receptors (SSTRs) −1, −2, −3 and −5, in a mouse model of MEN1. *Men1*$^{+/-}$ mice treated from 12 months of age with 40 μg/g pasireotide ($n=71$), or phosphate-buffered saline (PBS) ($n=73$), i.m. each month for 9 months, had magnetic resonance imaging (MRI) at 12 and 21 months of age, and received oral 5-bromo-2-deoxyuridine (BrdU), to assess tumour development and proliferation respectively. NETs were harvested at 21 months, and proliferation and apoptosis assessed by immunohistochemical quantification of BrdU-stained nuclei and TUNEL assays, respectively. Immunohistochemical analysis confirmed that the pancreatic and pituitary NETs expressed high levels of SSTR1, 2 and 5 and lower levels of SSTR3. Pasireotide-treated *Men1*$^{+/-}$ mice had increased survival (82% (pasireotide) vs. 64% (PBS), $P<0.05$), were associated with the development of fewer pancreatic (86% (pasireotide) vs. 98% (PBS)) and pituitary (52% (pasireotide) vs. 72% (PBS)) NETs (both $P<0.05$), and had smaller increases in pituitary NET volumes (pre-treated vs post-treated $=0.870\pm0.087\text{mm}^3$ vs $3.094\pm0.714\ \text{mm}^3$ (pasireotide) compared to $0.843\pm0.065\text{mm}^3$ vs $8.847\pm1.948\text{mm}^3$ (PBS), $P<0.01$). In addition, pasireotide-treated mice had fewer pancreatic NETs compared to PBS-treated mice (2.36±0.25 vs

3.72±0.32, respectively, $P<0.001$), had decreased proliferation in pancreatic (0.35±0.03% (pasireotide) vs. 0.78±0.08% (PBS)) and pituitary (0.73±0.07% (pasireotide) vs. 1.81±0.15% (PBS)) NETs (both $P<0.0001$), but had increased apoptosis in pancreatic (0.42±0.05% (pasireotide) vs. 0.19±0.03% (PBS)) and pituitary (14.75±1.58% (pasireotide) vs. 2.35±0.44% (PBS)) NETs (both $P<0.001$). Thus in summary, pasireotide treatment increased survival by ~20% and inhibited pancreatic and pituitary NET growth, indicating its potential as an anti-proliferative and pro-apoptotic therapy for pancreatic and pituitary NETs.

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## P147

**Steroid sulphatase and G-protein coupled oestrogen receptor in human colorectal cancer: correlation with late-stage disease and potential therapeutic targets**

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Steroid sulphatase (STS) liberates sulphated oestrogens into their active forms. In the colon, evidence suggests that although initially pro-apoptotic in healthy mucosa, once malignancy occurs, oestrogens may stimulate colorectal cancer (CRC) proliferation. Moreover, greater intratumoural oestrogen synthesis is negatively associated with survival outcomes in CRC patients. However, little is known about oestrogen metabolism pathways in CRC, and whether alterations in local oestrogen synthesis and actions relate to clinical and pathological features. Furthermore, it is unknown whether manipulation of oestrogen pathways has therapeutic potential. Therefore, using qRT-PCR and immunoblotting, in healthy human colorectal tissue matched with CRC samples (n=56) we correlate the dysregulation of key oestrogen synthesis enzymes (steroid sulphatase (STS), 17β-hydroxysteroid dehydrogenase (17βHSD) type-1, type-2, type-7, and type-12) and the G-protein coupled oestrogen receptor (GPER), with patient TNM staging, lymph node infiltration, and distant metastases. In addition, ELISA assays were undertaken to ascertain the effects of oestrogens on proliferation of CRC cell lines. STS activity, 17βHSD7, and 17βHSD12 expression all showed a positive correlation with TNM staging in patient CRC samples, indicating greater oestrogen availability is linked to advanced stage disease. Increased GPER expression also significantly ($P<0.05$) correlated with late-stage malignancy. In CRC cell lines, over-expression of STS significantly ($P<0.01$) increased cell proliferation when treated with sulphated oestrogens. This effect was completely ablated when treated in combination with the STS inhibitor STX64 ($P<0.001$). Furthermore, we show here for the first time that the GPER agonist, G1, also stimulated CRC proliferation; with both oestrogen and G1 effects significantly inhibited with the GPER selective antagonist G15 ($P<0.001$). Increased STS activity and GPER expression are associated with late-stage CRC, strongly suggesting a role for oestrogens in this malignancy. Thus, reducing the availability and action of oestrogens by inhibiting STS and GPER, respectively, may have therapeutic benefits for patients with CRC.

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## P148

**PTTG is phosphorylated at residue T60 and regulates p53 stability, in conjunction with PBF, in head and neck cancer**

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PTTG is a multifunctional proto-oncogene, overexpressed in thyroid, pituitary and other endocrine cancers. PTTG is also implicated in the pathogenesis of head and neck cancer, where high PTTG expression independently correlates with advanced tumour stage and reduced disease-free survival. Recently, abrogation of residue threonine-60 (T60) has been associated with altered PTTG half-life, chromosomal instability and cell invasion. We therefore generated a phospho-specific antibody against T60 PTTG. Transient transfection of wild-type PTTG resulted in a significant increase in T60-phosphorylated PTTG protein expression in HeLa cells (3-fold, p=0.001), which was blocked by a phospho-peptide. Detection was also lost with a PTTG-T60 mutant (T60A). Antibody specificity was further confirmed by immunoprecipitation assays. Paraffin-embedded formalin-fixed tumour sections were obtained for immunohistochemical analysis from patients with primary oropharyngeal squamous cell carcinoma. Abundant total PTTG protein expression was evident both in the cytoplasm and nucleus. In contrast, expression of T60-phosphorylated PTTG was predominantly nuclear. As interaction with its binding partner PBF facilitates PTTG nuclear localisation, and both proto-oncogenes alter p53 stability and function, we assessed the relative contributions of PTTG and PBF to p53 stability. Preliminary experiments demonstrated that transfection of wild-type PBF or PTTG into HPV-positive 93-VU-147T HNSCC cells decreased p53 protein levels compared to controls. Further, half-life studies demonstrated reduced p53 stability in 93-VU-147T cells transfected with either PBF or PTTG. Interestingly, transfection with a PBF mutant incapable of PTTG interaction, or a PTTG mutant unable to bind PBF, resulted in an initial decrease in p53 stability followed by subsequent stabilisation. These data indicate a potential role for both PTTG and PBF in modulation of p53 stability in head and neck cancers. Furthermore, PTTG is phosphorylated at residue T60 in head and neck tumours, which may alter its well described mitotic regulatory function.

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## P149

**Distinct p53 response profiles in transgenic mouse models of thyroid-specific PBF and PTTG expression**

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Functional disruption of the tumour suppressor p53 has a critical role in promoting the development of most cancers. The proto-oncogenes PBF and PTTG1 both regulate p53 activity, but the relative contribution of each gene in influencing p53 function has not been delineated, especially in thyroid cancer where both proto-oncogenes are commonly overexpressed. To better understand the interplay between PTTG1, PBF and p53 in vivo, we examined p53 responses in primary thyrocytes cultured from transgenic mice overexpressing PBF (PBF-Tg) and PTTG1 (PTTG-Tg), either singly or in combination in a bi-transgenic murine model (Bi-Tg). Western blotting showed that p53 and γ-H2AX protein levels were elevated in PTTG1-Tg and BI-Tg thyrocytes (>2-fold; $P<0.05$). In contrast, no significant increase was observed in p53 or γ-H2AX levels in PBF-Tg thyrocytes compared to WT (P=NS). Consistent with this, a greater proportion of a panel of p53-responsive DNA repair genes were significantly down-regulated in PTTG1-Tg (30/83 genes) and BI-Tg (30/83 genes) than in PBF-Tg thyrocytes (12/83 genes). A differential p53 response was further evident following gamma-irradiation of cells, with fewer significant mRNA changes occurring in PTTG1-Tg (0/10 genes; P=NS) and BI-Tg (4/10 genes; $P<0.05$) than in WT primary thyrocytes (10/10 genes; $P<0.01$). By comparison, irradiation of PBF-Tg thyrocytes gave the greatest reduction in mRNA levels (6/10 genes; $P<0.05$) for genes such as Chek1 (4.4-fold; $P<0.01$) and Rad51 (8.4-fold; $P<0.01$). We therefore examined potential associations between PBF and DNA repair genes in human thyroid tumours. Importantly, a significant correlation was apparent between PBF and Chek1 ($R=0.44$; $P<0.05$; N=22), Fancg ($R=0.78$; $P<0.001$; N=22) and Mutyh ($R=0.62$; $P<0.05$; N=22). Together our data reveal for the first time that PBF and PTTG1 mediate distinct p53 response profiles in vivo. These results offer important insights for understanding the impact of proto-oncogenes on thyroid tumorigenesis and for identifying new tumour biomarkers.

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## P150

**Follow up of differentiated thyroid cancer survivors during pregnancy: a retrospective analysis**

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Differentiated thyroid cancer (DTC) is common in female patients of reproductive age and generally has a good prognosis and so many patients may become

pregnant following treatment. Progression of DTC during pregnancy has been reported and may relate to thyroid stimulation by human chorionic gonadotropin (hCG). Until recently suppression of thyrotropin (TSH) by supraphysiological doses of levothyroxine was indicated for all patients following thyroidectomy and radioiodine ablation for DTC. We retrospectively analysed 31 patients during pregnancy between 2008 and 2014 following successful treatment of DTC to determine 1) changes in thyroglobulin (TG) levels during pregnancy and 2) the effect of TSH suppression on birth weight.

Thyroglobulin measurements during pregnancy were available in 28/31 patients, of which 26 remained suppressed, defined as TG $<0.90$ μg/l. One patient had elevated levels prior to pregnancy with no significant increase during follow up (2.35 μg/l to 2.94 μg/l) and one patient had a rise in TG (0.48 μg/l to 1.65 μg/l), with no evidence of recurrence on neck ultrasound and a return to baseline levels following delivery. TSH levels were lower in the third trimester compared to first and second, with 69%, 89% and 97% achieving levels $<0.2$miu/l respectively in each trimester. The mean free T4 levels were similar in each trimester ($19.6 \pm 0.6$ pmol/l, $18.5 \pm 1$pmol/l and $19.8 \pm 0.4$ pmol/l respectively). Delivery weight was available for 20 pregnancies with a mean birth weight of $3.5 \pm 0.6$ kg, which is similar to the UK national average.

In our cohort of patients there was no evidence of DTC relapse during pregnancy and routine monitoring of TG levels in pregnancy may not be necessary unless pre-conception TG levels are elevated. Suppression of TSH levels did not affect birth weight, and if required during pregnancy, should not adversely affect outcome provided free T4 levels are maintained within the normal reference range.

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## P151

### Pituitary-related outcomes of cranial radiotherapy (cXRT) in adults with gliomas

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Introduction

Radiation-induced hypopituitarism has been well-described in childhood-onset brain tumour survivors, however in adults has received less attention. The aim of this study was to assess the pituitary-related outcomes following cXRT in adults with extra-sellar gliomas.

Methods

We retrospectively collected longitudinal data regarding pituitary-related outcomes from medical records of 59 patients, diagnosed with extra-sellar gliomas in adulthood, for the entire duration of their endocrine follow-up. Patients were referred to endocrinology from a tertiary cancer referral centre. GH and HPA axes were assessed by ITT and/or GST, while gonadotropin, TSH and prolactin status were evaluated using basal values of the relevant anterior pituitary hormones.

Results

patients (32 males) diagnosed with gliomas (astrocytoma, oligodendroglioma, glioblastoma and ependymoma) were assessed. 54.2, 35.6 and 10.2% of tumours were localised in the anterior, middle and posterior cranial regions respectively. All patients received photon external beam cXRT (mean dose $53.3 \pm 6.0$Gy, mean number of fractions $28.7 \pm 3.3$). Patients' mean age at cXRT was $41.2 \pm 10.9$ years and the mean duration of follow-up was $8.7 \pm 5.1$ years. 76.3% of patients had brain surgery, while 54.2% received chemotherapy. The GH was the most commonly affected axis (severe GHD 55.9%, partial GHD 23.7%), followed by LH/FSH, ACTH and TSH deficiency (23.7, 17 and 6.8% respectively). Clinically significant ACTH deficiency necessitating glucocorticoid replacement was only present in two patients (3.4%). Hyperprolactinaemia was observed in 6 patients (10.2%), which was persistent in one case only. Longitudinal data analysis revealed gradual increase in the prevalence of pituitary hormone deficits throughout the follow-up period.

Conclusions

We observed high prevalence of pituitary dysfunction in adults with extra-sellar brain tumours following cXRT, which was comparable with the rates seen in childhood-onset brain tumour survivors. Long-term surveillance of these individuals in endocrine centres with appropriate expertise is required, given the evolving nature of radiation-induced hypopituitarism.

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## P152

### Phenotypic heterogeneity associated with proglucagon-expressing tumours is due to differential processing and secretion of proglucagon-derived peptides

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Context

Pancreatic neuroendocrine tumours (NETs) overexpressing glucagon are associated with phenotypic heterogeneity. Objective: To correlate clinical phenotype with detailed analysis of plasma levels of proglucagon-derived peptides (PGDPs) in subjects with proglucagon-expressing tumours using specific immunoassays and gel filtration profiles to elaborate molecular heterogeneity of PGDPs, before and after somatostatin analogues.

Case 1

A 57 year old women presented with necrolytic migratory erythema, refractory constipation, anorexia and hyperinsulinaemic hypoglycaemia. Liver biopsy demonstrated a grade 1 NET. CT revealed a pancreatic lesion with liver metastases and profound small bowel mucosal thickening. Biochemical investigations revealed hyperproglucagonaemia (3504 pmol/l). Octreotide treatment increased appetite, abolished hypoglycaemia and rapidly improved her rash.

Case 2

A 48 year old male presented with newly diagnosed diabetes mellitus, weight loss, vomiting and perineal rash. CT revealed a pancreatic lesion with widespread hepatic and bony metastases. Biopsy demonstrated a grade 1 NET. Biochemical investigations revealed hyperproglucagonaemia (786 pmol/l) and somatostatin analogues were commenced. Results: In both cases, plasma levels of detectable proglucagon, glucagon, GLP-1 and GLP-2 were elevated compared with healthy subjects, and attenuated by somatostatin analogues. In case 1, proglucagon processing by the tumour was similar to that of the intestinal L cell with increased production of intact GLP-1 and GLP-2 and manifesting clinically with hyperinsulinaemic hypoglycaemia and thickened small bowel, respectively. Unlike healthy controls, fifty percent of GLP-1 was glycine extended. In case 2, the tumour secreted a pancreatic α-cell profile of PGDPs and diabetes was the consequence of elevated intact glucagon. Case 2 had higher detectable levels of GLP-2-like immunoreactivity, however gel filtation profiling revealed the predominance of a lower molecular weight peptide with biological inactivity.

Conclusion

Differential processing of PGDPs by proglucagon-secreting pNETs explains the clinical heterogeneity associated with these tumours. Moreover, detailed phenotyping of such patients may provide improved understanding of PGDP biology in man.

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## P153

### Novel targeted treatment combinations for malignant neuroendocrine tumour olfactory neuroblastoma

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Background

Olfactory neuroblastoma (ONB), a neuroendocrine nasal tumour, exhibits a range of phenotypes from indolent to very aggressive. Even early disease is associated with high (60%) recurrence rates, while advanced disease has 1.5y disease-free and 2.5y overall mean survival. Medical treatments against primary and recurrent disease as well as prognostic biomarkers are urgently required. The few studies available suggest that mTOR/MAPK and Sonic Hedgehog signalling has a role in tumorigenesis in ONB. As cross-talk between pathways leads to drug resistance when single agent inhibitors are used, combined pathway inhibition makes rational sense.

Methods

The human ONB cell line TC268 was treated with IGF-1-inhibitor NVP-AEW541, dual PI3K/mTORC1/2-inhibitor NVP-BEZ235, VEGFR-inhibitor sunitinib, dual AKT/ERK-inhibitor lovastatin, sonic hedgehog-inhibitor itraconazole and novel S6K1-inhibitors FS115/147 alone and in combinations. Western blot and RT-qPCR are used to demonstrate altered pathway components for mTOR/MAPK and Hedgehog signalling respectively.

Results
Most effective inhibition occurred with vertical pathway blockade using AEW541&FS114 ($P<0.0001$). Similar results were obtained using sunitinib&FS115 ($P<0.0001$). Combined treatments were more effective than single drugs and at 10x lower doses. Further addition of itraconazole significantly reduced cell viability in combination with S6K1-inhibitors alone and S6K1-inhibitors with AEW541 ($P<0.0001$). BEZ235 was not as effective at reducing cell viability either alone or in combination, and when this was explored at the protein level we demonstrated unexpected increases in both AKT and ERK phosphorylation post treatment, which may represent a resistance mechanism. Lovastatin did not result in a statistically significant change to viability in any combination.
Establishment of a safe, effective, targeted, combined therapy could be validated on primary culture and a nude mouse xenograft model and then proposed for clinical trials, ultimately aiming at treating both primary and recurrent/inoperable disease.

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## P154

**Adrenal pigmentation in PPNAD is a result of melanin deposition and associated with upregulation of the melanocortin 1 receptor**

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Primary pigmented nodular adrenal disease (PPNAD) is a form of bilateral adrenocortical hyperplasia characterised by small to normal sized adrenal glands containing multiple small cortical pigmented nodules[1]. It may occur independently, but 90% of cases are a manifestation of the Carney complex. Most cases of PPNAD are diagnosed before age 30, and are the result of a germline mutation in PRKAR1A or PDE11A, leading to upregulation of cAMP signalling. It is a cause of ACTH-independent Cushing's syndrome. The cause of the black/brown pigmentation in PPNAD has not been definitively established, although some authors have ascribed it to lipofuscin, a product of lysosomal breakdown.
Paraffin-embedded bilateral adrenalectomy specimens from five paediatric patients with PPNAD were subjected to a series of histological and immunohistochemical staining techniques. Haematoxylin and eosin staining revealed intracellular deposits of a brown pigment that was removed by permanganate bleaching. The pigment was negative on Ziehl-Neelsen staining, and strongly positive with the PERLS and Masson-Fontana stains. Immunohistochemistry showed strong staining within the nodules for the melanosome marker HMB-45. These findings identify the pigment as melanin. The adrenal nodules in all five cases immunostained strongly positive for the melanocortin-1 receptor (MC1R), and for 11β-hydroxylase, the final enzyme in the steroidogenic pathway leading to cortisol production.
We suggest that the autonomously activated cAMP signaling pathway associated with PPNAD leads to upregulation of the MC1R, induction of steroidogenic enzymes, and generation of melanin within melanosomes. We hypothesise that the mechanism for hyperpigmentation in Carney complex skin lesions has a similar aetiology.
Reference
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## P155

**Primary cilia: a new player in phaeochromocytoma pathogenesis?**

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Introduction
Primary, non-motile, cilia are microtubule-based organelles that protrude from the cell membrane into the extracellular environment of virtually all nucleated mammalian cells. They function as signalling platforms involved in the transduction of extracellular stimuli and have an important role in cell cycle regulation. Disruption of primary cilia structure and therefore function has been identified in a range of cancers including kidney, breast, pancreatic and prostate, implying a role in tumorigenesis.
Primary cilia disruption is a cardinal feature of clear cell renal cancer; the subtype associated with von Hippel-Lindau (VHL) disease. One of the functions of protein VHL is to maintain the primary cilium by stabilising microtubules. There are no data regarding primary cilia and the other (non-renal) pathologies seen in VHL. We sought to investigate whether primary cilia are disrupted in phaeochromocytomas; both in the context of sporadic and familial (including VHL) cases.
Methods
Tissue/cells: tissue sections and primary cell culture from phaeochromocytomas and adjacent adrenal gland. Cilia detection: dual labelled immunofluorescence was performed against two components of the ciliary axoneme (acetylated α tubulin and Arl13b). Primary cilia incidence and length was quantified from maximum intensity projections generated from confocal Z stacks ($n>1000$ for incidence, $n>100$ for length measurements).
Results
Primary cilia structure is disrupted in phaeochromocytomas; both sporadic cases and those occurring in patients with germline mutations. Cilia are shorter and less frequent ($P<0.05$) in phaeochromocytomas compared to adjacent adrenal gland.
Discussion
Primary cilia structure is disrupted in phaeochromocytomas which may impact on cilia-mediated signalling, dysregulation of which is relevant to phaeochromocytoma pathogenesis.

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## P156

**Adverse metabolic profile in long-term survivors of adult and childhood-onset brain tumours: the role of growth hormone deficiency**

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Introduction
Childhood-onset brain tumour (CO-BT) survivors demonstrate elevated standardized mortality rates for cardiac disease. Adverse lipid profile and body composition contribute to the increased cardiovascular risk. Little is known about the metabolic changes in long-term survivors of adult-onset brain tumours (AO-BT).
Methods
We performed a cross-sectional study to compare cardiovascular risk parameters in CO-BT with AO-BT survivors and healthy controls. Measurements of lipid profile, fasting glucose and body composition were performed. Basal anterior pituitary hormone profile and dynamic tests (ITT or GST) were also undertaken.
Results
patients with AO-BT (mean age 39.5±13.2 years), 17 patients with CO-BT (mean age 20.4±4.9 years) and 36 healthy controls were assessed. All patients received cranial radiotherapy. 89.5% (17/19) of adult-onset and 94.1% (16/17) of childhood-onset patients developed growth hormone deficiency (GHD), partial or severe. No difference in the lipid profile and body composition was noted between the AO-BT and CO-BT groups. Fasting glucose was higher in the adult-onset compared with the childhood-onset group (5.07±0.99 mmol/l vs 4.56±0.33 mmol/l, P=0.04), however no difference was observed in the HbA1c. Patients with GHD, not on GH replacement, demonstrated significantly elevated total (5.64±1.06 mmol/l vs 4.66±0.9 mmol/l, P=0.004) and LDL cholesterol (3.28±0.66 mmol/l vs 2.76±0.82 mmol/l, P=0.016), BMI (28.3±6.6 vs 24.8±3.5, P=0.046), waist/hip ratio (0.89±0.07 vs 0.82±0.07, P=0.001) total (26.6±12.5 kg vs 15.4±7.5 kg, P=0.001) and truncal (14.76±6.35 kg vs 8.1±4.1 kg, P<0.001) fat mass and skinfold thickness (78.9±33.1 mm vs 41.32±17.2 mm P<0.001), compared with controls. On the contrary, patients on GH replacement did not differ for the above parameters from controls, with the exception of summative skinfold thickness, which was higher in the patient group (73.95±31.03 mm vs 45.38±14.18 mm, P=0.038).
Conclusions
Our results demonstrate an adverse cardiovascular risk profile in long-term brain tumour survivors of both adult and childhood onset. GHD has a pivotal role in patients' metabolic status, while GH therapy improves most of the metabolic parameters.

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## P157

**Ultrasound-guided percutaneous ethanol ablation for selected patients with papillary thyroid microcarcinoma: a novel, effective and well tolerated alternative to neck surgery or observation**

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Introduction
Perhaps due to a current global epidemic of "overdiagnosis", papillary thyroid microcarcinoma (PTM) is now the commonest endocrine malignancy (BMJ 348: 3045, 2014). Current management options vary from lobectomy or total thyroidectomy to "active surveillance". An alternative approach, used successfully for eliminating neck nodal metastases (JCEM 96: 2717, 2011), is ultrasound-guided percutaneous ethanol ablation (UPEA). Here we present our experience of treating with UPEA biopsy-proven tumour foci in ten PTM patients.
Subjects/methods
Study patients (7F, 3M) were aged, at time of UPEA, 36–86 years (median 52 years); three had significant co-morbidities. The twelve tumours varied from 4 to 10 mm diameter; tumour volumes ranged from 25 to 375 $mm^3$ (median 125). UPEA technique (local anesthetic, outpatient) and follow-up protocol was previously described (Surgery 154: 1448, 2013). The first patient had under ultrasound guidance only a single injection of 0.2 cc 95% ethanol directly into his tumour focus (8mm diameter; 232 mm3volume). Subsequent patients had two injections on consecutive days; ethanol volume injected ranged from 0.45 to 1.25 cc (median 0.9 cc). Three (33%), who had $<50\%$ tumour shrinkage, had a 3rd injection at 3–5 months. All patients were followed with neck ultrasound scans, with recalculation of tumour volume and assessment of tumour-associated Doppler flow at each visit.
Results
Patients were followed for 0.3–4.4 years (median 2.0). No patient developed after UPEA a painful thyroiditis; none had hoarseness or hypocalcaemia. All tumour foci have shrunk and Doppler flow eliminated. Average tumour volume reduction was 80% (range 52–100%); 4/12 tumours were no longer identifiable after 0.7–2.2 years (mean 1.5 years).
Conclusions
UPEA for PTM was well tolerated and was substantially ($>38$ 000 USdollars) cheaper than conventional surgery. Our results would suggest that, for PTM patients who do not wish neck surgery and are uncomfortable with "active surveillance", UPEA likely represents an attractive and "minimally invasive" definitive management option.

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## P158

**Clot structure analysis in survivors of acute lymphoblastic leukaemia: the role of growth hormone deficiency**

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Introduction
Childhood-onset cancer survivors demonstrate increased mortality rates due to cardiovascular and cerebrovascular events. Studies suggest that leukaemia and brain tumour survivors have a relative risk of 6.4 and 29.0 respectively for stroke. Adverse lipid profile, body composition, insulin resistance and hypertension have been implicated to the increased cardiovascular risk in these patients, however little is known about procoagulant factors and markers of vascular inflammation in these cases.
Methods
We undertook a pilot cross-sectional study in 6 patients with acute lymphoblastic leukaemia (ALL), compared with 6 age and gender-matched controls. Measurements included plasma fibrinogen using the Clauss method, high-sensitivity CRP using in-house ELISA and clot structure analysis, using turbidimetric analysis to measure *ex-vivo* fibrin polymerization and fibrinolysis speed.
Results
patients (mean age $27.5 \pm 7$ years), diagnosed with ALL at $6.7 \pm 3.1$ years of age, were assessed. All patients received cranial radiotherapy, while three patients had additional total body irradiation for bone marrow transplantation (total mean cranial radiation dose $28.6 \pm 8.3$Gy). All patients had severe GHD and 3 of them were on stable GH replacement at the time of the study. Fibrinogen levels were significantly higher in the ALL group compared with controls ($2.96 \pm 0.38$ mg/ml vs. $2.45 \pm 0.27$ mg/ml, $P=0.022$). All controls had undetectable CRP, while mean CRP level for patients was $1.8 \pm 2.1$mg/L; however difference failed to reach statistical significance ($P=0.065$). Patients had raised clot maximum absorbance ($0.42 \pm 0.08$ vs $0.31 \pm 0.03$ arbitrary units (AU), $P=0.009$), longer lysis time ($1336.8 \pm 653.1$ s vs. $893.8 \pm 65.22$ s, $P=0.009$) and larger lysis area ($915.4 \pm 578.9$ vs $521.1 \pm 71.1$ AU, $P=0.04$) compared with controls.
Conclusions
Our results demonstrate more compact clots and resistance to fibrinolysis ex-vivo in patients with ALL, which may contribute to the increased cardiovascular risk in these individuals. Similar changes in clot structure/lysis have previously been found in GHD, which may constitute the underlying mechanism responsible for the clotting abnormalities observed in ALL patients.

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## P159

**Use of Sunitinib in refractory hypercalcaemia in pancreatic neuroendocrine tumours**

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The orally administered targeted therapies, Sunitinib and Everolimus lengthen progression free survival in pancreatic neuroendocrine tumours (pNETs). Choice of therapy has traditionally been on avoidance of deterioration in established co-morbidities. Sunitinib has been recognised to induce hypocalcaemia and the subsequent need for calcium supplementation has been reported. However, little has been documented about the utility and therapeutic significance of this effect.
Case 1
A 45 year old lady presented with acute appendicitis and was found to have an incidental cystic pancreatic lesion, vascular liver metastases and lung metastases, pNET was confirmed. She was commenced on Lanreotide Autogel 120 mg deep sc injection monthly. Surveillance proved further progression and her corrected calcium (cCa) was 4.18 mmol/l, PTH 6.0 (NR 20–75 ng/l), PTH-rp $<0.7$ pmol/l (NR 0–1.8 pmol/l). Conventional treatment for hypercalcaemia failed and initiation of Sunitinib stabilised disease progression and rendered the cCa normal at 2.3mmol/l.
Case 2
An 81 year male presented with hypercalcaemia and a radiological diagnosis of pancreatic adenocarcinoma was made in 2004. Following splenectomy and distal pancreatectomy, histology confirmed pNET with a ki67 of 15%. Subsequent surveillance imaging confirmed liver and peritoneal metastases with recurrence in the tail of the pancreas. Sandostatin LAR 30 mg IM was commenced 4 weekly. The cCa was 3.24 falling to 2.5 mmol/l within 2 weeks of Sunitinib. Dose reduction from 37.5 mg to 25 mg has been made with success.
Case 3
A 73 year old male presented with hypercalcaemia. Staging CT and liver biopsy confirmed pNET. Pre-treatment cCa was 3.3 mmol/l but despite Everolimus this remained elevated at 3.34 mmol/l. Disease progression and diabetes mellitus has ensued. Sunitinib is due to be commenced and we would anticipate a calcium reduction.
Conclusion
Sunitinib induced hypocalcaemia could be given high consideration when refractory hypercalcaemia in progressive pNETs is a dominant feature of the disease.

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## P160

**Chromogranin B: a possible prognostic biomarker for neuroendocrine tumours?**

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Neuroendocrine tumours (NETs) are rare tumours that originate from neuroendocrine cells and their incidence has increased for the last 20 decades; partly because of advances in diagnostic tools, which have improved detection rates. Chromogranin A (CgA) is the most widely used biochemical tumour marker

for NETs, however, its prognostic utility has been questioned due to several non-neuroendocrine causes of elevated CgA. This is the first study to assess the prognostic utility of chromogranin B (CgB), a marker of NET that is less affected by these clinical drawbacks. As a result, we hypothesised that plasma CgB concentration is a more accurate and reliable prognostic indicator in NET patients. Data from 121 NET patients were collected retrospectively and submitted to statistical analyses. In univariate survival analysis, circulating CgB levels were associated with a significantly poorer survival time than CgA. In addition, multivariate analysis was performed by considering other prognostic indicators, including age, metastases at diagnosis; and, nonetheless, an elevated circulating CgB concentration was the most significant predictor of poor survival. Further comparisons of chromogranins with different measures of tumour burden demonstrated that plasma CgB levels were associated with a significant increased risk of disease progression. In conclusion, results show that CgB allows a more accurate distinction of patients with an increased risk of death and tumour progression in NET patients.

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## P161

### Imaging and evaluating side effects of antiandrogen therapy

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Androgens are required for normal development and fertility. They have a vital role in tissues such as the reproductive tract, the brain, muscle and bone. Prostate cancer (PCa) is the most prevalent malignancy in western males: it is dependent upon circulating androgens and the therapies currently available aim to reduce synthesis of circulating androgens and/or inhibit the pathway using antiandrogens. Therapies inhibiting androgen signalling, and in particular the androgen receptor (AR) can result in dangerous and/or debilitating side effects, including liver toxicity, impotence, osteoporosis. There is a wide interest in developing tissue-selective AR inhibitors (Selective Androgen Receptor Modulators, SARMs) which ideally should inhibit androgen action in the prostate but not affect or activate AR in other tissues. We developed a transgenic mouse model that expresses luciferase when the endogenous AR is activated. This model is extremely useful because AR activity can be assayed in both reproductive (testes, prostate) and non-reproductive (brain, gut) tissues, during the lifetime of the animals. In this study ARE-Luc mice are used to assess side effects of approved and novel agents that affect androgen signalling. Compounds were tested in cell lines first, to assess their effect on androgen activation. Subsequently, intact and castrated mice were treated with SARMs, imaged in vivo and ex vivo and tissues were collected for analysis. Our results identified novel tissues targeted by SARMs in vivo and characterise tissue-specific action of compounds that affect androgen signalling. Moreover in order to assess the role of AR signalling in the disease context, the compounds were tested in a transgenic mouse derived from the crossing of the ARE-Luc with a prostate cancer model. Overall this project will help predict possible side effects of treatments and their suitability for PCa treatment, as well as elucidate the role of the AR signalling in the progression of PCa.

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## P162

### Outcome of insulinomas diagnosed in a tertiary endocrine centre

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Background
Insulinomas, the most common functional neuroendocrine tumours of the pancreas, are usually sporadic, benign and solitary.

Aims
To assess biochemical data, localisation and treatment outcome of insulinomas diagnosed in a tertiary endocrine centre.
Patients and methods
Twenty-five patients (14 F/11 M), aged 49.1 ± 14.1 years, diagnosed with insulinoma in a Neuroendocrine Tumours centre between 2000 and 2014 were retrospectively reviewed. Prolonged (72-h) supervised fast was used for diagnosis (plasma glucose $<40$ mg/dl with simultaneous insulin level $>6$ μIU/ml). For tumour localisation, computed tomography (CT), magnetic resonance imaging, endoscopic ultrasonography and intra-operative ultrasonography were performed.
Results
Sporadic insulinomas were diagnosed in 23 patients (92%); two insulinomas developed in patients with multiple endocrine neoplasia type 1 (8%). Hepatic metastases were present in two patients (8%). Median duration between the onset of hypoglycaemic symptoms and diagnosis was 2 years. Glucose nadir during 72-h fast was 30.9 ± 7.1 mg/dl, with simultaneous median insulinaemia of 28 μIU/ml (25th percentile:15.2, 75th percentile:67.2 μIU/ml); mean C peptide during hypoglycaemia was 2.6 ± 1.8 ng/ml. Median duration to nadir glucose was 8 h (25th percentile:4.25, 75th percentile:12.25 h). Abdominal CT localized the insulinoma in 21 patients (84%) of patients. Mean tumour diameters were 22.3 ± 14.3 mm. Tumour's localisation was: cephalic ($n=7$), uncinate process ($n=2$), pancreatic neck ($n=3$), pancreatic body ($n=4$), pancreatic tail ($n=5$). From 20 patients underwent surgery (enucleation/cephalic duodenopancreatectomy/distal pancreatectomy), 16 patients were cured (80%); two patients refused surgery, in one patient surgery was contraindicated due to severe cardiac comorbidities and two patients were lost from follow-up. Medical treatment with diazoxide (200–300 mg/day) or somatostatin analogues were used in non-cured patients to control hypoglycaemia.
Conclusion
Partial pancreatectomy or enucleation provided a good cure rate in patients with pre-operative localised insulinomas.

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## P163

### Metastatic paraganglioma with isolated 3-methoxytyramine rise in a patient with SDHC mutation

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59-year-old gentleman with known succinate dehydrogenase complex subunit C (SDHC) gene mutation attended endocrine clinic with non-specific symptoms. Past medical history included glomus jugulare paraganglioma (PGL) surgically treated (1993, 2003) with residual disease, macroprolactinoma treated with cabergoline since 2010, and BPH treated with finasteride and tamsulosin. His brother has glomus vagale. Clinical examination was unremarkable. Plasma metanephrine ranged between 377 and 458 pmol/l (120–1180 pmol/l), and plasma normetanephrine ranged between 284 and 349 pmol/l (80–510 pmol/l). Plasma 3-MT was 1014 pmol/l (0–180 pmol/l) whilst on cabergoline 250 μg/wk. When cabergoline stopped for 4 weeks plasma 3-MT increased to 2185 pmol/l, and when cabergoline recommenced plasma 3-MT dropped to 1272 pmol/l. Possibly, as cabergoline as a dopamine D2 receptor agonist, it would be expected to exert negative feedback on the dopamine system and lower endogenous dopamine production, and therefore lower its metabolite (3-MT). MRI-whole-body showed stable appearances of the residual disease in the right jugular foramen with no other lesions. $^{(123)}$I-MIBG showed probable residual right jugular foramen disease without other avid lesions. $^{(68)}$Ga-DOTATATE PET/CT showed multiple foci increased avidity at the right skull base, and avid uptake in the sacrum suggestive of metastasis. Following MDT discussion $^{(177)}$Lu-DOTATATE ablation therapy was recommended. Co-existence of PGL and pituitary macroadenoma is a recognised rare syndrome, whether this is a coincidence or the result of a genetic defect, it remains unanswered. Isolated rise 3-MT is very rare, and it may the only positive biomarker in PGL, therefore in PGL patients 3-MT should be a part of the surveillance along with plasma metanephrine and noremetanephrine. $^{(68)}$Ga-DOTATATE PET/CT is superior to $^{(123)}$I-MIBG scan in the detection of metastatic PGL. To date we describe the first case in which elevation of 3-MT prompted further testing and the diagnosis of metastatic PGL in SDHC mutation.

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## P164

**Recurrent phaeochromocytomas in type2C variant of Von Hippel-Lindau syndrome**

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Phaeochromocytomas are rare neuroendocrine tumours with an incidence of 2–8/million persons per year. They occur as a sporadic entity or form part of a complex autosomal dominant familial genetic syndrome, mainly MEN2A, MEN2B, Neurofibromatosis Type1, Von Hippel-Lindau disease (VHL) and mutations in succinyl dehydrogenase. The mean age at diagnosis is in the third decade in the familial cases and the risk of recurrence has been described as high as 23%.

VHL is a hereditary syndrome caused by germ line mutation of the von Hippel-Lindau tumour suppressor gene and classically associated with retinal and CNS hemangioblastomas, clear cell renal carcinomas and phaeochromocytomas. The disease is heterogeneous in view of the varied gene mutants. The VHL Type2c presents with phaeochromocytomas as the only clinical manifestation as the gene mutation protein products can suppress renal carcinoma and the release of abnormal growth factors which contributes to abnormal blood vessels and hence angioblastomas.

We describe the case of a young patient in his late 1940s who presented with a recurrent phaeochromocytoma 14 years post left adrenalectomy. Further investigations revealed a recurrent catecholamine secreting lesion in the left adrenal bed. He had surgery. High urine and plasma metanephrines were detected during surveillance on follow up. He was found to have phaeochromocytoma in his right adrenal gland. Fluorescent sequence analysis has detected the missense mutation c.404G>C (p.Arg64Pro) in exon 1 of the VHL gene. This pathogenic mutation has been found to be particularly associated with the VHL Type 2C disease.

This case highlights the importance of genetic screening and regular follow up in patients presenting with phaeochromocytoma.

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## P165

**Lutetium ablation therapy as treatment for recurrent metastatic bladder paraganglioma**

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We present the case of a 53 year old gentleman with recurrent bladder paraganglioma. He presented initially in 2010 with headache and blurred vision when passing urine. The tumour was surgically removed, BP normalized and his postoperative MIBG-scan was normal. The patient presented with hypertensive crisis 4 years later with a suspicion of tumor recurrence, confirmed biochemically and radiologically. Treatment with phenoxybenzamine and bisoprolol resulted in adequate BP control. Plasma normetanephrine 5111 pmol/l (120–1180), plasma metanephrine 300 pmol/l (80–510) and Plasma 3-methoxytyramine 452 pmol/l (0–180). CT Abdomen and Pelvis showed a 15 mm bladder mass. No adrenal pathology. CT chest showed a soft tissue mass in left lung apex eroding left 3rd rib with pleural effusion. USS guided biopsy and histology was suggestive of neuroendocrine cells. Overall picture suggestive of metastasis. $^{(68)}$Ga-DOTATATE PET/CT showed avid soft tissue nodule associated with the bladder, compatible with phaeochromocytoma recurrence. There were multifocal avid deposits in the bones, the largest at the proximal left third rib with soft tissue extension. $^{(177)}$Lu-DOTA-TATE ablation therapy was commenced following MDT discussion. Post Lu-177 imaging still showed avid uptake in the ribs and awaiting further treatment cycle.

We present a case where Lutetium (Lu) ablation is used as a treatment option for metastatic paraganglioma of the urinary bladder. This is a rare presentation. Most tumors are benign. Patients with localized tumours have a favourable prognosis following treatment but lifelong follow up is essential as delayed recurrence could occurs as seen in our patient.When patients present with metastatic disease, survival is greatly reduced and palliative care is the norm (1) In patients with DOTATATE avid metastatic lesions, $^{(177)}$Lu-DOTA-TATE ablation therapy has emerged as a new modality of treatment with less toxicity leading to lasting remission in some cases.

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## P166

**Somatostatin responsive ACTH and precursor excess in a midgut mesentery NET**

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Introduction/Background

ACTH production from a midgut mesentery NET is extremely rare (1). A 62y old woman presented with hypokalaemia (2mmol/L) and hyperpigmentation, 11y after surgery of a pT3N1Mx non-functional G1 NET with SRS positive, non-resectable but stable, residual mass encasing mesenteric vessels. Serum cortisol (3261 nmol/L), ACTH (796 ng/L), CgA (530 pmol/L) and urine total cortisol metabolites (33920 µg/24h) including 14 sub-products indicated change of biological behaviour into a functioning NET. Pituitary or pulmonary sources were excluded.

Aims

To test somatostatin (SS) responsiveness on ACTH excess.

Materials and methods

A test dose of SS 100 µg s.c. was applied.

Results

SS resulted in rapid (300 min) improvement of ACTH (222.9 ng/l), cortisol (1700 nmol/l), urine cortisol metabolites (620 min; 4620 µg/24 h) and K+ (3.2 mmol/l; 48 h); followed by resistance after 3 wk (cortisol 6362 nmol/l, ACTH 617 ng/l; pro-ACTH and POMC 140; ULN <40 pmol/l), despite SS dose intensification and adding ketoconazole/metyrapone for cortisol excess; resulting in a L3 fracture, reduced mobility and subsequent fatal pneumonia.

Conclusion

Considering the 1–10% cross-reaction of ACTH precursors in ACTH assays (2), precursors were not sufficiently elevated to account for the observed ACTH excess, suggesting production of ACTH precursors by the NET and processing them to ACTH. Treatment with somatostatin was efficient in this context, at least in the short term.

References

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## P167

**Primary adrenal lymphoma**

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A 68 year old man presented with shortness of breath and was found to have a pulmonary embolus. During work up of the underlying aetiology he was noted to have bilateral adrenal masses. He was subsequently readmitted with pyrexia and feeling generally unwell. Imaging revealed the progression of adrenal masses and there was biochemical evidence of adrenal insufficiency with a random cortisol at the low end of normal. CT guided biopsy was consistent with the diagnosis of a high grade B cell lymphoma and after completing staging with bone marrow biopsy and PET scan R CHOP chemotherapy was instituted.

Primary adrenal non Hodgkin lymphoma is rare with fewer than 200 reported cases. It is more prevalent in men and has a peak incidence in the mid-1960s. Diffuse large B cell is the most common accounting for approximately 78% of reported cases. Up to 70% are bilateral at presentation. There is some association with EBV and mutations in p53 and c-kit oncogenes.

Symptoms of isolated adrenal lymphoma are variable and often non-specific. Factors associated with poor prognosis include presentation with adrenal insufficiency, large tumour size and an elevated LDH. The addition of Rituximab to the CHOP regimen has been associated with better outcomes. Adrenal lymphoma is also an indication for prophylactic intrathecal chemotherapy which has been used in this case for CNS prophylaxis. There has been a good response to therapy. The end of treatment PET scan showed residual uptake which is not thought to be clinically significant however this will continue to be monitored.

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## P168

**Metastatic bowel carcinoid associated bilateral carcinoid heart disease**

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A 63-year-old Caucasian female was admitted with a 12-month history of exertional breathlessness, anxiety attacks, syncopal episodes, diarrhoea, fatigue, reduced appetite, two stones weight loss, and dry facial and truncal flushing. Investigations revealed raised Urine 5-HIAA of 116 (RR <50 μmol/24 h) and raised Chromogranin A of 48 (RR <6 nmol/l). CT scan revealed an extensive soft tissue mass encasing the upper abdominal aorta, compressing the inferior vena cava and extending down along the retroperitoneum and iliac vessels. The ureters were obstructed with consequent bilateral hydronephrosis. Renal function was normal. Renogram showed a non-functioning right kidney and functioning left kidney. NM octreotide SPECT CT showed increased focal activity in mediastinal, bilateral retroperitoneal and mesenteric adenopathy with no increased activity in the liver, lungs, bowel or skeleton. Para aortic lymph node histology was consistent with metastatic gastrointestinal well-differentiated neuro endocrine tumour with Ki67 index of 1%. She was referred to cardiology as NT-proBNP was elevated at 3239 ng/l. Despite only modest elevation in five HIAA, trans-oesophageal echocardiogram and cardiac MRI confirmed severe mitral regurgitation, moderate aortic and tricuspid regurgitation, and mild pulmonary regurgitation. In view of frailty and heart failure she was considered too unwell for replacement of all four valves. Hence she underwent aortic and mitral metallic valve replacement. She also underwent right ureteric stenting to relieve obstruction but this unfortunately led to septicaemia and infective endocarditis.

Carcinoid syndrome is usually associated with the presence of liver metastases. It can also occur in the absence of liver metastases if there is large retroperitoneal nodal involvement, draining directly into the systemic circulation and bypassing the portal circulation as in this patient. It is unusual for left side heart valves to be affected predominantly, as serotonin is normally inactivated in the lungs to 5-HIAA.

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## P169

**Familial insulinoma in the absence of MEN**

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Case history

A 42 year old female presented with a history of collapse and seizure activity. Her blood glucose was noted to be 3.1 with the paramedics and subsequent hypoglycaemia was demonstrated on several occasions whilst in hospital. She described a family history of insulinoma in her mother. A 72 h fast was performed as an inpatient to look for insulinoma. The nadir serum glucose was 2.0 mmol/l with an inappropriate C-peptide level of 1006 pmol/l and an insulin level of 12 μ/l.

A CT and MRI of the pancreas showed no evidence of pancreatic or hepatic abnormalities. We proceeded to endoscopic ultrasound, which demonstrated a 14 mm lesion in the portal confluence of the pancreas. Calcium stimulation insulin venous sampling was strongly suggestive for hyperinsulinaemia in the superior mesenteric artery. She was commenced on diazoxide and later had a subtotal pancreatectomy. Histology delineated a grade one T1N0 neuroendocrine tumour, with Ki-67 of 4–5%. The patient's mother had had surgical resection for a histology ally proven insulinoma. As such, genetic testing was performed on this patient to exclude multiple endocrine neoplasia, which was negative for the MENIN gene.

Discussion

Familial insulinoma is very rare in the absence of MEN, however it does need to be considered. This has implications for family screening.

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## P170

**A young girl with bilateral adrenal tumours**

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18 year old female referred with 2 years history of hirsutism and secondary amenorrhoea. She was born at 37 weeks and was found to have neonatal hypothermia, hypoglycaemia and crossed hemihypertrophy, but no macroglossia. Due to the increased risk of Wilm's tumour she had regular ultrasound scans up to the age of 11 years. She attained menarche at the age of 13 but had only two periods within 18 months. By the age of 15 she developed hirsutism and acne. Biochemistry revealed serum Testosterone 5.3 nmol/l, SHGB 11 nmol/l, DHEA-sulphate >27 μmol/l, Androstendione >35 nmol/l. MRI scan showed bilateral adrenal masses measuring 8 and 6 cm in diameter. Staging CT scan confirmed no metastases. She underwent bilateral adrenalectomy. Post operatively she was commenced on Hydrocortisone and Fludrocortisone. She had her first menstrual cycle 2 months after surgery and serum androgens fell to undetectable levels. Histology of the adrenal masses showed adrenocortical neoplasm of uncertain malignant potential. The molecular basis of her condition is not yet known, and she did not have sufficient features to diagnose Beckwith-Weidemann syndrome, but adrenal tumours have been associated with overgrowth/hemihypertrophy syndromes, and continuing screening in adulthood should be considered.

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## P171

**Acromegaly in association with a bronchial carcinoid tumour**

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Bronchial carcinoid tumours are uncommon pulmonary neoplasms. Manifestation of a bronchial carcinoid with acromegaly secondary to extra-pituitary growth hormone releasing hormone (GHRH) production is rare, but bronchial carcinoid tumours are the most common cause of ectopic GHRH secretion.

We report the case of 60 year old female, ex-smoker with hypertension presenting with cough, dyspnoea and right lower lobe opacity on the chest X-ray. Patient was noted to have features of acromegaly and IGF1 was requested. Subsequent CT TAP showed a right lower lobe collapse, 4.5 cm mass obstructing the lower lobe bronchus which was visualised but could not be biopsied during bronchoscopy. 18-FDG PETCT scan showed only mildly avid right lower lobe mass, likely to be a slow growing carcinoid tumour, with chronic collapse of the right lower lobe. Pre-operative IGF1 level came back elevated at 67 nmol/l. Patient underwent right mid and lower lobectomies and the histology showed morphological appearance of grade 1 neuroendocrine carcinoma (classic carcinoid tumour) with no lymph node involvement. Patient underwent the oral glucose tolerance test (OGTT) with growth hormone (GH) levels reaching a nadir value of 0.82 μ/l. Patient's pituitary hormone profile and the MRI of the pituitary gland were both normal. The GHRH assay was not available. The most recent postoperative random GH level was 0.6 μ/l and IGF1 was 20 nmol/l. Bronchial carcinoid tumours are one of the commonest causes of ectopic GHRH production, with similar cases reported in the literature. In the view of possible insufficient normalisation of the GH levels post OGTT our patient will require further close follow up.

Our case represents the classic carcinoid tumour which is reported to have very good prognosis following surgical resection. The results of staining for GHRH on tissue samples are still awaited as the samples are being processed abroad.

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## P172

**Challenges in the diagnosis and management of a case of glucagonoma first presenting as a localised genital rash**

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Introduction

Glucagonoma syndrome is a rare paraneoplastic phenomenon characterised by necrolytic migratory erythema, diabetes mellitus and symptoms of gastrointestinal upset. It is often diagnosed late leading to a poor prognosis.

Case report

A 70 year old lady with type two diabetes mellitus and fibromyalgia presented in late 2011 to Gynaecology with an itchy erythematous vulval rash. Skin biopsy suggested lichen sclerosis and she was discharged with potent steroids. She later developed a similar rash on her left shoulder which resolved spontaneously. A few months later there was a deterioration in her glycaemic control, unintentional weight loss and non-specific abdominal pain. Investigations revealed iron deficiency anaemia and multiple liver lesions on CT abdomen. These were thought to be liver cysts when discussed in the surgical MDT. Fourteen months later her health declined, she now had an isolated elevated GGT of 103. A liver

ultrasound showed multiple lesions suggestive of metastatic disease. Staging CT revealed a small lesion in the tail of the pancreas and liver metastases. She had by this time developed an erythematous, erosive and scaling rash on her face, breasts and groin areas. A liver biopsy confirmed a neuroendocrine tumour. Octreotide was given but she remained symptomatic. Subsequent trials of sunitinib and then everolimus were also unsuccessful on account of severe oral mucositis, oesophagitis and development of bilateral pulmonary emboli. Her course was further complicated by a gastrointestinal bleed probably from her anticoagulant therapy. She currently remains stable on sustained release octreotride.
Conclusion
Glucagonoma is a rare neuroendocrine tumour. Necrolytic migratory erythema is a sensitive feature of glucagonoma syndrome, but can be difficult to diagnose in the initial stages. Treatment for non resectable and metastasized tumours are for symptomatic control but are often associated with significant side effects and complications as demonstrated by this case.

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## P173

**A management dilemma – emergency treatment of primary hyperparathyroidism in suspected but unconfirmed multiple endocrine neoplasia type 1**

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A 27 year old male patient with severe cardiomyopathy secondary to Epirubicin chemotherapy for rhabdomyosarcoma in infancy was noted to be hypercalcaemic during the course of admission for severe inotrope dependant congestive cardiac failure. Biochemical investigations confirmed a diagnosis of primary hyperparathyroidism (corrected Calcium 3.10 mmol/l, PTH 22 pmol/l, Vitamin D 77 nmol/l).
His brother had undergone trans-sphenoidal pituitary surgery for acromegaly and his paternal grandfather may have been diagnosed with a pituitary tumour. In a young person with primary hyperparathyroidism with family history of pituitary tumours, MEN 1 was considered to be highly likely. He was investigated for possible pituitary and pancreatic involvement, but pituitary profile, fasting hormonal gut profile, pituitary MRI and CT pancreas were normal.
He was on the waiting list for cardiac transplantation, but with development of hypercalcaemia could not be actively considered until its resolution. Urgent cardiac transplantation was deemed to be in his best interests given the severity of cardiac failure, but with insufficient time for genetic MEN-1 mutation analysis to plan further management, consideration was given to the extent of parathyroid surgery. Pre-operative imaging suggested a left lower parathyroid adenoma, and single gland vs subtotal parathyroidectomy were weighed as options. A decision was made in favour of subtotal parathyroidectomy with higher probability of normalising serum calcium and also to avoid second surgery if MEN-1 is later confirmed. A single left inferior parathyroid adenoma was noted during full neck exploration and three and a half glands were excised. Post-operatively, he remains normocalcaemic, PTH has normalized to 3.2 pmol/l and histology confirms the operative findings. The result of genetic test for MEN-1 is still outstanding.
This case highlights a management dilemma in which urgent surgical intervention had to be undertaken before a definitive diagnosis of MEN-1 could be made.

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## P174

**Promoter methylation signatures of BRCA2 and TP53 genes in the serum of some breast cancer patients attending radiotherapy clinic in Lagos, Nigeria**

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The prevalence of new cases of cancer in developing countries even though less than the developed countries have greater mortality rate, due to lack of access to treatment and early detection of the disease. Epigenetic alterations in BRCA2 and TP53, such as DNA methylation, maybe an effective approach in diagnosis, prognosis and therapy in breast cancer. This study examines the DNA methylation signature of BRCA2 and TP53 in Nigerian breast cancer patients. In this study, thirty seven (37) DNA samples were obtained of which 14 samples with Invasive Ductal Carcinoma (IDC) were assessed by methylation specific PCR and then observed by agarose gel electrophoresis. The methylation analysis shows that there were 8 (57.14%) of the 14 patients with methylated BRCA2 and 11 (78.57%) of 14 patients with methylated TP53 promoter region. This study shows a promising development for a blood - based epigenetics diagnostic approach in detecting breast cancer in Nigeria patients, but further work is required to establish the efficacy, as well as exploring the prognostic and therapeutic values.

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## P175

**Confusion in a patient with carcinoid syndrome**

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A 64 year old lady was admitted with a 2 day history of feeling generally unwell. Her relatives also reported new onset confusion over these 2 days. Her bloods showed raised inflammatory markers (CRP 110.3 mg/l, WCC $18.2\times10^9$/l) hypoalbuminemia (17 g/l), bilirubinemia (55 μmol/l) and a raised alkaline phosphatase (917 IU/l). She had recently been diagnosed with carcinoid syndrome; primary tumour situated in the terminal ileum with liver metastasis and had been deemed unfit for intervention by the specialist carcinoid team in the tertiary centre. She also suffered from chronic pancreatitis but had otherwise been well and living independently. Initial diagnosis was thought to be sepsis secondary to a urinary tract infection with delirium, with or without carcinoid crisis. She was commenced on short acting Octreotide to cover any carcinoid crisis and Tazocin for the urinary tract infection. This therapy resulted in slightly improved inflammatory markers but continued delirium. Patient started having diarrhoea and developed a rash; a diagnosis of Pellagra was postulated and treatment with Vitamin B complex and high protein diet was initiated after liaising with the specialist dietician. But unfortunately the patient passed away after a few days.
Tryptophan is an amino acid which is a precursor for both Niacin and Serotonin. There is diversion of tryptophan to making serotonin in patients with Carcinoid syndrome. This diversion causes decreased protein synthesis and Niacin deficiency leading to clinical manifestations of Pellagra.
Rare Causes of confusion like Vitamin deficiency should be considered in all cases when no other obvious cause is identified. Pellagra should be considered in patients with Carcinoid syndrome. Pellagra is due to deficiency of Niacin and if left untreated can lead to death.

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# Nursing practise

## P176

**Utilisation of nurse led clinics in endocrinology practice**

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Since the introduction of the reduction in hours for junior doctors in 1991, nurse led clinics (NLC) have increasingly become embedded into specialist practice. Whilst some NLC such as Diabetes and Respiratory have been established for many years, in the area of Endocrinology they are a newer evolving concept.
Currently there is limited research in this area. An online survey was distributed to 98 nurse members of the Society for Endocrinology. The questionnaire consisted of 35 multiple-choice questions, and also allowed an open response for additional comments. Questions sought to investigate if nurses were involved in nurse led clinics, types of clinic, knowledge and skills required, medico legal considerations and barriers and drivers of these services.
Initial responses have been received from 29% ($n=28$) nurse members (teaching and general hospitals and the independent sector). 85.7% of respondents ran nurse led clinics in a wide range of endocrine specialisms. Although 53.9% had worked in the speciality for 10 years or less, 67.8% had studied at a degree level standard or higher. NLC are developed for a number of reasons including organisational and medical colleague support, experience of the endocrine specialist nurse (ESN), service need and financial savings/income generation. 100% of those responding worked with some, significant or full autonomy in clinics. In these clinics a large number of patients are seen per month with 84% reviewing 11–150 patients. ESNs use a combination of verbal and written consent in NLC for dynamic function testing, clinical examination, radiological procedures, treatments and research. Whilst 84% stated they optimised or changed medications only 52% were non-medical prescribers. All respondents felt that their service was valued.

In conclusion; ESN are extensively involved in providing a valuable service, working with a significant level of autonomy, although further work is required to establish the extent of formalisation of the knowledge and skills required.

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## P177

**How is the Society for Endocrinology Competency Framework for Adult Endocrine Nursing used in practice?**

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The Competency Framework for Adult Endocrine Nursing was launched at the Society for Endocrinology (SfE) BES conference in March 2013 and was made available to Endocrine nurses and Endocrine centres in the UK. It was also made accessible via the SfE website. Questionnaires were sent out 6 months later to audit its use.

Results

80.4% of nurse respondents had heard of the document and 69.6% had read it. 100% of those who had read it found it easy to use and 89% were able to identify their personal level or that of staff they managed. 31% had used it for their appraisal, 27% had used it to benchmark their own practise and 17% had used it for service development. The document was presented to European nurses at the European Congress of Endocrinology (ECE) in May 2013. At the ECE in May 2014 the 15 nurse delegates were asked to complete a questionnaire about this document.

Results

100% thought it would be of benefit to nurses in their country. 87% thought it would most likely or definitely have a positive impact on their career development. 61% thought the document most useful to identify their developmental needs, 30% in benefiting career progression and 9% for appraisal. The 2nd edition of this document with four new competencies was published in *Endocrine Connections* in March 2015 and had 836 downloads in the first 3 months. It is proposed to continue to update this document and develop more competencies over the coming years.

To see current use of this document and its usefulness in developing both the individual nurse and endocrine nursing as a whole the authors are currently carrying out a further audit with the results to be included in the final presentation in November.

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## P178

**Pre-pituitary surgery patient satisfaction audit 2015**

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Background

Pre-pituitary surgery, patients attend a 'Pre-Pituitary Surgery Clinic' (PPSC), to discuss management options and risks. Clinicians in attendance are; pituitary surgeon (1) registrar (1) endocrine team consultants (1–3), registrars (1–2) nurses (1) senior house officer (1) visiting doctors/medical students (4–6). Patients are informed a number of clinicians attend the clinic due to the multidisciplinary nature (MDT) and unit teaching responsibilities. Formal audit of patients' views was performed following informal comments from a number of patients, who felt uncomfortable with the number of personnel present.

Aim

To determine patient satisfaction when attending the (PPSC).

Method

30 patients invited to complete an 8 part anonymous questionnaire, comprising yes/no answers or scores (1–10).

Results

(30/30) Departmental facilities met individual needs. 29/30 Received sufficient information. 1/30 Admission letter went missing. 30/30 Involved with decisions. 30/30 Benefits of treatment discussed. 27/30 Surgery risks discussed. 1/30 Not applicable. 2/30 Risks not discussed. 28/30 Able to discuss concerns. Scale 1 happy – 10 very unhappy regarding number of personnel in room. 18 $<3$ (54%) Very happy with number of personnel. 11 $>7$ (33%) Unhappy with number of personnel. Comments: Poor signage to endocrine department for drivers. Intimidating experience. Friendly, helpful, welcoming, well informed staff. Clinicians present at (PPSC) varied between 4 and 13.

Conclusion

All patients felt part of the decision-making process. Although all were aware of discussion of the benefits of surgery, 2/30 could not recall discussion of the risks. While 60% of patients were happy with the number of personnel in attendance 36.7% felt the number of people was intimidating. We have now assigned a separate room where patients will have the opportunity to see the core clinicians involved in their care if this is their preference. The survey will be repeated following introduction of this change.

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## P179

**Development of IT based nurse led endocrine tracking system**

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The Endocrine service is predominantly out-patient based looking after a range of conditions with strong dependence on biochemical monitoring for clinical safety. Due to capacity demands we needed to explore the options available to facilitate safe monitoring with potential to increase capacity.

The development of an IT based system allowed for the capacity increase, provided prompt communication with GP's, improved patient safety, included a diagnostic coding element and demonstrated a cost saving.

The tracker is run by the Endocrine Nurse Specialist and includes thyroid patients on block and replace or following radioactive iodine, monitoring following pituitary irradiation, conservatively managed hyperparathyroidism, and so on. Crucially the tracker prevents situations such as a hyperparathyroidism patient needing hospital admission to resolve hypercalcaemia as the GP had not checked the calcium as requested. The current caseload is 340 patients with a total 550 patients managed using the tracker since its inception in November 2013. The tracker has generated 1500 letters since June 2014 when letter templates were added.

Cost savings are difficult to quantify however clinic coding within the tracker and Trust IT systems differentiate face to face encounters and telephone consultations ensuring correct tariff remuneration. Prior to the tracker a new thyrotoxicosis tariff would be in the region of £370 – 470 for the first 12 months including a Consultant first visit and 2 or 3 more reviews by Consultant, Registrar or CNS. That cost has reduced since using the tracker to around £230 including the Consultant first visit. All follow ups are done by the CNS using phone consultations whenever possible up to the point of discharge.

The next step will be to audit patient satisfaction with the service development and enhance the tracker by adding patient cohorts such as thyroid cancer thyroglobulin monitoring.

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## P180

**A care service model for cost effective and structured individualised treatment choice for GH replacement therapy**

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Aim

To develop a cost effective and individualised service model for GH replacement therapy based on a joint decision making process with patients and commissioners.

Background

At University College London Hospital (UCLH), we have a caseload of about 300 adult patients treated with GH. Our in house structured GH treatment proforma is used at the patient's initial consultation for joint decision making on prescribing the most suitable GH, e.g. non-refrigerated, needle free, dexterity and visual impairment. Choice of device is also based on clinical judgement (patient's ability to handle the device) and cost-effectiveness. GH is prescribed and monitored by the Endocrine Team and reimbursed directly by CCGs; we send regular reports to GPs. Patients are registered with home service for device training, technical support and delivery of their GH and stores.

Outcomes and evaluation
Introduction of the home service in 2011 has provided a 20% cost saving on VAT which was previously applied on the drug dispensed by the Hospital Pharmacy. This also increased patient satisfaction through by reducing travelling time to collect GH. As GH products are PbR (payment by result) excluded they are charged in addition to the national tariff and ultimately funded by CCGs. The timeline from clinic consultation to treatment start has reduced to ~2 weeks compared to 4 months before we introduced in house tertiary prescribing and home service. When issuing a repeat prescription from UCLH, we ensure that up to date IGF1 and relevant other investigations are available for dose adjustment. This can be difficult to monitor when GH is prescribed in Primary Care. We regularly audit patients' satisfaction with and adherence to GH treatment, quality of life, wellbeing and their satisfaction with the homecare service using validated and in house developed questionnaires and re-design our service based on feedback.
Conclusion
We found that a streamlined and structured pathway provides significant cost savings, shorter waiting times for treatment start, more accurate and safer monitoring while maintaining individualised care and patient satisfaction.

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## P181

**Recurring abscesses at injection sites on the home injection programme**

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Acromegaly is a rare clinical condition resulting from prolonged and excessive release of GH from an adenoma within the pituitary gland. Somatostatin analogues are synthetic versions of Somatostatin, a hypothalamic peptide that inhibits pituitary GH release and is administered by monthly injection. This case describes a 31 year old gentleman with treated Acromegaly who was well established on a Somatostatin Analogue (Somatuline Autogel) on the home injection programme, suddenly developed abscesses at his injection sites. His mother and partner had received the appropriate training on how to administer the deep subcutaneous monthly injection, with assessment and supervision of their injection technique made by the Lead Endocrine Nurse Specialist. The first seven injections delivered through the home injection programme were uneventful. The next two injections resulted in abscess formation at the injection sites. This required hospitalisation for excision and drainage and treatment with both intravenous and oral antibiotics. The manufacturer of Somatuline Autogel were contacted to enquire if any known causes and cases were documented with this complication. On reviewing the gentleman it was discovered that he had excessive body hair particularly at the chosen site of the upper outer quadrant of the buttock. The medical department within the company were unaware of any previous incidences of abscess formation. In discussion with the gentleman, alternative injection sites were identified and preparation of the injection site by shaving the hair proposed. The next two injections were administered by the Lead Endocrine Nurse Specialist into the upper outer thigh, with the area shaved and cleansed with soap and water prior to injection. No further abscesses developed. Further training was given to his mother and he returned to the home injection programme.

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# Obesity, diabetes, metabolism and cardiovascular

## P182

**Testing causality in the association of plasma cortisol with risk of coronary heart disease: a Mendelian randomisation study**

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Background
Elevated morning plasma cortisol is associated with multiple cardiovascular risk factors in metabolic syndrome. Epidemiological studies have also reported a positive association between plasma cortisol and coronary heart disease (CHD), although not all estimates are statistically significant (OR 1.10 (95% CI 0.97 to 1.25)) (Davey Smith *et al*. Circulation 2005). Importantly, observational studies are unable to infer causality and results may be confounded.
Methods
A two-sample Mendelian randomisation approach was used to estimate the causal effect of plasma cortisol on risk of CHD. A genetic instrument for plasma cortisol comprised three SNPs which were associated with plasma cortisol in the recent Cortisol Network (CORNET) genome wide association meta-analysis ($n=12,597$) (Bolton *et al*. PLoS Genetics 2014). We investigated the association between this genetic instrument for plasma cortisol and risk of CHD in up to 22,223 cases/64,762 controls from the publicly available CARDIOGRAM consortium.
Results
Each standard deviation rise in genetically predicted plasma cortisol was associated with an odds ratio of 1.27 (95% CI: 1.01 to 1.60) for CHD.
Conclusions
These results are compatible with a causal effect for the observational association between plasma cortisol and CHD. The inconsistent results from observational studies may be explained by: the inverse association between cortisol and obesity, which confounded the positive association of cortisol with other cardiovascular risk factors; and the use of single snapshot plasma cortisol measurement rather than cumulative measure of cortisol exposure provided by genetic prediction. A bidirectional Mendelian randomisation analysis between plasma cortisol and BMI may yield greater clarity. Measurements of cortisol may add value to predictions of CHD risk.

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## P183

**Gender-related differences in circulating microparticles characteristics: implications for cardiovascular risk?**

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Introduction
Microparticles (MP) are circulating submicron particles released from cell membranes. They have an important role in intercellular endocrine signalling and may be increased in numerous conditions including cardiovascular disease. However, the influences of gender on MP characteristics are not fully understood.
Aim
To compare MP characteristics (size, concentration, cellular origin and inflammatory profile) in healthy males and females across the age and body weight spectrum.
Methods
Fasting blood samples were taken from healthy volunteers (males $n=14$; age $33\pm6$ years, BMI $28.1\pm7.2$ kg/m$^2$; females $n=16$; age $35\pm12$ years, BMI $26.4\pm6.5$ kg/m$^2$, $P=$NS). MPs obtained by ultracentrifugation were subjected to nanoparticle tracking analysis and time resolved fluorescence to determine MP concentration, size, cellular origin (CD41 platelet; CD11b leukocyte; CD235a erythrocyte; CD144: endothelial) and inflammatory profile (interleukin 6 (IL6), tumour necrosis factor alpha (TNFα), interferon gamma (IFNγ), fatty acid binding protein 4 (FABP4), peroxisome proliferator activated-receptor gamma (PPARγ)).
Results
Males had higher MP concentration than females ($5.7\times10^{11}/\text{ml}\pm3.3$ vs $3.8\times10^{11}/\text{ml}\pm1.2$, $P<0.05$) but no difference in mean size ($138\pm10$ nm vs $143\pm15$ nm respectively, $P=0.29$). Males had significantly higher fluorescence signals for platelet and endothelial-derived MPs (CD41:$4141\pm2309$ vs $2499\pm1087$, $P<0.05$; CD144: $3865\pm2365$ vs $1123\pm1247$, $P<0.05$) along with increased signal for IFNγ ($14160\pm6268$ vs $8885\pm4548$, $P<0.05$), IL6 ($2435\pm1689$ vs $1356\pm1195$, $P=0.05$) and FABP4 ($6597\pm3613$ vs $2915\pm2120$, $P<0.01$). TNFα signal was also higher in males but this did not quite reach significance ($1822\pm1900$ vs $921\pm649$, $P=0.085$).
Conclusions
Healthy men have increased circulating MPs compared to women, which are characterised by a more procoagulant and proinflammatory profile. These findings may contribute to gender-related differences in cardiovascular risk.

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## P184

**Longer duration of sitting down in pregnancy is associated with gestational diabetes, greater weight gain and depressive symptoms**

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Background
Studies have shown that interruptions in sedentary time in non-pregnant adults is positively associated with metabolic parameters, including abdominal obesity and glucose tolerance. However, there are no specific guidelines in the UK about recommended physical activity for pregnant women nor any validated tools to measure it. Our aim was to determine how much physical activity is carried out by pregnant women and how it relates to body anthropometry, glucose tolerance and depression.
Methods
A sub-study on physical activity levels during pregnancy was conducted as part of the multi-centre longitudinal PRiDE study, Physical activity was assessed by the International Physical Activity Questionnaire (IPAQ) at 2 time points and depression by the PhQ-9 questionnaire.
Results
Completed questionnaires were obtained from women in the late first (mean gestation 12+3 weeks, $n=1263$) and second trimesters (26+4 weeks, $n=982$) The frequency of doing any vigorous or moderate physical activity and walking for more than 10 min/day were 18.6, 38.9 and 83.7% in visit 1 and 23.8, 50.9 and 96.9% in the visit 2 respectively. Corresponding sitting times were 5.7 and 5.5 h/day. There was good correlation between the different components of the IPAQ during pregnancy with shorter duration of sitting being the only significant predictor of frequency of walking in the First trimester ($\beta=-0.40$, $P=0.01$). The onset of gestational diabetes was associated with longer sitting in early pregnancy (6.5 vs 5.7 h, $P=0.05$). At visit 2, duration of sitting per day was predicted by gestational weight gain (GWG) ($\beta=0.23$, $P=0.01$) and higher level of depression ($\beta=0.20$, $P<0.05$) after correcting for age, BMI and socio-economic status.
Conclusion
Self-reported sedentary behaviour is associated with depressive symptoms and GWG. If proven in objective assessments, reducing sitting time could improve metabolic risk in pregnancy.

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## P185

**Transcriptome profiles of human pancreatic islets following transient ischemia identify inflammatory and cell death responses**

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Islet transplantation, an effective treatment for Type I Diabetes, is compromised by transient ischemia. Ischemia reduces the viability and function of transplanted islets and may also promote signals that lower islet efficacy. In order to characterize the response of human islets to transient ischemia, high-throughput sequencing was employed to determine global gene expression. Islets isolated from healthy donors ($n=4$) were either pelleted in microcentrifuge tubes (ischemia) or cultured in vessels with oxygen-permeable membranes for 12 h at 37 °C, ambient $O_2$, and 5% $CO_2$ in a paired fashion. Isolated mRNA ($n=4$) was sequenced using Illumina RNAseq and analyzed for differentially expressed transcripts with edgeR. RNA transcripts were queried for enrichment and modelled to functional pathways previously defined using Ingenuity IPA and public databases. Network analysis of differentially expressed genes was performed to measure the connectivity of protein products in physical space to iterate functional subnetworks. RNAseq identified over 22 000 transcripts of which 650 were differentially expressed (FDR $<0.05$) in ischemic islets. Eight canonical pathways were enriched (corrected $P<0.05$), with the highest scoring pathways; TNF signalling, HIF-1alpha transcription factor network, and cellular senescence. Upstream analysis with IPA identified IL1B, TNF, TGBF1, and PDGF BB (activation $z$-score $>6$) as upstream regulators based on the differentially expressed genes. Network analysis revealed highly connected sub-networks involved in cell death, injury and tissue morphology, and amino acid metabolism. These findings are consistent with expectations following ischemic insult, however illustrate the dynamic response of cell clusters to hypoxia and potentially nutrient deprivation. Isolated human islets exposed to ischemia had differentially expressed cytokine profiles consistent with increased inflammation. Ischemia also activated TNF and likely IL1B, both of which are established stress response and survival signals in islets. Identification of tangible molecular targets provides the framework for intervention strategies in order to improve islet health during isolation and transplantation procedures.

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## P186

**Studies of Nuf mice with an activating calcium-sensing receptor (CaSR) mutation demonstrate the CaSR to regulate pancreatic beta-cell mass and glucose homeostasis**

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The modulation of pancreatic islet mass represents a novel therapeutic approach for the management of diabetes mellitus. G-protein coupled receptors (GPCRs) regulate beta-cell expansion and proliferation, and the objective of this study was to assess whether the calcium-sensing receptor (CaSR), which is an abundantly expressed beta-cell GPCR, may influence islet mass and systemic glucose homeostasis, and thus represent an exploitable drug target in some forms of diabetes. We characterised the islet mass, glucose tolerance and insulin secretory capacity of *Nuf* mice, which harbour an activating CaSR mutation, and evaluated whether a targeted negative allosteric modulator, known as ronacaleret, may rectify any disturbances of glucose homeostasis. All studies were conducted using WT and homozygous affected (*Nuf/Nuf*) mice aged 20–28 weeks and in accordance with institutional welfare guidelines. Islet mass and beta-cell proliferation rates were measured by pancreas histology and immunofluorescent labelling for insulin and KI-67. I.p. glucose tolerance and insulin secretion were assessed in mice that received either 90 mg/kg ronacaleret or drug vehicle by oral gavage over 5-days. These studies revealed *Nuf/Nuf* mice to have a 31% reduction in islet mass and 39% reduction in beta-cell proliferation rates when compared to wild-type littermates ($P<0.05$), with no derangement of islet architecture. *Nuf/Nuf* mice also displayed significantly impaired glucose tolerance ($P<0.001$) compared to wild-types and an almost complete absence of insulin secretion in response to an i.p. 2 g/kg glucose bolus injection. Administration of ronacaleret significantly ($P<0.05$) lowered plasma glucose concentrations in *Nuf/Nuf* mice, but did not increase insulin secretion, and had no significant effect on beta-cell mass or proliferation rates. These findings demonstrate that the CaSR is a determinant of pancreatic islet mass and novel target for the modulation of plasma glucose concentrations, and also highlight that this GPCR may have additional insulin-independent effects on glucose homeostasis.

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## P187

**Role of oral cholecalciferol as adjuvant therapy in type 1 diabetes mellitus: randomised controlled trial**

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Introduction
The vitamin D hormone system has been implicated in the pathogenesis of several autoimmune diseases, including Type 1 Diabetes Mellitus, as an adaptive immune system modulator.
Objectives
The objective of this study is to examine the role of cholecalciferol in modulating the altered immune response in T1DM, thereby improving glycemic control and residual pancreatic Beta-cell function, measured objectively by Haemoglobin A1c levels, GAD65 antibody titres and C-peptide levels.
Method
Fifty two T1DM patients aged 1-18 years attending JIPMER Pediatrics department in year 2014 were randomised into two groups. High dose oral cholecalciferol therapy (1.2 lakh IU per month) was instituted in addition to insulin in intervention arm, while only insulin was continued in other arm for 6 months.
Results
Prevalence of Vitamin D deficiency was as high as 63.5% among T1DM patients in our study. The Cholecalciferol group achieved significantly lower HbA1c

levels at 3 and 6 months follow-up than controls ($P<0.05$). The mean C-peptide levels were significantly greater ($P<0.05$) in cholecalciferol group as compared to controls at end of 6 months. Significant difference in GAD65 antibody levels was not found between the groups at the end of 6 months. No adverse events due to cholecalciferol therapy were reported.

Conclusions

Our study shows that high dose oral Cholecalciferol concomitant with insulin therapy is safe and is related to slow decline of residual Beta-cell function in T1DM patients, thereby enhancing glycemic control. An affordable, safe and easily obtainable vitamin may serve as a novel approach in the fight against a costly, debilitating, chronic disease.

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## P188

**Prevalence and determinants of metabolic syndrome among HIV infected patients receiving highly active antiretroviral therapy in Kano, northwestern Nigeria**

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Background

We aimed to determine and compare the prevalence and associated factors of metabolic syndrome (MS) among HAART exposed versus HAART naïve HIV infected patients at the Aminu Kano Teaching Hospital, Kano (AKTH).

Method

In this cross-sectional comparative study we evaluated 300 HIV infected persons who were divided into two age and sex matched groups – HAART-exposed and HAART-naïve – attending AKTH. MS was diagnosed using ATP-III criteria. Insulin resistance (IR) among participants with MS was estimated using HOMA-IR.

Results

The mean ± S.D. age of HAART-exposed and HAART-naïve subjects was 35.7 ± 10.0 and 34.0 ± 9.7 years respectively ($P=0.152$). The prevalence of MS among HAART exposed participants was 19.3%, while it was 5.3% among HAART-naïve controls, ($P<0.001$). Gender wise, 8.7% of males and 10.7% females in the HAART exposed group had MS, while 1.3% of males and 4.0% of females in the HAART naïve group had MS, with no statistical significant difference ($P>0.05$). Advanced age, longer duration of HIV and HAART exposure, increased BMI, weight gain after HAART exposure, exposure to PIs and increased mean CD4 cell count were found to be significantly associated with MS ($P<0.05$). However, only age (OR 4.3, 95% CI 1.6–11.8, $P=0.005$) and BMI (OR 4.2, 95% CI 1.5–11.9, $P=0.007$) were found to be independently associated with the development of MS. The prevalence of IR among participants with MS that were HAART exposed was 79.3% while that among HAART naïve with MS was 25.0% ($P=0.008$).

Conclusion

MS is common among HIV patients on HAART mostly related to use of PIs. Advanced age and increased BMI are independent determinants of MS in HAART treated patients. Life style measures and regular monitoring of anthropometric indices of HIV patients will help to reduce the metabolic problems associated with drug treatment.

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## P189

**The effect of L-arginine on anorectic gut hormone release in humans**

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High protein diets suppress appetite and facilitate weight loss, but are difficult to adhere to in the long-term. Understanding the mechanisms by which protein suppresses appetite may establish targets for more acceptable interventions to treat or prevent obesity. Of particular interest is the concept of functional foods or novel products which aim to potentiate satiety. However, the specific mechanisms regulating protein-induced satiety are unknown. Protein has a satiating effect greater than that of other macronutrients. A high protein meal increases circulating concentrations of the anorectic gut hormones peptide YY (PYY) and glucagon-like peptide-1 (GLP-1). The effects of a high protein diet are thought to be mediated by PYY. Receptor systems that respond to amino acids have been identified. Previous work has investigated the effect of specific amino acids which act as ligands for the following G-protein coupled receptors: CaR, T1R1/T1R3 and GPRC6A on appetite in rodents. These receptors are present within the gut, where they are thought to be responsible for amino acid sensing. L-arginine activates the T1R1/T1R3, the GPRC6A and the CaR. Animal studies showed that L-arginine can significantly reduce food intake and elevate plasma GLP-1 and PYY, whilst glycine had no effect.

The role of L-arginine on anorectic gut hormone release in humans was thus investigated. Following an overnight fast, nine healthy volunteers were given capsules containing a dose of L-arginine, glycine or vehicle control (17.1 μmol) in a double-blinded randomised manner. Following administration of the amino acid or control, visual analogue scales were completed and gut hormone analysis carried out every 15 min over a 2.5 h period. At $t=60$ min, an ad libitum meal was presented to the volunteers in order to assess food intake. Oral administration of L-arginine significantly increased circulating levels of GLP-1 ($P<0.01$) compared to vehicle or glycine controls. In addition, following the ad libitum meal there was a significant increase in PYY ($P<0.05$) following L-arginine administration compared to vehicle or glycine controls. However, L-arginine had no significant effect on subjective measures of appetite or food intake compared to vehicle or glycine controls. These results suggest L-arginine can modulate gut hormone release in humans.

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## P190

**Generation of a long acting GCSF for treatment of neutropenia and stem cell harvest**

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Rationale

Over the last 20 years, granulocyte colony-stimulating factor (GCSF) has become a recognised therapy in the treatment of patients suffering from neutropenia. Current therapies require daily injections of GCSF to stimulate stem cell production and response to treatment is often unpredictable as GCSF is rapidly cleared. A number of approaches to reducing GCSF clearance have been tried mainly through conjugation with another moiety. The technologies already being employed, included PEGylation, immunoglobulins and glycosylation to increase the half-life of rhGCSF, however although these approaches have reduced clearance the pharmacokinetic profile has remained unpredictable. The aim of this project is to create a long acting GCSF with predictable pharmacokinetic profile to provide a more effective treatment for generating stem cells for bone marrow transplantation.

Hypothesis

The incorporation of variable glycosylated linkers between two GCSF molecules will create a construct with high molecular weight and protected from proteolysis resulting in reduced clearance while retaining bioactivity.

Methodology

GCSF tandem molecules with linkers containing between 2 and 8 NAT glycosylation motifs and their respective controls (Q replaces N in the sequence motif NAT) were cloned, and sequenced. Following expression in Chinese hamster ovary (CHO) cells, expressed protein was quantified by ELISA and analysed by SDS–PAGE and western blot to confirm molecular weights. *In vitro* bioactivity was tested using an AML-193 proliferation assay. Immobilised Metal Affinity Chromatography (IMAC) was used to purify the protein. Pharmacokinetic and pharmacodynamic of the GCSF tandem proteins were measured in normal Sprague Dawley Strain rats with full ethical approval.

Results

Purified glycosylated tandem molecules show increased molecular weight above that of controls when analysed by SDS–PAGE. All GCSF tandems show increased bioactivity in comparison to rhGCSF. Following intravenous administration to rats, GCSF2NAT, GCSF4NAT and GCSF8NAT containing 2, 4 and 8 glycosylation sites respectively displayed a reduced rate of clearance compared to both rhGCSF and non-glycosylated GCSF tandem control. Both GCSF2NAT and GCSF4NAT show a significant increase in the percentage of neutrophils over controls at 12 h post injection. This effect however is short lived as the counts at 24+ h are not significantly different to controls. GCSF8NAT shows an increase in the percentage of neutrophils that is only significant at 48 h.

Conclusion

Results show that the use of glycosylated linkers to generate GCSF tandems results in molecules with increased molecular weight, improved *in vitro* bioactivity, longer circulating half-lives and enhanced neutrophilic population when compared to both rhGCSF and the non-glycosylated tandem protein.

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## P191

**Hypercortisolaemia increases femoral adipose tissue blood flow but not net fatty acid release *in vivo***

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Hypercortisolaemia (Cushing's Syndrome) is characterised by abdominal fat accumulation and gluteofemoral fat loss. The mechanisms underpinning this fat mass redistribution are unknown. Adipose tissue blood flow (ATBF) and net fatty acid release rate (lipolysis) are important determinants of depot-specific fat mass. We hypothesized that hypercortisolaemia may result in increased femoral ATBF and lipolysis promoting gluteofemoral fat mass loss. We recruited nine healthy male volunteers (median age 28 years, range 19–57; BMI 26.5 kg/m$^2$, range 22.7–30.4) who underwent *in vivo* assessment of femoral ATBF and lipolysis using radioactive Xenon washout and the arterio-venous difference technique. All subjects were studied twice, following a preceding infusion period with hydrocortisone (0.2 mg/kg per min for 16 h) and saline, respectively. Infusions were given in a randomised double-blind order. On each of the two study days ATBF and lipolysis were studied in the fasting state and following a 75 g glucose drink. Hydrocortisone infusion increased plasma cortisol significantly (AUC 2009$\pm$565 vs 77$\pm$5 nmol/l, $P=0.012$). Compared to saline, hydrocortisone increased systemic plasma non-esterified fatty acids (NEFA) by 62$\pm$13% during the fasting state (AUC 944$\pm$53 vs 597$\pm$39 μmol/l, $P=0.008$). Expectedly, glucose suppressed systemic lipolysis, although postprandial NEFA remained higher after hydrocortisone (AUC 341$\pm$23 vs 178$\pm$11 μmol/l, $P=0.008$). Hydrocortisone infusion induced a steady increase in femoral ATBF during the postabsorptive phase (AUC 4.4$\pm$0.6 vs 2.4$\pm$0.4 ml/min$\times$100 g/tissue, ANOVA $P=0.009$ infusion type$\times$time). Following oral glucose load, femoral ATBF increased further compared to saline (AUC 7.9$\pm$1.4 vs 3.3$\pm$0.5 ml/min$\times$100 g/tissue, $P=0.011$). During control conditions, fasting femoral NEFA release rate was high (AUC 671$\pm$232 nmol/min$\times$100 g/tissue), and was inhibited following glucose (AUC 413$\pm$162 nmol/min$\times$100 g/tissue). Hydrocortisone did not have any effect on femoral fasting or postprandial lipolysis ($P=0.285$ and $P=0.447$ compared to saline, respectively). Hypercortisolaemia increases femoral ATBF, but not lipolysis, suggesting differential glucocorticoid effects in the vasculature and adipocytes *in vivo*. Further research should focus on the effect of hypercortisolaemia on depot-specific fatty acid uptake.

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## P192

**Central IL6 signalling and the development of impaired insulin secretion in type2 diabetes**

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Obesity and the metabolic syndrome are chronic inflammatory states that are now understood to be involved in the development type 2 Diabetes. Hypothalamic cytokine signalling is thought to influence neuroendocrine networks critical to the dynamic maintenance of glucose homeostasis. Interleukin 6 (IL6) is a cytokine that displays tissue-specificity and is instrumental in the mounting of the acute phase response, as well as in the subsequent resolving and adaptive phase. IL6 is a strong biomarker of disease progression and its receptor displays extensive expression centrally. To examine the role of central IL6 signalling in diabetes, we generated brain-specific receptor knock-down mice (NesCre IL-6R KD) by crossing NesCre1 and IL-6R$^{flox/flox}$ mice, and proceeded to validate and metabolically phenotype their KD and control offspring on standard chow (SC) and high fat diet (HFD). KD mice on SC exhibited a 15% decrease in body mass compared to their controls and appeared to harbor increased musculature and decreased body fat at 3 and 20 weeks of the study. Unsurprisingly, HFD KDs only displayed significant differences in body fat following 20 weeks. Intriguingly, KD animals on SC were found to be less active in the feeding phase of their cycle, with no significant differences observed in the HFD groups. Similarly, no significant differences in terms of food consumption were observed, however comparison of mean RER over 48 h using the CLAMS monitoring system, revealed a preference to glucose oxidation in KD animals compared to control animals on SC and HFD. Most strikingly, dramatic deficits in basal and glucose-stimulated insulin secretion were observed in both SC and HFD KD mice, while HFD KDs displayed significantly elevated resting blood glucose by 10 weeks of diet. This would suggest central classical IL6 signaling to regulate insulin release in the periphery as well as the adaptive response to HFD via promoting peripheral fat oxidation.

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## P193

**Women with idiopathic intracranial hypertension have a distinct andro-metabolic signature compared to polycystic ovary syndrome and simple obesity**

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Context
Idiopathic intracranial hypertension (IIH) is characterised by elevated intracranial pressure and occurs predominantly in obese premenopausal women. Signs and symptoms of polycystic ovary syndrome (PCOS) often coexist in IIH. Here we compared the androgenic and metabolic phenotypes in IIH, PCOS and simple obesity.
Patients and methods
We studied 25 patients with IIH (mean age 34.4$\pm$9.2 years; mean BMI 37.8$\pm$5.2 kg/m$^2$), in comparison to 31 women with PCOS and 15 with simple obesity; all three groups were matched for age and BMI. Women with IIH were studied before and after a weight loss intervention (mean BMI change $-5.8\pm3.0$ kg/m$^2$). In all participants we performed comprehensive metabolic phenotyping and steroid profiling. Serum androgens were measured by liquid chromatography-tandem mass spectrometry); 24-h urinary steroid excretion was analysed by gas chromatography/mass spectrometry. Urinary steroid profiles were correlated with clinical parameters of IIH severity.
Results
Serum testosterone in IIH was comparable to PCOS and significantly higher than controls ($P=0.01$). Serum androstenedione was significantly increased in PCOS ($P=0.008$) but IIH did not differ from controls. Insulin resistance as assessed by HOMA-IR did not differ between IIH and controls; PCOS women had a trend towards significantly higher HOMA-IR values ($P=0.08$). Total glucocorticoid excretion was significantly higher in IIH compared to controls ($P=0.01$) and decreased after weight loss ($P=0.02$). Similarly, the urinary ratio of 5α-THF/THF, a marker of systemic 5α-reductase activity, was significantly increased in IIH compared to controls ($P=0.04$). The An/Et ratio correlated significantly with baseline markers of ocular papilloedema in IIH ($R=0.47$, $P=0.02$).
Conclusion
These results indicate a distinct andro-metabolic signature in IIH, with increased testosterone but normal androstenedione and HOMA-IR values. We propose the new term of 'andrometabolic syndrome' that regularly features androgen excess and obesity and variably presents with additional features such as anovulation (PCOS) and raised intracranial pressure (IIH).

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## P194

**Differences between men and women in markers of dietary fatty acid oxidation**

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Men have a higher prevalence and risk of non-alcoholic fatty liver disease (NAFLD) than age-matched women and this may, in part, be related to differences in fatty acid oxidation. Fasting and postprandial fatty acid oxidation was investigated in 11 healthy men and 11 healthy women matched for age (46$\pm$2 years vs 46$\pm$2 years (mean$\pm$S.E.M.)), BMI (28.0$\pm$1 vs 27.0$\pm$1 kg/m$^2$) and liver fat content (3.4$\pm$0.9 vs 3.9$\pm$0.7%) measured using magnetic resonance spectroscopy (MRI). Plasma acetoacetate and 3-hydroxybutyrate (3OHB) concentrations were measured as markers of hepatic fatty acid oxidation. Postprandial fatty acid oxidation was investigated by feeding a mixed test meal labelled with a stable-isotope tracer (U13Cpalmitate) and measuring incorporation into expired $CO_2$ (as a marker of whole body oxidation) and 3OHB (as a marker of hepatic fatty acid oxidation). Men and women had a similar amount of liver fat content despite men having a significantly greater amount of visceral fat ($P<0.05$). However, overall visceral fat content was related to liver fat content ($rs=0.57$, $P<0.05$). Men had higher plasma triglyceride (TG) concentrations than women in the fasting and postprandial state ($P<0.05$). Although there was

no difference in fasting plasma acetoacetate and 3OHB concentrations there was a divergence between men and women with consumption of the test meal with males having notably lower ($P<0.05$) postprandial concentrations. In contrast, women had a greater appearance of plasma 13C-3OHB and breath 13CO2 than men, indicating that recently ingested dietary fatty acids were entering oxidation pathways to a greater extent in women. These results show differences in dietary fatty acid oxidation between genders where women tend to favour oxidation pathways which may, in part, explain the lower prevalence of NAFLD noted in women.

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## P195

**Establishing human liver cell models to investigate the effects of exogenous metabolic substrates on fatty acid partitioning**

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Hepatic steatosis, accumulation of intracellular triglyceride (TG) (>5% of hepatic tissue), is the prerequisite for the development of non-alcoholic fatty liver disease (NAFLD). NAFLD represents a spectrum of liver diseases and is associated with obesity and obesity-related metabolic diseases such as type 2 diabetes and cardiovascular disease. Steatosis occurs due to an imbalance between fatty acid input and removal (fatty acid partitioning) which can be affected by genetics, diet, hormones and drugs; making the cause of NAFLD difficult to elucidate. Available cell models provide insight however, development of human cell models that are characterized in terms of culture conditions and the effect this may have on intracellular fatty acid partitioning are required. We set out develop a model of TG accumulation using a physiological mixture of exogenous fatty acids (representative of dietary or non-esterified fatty acids) and assess fatty acid partitioning in a novel human liver cell line (LIVOAPOLY). Under basal conditions LIV0APOLY cells do not have notable lipid accumulation. Short-term exposure of exogenous fatty acids promoted storage, oxidation and secretion of TG. Additionally, when exposed to higher glucose concentrations intracellular TG content increased, indicative of an increase in *de novo* lipogenesis (DNL) as would be predicted in vivo. Stable isotopes were used to assess the fate of exogenous fatty acids in LIV0APOLY cells compared to primary human hepatocytes, the gold standard liver cell model. Both cell types stored similar amounts of labelled fatty acids TG, however, oxidation and secretion of FA varied between the cell types. The differences observed maybe due to variations in culturing formulations between the cells. LIV0APOLY cells can be considered a useful human liver cell model for studying the effects of exogenous metabolic substrates on fatty acid partitioning in order to delineate the mechanisms which may influence the development and progression of NAFLD.

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## P196

**Evidence for 11β-HSD1 regulation of brain energy metabolism following systemic inflammation**

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Background
Chronically elevated brain glucocorticoid (GC) levels impair cognition. In rodents, raised GC levels prior to lipopolysaccharide (LPS) administration potentiate neuroinflammation although GC suppresses neuroinflammation if administered after LPS. 11β-hydroxysteroid dehydrogenase-1 (11β-HSD1) activity can increase intracellular GC levels, including in the brain, without alteration in circulating levels. 11β-HSD1 deficiency/inhibition protects against age-related cognitive impairment. However, the underlying mechanism remains unclear. 11β-HSD1 activity is coupled to hexose-6-phosphate dehydrogenase activity, itself dependent on cellular energy status. Processes affected by deficiency/inhibition of 11β-HSD1 (e.g. acute inflammation, angiogenesis) are associated with increased aerobic glycolysis.

Hypothesis
11β-HSD1 deficiency alters hippocampal energy homeostasis following inflammation.
Methods
Sterile peritonitis was induced (i.p. injection of 100 μg/kg LPS or 0.9% saline; culled 3, 6 or 9 h later) in male *Hsd11b1*$^{-/-}$ and C57BL/6 mice. A burrowing test was performed prior to cull to assess 'sickness' behaviour[5]. Levels of transcripts encoding inflammatory markers, metabolic transporters and enzymes were quantified by qRT-PCR. Inflammatory cells, plasma cytokines and GC levels were quantified by flow cytometry, ELISA and RIA respectively.
Results
Peripheral inflammation (inflammatory cells numbers in blood, plasma cytokine levels and 'sickness' behaviour) did not differ between genotypes ($P>0.05$; $n=3$–8). However, the brain inflammatory response differed between genotype with lower expression of *Tnfa*, *Il1b* and *Il6* in *Hsd11b1*$^{-/-}$ mice ($P<0.05$, $n=7$) 3 h post LPS. At 6 h (peak of brain inflammatory response), hippocampal *Tnfa*, *Il1b* and *Il6* expression levels did not differ between genotype ($P>0.05$; $n=7$–8) but principal component analysis of mRNA encoding 18 key metabolic transporters and enzymes showed a distinct cluster for *Hsd11b1*$^{-/-}$ mice post LPS (reduced glucose transporters and increased mitochondrial enzymes mRNA levels) (cumulative variance=54%). Hippocampal *Hsd11b1* was itself down-regulated in C57BL/6 mice ($P<0.05$; $n=7$–8) 9 h following inflammation.
Conclusion
These data suggest that 11β-HSD1 deficiency reduces the hippocampal cytokine response and attenuates the switch to aerobic glycolysis following peripheral inflammation.

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## P197

**Impact of number of transplanted human islet equivalents on successful return to normoglycaemia in an NSG diabetic mouse model**

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Introduction
Islet transplantation (ITx) is a therapeutic option for patients with Type 1 diabetes. However there is attrition of graft function and the majority of patients require exogenous insulin injections and/or further transplants, within five years. Mouse models help elucidate mechanisms of islet graft failure and the NOD/Lt-*scid IL2r*$\gamma^{null}$ (NSG) mouse is useful to study human islet engraftment but few published studies exist. As different numbers of islets are necessary to render different mouse strains euglycaemic, our aim was to determine differences in transplant success based on number of islet equivalents (IEQ) in this specific mouse model.
Methods
Either 2000 ($n=4$) or 4000 ($n=3$) human IEQs were transplanted under the kidney capsule of male streptozotocin-induced diabetic NSG mice. Blood glucose levels were monitored for 6-weeks post-ITx, glucose tolerance tests (GTT) performed at 2 weeks (2 g glucose/kg body weight, i.p.) with human c-peptide level measurements, before histological processing of kidney capsule for islets/insulin staining.
Results
Mice transplanted with 4000 IEQs returned to normoglycaemia (non-fasting blood glucose <12 mmol/l) after (mean ± S.E.M.) 6.3 ± 1.8 days, but those receiving 2000 IEQs had not after six weeks. Following the GTT, glucose concentrations were diminished and stimulated human c-peptide concentrations greater with 4000 vs 2000 IEQs (600 ± 138 vs 2769 ± 326 mmol/l × min ($P<0.001$) and 1447.8 ± 1039.8 vs 24.8 ± 13.2 pmol/l respectively). Histology showed the presence of islets and positive insulin staining under the kidney capsule.
Discussion
We have demonstrated that the number of islets transplanted is critical to transplant success and a return to normoglycaemia. In our study, the large number of islets transplanted suggests the NSG mouse is highly resistant to insulin, compared to other mouse models. This should be taken into account in further studies, and further human islet 'dose' finding studies performed to determine the optimal number for ITx success.

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## P198

**Vascular dysfunction in horses with insulin resistance**

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Background
Vascular impairment, most commonly due to endothelial dysfunction, is associated with cardiovascular risk in humans with obesity, insulin resistance or Cushing's syndrome. Similar endocrine disorders in horses, resulting in insulin resistance, are associated with laminitis, a dysfunction of the vasculature supplying the hooves, the mechanism of which is unclear. We hypothesised that horses with insulin resistance have both local and systemic evidence of vascular dysfunction.
Methods
In a case control study, healthy horses ($n=6$) and horses with insulin resistance ($n=6$) destined for euthanasia were recruited. Small vessels ($<1$ mm) were harvested from the hooves (laminar artery, laminar vein) and facial skin (facial artery). Vascular function was investigated using wire myography, assessing the response to vasoconstrictors phenylephrine ($10^{-9}$–$10^{-5}$M) and 5-hydroxytryptamine (5HT;$10^{-9}$–$10^{-5}$M) and the vasodilator acetylcholine ($10^{-9}$–$10^{-5}$M).
Results
Laminar arteries and veins and facial arteries from healthy horses contracted in response to KPSS, phenylephrine and 5HT (Emax:laminar arteries > facial arteries > > laminar veins). Laminar veins were more sensitive to 5HT than either laminar arteries or facial arteries ($pD_2$ $7.11\pm0.16$ vs $6.47\pm0.19$, $P=0.02$). Acetylcholine induced a relaxation in all vessels (Emax;laminar arteries ≫ veins = facial arteries; $P=0.02$) which was abolished by removal of the endothelium. In comparison with healthy controls, acetylcholine-induced relaxation was dramatically reduced in vessels from horses with endocrine disease (% relaxation of healthy laminar arteries $313\pm94.1$% vs diseased $129\pm14.2$%, $P=0.014$). In addition, contractile responses to phenylephrine ($P=0.005$) and 5HT ($P=0.0007$) were increased in laminar veins from horses with endocrine disease; these differences were still apparent following removal of the endothelium. Sensitivity to phenylephrine was reduced (in an endothelium-independent manner) in laminar arteries ($P=0.006$) and veins ($P=0.009$) from horses with endocrine disease.
Conclusion
Horses with insulin resistance exhibit significant local and systemic vascular dysfunction. Systemic endothelial dysfunction may be a key determinant of the vascular disease associated with insulin resistance in horses.

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## P199

**Evidence for improved mitochondrial efficiency in adipose tissue of T2DM women after malabsorptive but not restrictive bariatric surgery**

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Aims/hypothesis
Genetic and functional defects in mitochondria are linked with phenotypes of obesity, hypercholesterolemia, and type 2 diabetes (T2DM). However, the impact on mitochondria during surgery-induced metabolic recovery is unknown. We therefore studied the effect of restrictive versus malabsorptive bariatric procedures on indicators of mitochondrial efficiency and control of biogenesis/function.
Methods
Forty-three morbidly obese, T2DM consented women underwent restrictive (laparoscopic adjustable gastric banding, $n=14$; gastric plication, $n=16$) or malabsorptive (bilio-pancreatic diversion, $n=13$) operations in an ethics-approved study. Abdominal subcutaneous adipose tissue (AbScAT) biopsies taken before and 6 months after surgery were used to assess mitochondrial number (mtDNA copy number) and mRNA expression of biogenesis (PGC1α, POLG, TFAM), oxidative phosphorylation (mtND6, SDHA, COX4I1, mtATP6), and antioxidant related genes (SOD1, UCP2).
Results
Metabolic health of patients was significantly improved in both surgical interventions, although more marked in malabsorptive surgery, where 69% achieved T2DM remission (HbA1c < 42 mmol/mol off T2DM medication) compared to only 33% in restrictive surgery. Mitochondrial efficiency in AbScAT was positive across all genes assessed in the malabsorptive surgery (mtATP6 $r=0.898$, $P=0.0004$), evidenced by correlation of change in mitochondrial number versus mRNA expression, whilst negative in the restrictive surgery ($r=-0.316$, $P=0.123$). Further analysis of mitochondrial inefficiency identified that PGC1α (29.5%, $P=0.004$), SOD1 (11.7%, $P=0.019$) and HDL (18.3%, $P=0.008$) levels were significant independent predictors of this event. Finally, T2DM remission was also associated with tighter control of UCP2 and mitochondrial number in response to nutrient excess, as denoted by correlation with BMI ($P=0.004$) and HbAlc ($P=0.0003$) respectively; again more marked in malabsorptive surgery.
Conclusions/interpretation
Malabsorptive surgery led to marked improvement in metabolic health, enhanced mitochondrial efficiency and higher T2DM remission than restrictive surgery. Taken together this data suggests that AbdScAT may act as an important site for mitochondrial improvement supporting metabolic recovery, particularly in cases where nutrient absorption is severely limited rather than purely restricted.

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## P200

**AMPK activators modulate pro-inflammatory responses in human adipocytes**

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Adipose tissue inflammation may be an important mechanism in the development of systemic insulin resistance, such that modulation of this system may be a therapeutic target for type 2 diabetes and obesity-related disorders. AMPK has anti-inflammatory properties, yet the anti-inflammatory actions of AMPK in adipocytes remain poorly characterised. We questioned whether AMPK activation influences pro-inflammatory responses in cultured adipocytes in basal and stimulated conditions. Expression of pro-inflammatory chemokines and cytokines were studied in SW872 cells (human adipocyte cell line). Adipocytes were exposed to the AMPK activators AICAR (1 mM, 1 h) or A769662 (100–300 μM, 30 min) and subsequently stimulated with pro-inflammatory mediators TNF-α (10 ng/ml, 8–24 h) or Interleukin-1β (IL-1β, 10 ng/ml, 6 h). Effects on mRNA expression of MCP-1, CXCL-1, CXCL-10 and IL-6 were examined by RT-PCR. Immunoblotting confirmed AMPK activation with phosphorylation of AMPK-Thr172 and downstream ACC-Ser79. AICAR, a non-specific AMPK activator, decreased basal expression of MCP-1 (90%), CXCL-1 (71%) and CXCL-10 (93%) after 24 h and attenuated TNF-α- and IL-1β- stimulated increases in gene expression of CXCL-1 (IL-1β 11.7 vs AICAR + IL-1β 3.3 fold increase) and CXCL-10 (IL-1β 39.6 vs AICAR + IL-1β 4.2 fold increase). The selective AMPK activator A769662 however increased basal IL-6 (28 fold) and MCP-1 (3.8 fold) and exacerbated TNF-α- and IL-1β-induced IL-6 (TNF-α 5.8 vs A769662 + TNF-α 29.8 fold increase) and MCP-1 gene expression (TNF-α 2.5 vs A769662 + TNF-α 5.3 fold increase). Conversely, A769662 decreased basal (52%) and agonist-stimulated increases in CXCL-10 gene expression (TNF-α 11.5 vs A769662 + TNF-α 0.56 fold change). These data demonstrate that pharmacological activators of AMPK influence pro-inflammatory responses in human adipocytes. Depending on the signalling pathways targeted by the activators, responses seem to differ. Selectively activating AMPK with A769662 promotes inflammation by increasing expression of cytokines. The fact that AMPK activators influence basal cytokine expression and production, suggests that these processes are constitutively active in adipocytes. Our findings highlight an interaction between AMPK and inflammation.

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## P201

**eNAMPT-monomer plays a critical role in pathophysiology of experimental diabetes and represents a novel target for treatment of type 2 diabetes**

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Extra-cellular nicotinamide phosphoribosyltransferase (eNAMPT; also referred to as visfatin/PBEF) concentrations are elevated in serum of patients with type 2

diabetes (T2D). However, the relationship between abnormally elevated serum eNAMPT and the pathophysiology of T2D is unclear. eNAMPT circulates in functionally and structurally distinct monomer and dimer forms. eNAMPT-dimer exerts NAD-biosynthetic activity. The role of eNAMPT-monomer is unclear but may exert NAD-independent pro-inflammatory effects. However studies of eNAMPT in T2D have not distinguished between monomer and dimer forms. Since T2D is characterised by chronic inflammation, we hypothesized a selective role for eNAMPT-monomer in T2D pathophysiology.

Two experimental models were used to examine the role of eNAMPT-monomer in T2D; (A) diabetic high-fat fed mice (HFD; 10 weeks) were administered a neutralizing anti-eNAMPT-monomer antibody; (B) lean mice were administered recombinant eNAMPT-monomer daily for 14 days, to induce a serum concentration of 5 ng/ml, similar to levels in HFD mouse serum.

Serum eNAMPT-monomer levels were elevated in diabetic HFD mice, whilst eNAMPT-dimer levels were unchanged. Increased eNAMPT-monomer levels occurred in part due increased secretion from dysfunction visceral white adipose tissue and liver. Strikingly, neutralization of eNAMPT-monomer in HFD mice resulted in lowered blood glucose, amelioration of impaired glucose tolerance (IGT) and whole-body insulin resistance, improved pancreatic islet function and hepatic insulin sensitivity and reduced systemic and tissue inflammation. These effects were maintained at least 3 weeks post-dosing. Finally, eNAMPT-monomer administration induced a diabetic phenotype in mice, characterised by elevated blood glucose, IGT, whole-body insulin resistance, impaired pancreatic insulin secretion and presence of systemic inflammation. In contrast administration of eNAMPT-dimer led to a modest improvement in glycaemic control.

In summary, we demonstrate that chronic elevation of eNAMPT-monomer plays a crucial role in the pathogenesis of diet-induced T2D via pro-inflammatory mechanisms. These data provide proof-of-concept evidence that eNAMPT-monomer represents a novel therapeutic target for T2D.

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## P202

### Predictors of glycaemic response and impact on weight and BP of dapagliflozin therapy in type 2 diabetes

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Background

Dapagliflozin was the first SGLT2 inhibitor licensed in UK to improve glycaemic control in type 2 diabetes. The study aimed to assess impact of dapagliflozin on glucose, weight and BP and identify factors predictive of glycaemic response in a large cohort.

Methods

Observational retrospective data collected on all patients prescribed dapagliflozin across primary and secondary care in two Scottish health board areas. HbA1c, weight, BP and concomitant therapy pre and post initiation of therapy and rate of and reasons for discontinuation of therapy were extracted from electronic patient records (SCI-diabetes). HbA1c response by intention to treat was investigated compared to baseline for subgroups by concomitant treatment, baseline BMI and baseline HbA1c. Weight and BP change at 12 months were compared to baseline.

Results

Five hundred and ninty seven patients were included and 521 had follow up data available. 36 patients (6%) discontinued treatment. HbA1c (mmol/mol) at initiation was 80 (IQR 67–94) and at 6,12, and 24 months were 68 (57–76.5), 66 (56–78) and 66.5 (57–76) ($P \leq 0.001$). Baseline Systolic BP was 136 mmHg (IQR 124–146) and at 12 months was 131 mmHg (120–142) Baseline diastolic BP was 80 mmHg (72–84) and at 12 month 77 mmHg (70–82) ($P \leq 0.001$). Complete weight data at baseline and 12 months was available in 181 patients and was 100.3 kg (IQR 88–118.1) at baseline reducing to 97 kg (IQR 84.4–114) at 12 months ($P = 0.07$). Glucose lowering efficacy was seen in patients treated with concomitant insulin, GLP-1 and dual and triple oral therapy. Glycaemic reduction was greater in patient group with higher baseline HbA1c. Significant reduction in HbA1c from baseline was seen across all BMI ranges.

Conclusions

In a large cohort Dapagliflozin effectively reduces HbA1c, weight and BP. As predicted by the mode of action by SGLT2 inhibition, glycaemic efficacy is independent of concomitant therapy type and patient phenotype.

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## P203

### Pre-operative assessment for Sleep apnoea in patients referred for bariatric surgery: the usefulness of various clinical screening tools

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Aim

Obstructive sleep apnoea (OSA) is widely prevalent in patients with obesity, referred for bariatric surgery. Various clinical tools are clinically used for screening pre-operatively before referring for formal sleep studies. The aim of our observational analysis was to assess the usefulness of various screening methods for OSA used in clinical practice.

Methods

Records of all patients who had been referred to bariatric services in 2014 were reviewed. Various screening methods were used during clinical visits to justify investigating for OSA. Patients were referred for formal sleep studies to confirm OSA.

Results

Two hundred and twenty seven new patients were seen in medical bariatric clinics. Mean BMI was 47.6 (28–82). 39 patients had already established CPAP treatment for OSA, hence excluded.104/188 had been assessed for OSA (and a further 22 have been referred and awaiting assessment). 35/104 (34%) did not have OSA; a further 13/104 (12%) had only mild OSA. 54% had severe OSA needing CPAP treatment: The sensitivity, specificity, positive and negative predictive values of various tests, with numbers tested are:

| Tests | $n=$ | Sensitivity (%) | Specificity (%) | PPV (%) | NPV (%) |
|---|---|---|---|---|---|
| Sats on air $<92\%$ | 72 | 8 | 87 | 57 | 30 |
| $PCO_2 > 6.0$ | 77 | 20 | 86 | 79 | 30 |
| $PO_2 < 8$ | 77 | 11 | 87 | 67 | 28 |
| $PO_2 < 10$ | 77 | 71 | 55 | 80 | 43 |
| STOP-BANG $\geq 3$ | 39 | 100 | 7 | 66 | 100 |
| STOP BANg $\geq 5$ | 39 | 76 | 50 | 73 | 54 |
| Epworth score $> 10$ | 74 | 33 | 59 | 31 | 21 |
| FEV1/VC $<70$ | 43 | 7 | 77 | 40 | 26 |

There was positive correlation between the BMI and presence of OSA ($r = +0.2$) and the severity of OSA ($R = +0.15$).

Conclusion

The prevalence of OSA is high in obesity. Various commonly used respiratory screening tests may not accurately provide a pre-test probability for diagnosis of OSA. Formal overnight oximetry and sleep studies are required to diagnose this comorbidity in obesity, as it has implications during anaesthesia and post-op follow up.

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## P204

### Effect of vitamin D supplementation on cardiovascular risk factors and exercise performance in healthy subjects; a randomised placebo controlled pilot study

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Background

Evidence suggests associations between vitamin D deficiency and cardiovascular risk factors, including hypertension and excessive cortisol levels. Also, vitamin D levels may be associated with exercise performance. Thus, we aimed to investigate the effects of vitamin D intake on cardiovascular risk factors, free urinary cortisol and exercise performance.

Methods

A randomised placebo controlled single-blinded parallel trial was conducted in healthy subjects. They received 2000 IU vitamin $D_3$ per day ($n=8$) or placebo ($n=5$) for 14 days. Body composition, BP and arterial elasticity (PWV) were recorded at baseline, day 7 and day 14 of intervention. Two 24 h urine samples were collected to estimate free cortisol and cortisone levels. Exercise performance was assessed at baseline and day 14 of intervention using a bike ergometer in which BP and PWV were measured before and after exercise. The distance cycled in 20 min and Borg rate of exertion scale were recorded.

Results

In the intervention arm, vitamin D supplementation significantly reduced systolic and diastolic BP; from $114.65 \pm 16.41$ and $78.58 \pm 12.65$ to $105.41 \pm 11.12$

($P=0.022$) and 66.25±11.69 mmHg ($P=0.014$) respectively. However, PWV was only reduced slightly ($P=0.085$). Urinary free cortisol levels were significantly reduced from 162.59±58.9 to 96.4±37.25 nmol/day ($P=0.044$), and cortisol/cortisone ratio from 2.22±0.7 to 1.04±0.42 ($P=0.017$). Exercise-induced systolic and diastolic BP were significantly reduced post vitamin D intake from 128.2±14.67 to 117.45±8.6 ($P=0.049$) and from 75.20±8.35 to 70.12±7.28 mmHg ($P=0.045$) respectively. The distance cycled in 20 min significantly increased from 4.98±2.65 to 6.51±2.28 km ($P=0.020$), whilst the Borg rate of exertion scale reduced from 5.13±1.36 to 4.25±0.71 RPE ($P=0.021$). In the placebo arm, no significant effects on CVD risk factors and exercise performance were observed.

Conclusions

These results suggest that daily vitamin D supplementation may ameliorate CVD risk factors including a decrease in 11β-HSD 1 activity and improve exercise performance in healthy individuals. However, large scale studies are required to verify our findings.

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## P205

### Evaluation of adipocytokines and traditional cardiometabolic risk factors in young male cancer survivors: an age-matched control study

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Background

Life expectancy of cancer survivors has doubled in the past four decades; however, death due to cardiovascular disease is more prevalent in survivors than the general population.

Objective, design and methods

We evaluated novel and traditional cardiometabolic risk factors in young male cancer survivors in a cross-sectional study of male cancer survivors aged 25–45 years compared with age-matched non-cancer controls. Demographic and anthropometric data were recorded and biochemical and hormonal parameters assayed from fasting blood samples in 176 survivors and 213 controls (lipids were measured in all survivors and 97 controls).

Results

Compared with controls, survivors had significantly higher BMI, adipocytokines, insulin resistance, total cholesterol and triglyceride levels and lower free androgen index (FAI). Handgrip strength, smoking, alcohol consumption, free estrogen index, insulin-like growth factor 1 and high-density lipoprotein cholesterol levels did not differ between cancer survivors and controls. Risk factors were analysed simultaneously using stepwise multi-variable logistic regression, and this showed that high leptin:adiponectin ratio (odds ratio=2.63; 95% CI 1.34–5.15; $P=0.005$), hypercholesterolaemia (odds ratio=1.85; 95% CI 1.08–3.17; $P=0.025$) and low FAI (odds ratio=2.01; 95% CI 1.07–3.79; $P=0.030$) were independently more common in survivors. The odds ratio in survivors for having at least two of these three risk factors rose to 6.58 (95% CI 3.30–13.12; $P<0.001$). Among survivors, risk factors were not different between cancer therapies but worse in survivors who had radiotherapy involving the testes (hyperleptinaemia and insulin resistance) or age at diagnosis above group median (hypertriglyceridaemia and hypercholesterolaemia).

Conclusions

A high leptin:adiponectin ratio, hypercholesterolaemia and low FAI are observed in young male cancer survivors, especially those who received radiotherapy involving the testes or were diagnosed at a later age. In view of their youth and known increased risk of cardiovascular death, treatment strategies are required to address this cardiovascular risk.

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## P206

### Low vitamin B12 in pregnancy is associated with maternal obesity and gestational diabetes

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Aims

Vitamin B12 insufficiency has been linked with adiposity and insulin resistance. A clinical study from India showed that B12 insufficiency in pregnancy was associated with higher risk of gestational diabetes (GDM), mediated by maternal BMI. It is not known whether the same association exists among pregnant women in the UK.

Methods

A retrospective study was done on women attending our antenatal clinic from 2010–2013. Information including maternal demographics, first trimester BMI, B12, folate and glucose were collected and multivariate regression models applied.

Results

Two hundred and ninty four women (132 GDM, 162 controls) who had B12 levels checked in the early third trimester were included. Overall, 26.9% had B12 values <150 pmol/l. GDM mothers were older, more obese and had significantly lower mean B12 values than controls (184 vs 225 pmol/l, $P=0.03$) with similar folate levels. In all women, first trimester BMI had a significant negative correlation with third trimester B12 ($r=-0.21$, $P<0.001$). Linear regression showed that first trimester BMI was the only significant predictor of B12 ($\beta=-0.2$, $P=0.002$), after adjusting for age, parity, ethnicity, folate and gestation. Women in the lowest B12 tertile had significantly higher odds of a diagnosis of GDM than those in the highest tertile (AOR: 2.45, 95% CI 1.24, 4.84), after adjusting for BMI and other variables as above.

Conclusion

This study confirms, for the first time in a UK population, that obese pregnant women are at risk of B12 insufficiency, which in turn is associated with over two-times higher probability of GDM. Further longitudinal studies are urgently needed to determine whether early pregnancy B12 insufficiency predicts the onset of GDM and establish the mechanisms underlying the link between insulin resistance and B12.

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## P207

### Adrenaline mediated metabolic and functional quiescence protects insulin producing cells from hypoxia

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Pancreatic islets are susceptible to hypoxia during isolation, culture, and transplantation. An intervention that protects against hypoxia while preserving β-cell function will improve islet quality. Adrenaline suppresses insulin secretion and oxygen consumption in pancreatic islets. These findings suggest that adrenergic action creates metabolic quiescence in β-cells. Our objective was to determine whether adrenaline is protective during hypoxic culture in an insulinoma cell line. Min6 cells were cultured for 72 h in four groups (n=3/group): normoxia, normoxia+100 nM adrenaline, hypoxia (1% $O_2$), hypoxia+100 nM adrenaline. Adrenaline was removed for 1 h or 48 h before functional assessments. Stimulated insulin release was determined in 16.7 mM glucose and fold change calculated from 0 mM glucose. RT2-PCR hypoxia arrays were used to determine mRNA expression. PCR arrays confirmed Min6 cells in 1% $O_2$ have a hypoxic gene signature. Immediately after treatment, adrenaline supplementation increased stimulated insulin concentrations in hypoxic (40.6±5.3 ng/ngDNA) and normoxic (39.4±3.8 ng/ngDNA) cultures compared to normoxic (14.1±1.2 ng/ngDNA) and hypoxic cultures (15.2±1.1 ng/ngDNA). After 48 h recovery, stimulated insulin concentrations were increased 46±7% ($P<0.05$) in normoxia+adrenaline compared to normoxia. Hypoxia reduced ($P<0.05$) glucose stimulated insulin secretion to 1.8±0.3 fold compared to normoxia at 3.4±0.6 fold, but hypoxia+adrenaline (2.6±0.4 fold) was similar to normoxia. Oxygen consumption rate was not different between the groups, but glucose-stimulated NADH concentrations were greater in normoxic cells. Hypoxia and adrenaline reduced cell density compared to normoxia and the number of non-adhered cells was similar in all conditions, indicating slower replication rates, not cell death. Adrenaline supplementation to hypoxic β-cell cultures preserved insulin secretion. Although adrenaline reduced proliferation of insulinoma cells, the impact will likely be less in mature islets because viability was maintained. These data identify the potential use of adrenergic agonists as a protective agent against hypoxia during islet isolations procedures to improve β-cell viable and function.

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## P208

**Bile acids stimulate GLP-1 release predominantly by accessing basolateral GPBAR1 (TGR5)**

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Background
Glucagon-like peptide 1 (GLP-1) is an incretin hormone released from enteroendocrine L-cells in the gut. GLP-1 analogues and dipeptidyl-peptidase-4 inhibitors are currently used to treat type-2 diabetes. A greater understanding of the mechanisms underlying the release of GLP-1 may facilitate the development of therapeutics to stimulate the release of endogenous GLP-1. Bile acids have been shown to induce GLP-1 release via the G protein-coupled bile acid receptor 1 (GPBAR1/TGR5) and increased cAMP. The apical sodium-dependent bile acid transporter (ASBT) and nuclear farnesoid X receptor (FXR) may also be involved.
Aim
To identify pathways of bile acid-stimulated GLP-1 secretion and whether these are activated by bile acids from the apical or basolateral direction.
Methods
Intracellular cAMP and Ca2+ were monitored in cultured primary murine L-cells using transgenic mice expressing fluorescent sensors (Epac2camps or GCaMP3) specifically in L-cells. GLP-1 release was measured from primary murine intestinal cultures, tissue segments mounted in Ussing chambers and perfused rat intestine.
Results
Bile acids increased intracellular cAMP and Ca2+ in L-cells and stimulated GLP-1 secretion from intestinal cultures. GPBAR-A, a specific GPBAR1 agonist, also stimulated GLP-1 secretion but GW4064, a specific FXR agonist, did not. Bile acids stimulated GLP-1 secretion from intestinal tissue segments mounted in Ussing chambers when applied to either the apical or basolateral side. However, stimulation by apically applied bile acids was blocked by an ASBT inhibitor. GPBAR-A was only effective when applied basolaterally. Furthermore, in perfused rat intestine, vascular application of TDCA elicited a greater secretory response than when luminally applied.
Conclusion
Bile acids stimulate GLP-1 secretion primarily via activation of GPBAR1 on the basolateral surface of intestinal L-cells. This has implications for the design of therapeutics to target GPBAR1 and suggests the stimulation of gut hormone secretion may include post-absorptive mechanisms.

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## P209

**Vitamin B12 deficiency alters adipogenesis and associated microRNA's in human adipocytes**

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Background
Human cohorts and animal models provide compelling evidence suggesting the role of vitamin B12 in modulating the risk of diabetes and adiposity via developmental programming. We recently demonstrated that neonates born to mothers with low B12 levels in pregnancy have adverse cord blood lipid profile and *in vitro* studies in human adipocytes with low B12 showed increased cholesterol biosynthesis. Therefore, we hypothesized that vitamin B12 deficiency may play an important role in the regulation of adipogenesis. We chose adipocytes as an *in-vitro* model system as they undergo several cycles of differentiation and maturation, mimicking *in-vivo* intergenerational effect (pregnancy-offspring) and investigated the role of vitamin B12 deficiency on adipogenesis.
Methods
Primary human pre-adipocytes were cultured and differentiated in various B12 concentrations (1) Control: (B12 – 500 nM); (2) Low B12 (B12 – 0.15 nM) (3) No B12: (B12 – 0 nM). Maternal venous blood samples ($n=91$) and adipose tissue ($n=42$) were collected at delivery.
Results
Adipocytes cultured in B12 deficient conditions showed increased lipid accumulation and triglyceride levels. In our clinical study, mothers with low B12 status had higher BMI and adverse lipid profile. Gene expression of adipogenic regulators (PPARγ, CEBPα, RXRα), lipid coating protein (perilipin), lipogenesis (FASN, ACC1) and development-related genes (Zfp423, EZH2, WISP2) were altered in adipocytes cultured in B12 deficient conditions and in adipose tissue from mothers with low B12. Expression of anti-adipogenic microRNAs (miR-27b and miR-195a targeting PPARγ and Zfp423, respectively) were down-regulated in adipocytes cultured in B12 deficient conditions.
Conclusion
Our study highlights that low B12 enhances adipogenesis and triggers specific microRNAs. Thus suggesting B12 deficiency alters adipocyte commitment and differentiation via epigenetic alterations leading to the development of obesity and insulin resistance in later life.

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## P210

**Proteomic detection of extracellular matrix proteins in insulin-resistant skeletal muscle**

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Increased deposition of extracellular matrix (ECM) components such as collagens and hyaluronan is associated with diet-induced insulin resistance (IR) in skeletal muscle. Studies of the role of specific ECM components in muscle IR have been pivotal but incomplete. Here we used an approach (i.e. ECM-specific mass spectrometry-based proteomics) that gives a more complete view of ECM protein abundance with muscle IR. Male C57BL/6 mice were fed with either a chow or high fat (HF) diet, which contains 60% calories as fat for 20 weeks before gastrocnemius muscle was collected. Muscle samples were incubated in 1% sodium dodecyl sulfate for 3 days to remove cytosolic proteins and the remaining extracellular and fibril compartments were frozen in liquid nitrogen, grounded, and applied to quantitative proteomics using isobaric tags for relative and absolute quantitation (*iTRAQ*) technique. Over 80 proteins were detected by proteomics and >90% of these proteins were ECM proteins or contained an extracellular domain, of which collagen (COL) XXIV was the most elevated ($10.1\pm0.80$) ECM protein in muscle from HF-fed mice compared to chow-fed mice. In addition, protein expression of COLIα1, COLIα2, and COLIIIα1 were increased by HF feeding by $2.86\pm0.80$, $2.86\pm0.79$, and $2.56\pm0.64$ fold respectively. We further verified these proteomic results in gastrocnemius homogenates by real-time PCR and immunohisochemistry. We showed that mRNA levels of COLXXIV ($2.15\pm0.44$ fold), COLIα1 ($2.20\pm0.53$), COLIα2 ($1.88\pm0.24$) and COLIIIα1 ($1.97\pm0.17$) were increased in muscle of HF-fed mice and protein staining of COLIII was increased by $2.08\pm0.54$ fold in HF-fed mice. These results were consistent with results from proteomics and suggest that the ECM-specific proteomics is a powerful tool to identify new ECM targets for muscle IR. COLXXIV was shown for the first time to be increased in insulin-resistant skeletal muscle and may represent a new ECM target for treating muscle IR.

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## P211

**Longitudinal changes in adipose tissue gene expression profile are asociated with deteriorating glucose tolerance**

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Increased adiposity is associated with insulin resistance, type 2 diabetes and metabolic dysfunction. Altered adipokine secretion, changes in local and systemic glucocorticoid metabolism and increased inflammation have all been postulated as contributory mechanisms. We have previously described changes in subcutaneous adipose tissue (SAT) gene expression that are associated with obesity and glucose intolerance in cross-sectional studies. However, the effects of longitudinal changes of gene expression to modulate metabolic phenotype have not been determined. 65 obese or overweight individuals (women=42) underwent oral glucose tolerance testing (OGTT), body composition analysis

using DXA, 24-urinary steroid metabolome analysis and SAT biopsy. Participants were reinvestigated after a median of 5 years (IQR 3.5–5). Area under the curve glucose across the OGTT was used to stratify the cohort into those whose glucose tolerance improved over the duration of the study ('improvers', $n=30$) and those in whom it deteriorated ('deteriorators', $n=35$). SAT gene expression profiles ($n=30$) were determined using the Fluidigm™ platform. At baseline, there were no differences in fat mass or SAT gene expression profile. However, in the deteriorators, total body weight (91.5±17.1 vs 97.5±18.8 kg, $P<0.0001$), total fat mass (36325±8134 vs 39588±10190 g, $P=0.0005$) and trunk fat mass (18766±4786 vs 20753±5713 g, $P<0.0001$) increased, whilst these did not change significantly in the improvers. SAT gene expression profiles were markedly different between the two groups. In the deteriorators, the expression of genes associated with adipocyte lipid metabolism, including FAS (12.1±4.0 vs 10.4±1.9Δ*C*t, $P=0.03$), LPL (11.5±3.1 vs 9.6±1.5Δ*C*t, $P=0.006$), CD36 (9.4±2.8 vs 7.4±1.4Δ*C*t, $P=0.002$) and DGAT2 (11.6±3.5 vs 10.0±1.2, $P=0.04$) all increased during the study, whereas in the improvers, they remained unchanged. A similar pattern of expression was observed for those genes involved in insulin signaling, adipokine production and adipocyte differentiation. We have defined longitudinal changes in SAT gene expression that are associated with worsening glucose tolerance. This suggests a crucial role for SAT in the progressive development of glucose intolerance and highlights specific molecular markers that may drive this process.

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## P212

**Distinguishing different subtypes of aldosterone-producing adenoma by histological, immunohistochemical and radiological features; a basis for individualised treatment strategies in primary aldosteronism?**

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Background
Primary aldosteronism (PA) is now recognised to account for 5–10% of all cases of hypertension (and 20–25% of refractory hypertension). For patients with a demonstrable unilateral cause, adrenalectomy offers the potential for cure of PA, although resolution of hypertension occurs in only ~50% of patients. We have examined histological appearances and radiological features in patients with APAs undergoing adrenalectomy in an attempt to further our understanding of the heterogeneity of PA.
Methods/results
We report 2 years' experience since our retrospective analysis of >50 APAs, after which we introduced routine post-operative immunohistochemistry, and during which time PET-CT has started to provide an in vivo assessment of function. Histological examination of adrenalectomy specimens identified two distinct subtypes of APA; those resembling cells of the zona fasciculata (ZF) (Fig. 1a), and those harbouring more compact cells reminiscent of the zona glomerulosa (ZG) (Fig. 2a). ZF tumours are larger, with lipid-laden cells, and may represent the original Conn's adenoma. In contrast, ZG tumours are often smaller (and may not be easily visualised on CT or MRI), with lipid-deplete cells. IHC using CYP11B1 and CYP11B2 selective antibodies confirms predominant CYP11B1 expression in ZF tumours (Fig. 1b); in contrast, ZG tumours express mainly CYP11B2 (Fig. 2c). Preliminary findings suggest that these different tumour subtypes may exhibit differing uptake of the PET tracer 11C-metomidate (ZG>ZF).

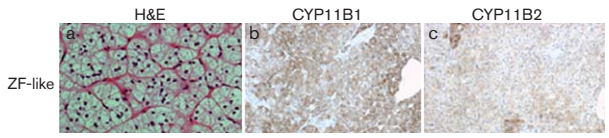


**Fig. 1**

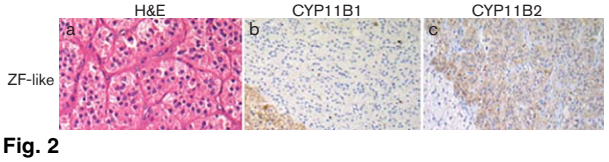


**Fig. 2**

Conclusions
The identification of different histological subtypes, together with recent advances in tumour genotyping and functional PET imaging, may now provide an opportunity to investigate stratified decision-making in the management of PA, thus targeting limited resources towards those patients who are likely to benefit most from adrenalectomy.

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## P213

**Adrenal insufficiency following bariatric surgery**

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Introduction
Bariatric surgery is now a common surgical procedure for weight management recommended by NICE. Complications such as dumping syndrome, micronutrient deficiencies and cholelithiasis are well documented in the literature. Here we discuss the lesser reported complication of adrenal insufficiency seen in three patients following roux-en-y gastric bypass surgery for morbid obesity.
Case reports
Three patients were seen in the outpatient clinic post bariatric surgery. Patient A: complained of symptoms suggestive of dumping, nausea, sweating and lethargy. Patient B presented to clinic five years following bariatric surgery complaining of cramps, thinning hair, constipation, lethargy and anxiety. Patient C complained of excessive tiredness a year following surgery. Baseline cortisol was very low in all three patients. All three patients had poor cortisol response following a challenge with synacthen, pituitary function was normal, adrenal antibody was negative and imaging of the pituitary gland did not reveal any abnormality. All three patients were started on hydrocortisone with resulting improvement in their symptoms.
Discussion
The cause of adrenal insufficiency in these cases remains unexplained. Potential mechanisms include disruption to absorption of bile and absorption of vitamins and trace elements affecting steroid synthesis; weight loss causing alteration to the hypothalamo-pituitary-adrenal axis; damage to the pituitary/adrenal glands as a result of stress; blood loss during surgery resulting in pituitary/adrenal insufficiency or reduction in steroid metabolites produced by adipose tissue.
Conclusion
These cases highlight the importance of long-term follow-up of patient's post-bariatric surgery and the importance of checking the cortisol level in patients who are symptomatic with dizziness and lethargy. Rapid weight loss which is expected with bariatric surgery may mask symptoms of adrenal insufficiency.

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## P214

**Discovery and localisation of the expression of calcitonin-type and CRH-type neuropeptide precursors in an echinoderm**

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Calcitonin and corticotropin-releasing hormone (CRH) are members of a family of neuropeptides that exert effects by activating secretin-type G-protein coupled receptors. Calcitonin lowers blood calcium levels and acts to protect against calcium loss from the skeleton during periods of calcium mobilisation. CRH is a key player in the stress response mediated by the hypothalamic-pituitary-adrenal axis. Analysis of the phylogenetic distribution of calcitonin-type and CRH-type peptides and their receptors indicates that the evolutionary origin of these signalling systems can be traced to the common ancestor of the Bilateria. Furthermore, it has been discovered that calcitonin-type and CRH-type peptides act as diuretic hormones in insects. However, little is known about the physiological roles of these peptides in other invertebrates. We are using the starfish Asterias rubens (phylum Echinodermata) as a model system to investigate the evolution and comparative physiology of neuropeptides. As deuterostomian invertebrates, echinoderms bridge the 'evolutionary gap' between protostomian invertebrates (e.g. insects) and the vertebrates. Furthermore, the pentaradial symmetry of echinoderms provides a unique context for investigation of neuropeptide function. Here we report the cloning and sequencing of cDNAs from A. rubens that encode a calcitonin-like peptide precursor (ArCTLPP) and CRH-like peptide precursor (ArCRHLPP). Analysis of the expression of these precursors in A. rubens using mRNA *in situ* hybridisation revealed that

ArCRHLPP is expressed by cells in the ectoneural region of the radial nerve cords and circumoral nerve ring, marginal nerves, coelomic epithelium, cardiac and pyloric stomach and pyloric caecae, whilst ArCTLPP is expressed in the ectoneural and hyponeural regions of the radial nerve cords and circumoral nerve ring, cardiac and pyloric stomach and pyloric caecae. These data provide a basis for investigation of the physiological roles of calcitonin-type and CRH-type neuropeptides in starfish, which may provide novel insights on the evolution of neuropeptide function in the animal kingdom.

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## P215

### The role of vitamin B12 deficiency in molecular mechanism of metformin

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The anti-diabetic drug, metformin, is associated with progressive decrease in serum vitamin B12 levels whereas vitamin B12 deficiency is related to increased insulin resistance and dyslipidaemia by altered methylation. As the risk of vitamin B12 deficiency is increased among metformin users, it is of utmost importance to examine how metformin response in vitamin B12 deficient population.

We investigated the cellular mechanism of metformin in vitamin B12 insufficient human hepatocellular cell line (HepG2) and examined the efficacy of metformin response in vitamin B12 deficient ($<$150 pmol/l) gestational diabetes mellitus (GDM). HepG2 was cultured in different vitamin B12 conditions (0, 10, 100, 1000 nM) for 24 days (passaged on every 6th day for four passages). Then, they were treated with insulin 100 nM for 15 min or with metformin 2 mM for 24 h. Protein and RNA extracts were quantified for insulin signaling molecules and AMP-activated protein kinase (AMPK) and its downstream signals in lipid metabolism.

In HepG2 culture, decreased phosphorylation of insulin signaling molecules (Akt, GSK) were observed across decreasing B12 conditions. In metformin-treated hepG2 cells, Thr-172 phosphorylation of AMPK and Ser-79 phosphorylation of acetyl-CoA carboxylase (ACC) were increased in 1000 nM B12 compared to 0 nM B12 condition. Similarly, there was decreased gene expression levels of Fatty Acid Synthase (FAS) and 3-Hydroxy 3-Methylglutaryl CoA Reductase (HMGCR) enzymes in 1000 nM B12 compared to 0 nM B12 condition. There is no significant difference in the risk of metformin failure (metformin-treated GDM with additional insulin) between vitamin B12 deficient and normal GDM (odds ratio 0.91 (95% CI 0.42,2.00)).

Our preliminary results indicate that in hepatic cells differentiated in low B12 conditions, metformin phosphorylation on AMPK and its downstream molecules are reduced. This noble finding suggests the future exploration of mitochondrial function in B12 deficient cell and highlights the importance of vitamin B12 sufficiency for full potency of metformin. It is also suggested that more precise definition of metformin failure should be used for clinical studies.

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## P216

### Enhanced orbitofrontal cortex activation following sympathetic neural stimulation in young women with polycystic ovary syndrome: an fMRI study

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Introduction

Polycystic ovary syndrome (PCOS) is associated with increased cardiovascular risk, which may relate to enhanced sympathetic nervous system (SNS) activation. The cerebral pathways involved in this process are not known.

Aims

i) To compare SNS activation in response to isometric forearm contraction (IFC) in patients with PCOS and controls. ii) To identify and compare the neuronal signatures of this response.

Methods

15 PCOS (age 30.6 years, BMI 24.7 kg/m$^2$) and 15 matched controls (age 29.1 years, BMI 25.7 kg/m$^2$; $P$ = NS) were studied. Out-of-scanner tests: measurement of mean blood pressure (MAP) and heart rate (HR) responses to 30% IFC for 180 seconds; baseline and post-task catecholamines. In-scanner: Blood oxygen level dependent (BOLD) fMRI using an identical block paradigm design for IFC. BOLD signal changes were measured during the task and a general linear model (www.fmrib.ox.ac.uk/fsl FEAT) was used to identify BOLD signal correlating to MAP responses, (threshold $Z > 2.3$, corrected cluster threshold $P = 0.05$).

Results

IFC elicited an increase in HR and MAP in PCOS and controls but these did not differ between groups ($P = 0.45$ (HR) and $P = 0.41$ (MAP)). Adrenaline increased significantly post-IFC in PCOS (0.8–1.4 ng/ml $P = 0.01$) but not in controls (0.7–0.8 ng/ml $P = 0.3$). Brain activation indexed by the BOLD signal in response to IFC was significantly greater in the PCOS group compared to the control group in the right orbitofrontal cortex ($P < 0.0001$), left angular gyrus and lateral occipital cortex ($P = 0.04$).

Conclusions

PCOS is associated with enhanced SNS activation and increased regional brain activation in response to IFC. These observations may explain some of the increased cardiovascular risk evident in this patient group, and may offer new cerebral targets for intervention associated with enhanced SNS activity.

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## P217

### Enhanced insulin secretion and insulin action in young lambs with intrauterine growth restriction

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Intrauterine growth restriction (IUGR) is associated with postnatal metabolic complications, which include glucose intolerance in adulthood. However, information is limited on the progression of insulin secretion and its action in young IUGR infants. The objective of this study was to determine glucose stimulated insulin secretion and insulin action in young lambs with placenta insufficiency-induced IUGR.

Placental insufficiency-induced IUGR was created by exposing pregnant ewes to high ambient temperatures during mid gestation, and the IUGR lambs ($n = 5$) were compared to lambs ($n = 8$) from pair-fed, thermoneutral ewes. Lambs were reared on milk replacer. At $8 \pm 1$ days of age, a square-wave hyperglycaemic clamp was performed after 3 h fast to measure glucose stimulated insulin secretion. At $15 \pm 1$ days insulin sensitivity was determined with a hyperinsulinaemic-euglycemic clamp. Basal and hyperinsulinaemic glucose utilization rates were measured with [$^{14}$C(U)]-D-glucose.

IUGR lambs had lighter birthweights than control lambs ($3.8 \pm 0.7$ kg vs $4.7 \pm 0.9$ kg; $P = 0.04$). Fasting plasma glucose and insulin concentrations were not different. During the square-wave hyperglycaemic clamp, insulin secretion for the first 20 min of hyperglycaemia was greater in IUGR lambs than in controls ($159 \pm 30$ vs $68 \pm 24$ μg$\times$min/l). No differences were found between treatments for second phase insulin secretion or glucose-potentiated arginine stimulated insulin secretion. Fasting and hyperinsulinaemic glucose utilization rates were not different between treatments, but IUGR lambs had greater insulin sensitivity compared to control lambs in both periods (basal $5.36 \pm 1.00$ vs $3.23 \pm 0.36$ μmol$\times$l/mU per min per kg; hyperinsulinaemic $2.04 \pm 0.64$ vs $0.85 \pm 0.08$ μmol$\times$l/mU per min per kg). Insulin disposition index, the product of insulin sensitivity and acute insulin secretion, was increased 4.7 fold at basal and 6.5 fold with hyperinsulinemia in IUGR lambs.

A greater insulin disposition index demonstrates that young IUGR lambs have inappropriate insulin secretion for their insulin sensitivity. This imbalance between secretion and sensitivity of insulin may promote a loss of function that results in glucose intolerance.

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## P218

### Glucocorticoid excess increases hypothalamic AgRP and results in obesity and hyperinsulinaemia in mice

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Glucocorticoids (Gcs) are widely prescribed to treat a number of conditions, such as arthritis and asthma. However, patients receiving Gcs often develop metabolic complications such as obesity and hyperglycaemia. The aim of this study was to

investigate the molecular mechanisms in the hypothalamus which drive these adverse effects. Male C57BL/6J mice were given *ad libitum* access to either corticosterone (CORT; 75 μg/ml) or vehicle (V; 1% ethanol) in their drinking water in combination with chow (C) or high fat (HF) diet (60% of calories from fat). Exogenous Gcs in the drinking water produced a robust and reproducible metabolic syndrome-like phenotype. After 4 weeks, body weight was increased to a similar extent in CORT-C, CORT-HF and V-HF animals compared to V-C mice. These three groups all had increased adiposity with a shift from epididymal to inguinal and mesenteric deposition with CORT. CORT treatment led to fed (25-fold) and fasting (fivefold) hyperinsulinaemia. Similarly, postprandial hyperglycaemia was present in CORT-C and CORT-HF groups (approximately twofold); however this only progressed to fasting hyperglycaemia (80% increase) in CORT-HF mice. Food intake was increased in the CORT-C group, leading us to assess the expression levels of hypothalamic neuropeptides known to control food intake. NPY and POMC remained unaffected, but AgRP increased (threefold) in the CORT-C group, providing a likely explanation for the increased food intake. In summary, we have demonstrated that a metabolic syndrome-like phenotype can be rapidly induced in mice using exogenous CORT treatment, and this is exacerbated when combined with HF diet. This suggests that Gcs acting in the hypothalamus contribute to the overall development of metabolic complications seen with Gc therapy.

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## P219

**Non-selective beta-adrenergic agonist infusion acutely stimulates the temperature of brown adipose tissue in adult males**

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Brown adipose tissue (BAT) is present in small quantities in human adults (~100 g) and can have a significant influence on metabolism. This effect is mediated by rapid activation of the BAT specific uncoupling protein 1 (UCP1), following stimulation of β-adrenergic receptor (AR) by the sympathetic nervous system. AR agonists stimulate rodent BAT, but have so far provided inconsistent findings in humans when administered orally (Vosselman *et al*. 2012). Rapid activation of UCP1 at birth is mediated in part by the prepartum surge in cortisol (Mostyn *et al*. 2003), but whether this has any influence on BAT function in adults remains to be fully established. Our study therefore aimed to determine whether the non-selective AR agonist isoprenaline (ISO) increased the temperature of supraclavicular BAT in adult humans, and whether this could be modulated by infusion of hydrocortisone.

Eight healthy young males (18–30 years) of normal body weight (BMI: 18–24.9 kg×m$^2$) were recruited. Participants underwent the study twice, after a hydrocortisone infusion (0.2 mg/kg per min for 16 h) or saline (control). Infusions were given in a randomised double-blind order with at least 2 weeks between treatment regimes. BAT activity was continuously measured by thermal imaging (Symonds *et al*. 2012), together with heart rate, at baseline and during a 1 h systemic infusion of ISO (25 ng×kg fat-free per mass per min). Environmental temperature remained constant at 22–23 °C.

ISO resulted in a significant increase in temperature of the supraclavicular region (35.61±0.15 °C to 36.01±0.15 °C, $P<0.001$) and heart rate (62±4 bpm to 108±5 bpm, $P<0.001$). Peak responses occurred between 20 and 40 min, while responses were unaffected by hydrocortisone ($P=0.56$).

Infusion of a β-agonist stimulates the temperature of the main site of BAT in humans. This response appears to be unaffected by short-term infusion of hydrocortisone, suggesting raised cortisol has limited influence on BAT activity in young adult males in this model.

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## P220

**Endogenous ARX knockdown enhances beta-cell lineage specification and maturation during *ex vivo* transdifferentiation of human exocrine pancreatic tissue**

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The shortage of donor material has driven research towards finding a replenishable supply of islets for transplantation. We have previously shown that the exocrine material that results as a by-product of the islet isolation procedure can be transdifferentiated towards functional islet-like structures by overexpression of the pancreatic transcription factors (TFs) Pdx1, MafA, Ngn3 and Pax4 followed by culture in the presence of betacellulin, nicotinamide and exendin-4. These cells secreted insulin in a glucose responsive manner and rescued diabetes in a streptozotocin-induced diabetic mouse model, but expressed 1% of the insulin levels of mature islets. Modification of the culture conditions to include laminin and a low glucose concentration has further enhanced transdifferentiation towards endocrine lineages.

A major increase in insulin production was observed upon siRNA knockdown of the α-cell TF ARX at the later stages of the protocol. Specific ELISAs for human proinsulin, insulin and C-peptide and ultrastructural analysis demonstrated that the newly transdifferentiated cells were able to efficiently store (33.5±7.3 pg/μg protein) and process insulin, secreting C-peptide in a regulated glucose-responsive manner, with insulin levels rising to 15% of those found in human islets. ARX knockdown further decreased glucagon expression. Removal of Pax4 abolished regulated glucose-response, supporting its critical role for functionality and maturation of the transdifferentiated cells. This population was monohormonal, comprising 40% C-peptide$^+$, 4% glucagon$^+$, and <2% somatostatin$^+$ cells. Grafted in diabetic Scid/Bg mice, these cells had an immediate and prolonged (100 days) effect in normalising blood glucose levels and body weights.

We have shown for the first time that late inhibition of ARX, along with Pax4 overexpression, is crucial for the transdifferentiation of human exocrine cells towards mature beta-like cells. We estimate that ~3 billion of these cells would have an immediate and therapeutic effect following transplantation in people with type 1 diabetes.

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## P221

**Altered adipocytes in an ovine model of polycystic ovary syndrome**

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Women with Polycystic Ovary Syndrome (PCOS) are at increased risk of developing insulin resistance, obesity and dyslipidemia, however obesity *per se* does not explain the higher incidence of insulin resistance in PCOS women when compared to the general population. Altered adipose tissue morphology and function may be a central factor contributing to metabolic disturbances in PCOS. Using a clinically realistic ovine model of PCOS we reported hyperinsulinaemia and early fatty liver changes, with no difference in body weight and adiposity, in adolescence.

Here we aimed to examine adipose tissue development during transition from adolescence to adulthood. Pregnant Scottish Greyface ewes were treated biweekly with either 100 mg of testosterone propionate (TP) or vehicle control (C) from day 62–102 of gestation. Two cohorts of animals, adolescent 11 months old (C=5; TP=9) and adult 30 months old (C=11; TP=4), were investigated. *PPARG* expression, the master regulator of adipogenesis, was downregulated ($P<0.01$) in subcutaneous adipose tissue (SAT) but not visceral adipose tissue (VAT) of adolescent TP-treated animals. This suggests decreased differentiation of preadipocytes into mature adipocytes. As adults, TP-exposed animals had increased body weight ($P<0.05$), increased fasting insulin concentration ($P<0.05$), decreased fasting glucose to insulin ratio ($P<0.05$) and increased fasting non-esterified fatty acid concentration ($P<0.05$). Histological analysis revealed that TP-exposed animals have decreased total number of adipocytes ($P<0.05$) and increased mean adipocyte size in SAT ($P<0.05$) but not in VAT. In summary, impaired preadipocyte differentiation into adipocytes in SAT in adolescent prenatally androgenized female sheep results in decreased numbers of adipocytes and increased mean size of adipocytes in adult SAT. This consequently lowers capacity of SAT to safely store fat and potentially explains presence of fatty liver and hyperinsulinaemia in TP-treated animals, due to increased release of FFA into circulation, subsequent lipotoxicity and decreased insulin sensitivity in peripheral tissue.

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## P222

**Transcriptional profiles explain insulin hypersecretion following chronic adrenergic stimulation in foetal sheep islets**

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Prolonged increases in foetal plasma noradrenaline occur in pathologies, including intrauterine growth restriction (IUGR). Infusion of noradrenaline into healthy sheep foetuses showed continuous suppression of insulin secretion but produced a compensatory beta-cell response after noradrenaline infusion was terminated, similar to findings with adrenergic antagonists in IUGR foetuses. Hyper-insulin secretion responsiveness was confirmed in isolated foetal islets. To identify molecular mechanisms that explain islet programing from chronically elevated noradrenaline, we performed high throughput mRNA sequencing. Noradrenaline (1–4 μg/min per kg) or vehicle was intravenously infused into foetal sheep for 7 days beginning at 131 days of gestation. Islets ($n=4$/group) were isolated with collagenase procedures. The extracted RNA was sequenced with the Illumina hiseq2500 and analysed with Tuxedo Software using the sheep genome (Oarv3.1). Differentially expressed transcripts were queried for enrichment and modeled to functional pathways. Plasma noradrenaline was increased tenfold in noradrenaline-infused foetuses compared to vehicle-infused controls. In noradrenaline-islets, 321 genes were differentially expressed. These genes were involved in an array of biological processes including: cell death, signal transduction, inflammation, adhesion, and growth. Molecular functions and cellular locations of differentially expressed genes were related to neuronal development, hormone activity, calcium binding, and transcription factor binding. Noradrenaline increased expression of genes that regulate pancreatic endocrine cell function: including; somatostatin (2.9-fold) and death associated protein like 1 (DAPL1, 5.8-fold). Gene shifts that explain insulin hypersecretion include: increases in glycolytic genes aldolase (ALDOB, 2.2-fold) and fructose-1,6-bisphosphatase (FBP, 1.7-fold), as well as NK6 homeobox 3 (NKX6-3, 2.8-fold), phosphatase 2A inhibitor (PP2A, 2.5-fold) and inward rectifying potassium channel (KCNJ8, 3.2-fold) with decreases in synaptic regulatory genes (RIMS4, −1.9-fold). Noradrenaline-induced gene expression describes proximal and distal mechanisms for the observed insulin hyper-secretion. The specific pathways promote glucose metabolism and potassium transport, consistent with better glucose sensitivity, as well as calcium regulation and exocytosis, consistent with more efficient insulin release.

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## P223

**Measuring the contribution of de novo lipogenesis to fatty liver disease: a mouse and translational human study using high resolution mass spectrometry**

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*De novo* lipogenesis (DNL) is recognised as potentially contributory to pathogenesis of non-alcoholic fatty liver disease (NAFLD), and could be a factor in the progression of simple steatosis to steatohepatitis, although it contributes a relatively minor amount of whole body fat compared to dietary lipid. Consequently understanding the lipidome during induced DNL may provide insight into potential biomarkers of DNL that could be clinically relevant in those at risk of NAFLD and the metabolic syndrome, particularly those of moderate BMI but predisposed to developing fatty liver. Initially to study DNL *in vivo*, 7 ob/ob mice were fed either high fat diet, to inhibit DNL, or regular chow diet, to stimulate DNL, along with deuterium enriched drinking water. The high resolution liquid chromatography mass spectrometry (LC-MS) and isotope ratio MS demonstrated an overall increase in saturation of fatty acids (FA) in triglycerides, with increased deuterium incorporation into the triglycerides predominantly containing C16:0, C16:1 and C18:1. To translate these results into humans ten healthy men from 21 to 30 years-old were recruited. On the first day participants consumed deuterium-enriched water, labelling lipids produced during DNL. The following day blood samples were drawn before and after a carbohydrate-rich (62% by energy content) meal constituting 110% of calculated basal metabolic rate. Consequently intact lipidomics was performed by LC-MS, and FA composition analysis by GC-MS with and without lipid class separation by solid phase extraction. The rate of *de novo* lipogenesis was analysed using the incorporation of deuterium into the FA pool of the blood plasma, and this data was integrated with the concomitant changes in intact lipids. In conclusion, these murine experiments demonstrated that intact lipids may be a viable marker of DNL *in vivo*. Additionally the established deuterium incorporation method for DNL parallels the novel intact lipid markers in the human intervention study.

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## P224

**The hypoglycaemic and antioxidant properties of oleanolic acid ameliorate blood pressure and kidney function of experimental animals**

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The wide range of phytochemicals in medicinal plants serves as sources of bioactive compounds for treatment diabetic complications. We investigated whether Syzygium aromaticum-isolated oleanolic acid (OA) has beneficial effects on some diabetic complications in STZ-induced diabetic rats. We used experimental models of hypertension [spontaneously hypertensive (SHR) and Dahl salt-sensitive (DSS)] rats to explore the effects of OA on mean arterial blood pressure (MAP) and renal function. Selected biochemical parameters were also assessed in blood, muscle and liver samples from diabetic rats treated with OA for 5 weeks. The effects of a 9-week OA administration on MAP and kidney function were monitored in normotensive Wistar, SHR and DSS rats. In addition, anti-oxidative effects of OA on cardiac, hepatic and renal tissues were examined in all groups. Renal proximal tubular effects of OA on $Na^+$ handling were studied in anaesthetized Wistar rats challenged with hypotonic saline after a 3.5 h equilibration for 4 h of 1 h control, 1.5 h treatment and 1.5 h recovery periods using lithium clearance. OA was added to the infusate during the treatment period. Untreated diabetic rats exhibited hyperglycaemia, oxidative stress, depleted hepatic and muscle glycogen concentrations which were restored to near normalcy by OA treatment. OA administration elicited hypotensive responses in all groups of animals which were more marked in hypertensive animals and correlated positively with increased urinary $Na^+$ excretion. Acute infusion OA increased $FE_{Na}$ and $FE_{Li}$ without influencing GFR indicating that at least part of the overall natriuretic effect involved inhibition of $Na^+$ reabsorption in the proximal tubule. OA treatment reduced malondialdehyde (a marker of lipid peroxidation) and increased activities of antioxidant enzymes; superoxide dismutase and glutathione peroxidase in hepatic, cardiac and renal tissues in all groups. These results suggest that the hypoglycaemic and antioxidant effects of OA improve some metabolic complications of diabetes.

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## P225

**Dietary cocoa (*Theobroma cacao*) and turmeric (*Curcuma longa*) consumption: a comparison of metabolic effect in high-fat fed rats**

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A growing number of studies have reported beneficial health effects of cocoa and turmeric, including atherosclerosis, hypertension and insulin resistance. Relatively few studies have investigated the preventive or therapeutic effects of cocoa and turmeric against obesity-related metabolic disorders and co-pathologies. The study was undertaken to determine the effects of cocoa and turmeric powder supplementation on glucose tolerance, insulin sensitivity and lipid profile in rats fed with high-fat diet (HFD). Twenty-four (24) male Sprague-Dawley rats were initially divided into two groups of six and eighteen rats; the group of 18 rats was fed with HFD while the other group of 6 rats consumed the control diet. After seven weeks on the dietary regimen, 12 rats from the HFD group were shifted to either cocoa-supplemented (50 mg/kg diet) or turmeric-supplemented (100 mg/kg diet) with six rats in each group, while the remaining rats continued on the HFD for another 7 weeks. Throughout the study, food intake and body weights were measured and recorded. Thereafter, OGTT and ITT were performed; fat pads were excised and weighed immediately. Blood samples were also collected via

the retro-orbital sinus to measure the levels of cholesterol, triglycerides, HDL and LDL. Data obtained from this study showed that dietary cocoa and turmeric supplementation reduces body weight gain, retroperitoneal and testicular fat accretion, improves lipid profile, ameliorates glucose intolerance and enhances insulin sensitivity in the HFD fed- obese rats. Dietary supplementation with cocoa and turmeric ameliorates obesity-related hyperglycemia, glucose intolerance and insulin resistance in HFD fed obese rats. Notably, both nutriceuticals were capable of improving glucose tolerance by increasing insulin sensitivity.

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## P226

### Glucose tolerance, insulin sensitivity and adiponectin level in niacin-treated obese rats

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We examined the effect of dietary niacin supplementation on fat mass, glucose control, insulin sensitivity, lipid profile, and adiponectin level in diet-induced obese rats. Twenty-one male Sprague-Dawley rats (n = 21) were initially divided into two (2) groups of seven and fourteen rats; the group of 14 rats was fed with a high-fat-diet (HFD) and the other group of 7 rats consumed the control diet. Eight weeks after the diet regimen started, half of the rats from the HFD group were shifted to the niacin-supplemented diet (HFND; 1mg niacin/kg diet) while the remaining rats continued on the HFD for another 6 weeks. Throughout the experimental period, the food intake and body weights were measured and recorded. Thereafter, OGTT and ITT were conducted. Fat pads were excised and weighed immediately. Blood samples were also collected before killing to measure the levels of cholesterol, triglycerides, HDL and LDL. HFD-induced obese rats showed significant increase ($P < 0.05$) in body weight gain, reduced glucose tolerance, insulin sensitivity and increase adiposity as well as altered lipid profile after 8-week of feeding, compared with the controls. However, niacin-supplemented rats had reduced ($P < 0.05$) weight gain and/or body weight compared with HFD-induced obese rats even in the absence of a significant difference in the food intake among the groups in the experiment. In addition, the rats showed an improved time-course glucose control, insulin sensitivity as demonstrated by a significantly lower AUC values for the glucose curves. The plasma levels of cholesterol, triglycerides and LDL returned towards control values in rats supplemented with niacin compared with obese rats. The findings suggest that niacin exerts beneficial effect on adiposity, glucose tolerance and insulin sensitivity, plasma lipids, and that it specifically modulates the level of serum adiponectin under obese condition.

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## P227

### Circadian rhythm of ambulatory blood pressure in rotating night shift nursing professionals and its relation with 6- sulfatoxy melatonin as neuroendocrine chronomolecule

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Background & aim
Night shift work is associated with a disruption of circadian rhythms, where a person's internal body clock is in conflict with the rotating shift schedule. The circadian rhythm of the human body is characterized with an alternating sleep-wake cycle. Shift work has been associated with increased risk of hypertension, cardiovascular diseases and hormonal disturbances. The Present study was aimed to investigate the effects of rotating night shift on 24 h chronomics of BP/HR and its relation with 6-sulfatoxy melatonin levels.
Material & methods
healthy nursing professionals, aged 20–40 year, performing day and night shift duties were recruited. Each study subject had a monthly scheduled of regular nine night shifts (12 h night shift, from 2000 to 0800 h) followed by remaining 17–18 day shifts (6 h day shift, from 0800 to 1400 h) with a total of 4 days off in between. Subjects were recruited from the Trauma Center, GM and Associated Hospitals, King George's Medical University, Lucknow, UP, India. The duration and pattern of shift work were the same among all the subjects. Ambulatory BP and HR were recorded at every 30 min intervals in day time and each hour in night time synchronically with circadian pattern of 6 sulfatoxy melatonin during shift duties. 6-sulfatoxy melatonin (melatonin sulphate) was estimated by Competitive ELISA method (IBL international melatonin sulphate ELISA kit).
Results
Highly Significant difference was found in double amplitude (2DA) of blood pressure between night and day shift ($P < 0.001$). Ecphasia (odd timing of circadian pattern of blood pressure not of heart rate) was also found in few subjects. In night shift, hyperbaric index (HBI) of mean systolic blood pressure was found to be increased at 2400–0300 h (midnight) while during day shift, peak was found at 0600–0900 h. Peak melatonin was to be found in early morning as compared to mid night in both the shift.
Conclusion
The present study concluded that the desynchronisation appeared during night shift and entrainment of circadian rhythm in the day shift.

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## P228

### Characterising fat distribution and response to weight loss in idiopathic intracranial hypertension

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Introduction
Idiopathic intracranial hypertension (IIH) occurs in young obese women (>90%) but little is known about the metabolic characteristics in these patients. We aimed to characterise IIH fat distribution, metabolic phenotype and evaluate alterations following weight loss.
Methods
IIH and matched (BMI/sex) healthy obese controls were recruited. Metabolic indices (fasting lipid, glucose, insulin), anthropological measures and body composition were assessed (dual energy X-ray absorptiometry). IIH patients then underwent a therapeutic diet over 3 months followed by re-evaluation. The diet is a previously validated and nutritionally complete very low calorie total meal replacement liquid (Lipotrim, Howard Foundation, Cambridge, UK), providing 425 Kcal/day.
Results
IIH patients (n = 29) had a similar centripetal fat distribution to simple obesity patients (n = 47), which is contrary to previous reports of fat distribution measured by waist hip ratios. Lipid and glucose profiles were similar in IIH and normal obesity. Weight loss intervention resulted in a significant loss in body weight ($-14.2 \pm 7.8\%$), BMI ($-5.8 \pm 3.0$ kg/m2), and waist circumference ($-9.8 \pm 5.4$ cm) (all $P < 0.001$). Importantly, weight loss resulted in significant amelioration of clinical signs and symptoms of IIH, namely a decrease in intracranial pressure ($-8.3 \pm 4.1$ cm $H_2O$; $P < 0.001$). Following weight loss intervention there was a significant reduction in total fat mass ($-9.10 \pm 4.7$ kg; $P < 0.001$). Interestingly, fat loss occurred predominantly from the truncal regions compared to the limbs ($-4.7 \pm 37$ vs $-1.1 \pm 2.1$; $P < 0.01$).
Conclusions
Fat distribution in IIH patients is centripetal, akin to simple obesity. Clinical resolution of IIH is associated with preferential loss of truncal fat, potentially suggesting a pathogenic role for central adiposity in IIH.

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## P229

**Anti-inflammatory effects of metformin and their relationship to the therapeutic action of the drug**

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Improvements in circulating cytokine levels have occurred in patients with endometriosis and chronic heart disease when prescribed the type 2 diabetes (T2D) drug metformin. However it is unclear how metformin exerts these effects and whether or not they are related to antihyperglycaemic effects in T2D. The purpose of this study is to investigate the relationship between anti-inflammatory and anti-hyperglycaemic effects of metformin in the liver, the main target tissue of the drug. Hepatic responses to metformin were studied using primary mouse hepatocytes. Metformin exhibits anti-inflammatory effects in hepatocytes alongside anti-hyperglycaemic responses, co-ordinated by AMPK and NF-κB. Comparing metformin with the IKKβ inhibitor BI605906, we found that both drugs inhibited TNFα-dependent IκB degradation and expression of pro-inflammatory cytokines IL-6, IL-1β, VEGF & CXCLl/2, whilst metformin but not BI605906 suppressed lipogenic genes SREBP1, PPARγ, FASN and glucose production. These results suggest NF-κB mediates effects of metformin on hepatic pro-inflammatory cytokines but is insufficient by itself for effects on hepatic glucose production and lipogenesis, which are likely to be more closely related to the drug's ability to inhibit mitochondrial enzymes. Studies in obese pre-diabetic and db/db mice are currently underway and will provide signalling knowledge on how metformin behaves in pathophysiological contexts more relevant to diabetes. Initial results indicate differences in hepatocyte responses between obese and control when treated with differing doses of metformin. Hepatocytes extracted from obese animals require a higher concentration of metformin to elicit responses, including glucose output and ATP production. We believe that hepatocytes extracted from obese animals may be impaired in their ability to take up metformin.
This study indicates that metformin exhibits a dual anti-inflammatory and antihyperglycaemic effect which might contribute to its therapeutic advantage over other T2D treatments. Importantly, the separation of these properties that we have identified suggests that metformin's immune modulatory properties merit investigation in patients without diabetes.

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## P230

**Maternal overnutrition programs hypothalamic neuropeptides and metabolic syndrome in offspring**

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Background and objective
The prevalence of obesity is increasing worldwide and it is known that intra-uterine experience can program metabolic disorders. The hypothalamic appetite regulatory system is a key target of developmental programming by maternal nutrition. Therefore, the aim of this study was to investigate the effects of maternal overnutrition on the expression of hypothalamic genes controlling energy homeostasis.
Research design
Eight week old female Sprague-Dawley rats were fed high fat diet (HFD) or low fat diet (LFD) for 6 weeks before mating, throughout gestation and lactation. At postnatal day 21, hypothalami were collected from half of the offspring and the remaining were weaned onto LFD for 5 weeks, after which they were divided into LFD or HFD fed for 12 weeks.
Results and conclusion
Offspring from HFD fed dams were 32% heavier at birth and gained more weight with increased adiposity compared to those from LFD fed dams, independent of post-weaning diet. Maternal HFD also led to glucose intolerance in offspring at 8 weeks of age. Maternal diet interacted with offspring HFD to cause hyperphagia, increased body weight and epididymal and subcutaneous fat mass. At postnatal day 21, offspring of HFD fed dams had reduced mRNA expression of hypothalamic NPY (approximately twofold), AgRP (approximately twofold) and increased NPY-1 receptor (1.3 fold) but no change in the levels of POMC. The differential expression of AgRP (approximately twofold) and NPY1R (1.6 fold) persisted into adulthood with an increase in POMC expression (1.4 fold) in the offspring fed HFD. However, no change in the mRNA levels of MC4R and orexigenic peptides galanin, enkephalin and dynorphin were observed. These results suggest that maternal overnutrition alters central appetite neuroendocrine circuitry and exerts a detrimental effect on the offsprings' metabolic homeostasis. Postnatal overnutrition has additive effects with maternal programming to increase metabolic disorders.

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## P231

**'Thin-fat' phenotype in early foetal life in GDM pregnancies – novel evidence from an Indian Cohort**

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Research question
What are the differences in foetal size in early pregnancy between GDM and control.
Methods
Serial foetal biometry was performed for GDM women and controls at 18–20 weeks and 28–32 weeks. At each visit, abdominal and head circumference (AC) & (HC), femur length (FL) and abdominal wall thickness (AWT) was measured along with collection of maternal data.
Results
women with GDM and 184 controls had complete anthropometric and maternal demographic data. Scans were performed at 20.5($\pm$1.7) and 32.7($\pm$1.4) weeks. At 20-weeks GDM group had higher AWT despite smaller measures of all other anthropometric variables. Mean (S.D.)mm GDM vs Control: AC: 148.9(29) vs 155.1(19.7), $P=0.01$, HC: 171.8(32.9) vs 179.7(21.9), $P=0.006$, FL: 32.9(7.1) vs 34.5(4.5), $P=0.01$, AWT: 2.6 (0.5) vs 2.3 (0.4) $P<0.001$. These differences persisted despite adjustment for age, BMI, height, gestational age, sex and FPG. Similar significant differences persisted at 28–32 weeks. Gestational age and sex adjusted birth-weights (BW) of the two groups were similar ($P=0.56$).
Conclusion
Smaller size with increased abdominal fat is seen as early as 20 weeks in foetuses of GDM mothers, even prior to GDM diagnosis. Adiposity may potentially be a significant contributor to BW in GDM. AWT could serve as an early marker of GDM.

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## P232

**Functional significance of renal gene expression changes in a mouse model of ACTH-dependent Cushing's syndrome**

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Renal functions in a mouse model of Cushing's Syndrome have been characterised by analysing RNA expression complemented by immunohisto-chemistry studies. A microarray of kidneys from mice infused with ACTH for two weeks identified gene transcripts that were up-regulated (70) and down-regulated (49) more than two-fold. Four separate clusters of closely correlated genes ($r>0.97$; $P<0.001$) were investigated in more detail. One down-regulated cluster included histocompatibility genes (H2-Aa, H2-Ab1, H2-Eb1, ii) and others associated with hypertension-induced inflammation (adipsin, apelin, angiotensin converting enzyme). This anti-inflammatory phenotype was confirmed by immunostaining for the macrophage marker F4/80: fewer positive cells were associated with renal medulla and glomerular regions. A separate down-regulated cluster included bcat, p311, rnf24 and tgm1 which could be linked to a decrease in tubular cell proliferation and/or fibrosis. Numbers of Ki67 labelled proliferating cells were significantly less in the outer cortex of ACTH-treated kidneys but there was no evidence of glomerulosclerotic changes. One cohort of up-regulated genes included several linked to mineralocorticoid excess (p21, ztbt16) and others known to be associated with nephropathy (fgg, fga, tpa). Immunohistochemistry confirmed protein matched mRNA patterns for representatives of this group (arg2, slc13a1) and localised expression to proximal tubules. The second up-regulated cluster included those known to be controlled by glucocorticoids (fkpbp, gilz, sult1a1, agpt3 and 4). Based on previous studies, this up-regulated cluster might reflect glucocorticoid-induced changes in podocytes which in turn would explain the albuminuria which has been noted in this model of Cushings. In summary ACTH inhibited expression of some genes commonly associated with inflammation and fibrosis and promoted the expression of others that cause nephropathy and proteinuria. There is evidence that these positive and negative

outcomes are, in part, differentiated as mineralocorticoid and glucocorticoid-regulated processes.

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## P233

**Brown adipose tissue plays a role in regulating fetal growth by undergoing a gestational-dependent phenotypic change**

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Pregnancy is associated with increased maternal plasma lipid levels, an important physiological response to support the nutritional demand of the developing fetus and mother. The homeostatic controls of metabolically active organs are known to change in response to pregnancy signals. We hypothesised that pregnancy affects brown adipose tissue (BAT) phenotype and function and as a consequence plays a role in the regulation of fetal development and growth.

Using mice at gestational day 14 of pregnancy (GD14) or non-pregnant controls, we showed that the interscapular BAT (iBAT) was hypertrophied and exhibited a loss of BAT phenotypic markers (e.g. *Ucp1*) at GD14. In parallel, the expression of white adipose markers was increased at GD14, accompanied by lipid droplet accumulation. At a functional level, norepinephrine-mediated energy expenditure and increase in respiratory quotient observed in terminally anaesthetised non-pregnant mice were both abrogated in GD14 mice. This suggests that inducible thermogenesis and fuel utilisation in thermogenic tissue are modified by pregnancy.

To study the impact of the pregnancy-dependent phenotypic change on maternal and fetal parameters during the growth phase of the fetus (GD14-GD19), the iBAT was surgically ablated in female mice prior to mating and the mice were sacrificed at GD18. Ablation of iBAT resulted in a significant increase at GD18 in: i) normalised maternal body weight, ii) fetal weight, iii) placental weight, and iv) fetal serum FFA and hepatic cholesterol concentrations. This indicates that the removal of the main BAT depot results in the dysregulation of fetal lipid metabolism and growth.

These data shed new light on a pregnancy-brown adipose tissue axis that appears to play a role in the regulation of fetal growth driven by a gestational reduction in classical BAT phenotype.

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## P234

**Seasonal variation of $HbA_{1c}$**

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The aim of this study was to investigate seasonal variation in haemoglobin A1c ($HbA_{1c}$) using a retrospective population analysis linking $HbA_{1c}$ values to the time of year. The study cohort included 171 442 NHS patients from Scotland, UK. Patients were divided into three groups. The 'stable group' were defined as having a HbA1c that varied less than the tertile either side of the mean $HbA_{1c}$. The 'high summer' was comprised of patients with a $HbA_{1c}$ that varied by the second and third tertile above the mean $HbA_{1c}$ in summer. A 'high winter' group was defined similarly but $HbA_{1c}$ being higher during the winter months. There was a significant temporal trend in the mean $HbA_{1c}$ levels for all patients in the study with a maximal variation of 2.4 mmol/mol ($P<0.01$) between May and August. In high summer patients, there was a variation of 7.5 mmol/mol ($P<0.01$) between January and October. In high winter patients, there was a variation of 8.7 mmol/mol ($P<0.01$) between March and September. Overall 45.3% of all patients ($n=77\,720$) were in the high winter $HbA_{1c}$ group, 31.3% ($n=53\,656$) were in the high summer HbA1c group, and 23.4% ($n=40\,066$) were in the stable $HbA_{1c}$ group. Associations with seasonal variation of $HbA_{1c}$ included social deprivation, male gender, T1DM, increasing BMI and increasing diastolic blood pressure (DBP). The most likely explanation for this is treatment adherence, however this could not be measured in this retrospective population analysis. More than half the patients have a clinically relevant annual variation in $HbA_{1c}$, which could impact on clinical decision making. Both clinicians and researchers need to be aware of the influence of time of year on the $HbA_{1c}$ value, especially in the context of meeting treatment target goals for $HbA_{1c}$.

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## P235

**Relationship between high-sensitivity C-reactive protein and anthropometric indices of obesity**

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Background

Obesity contributes to the occurrence of many metabolic and cardiovascular complications including Type 2 diabetes, hypertension etc. Body adiposity is not just an energy store, but also an endocrine organ with elaboration of hormones and cytokines. Some of these cytokines have been implicated in the initiation and propagation of inflammation, and hence atherosclerosis.

Aim

This study examined the relationship between high-sensitivity C-reactive protein (hs-CRP), a marker of inflammation and anthropometric indices of obesity.

Method

This is a cross-sectional study carried out at Obafemi Awolowo University Teaching hospital, Nigeria, involving 108 apparently healthy individuals who presented voluntarily following an advert for research placed at public places. Ethical approval was granted by the hospital's ethical committee and informed content was given by the participants. Relevant history was obtained and anthropometric measurements were taken. Obesity/abdominal obesity was defined as body mass index (BMI) $\geqq 30 kg/m^2$, waist-to-hip ratio (WHR) of $\geqq 0.9$ in men and $\geqq 0.85$ in women, waist circumference (WC) of $>94$ cm in men and $>80$ cm in women, or waist-to-height (WHtR) ratio of $>0.5$ respectively. Hs-CRP measurement was done. Data was analysed using SPSS 20 version. Correlation between markers of inflammation and obesity indices was performed. Statistical significance was defined as $P$ value $<0.05$.

Results

Mean age of participants was $55.59 \pm 7.75$years. Obesity was present in 59(54.6%), 83(76.9%), 75(69.4%) and 20(18.5%) using WC, WHR, WHtR and BMI respectively. There was significant positive correlation between hs-CRP and WC ($r=0.220$; $P=0.022$), WHR ($r=0.243$; $P=0.011$), WHtR ($r=0.269$; $P=0.005$) and BMI ($r=0.246$; $P=0.010$) respectively.

Conclusion

High sensitivity C-reactive protein is positively correlated with all anthropometric measures of obesity studied, with the highest correlation occurring between high-sensitivity-CRP and waist-to-height ratio.

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## P236

**2β-hydroxybetulinic acid 3β-caprylate: an active principle from euryale ferox salisb. seeds with antidiabetic, antioxidant, pancreas & hepatoprotective potential in streptozotocin induced diabetic rats**

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Introduction

The aim of the present study was to evaluate the glycaemic control, antioxidant, pancreas and liver protective effect of 2β-hydroxybetulinic acid 3β-caprylate (HBAC) from *Euryale ferox Salisb*. seeds on streptozotocin induced diabetic rats.

Materials & methods

The active principle was isolated from *Euryale ferox Salisb*. seeds extract by utilizing chromatographic techniques. The rats were divided into seven experimental groups: Gp 1-normal; Gp2- normal + HBAC (60 mg/kg p.o.); Gp3- diabetic control; Gp 4- Diabetic + HBAC (20 mg/kg p.o.); Gp5- Diabetic + HBAC (40 mg/kg p.o.); Gp6- Diabetic + HBAC (60 mg/kg p.o.) and Gp 7- Diabetic + Glibenclamide (10mg/kg p.o.). Biochemical estimation, free radical scavenging examination and histopathological study was performed at the end of experimentation i.e. on 28th day.

Results

The active principle isolated and identified with spectral data as 2β-hydroxybetulinic acid 3β-caprylate (HBAC). It was detected for the first time that HBAC has improvised the glycaemic control in streptozotocin induced diabetic rats. Furthermore, it is remarkable to note that it exhibited excellent free radical scavenging property and pancreas and hepatoprotective property as well, supported by histopathological examination. One of the mechanisms of action of HBAC appears to be stimulating the release of insulin from pancreatic β-cells.

Conclusion
HBAC improved the glycaemic control, reduced the free radical activity along with corrected glycemic control, lipid profile, and enhanced level of insulin along with improvement in pancreas and hepatoprotective architecture. Considering the above results, HBAC shows potential to develop a medicine for diabetes as combinatorial or mono-therapy.

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## P237

**Clinical profile and outcome of patients with hyperglycaemic emergencies presenting at a rural hospital in southern Nigeria**

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Background
Hyperglycaemic emergencies (HEs) continues to be important causes of morbidity and mortality among persons with diabetes mellitus. Hyperglycaemic hyperosmolar state (HHS) and diabetic ketoacidosis (DKA) are the two most common acute metabolic complications seen in persons with diabetes mellitus.
Objective
To describe the clinical presentation and outcome of patients with hyperglycaemic emergencies.
Materials and methods
This was a retrospective descriptive study. Records of patients admitted with hyperglycaemic emergency between April 2013 and March 2015 were retrieved. Data was extracted using a standardized questionnaire which included demographic, clinical and laboratory parameters and outcome at discharge.
Results
A total of 88 patients with HE admitted during the period, 61.4% were females. The mean (SD) age for male and female were 60.2 (12.8), 52.2 (18.3) respectively and the $P=0.03$. Forty seven (53.4%) had HHS, 34 (38.6%) had DKA and 7 (8%) had mixed type of HE. Out of these patients, 28 (31.8%) were newly diagnosed. Sepsis (30.7%) was the commonest precipitant. Other rare precipitants were Stroke (8%) and myocardial infarction (3.4%). The mean (S.D.) values for random blood glucose, osmolality for HHS and DKA were 34.7 (6) mmol/l, 339.0 (19) mmol/kg and 23.9 (7.6) mmol/l, 312 (14.1)mmol/kg respectively. The mean (SD) serum sodium, potassium and urea were 137.5 (6.7) mmol/l, 4.6 (0.95) mmol/l and 12.8 (8.4) mmol/l respectively. Seven (8%) patients developed complications and the commonest was acute kidney injury. Overall case fatality rate was 34.1%. Age, gender, electrolyte derangement and complications did not predict outcome ($P>0.05$).
Conclusion
Management of HE constitute a great challenge in poor resource countries, hence emphasis should be made on prevention through adequate glycaemic control and prompt implementation of effective treatment guideline.

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## P238

**Diabetic neuropathy: an evaluation using the ntss-6 questionnaire and biothesiometry in type 2 DM patients**

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Background & objectives
Vibration perception threshold (VPT) is the gold standard for diagnosis of diabetic peripheral neuropathy. The relationship between symptom severity and VPT remains to be determined. This study evaluated the diagnostic utility of symptoms using the NTSS-6 symptom score vis a vis Bio-thesiometry.
Methods
diabetic patients with symptoms suggestive of peripheral neuropathy and 40 apparently healthy controls were studied. Detailed clinical history and physical examination were performed. Peripheral neuropathy was assessed using the Neuropathy total symptoms score-6 questionnaires and testing vibration perception threshold (VPT) with a biothesiometer.
Results
The prevalence of peripheral neuropathy using the NTSS-6 scoring system was 50% and 45.5% using biothesiometer. Numbness was the most predominant symptom followed by burning sensation both amongst those with clinically significant neuropathy.The NTSS-6 scoring had a sensitivity of 66% and a specificity of 66.3%. Age and BMI were statistically significantly associated with clinically significant neuropathy. Significant correlations were observed between the NTSS-6 score and the VPT score. ($P$-value $=0.002$, $r=0.588$).
Conclusions
It is apparent that though symptoms suggestive of peripheral neuropathy (PN) in persons with diabetes mellitus (DM) may not always indicate the presence of pathological PN, symptom scores remain useful tools for assessing DM peripheral neuropathy and correlate well with biothesiometry findings.

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## P239

**Hypothalamic glucocorticoid levels increase in an in vivo model of glucocorticoid-induced obesity and hyperinsulinaemia**

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Glucocorticoid (Gc) excess, either endogenously produced (Cushing's syndrome) or as a result of exogenous steroid treatment, can cause metabolic disorders such as obesity and hyperglycaemia. The contribution of centrally acting corticosterone in the development of these metabolic disorders is unknown. Gcs regulate the anorexigenic and orexigenic neuropeptides (POMC and AgRP) within the arcuate nucleus of the hypothalamus to modulate energy balance. This study investigated the effects of exogenous corticosterone treatment on central corticosterone levels and evaluated the consequences on Gc-target genes and genes regulating Gc levels. Mice were administered corticosterone (75 μg/ml, CORT) or ethanol (1%, Veh) in their drinking water with high fat diet (HFD, 60% energy from fat diet) or chow for 4 weeks. The CORT and CORT/HFD generated a metabolic syndrome-like phenotype. Increased circulating corticosterone levels were found at the nadir and peak, and caused ablation of the corticosterone diurnal rhythm. LC-MS/MS analysis showed increased hypothalamic corticosterone levels in both the CORT and CORT/HFD groups. POMC and GR mRNA expression levels remained unchanged in the pituitary indicating the increases in corticosterone levels are a result of exogenous administration. There was a twofold decrease in the glucocorticoid receptor (GR) mRNA in the hypothalamus, and *in situ* hybridisation indicated that this was in a region other than the ARC or PVN. The GR was still active as CORT caused greater than twofold increase in both glucocorticoid-induced leucine zipper (GILZ) and fkbp5 expression. Exogenous CORT treatment resulted in a decrease in mRNA expression of 11β-hydroxysteroid dehydrogenase (11βHSD1), the enzyme which regenerates active Gcs, while the expression of the efflux transporter MDR-PGP was unaltered. In summary, exogenous Gc treatment increases hypothalamic corticosterone and this results in increases in Gc target genes, which in turn may be associated with the development of metabolic disturbances seen with GC excess.

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## P240

**Effects of androgen on the adipokinome and metabolic genes in white and brown adipose tissue**

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Polycystic ovary syndrome (PCOS) is a common endocrinopathy that is associated with an adverse metabolic profile including insulin resistance and dyslipidaemia. Hyperandrogenism is the hallmark of PCOS and obesity increases androgen synthesis, partly due to accompanying hyperinsulinemia but also as a result of adipokines affecting ovarian steroidogenesis (Comim *et al*., 2013, PLoS ONE 8(11). Adipokines are factors secreted by adipose tissue and while a clear link between obesity and the severity of PCOS exists, the relationship between hyperandrogenism and adipose tissue is less clear. In this study, a systems approach was used to investigate effects of androgen treatment on the adipokinome. Immortalised mouse white and brown preadipocytes were fully differentiated for 14 days and then treated with either dihydrotestosterone (DHT) or control for 24 h. Quantitative PCR was then used to analyse differential gene expression for a panel of 25 adipokines. Our results show that hyperandrogenism leads to dysregulation of the adipokinome in adipose tissue with potential impact

on the secretory function of adipocytes. Interestingly, brown adipocytes (BAT) were far more responsive to androgen treatment than white adipocytes (WAT). Several of the affected genes are involved in BAT identity and thermogenesis as well as adipokine production. In the light of this we extended our investigation and treated explants of mouse inter-scapular BAT with either DHT or control for 24 h. The results show that DHT treatment reduces expression of key markers of BAT identity and genes implicated in thermogenesis, including UCP1 ($P<0.05$), PGC-1 ($P<0.05$) and Cidea ($P<0.05$). Furthermore, androgen treatment was shown to significantly attenuate ($P<0.05$) the β-adrenoceptor-stimulated increase in UCP1 expression in brown adipocytes. These results show that androgens not only affect WAT but also have significant effects on BAT with respect to genes that are involved in both adipokine synthesis and in energy expenditure.

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## P241

**From the bronze age to the iron age**

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A 53 year old man, without any previous medical history, presented acutely to the Accident and Emergency Department with a 5 day history of vomiting and abdominal pain on a background of weight loss, fatigue, polyuria and polydipsia for 4 weeks. On examination he was obviously tanned and thin. He was clinically dehydrated and had palpable hepatomegaly. Immediate biochemical testing revealed hyperglycaemia with a venous blood glucose of 20.7 mmol/l, a metabolic acidosis with a pH of 7.1 (and venous bicarbonate of 11.3 mmol/l) and significant ketonuria (++++) thus confirming the clinical suspicion of diabetic keto-acidosis (DKA). His serum ferritin levels were markedly raised at 11346 ug/l with a raised transferrin saturation of 92%. His initial basal pituitary function showed evidence of partial anterior hypopituitarism. This was manifest by hypogonadotrophic hypogonadism with an FSH of <1 IU/l (2–12),LH of <1 IU/l (2–9) and testosterone of 0.5 nmol/l (9–29). He also had evidence of secondary hypothyroidism (TSH 2 mU/l (0.27–4.2) and free T4 9 pmol/l (12–22)) though his cortisol levels were physiological (820 nmol/l at 9 am). He was treated with intravenous fluids and insulin initially and this was later converted to subcutaneous insulin therapy. His HFE gene testing proved positive with him being homozygous for the C282Y mutation thus confirming the diagnosis of Hereditary Haemochromatosis (HH). HH is a disorder of iron metabolism with iron deposits in different organs including within the endocrine system. Although diabetes is a common association of HH, it is unusual for patients to present with DKA. This case serves to highlight this very issue thus serving as a reminder to be aware of potential causes of secondary diabetes when assessing patients with DKA. It also aims to demonstrate the multi-gland nature of the endocrinopathy encountered by patients with HH.

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## P242

**Characterisation of the astrocytic response to acute and recurrent hypoglycaemia**

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Background
In diabetes, exposure to frequent episodes of hypoglycaemia diminishes a persons awareness of hypoglycaemia, by poorly defined mechanisms. Evidence suggests that, at least in part, changes in cell behaviour within the ventromedial hypothalamus may contribute to this defect and that changes in AMP-activated protein kinase (AMPK), a key glucose-sensing enzyme are involved. Evidence suggests there are (mal)adaptations within neurons, however, little is known about changes within the astrocyte, which make up at least 50% of the cells within the hypothalamus.
Methods
Human U373 cells and primary mouse hypothalamic and cortical astrocytes were exposed to 2.5 or 0.1 mM glucose containing DMEM for up to three hours. Supernatants were collected for further analysis and cellular lysates were analysed by Western blotting.
Results
Application of low glucose (0.1 mM) increased AMPK (thr172) and acetyl CoA carboxylase (ser79) phosphorylation, indicating an increase in AMPK activity when compared to control (2.5 mM). Extracellular lactate (eATP) levels decreased with low glucose by ~60% (2.5 vs 0.1 mM), which was much less than the 25-fold reduction in glucose availability, indicating that under low glucose conditions, astrocytes may be preferentially more glycolytic. Acute low glucose application also increased extracellular ATP levels, a universal "danger" signal. Astrocytes were exposed to three episodes of low glucose to mimic recurrent hypoglycaemia (RH). The change in eATP mediated by low glucose was similar between control and RH. There was a trend toward reduced AMPK activation following exposure to low glucose following RH, which did not reach significance. However, there was a significant attenuation of decrease in lactate release by low glucose following RH, suggesting a relative enhancement of lactate release.
Conclusion
Astrocytes are functionally altered by exposure to acute and recurrent low glucose suggesting that changes within the astrocyte may contribute to defective glucose counterregulation in diabetes.

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## P243

**Markers of adipose tissue hypoxia are elevated in subcutaneous adipose tissue of morbidly obese patients with hypoventilation syndrome and obstructive sleep apnoea syndrome but not in the moderately obese**

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Adipose tissue (AT) dysfunction is thought to be a central component in the pathophysiology of obesity-associated metabolic disease. Low AT oxygenation (hypoxia) is suggested to be a driver of this dysfunction, yet studies in humans have resulted in conflicting data. Therefore, we aimed to investigate if markers of AT hypoxia were present in the subcutaneous AT of morbidly obese individuals who had hypoxia from obesity hypoventilation syndrome (OHS), with or without obstructive sleep apnoea (OSA), two extreme phenotypes with the potential to produce a proof of principle type result.
To provide a methodological positive control for the detection of cellular makers of hypoxia in human AT, we undertook *in vitro* cellular studies in which human primary adipocytes were incubated in normoxic (21% $O_2$; 5% $CO_2$) or hypoxic (1% $O_2$; 5% $CO_2$) conditions. As expected, hypoxia significantly increased HIF1A protein in primary adipocytes and gene expression of both classical HIF1A target genes (GLUT1 and VEGFA) and hypoxia-sensitive pro-inflammatory genes (IL6 and PAI1). We then measured these markers in human subcutaneous AT from subjects of 4 distinct phenotypes: lean (BMI = 24.2), moderately obese (BMI = 32.5), morbidly obese with OHS (BMI = 45.3) and morbidly obese with OHS and OSA (BMI = 45.3).
We found no significant differences in either AT HIF1A protein levels or AT expression of hypoxia-sensitive genes between lean and moderately obese subjects. In contrast, subjects with either OHS, or OHS with OSA, exhibited significantly higher HIF1A protein levels versus moderately obese and lean controls (OHS fivefold compared to moderately obese; OHS with OSA sixfold compared to moderately obese), and this was correlated with expression of hypoxia-sensitive genes.
Our results suggest that markers of AT hypoxia only become evident in individuals with both extreme levels of adiposity and systemic hypoxia. In addition, there is an additive effect on hypoxia markers in patients with OSA over and above OHS alone. Although our data are not consistent with the view that AT hypoxia drives metabolic dysfunction in moderate obesity, AT hypoxia may contribute to the development of metabolic dysfunction in OHS and OSA.

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## P244

### Prevalence of diabetes mellitus among HIV patients in Kano, northwestern Nigeria

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Background
Highly active antiretroviral therapy (HAART) particularly protease inhibitors cause hyperglycaemia through insulin resistance. There is raising number of HIV patients on HAART attending the diabetic clinic in Nigeria and very few studies were done to determine their risk of developing diabetes. We aimed to determine the prevalence and factors associated with diabetes mellitus (DM) among HIV patients.
Methods
In a cross sectional study of 300 HIV patients at the AKTH Kano, we assessed the glycaemic parameters and anthropometry of study participants. The patients were evaluated as per HAART-treated and HAART-naïve groups and the results compared for the two groups. Fasting plasma glucose (FPG) was determined using glucose oxidase method after an overnight fast and WHO criteria was used to define diabetes mellitus as FPG $\geq$7.0 mmol/l.
Results
The mean $\pm$ s.d. age of the study participants was 34.8 $\pm$ 9.9 years with a male to female ratio of 1:2. The prevalence of DM was found to be 7.3%. Prevalence among HAART-treated was 12.0% while among HAART-naive participants was 2.7% ($P=0.001$). Among the HAART-treated, older age, exposure to protease inhibitor based regimen, longer duration of HIV diagnosis, and HAART exposure, increased CD4 cell count, abnormal waist circumference, and presence of hypertension were found to be associated with the occurrence of DM ($P<0.05$). Among the HAART-naïve longer duration of HIV infection and the presence of abnormal waist circumference were found to be associated with the development of DM. Increased waist circumference (OR 4.4; 95% CI, 1.5–12.7; $P<0.05$) and presence of hypertension (OR 5.4; 95% CI, 1.9–15.1; $P<0.05$) were found to be independent predictors for the development of DM.
Conclusion
HAART exposure causes disturbances of glucose metabolism among HIV patients. Plasma glucose evaluation should form part of the routine investigations among HIV patients particularly those on HAART.

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## P245

### Obstructive sleep apnoea and bariatric surgery: the need for more universal screening and post-operative follow up

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Aim
Bariatric surgery can provide significant weight loss but a complete resolution obstructive sleep apnoea (OSA) cannot be predicted. The aim of our audit was to assess the prevalence of OSA in bariatric clinics and outcomes after malabsorptive bariatric surgery.
Methods
Retrospective observational analysis was performed on patients who had undergone bariatric surgery (laparoscopic gastric bypass and sleeve gastrectomy) and completed a minimum of 6 months of follow up. Information from clinical letters, from first review in bariatric service and latest follow up appointment were compared. Referral for assessment of OSA was based on clinical index of suspicion.
Results
Records of 230 patients were analysed. Pre-operative mean age was 45 years (20–69); mean weight 136 kg (85–250); BMI 48.5 kg/m$^2$ (35–83); and 31% were men. Pre-op: 13.5% ($n=31$) had pre-established OSA on CPAP; 27.4% ($n=63$) had clinical possibility of OSA and referred for sleep studies. 21.7% ($n=50$) had no features to suggest OSA. There was no documentation on the rest of 86 patients. Respiratory assessment: Of the 63 patients referred, 57% ($n=36$) had no or mild OSA; 8% ($n=5$) had moderate OSA; 35% ($n=22$) have severe or very severe OSA. The BMI of those without OSA was lower than those with OSA (46.3 vs 52.0, $P=0.014$). Post-op follow-up: mean period of follow up was 236 days. 40 of the 58 patients (who had moderate to severe OSA or established CPAP treatment) had further respiratory follow-up. Mean BMI was 27.9 (25–66). 15/40 had OSA resolved and hence CPAP discontinued; 22/40 had improvement in OSA but still warranted CPAP continuation; three patients were not compliant with CPAP. Comparing continued vs resolved OSA group: pre-op BMI (52 vs 49.6), latest BMI (38.5 vs 35.7), and weight loss achieved (44.0 vs 40.5) were comparable.
Conclusion
i) Screening for OSA needs to be universal in bariatric pathways (especially in patients with higher BMI) as clinical suspicion may not be accurate. ii) Prevalence of OSA is high in patients awaiting bariatric surgery (58 of 144 tested – 40%). iii) 55% continue to have OSA after bariatric surgery and requiring CPAP despite good weight loss. iv) Periodic reassessment of OSA needs to be arranged post-bariatric surgery and spontaneous resolution should not be assumed.

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## P246

### The effect of soy protein with and without isoflavones in men with type 2 diabetes mellitus and subclinical hypogonadism: a randomised double-blind parallel study

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Isoflavones are a subgroup of phytoestrogens and have a chemical structure similar to oestrogen, leading to concerns regarding possible adverse effects of isoflavones in men. Testosterone levels in men with type 2 diabetes mellitus (T2DM) are lower.
Materials and methods
A randomised double-blind, parallel study was undertaken with 200 men with T2DM, of median age of 50 years (25th/75th centiles; 50, 55) years with a total testosterone level $\leq$12 nmol/l and normal gonadotrophins. They were randomised and administered either 30 g soy protein with 66 mg isoflavones (SPI)/day, or 30 g soy protein alone without isoflavones ($<$300 parts/billion) (SP) for 12 weeks.
Results
There was no significant change in serum total testosterone with either SPI or SP supplementation. There was no significant changes in absolute free testosterone levels with either SPI or SP. DHEAS reduced significantly with SPI and increased with SP suggesting SPI reduces adrenal androgens rather than gonadal androgens. Regarding glycaemic control, there was a significant reduction in HbA1c, fasting glucose, fasting insulin, and HOMA-IR after SPI that were significantly greater than the changes observed in SP. Triglycerides and hsCRP reduced significantly with SPI that was not seen with SP supplementation. Diastolic blood pressure reduced significantly after both SPI and SP. There were no changes in systolic blood pressure in either group. There was a significant improvement in endothelial function with an increased reactive hyperemia index (RHI) with SPI whereas there was a significant reduction with SP administration.
Conclusions
It is reassuring that soy protein with and without 66 mg isoflavone/day do not have any effects on total and free testosterone levels in men with T2DM, and who had compromised gonadal function with low testosterone levels, after 12 weeks. There was a significant improvement in both glycaemic control and cardiovascular risk markers including lipids, diastolic blood pressure, and hsCRP with the soy protein/isoflavone combination compared to soy protein alone.

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## P247

**Is polycythaemia a marker of an increased cardiovascular risk in transmen?**

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A retrospective single centre audit was conducted on the effects of polycythaemia in trans-male and native male.

Polycythaemia is a recognised side effect of testosterone treatment. It could be viewed as a maker of excess testosterone action. We examined whether lipid profiles as a marker of cardiovascular risk differed in a population of polycythaemic transmen (TM) and native males (NM).

Forty-six NM and 12 TM were identified to have polycythaemia (haematocrit $\geq$0.48). The mean age of TM was younger than NM (43.75 years vs 56.36 years, $P>0.05$). Baseline haematocrit (0.44 NM vs 0.45 TM) and polycythaemic stage haematocrit (0.52 NM vs 0.51 TM) were not different between groups but the rise of haematocrit compared to baseline was significant in both groups ($P<0.01$). Following either conservative management or venesection, post treatment haematocrit improved in both groups (0.47 NM vs 0.48 TM, $P$ 0.0001).

TM have higher baseline total cholesterol (5.4$\pm$1.3 TM vs 4.5$\pm$1.2 NM mmol/l, $P$ 0.08) and LDL (3.33$\pm$1.0 mmol/l vs 2.67$\pm$0.87 mmol/l, $P$ 0.09). At polycythaemic stage, TM had higher total cholesterol (5.5$\pm$1.03 TM vs 4.5$\pm$0.85 NM mmol/l), LDL (3.27$\pm$0.89 TM vs 2.46$\pm$0.73 NM mmol/l), and HDL (1.34$\pm$0.19 TM vs 1.13$\pm$0.32 NM mmol/l) all $P<0.01$, which was also seen in post treatment total cholesterol (6.0$\pm$1.10 TM vs 4.2$\pm$0.90 NM mmol/l), LDL (3.72$\pm$0.87 TM vs 1.10$\pm$0.31 NM mmol/l), and HDL (1.39$\pm$0.23 TM vs 1.11$\pm$0.31 NM mmol/l) all $P<0.01$. In NM, the lipid profile improved once the polycythaemia was treated, in contrast the lipid profile deteriorated in TM (total cholesterol 5.53 mmol/l vs 6.00 mmol/l, triglyceride 1.85 mmol/l vs 2.14 mmol/l (both $P<0.001$), polycythaemic stage vs post-treatment).

Our data suggests that there are no differences between TM and NM haematological responses to the treatment of polycythaemia. However, the lipid profile of TM appears to deteriorate with the treatment of polycythaemia which is not seen in NM which could suggest that polycythaemia is a marker of higher cardiovascular risk in transmen.

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## P248

**Cost of illness among patients with diabetic foot ulcer in secondary and tertiary health facilities in Kano, northwestern Nigeria**

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Background

In Nigeria, the economic burden of diabetes foot ulcer is enormous for several reasons and there are very few studies that look at the economic cost of diabetes foot care. This study estimated the cost of illness among patients with diabetes foot ulcer in secondary and tertiary health facilities in Kano.

Methods

The study was a cross-sectional evaluation involving ninety patients with diabetes having various degrees of foot ulcerations. A structured questionnaire was used to estimate the direct medical, non-medical, and indirect costs of illness. Ulcer was examined clinically. HbA1c was done to determine the glycaemic control of subjects.

Results

The mean$\pm$S.D. age of the subjects was 59.3$\pm$15.1 years with M:F ratio of 4:1. Among the male participants, 68.1% were the breadwinners of their families. About 60% of the participants earn <$100 monthly. The total cost of illness of diabetic foot ulcer for the 90 participants was $164 484.38 (average=$1827.61). The direct cost of illness was $125 987.81 (average=$1399.86) making up 76.6% of the total cost of care. Direct medical cost was $85 086.88 (average=$945.41). The direct non-medical cost was $40 900.94 (average =$454.49). The total indirect cost was $38 497.38 (average=$427.74). Drugs accounted for the largest share of the total cost (21.9%). Out of pocket payment accounted for 90% of payment. The duration and location of foot ulcer, duration and frequency of hospital admission, average monthly income and presence of co-morbidities were significantly associated with increase cost of illness, $P<0.05$.

Conclusion

The cost of diabetic foot ulcer in Kano is prohibitive and the patients mostly affected are poor, unemployed, and the breadwinners of their families. Improved healthcare funding for diabetes care and subsidy on DM medications is advised to stem the tide of the disease.

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## P249

**Impact of DAFNE and subsequent CSII therapy on glycaemic control in type 1 diabetes mellitus**

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Background

Dose adjustment for normal eating (DAFNE) structured education programme is an effective tool in improving glycaemic control in patients with type 1 diabetes while reducing the frequency of hypoglycemic episodes. DAFNE however, does not solve all glucose related problems and patients often request or are recommended continuous subcutaneous insulin infusion (CSII) therapy post DAFNE.

Objective

Out of our 370 DAFNE graduates, 46 have subsequently gone on to CSII therapy. The aim of our audit was to examine glycaemic control of these 46 graduates before and after they completed the DAFNE and then before and after starting CSII therapy and reason for CSII therapy.

Methods

The data was collected using the hospital's electronic data base (Cellma and Pipe) and by contacting the patients via phone for further details.

Results

56% patients were female. Mean age was 40$\pm$9.4 (mean$\pm$S.D.) years with BMI of 26.6+4.5 kg/m$^2$ and duration of diabetes was 17.2$\pm$8.5 years. Duration since completion of DAFNE at the time of study was 5.8$\pm$2.2 years and duration of CSII therapy was 4.0$\pm$2.2 years. HbA1c before DAFNE was 8.3$\pm$1.2% (67.2$\pm$9.8 mmol/mol) compared to 8.05$\pm$1.0% (64.5$\pm$11 mmol/mol) 12 months post DAFNE–$P$ value=0.31. The indication of commencement of CSII therapy was to improve overall glycemic control in 45% patients, impaired awareness of hypoglycaemia in 26% and patient preference, felt it would suit their lifestyle, in 23%. HbA1c before commencement of CSII was 8.3%$\pm$1.07 (67.2$\pm$11.7 mmol/mol) compared to 7.9%$\pm$0.9 (62.8$\pm$9.9 mmol/mol) after 12 months of CSII therapy–$P$ value=0.04.

Conclusion

DAFNE is an effective education programme for patients with type 1 diabetes but may not improve glycaemic control in all. Selected patients benefit from going on to CSII therapy post DAFNE and this can be associated with an improvement in HbA1c.

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## P250

**Lifelong exposure to sewage sludge chemicals causes proteome-wide and sex-specific disturbances in the liver**

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Introduction
A complex cocktail of endocrine-disrupting and other chemicals is ubiquitous in the modern environment. Exposure to such chemicals contributes to diseases including metabolic syndrome and infertility. The liver is the primary defence organ against xenotoxicants, but also the source of major of plasma proteins, and growth factors/hormones.
Aim
To understand how chronic exposure to complex mixtures of chemicals at human and environmentally relevant concentrations affects liver function and contributes to disease outcomes.
Methods
Liver protein extracts from adult sheep that grazed on control or sewage sludge-fertilised pastures throughout their lives (gestation and lactation via the mother and post-weaning grazing) were divided in four groups ($n=10$–12/group) according sex and treatment. Proteins were resolved using 2D differential in-gel electrophoresis and compared using SameSpots Software. Differentially expressed protein spots were identified by liquid chromatography/tandem mass spectroscopy (LC–MS/MS). Western blots were used to measure total protein levels and post-translational modifications.
Results
Chronic exposure to sewage sludge grossly altered the liver proteome in a sex-specific manner. Out of the observable 445 protein spots, 143 spots in ewes, and 94 spots in rams (with an overlap of 44 spots) showed statistically significant ($P<0.05$) spot volume alterations compared to controls. Proteins identified in 29 treatment-affected spots included: major plasma-secreted proteins (albumin, transferrin, and apolipoprotein A1), detoxification enzymes (GSTM, GSTT, and INMT), and fatty acid β-oxidation enzymes (ACAA2 and ECHS1). Albumin and transferrin were increased in a subset of treated rams and transferrin glycosylation patterns were altered in ewes. Reproductive system development and function, endocrine system and cancer pathways affected in males and cell death and survival, developmental disorders, haematological disease pathways affected in females.
Conclusions
Major alterations by environmental chemical exposure to liver function are likely to affect multiple systems in the body thereby providing a strong link between exposure and disease progression and/or development.

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## P260

**GLP1 agonist based therapy has modest effect on weight and glycaemic control among UAE patients with diabetes: analysis of data on 3725 patients**

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Background and aims
GLP1 agonists are used in UAE for diabetes and obesity management (prevalence of 19 and 74% respectively). Few outcome data on GLP1 agonist therapy in this population is available. We investigated the effects of two commonly used GLP1 agonists on weight and HbA1c among UAE patients with diabetes.
Materials and methods
Data was retrieved from a computerized database of patients attending a large diabetes center in UAE. Patients on exenatide 2 mg weekly ($n=1223$) or liraglutide 0.6–1.8 mg daily ($n=2502$) for 12 months were included in the study. Data on weight and HbA1c were collected at three different time points of treatment; baseline, 6 months (range 5–7) and 12 months (range 10–14). Two-sample *t*-test and analysis of response profile were performed (Stata 13, Stata Corp LP, TX, USA).
Results
Mean weight reductions over 6 months of treatment were 1.5 and 2.7 kg for weekly exenatide and daily liraglutide respectively ($P<0.001$). There was no further significant weight reduction with either drug at 12 months after treatment initiation. Mean HbA1c reductions after 6 months of treatment were 0.79 and 0.75 for weekly exenatide ($P<0.001$) and daily liraglutide ($P<0.001$) respectively ($P=0.42$). No significant change in HbA1c at 12 months with either drug was found. Analysis of response profile showed statistically significant difference in rate of change over time for both weight and HbA1c in the patients who received daily liraglutide compared to weekly exenatide ($P<0.001$).
Conclusions
In UAE diabetic patients, the studied GLP1 agonists resulted in modest weight reduction (more with liraglutide) and HbA1c after 6 months of treatment with no further change at 1 year post treatment. Further studies are warranted to investigate specific effects of GLP1 agonists among the study population.

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## P261

**Extracts of *Hymenocardia acida* ameliorate insulin resistance in skeletal muscle cells**

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There are a limited range of drugs available to treat type 2 diabetes (T2D) and insulin resistance (IR) that are efficacious and inexpensive. In West Africa, extracts of *Hymenocardia acida* are frequently used. However, few objective studies have yet attested to their efficacy. Here we evaluated the potential for extracts of *H. acida* to overcome IR in muscle *in vitro*.
Crude extracts of *H. acida* were prepared in methanol (MeOH) and chloroform ($CHCL_3$). The methanolic extract was also subjected to a further solid phase extraction (SPE) on C18 silica using ratios of MeOH:$dH_2O$ from 0 to 100%, yielding 15 sub-fractions. Sub-fractions were dried and analysed using proton nuclear magnetic resonance ($^1$H-NMR) spectroscopy. The effect of crude extracts or SPE sub-fractions 1–3 on the viability of differentiated L6 myotubes was then assessed using resazurin. Subsequently, myotubes were treated with palmitic acid (PA) to generate IR and incubated with extracts or sub-fractions at concentrations across a 100-fold range that did not impair viability. Their effects on insulin sensitivity were assessed using uptake of $^3$H-2-deoxyglucose uptake ± insulin.
Treatment with PA had the expected effect to abolish insulin-stimulated glucose uptake in L6 myotubes. Co-incubation with the MeOH extract at 200 μg/ml significantly restored insulin sensitivity of glucose uptake by 63% ($P=0.041$), while the $CHCL_3$ extract was not effective. SPE sub-fractions 1–3 were identified as potentially containing bioactive compounds, due to the presence of peaks on $^1$H-NMR trace, indicative of the presence of un-/partially-substituted benzene rings in their chemical structure. However, treatment with these sub-fractions did not modify insulin-stimulated glucose uptake in myotubes.
Thus, methanolic extracts of *H. acida* have shown promise in ameliorating IR in skeletal muscle cells, representing the key tissue mediating insulin-stimulated glucose disposal. Further work must aim to identify the active substance(s) responsible and their mechanism of action.

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## P262

**A study of metabolic syndrome among HAART naïve HIV patients in Kano, northwestern Nigeria**

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Background
Most studies of the metabolic syndrome (MS) among HIV patients focus on those receiving HAART. This study determined the prevalence and factors associated with MS among HAART naïve HIV patients in Kano, Nigeria.
Methods
In this descriptive study, we evaluated 150 consecutive HAART naive HIV patients attending the HIV clinic of Aminu Kano Teaching Hospital. Data on socio-demography, relevant clinical history, anthropometric indices, and blood pressure (BP) were recorded for each participant. Fasting plasma glucose and lipid profile were assessed. MS was defined using NCEP/ATP III and IDF criteria. Insulin resistance (IR) for those with MS was calculated using HOMA-IR.
Results
The mean $\pm$ S.D. age of the study participants was $34.0 \pm 9.7$ years. There were 54 (36%) males and 96 (64%) females, $P = 1.00$. The prevalence of MS was 5.3 and 9.3% using the NCEP/ATP III and 9.3% IDF criteria respectively. According to gender, 3.7% of males and 6.3% of females had MS (NCEP/ATP III), while according to IDF criteria, 7.4% of males and 10.4% of females had MS, $P > 0.05$. Using the NCEP-ATP III, 87.5% satisfied three criteria while 12.5% met all five components of the criteria. When IDF criteria were used, 78.6, 14.3, and 7.1% met three to five components respectively. Elevated BP was the commonest component of MS using both criteria. Using ATP III criteria, the factors associated with MS were abnormal waist circumference and increased waist:hip ratio ($P < 0.05$). When IDF criteria were used, the factors associated with MS were advanced age, increased BMI and waist:hip ratio ($P < 0.05$).The prevalence of IR among participants with MS was 25.0%.
Conclusion
HIV patients are not spared from the emerging epidemic of MS even in the absence of HAART. Metabolic assessment should be a routine in all HIV patients regardless of HAART treatment status.

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## P263

**Anthropometric indices: optimal cut-off values for abdominal obesity in adult Nigerians**

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Introduction
Central obesity has been linked more to adverse cardiovascular risks than general obesity. The cut-off values for BMI, waist circumference (WC), waist:hip ratio (WHR), and waist:height ratio (WHtR) have been shown to vary with ethnicity. This study sets out to determine the optimal cut-off values for obesity in adult Nigerians using four anthropometric indices.
Method
In a cross-sectional study of adults in Delta State, Nigeria, data on weight, height, waist circumference, and hip circumference were obtained and BMI, WC, WHR, and WHtR calculated. Using BMI as the standard method for diagnosing obesity, the receiver operating characteristic (ROC) analysis was used to optimise the sensitivity and specificity of the other anthropometric indices.
Results
A total of 866 participants aged 18 years and above were studied, 381 (44.0%) males and 485 (56.0%) females. The prevalence of obesity was 11.2% using a BMI $\geq 30$ kg/m$^2$. On the ROC curve, WHtR had the largest area under curve of 0.862, 0.824 for WC, and 0.655 for WHR. Optimal cut-off values being proposed among adult Nigerians is WHtR $\geq 0.6$, WC $> 104$ cm, and $> 90$ cm for males and females, and WHR $> 0.96$ and $> 0.91$ for males and females respectively.
Conclusion
WHtR is the best screening tool for diagnosis of abdominal obesity in this setting. Optimal cut-off values for WHtR, WC, and WHR may need upward reviews in this setting compared to existing cut-off values for western populations.

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## P264

**Influence of gender on the distribution of type 2 diabetic complications at the Obafemi Awolowo Teaching Hospital, Ile-Ife, Nigeria**

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Background
Gender specific differences appear particularly relevant in the overall management and outcome of type 2 diabetes mellitus (T2DM). We determined gender specific differences in cardio-metabolic risk, microvascular, and macrovascular complications in patients with T2DM.
Method
Four hundred T2DM patients matched for age and disease duration were studied. Relevant clinical information, physical examination and laboratory data were analysed.
Results
Of the 400 patients with T2DM, 190 (47.5%) were males and 52.5% were females. The mean age of the study population was $60.6 + 9.93$ years while the mean duration of DM was $7.81 + 5.76$ years. Women had higher prevalence of hypertension (83.3% vs 72.1%) and obesity (34.8% vs 14.7%) than men $P < 0.05$. Mean total cholesterol was significantly higher in women (4.45 mmol/l) than in men (4.08 mmol/l); $P = 0.001$. More women (31.4%) reached target glycaemic goals of HbA1c of $< 7.0\%$ than men (16.8%). There were no gender differences in microvascular and macrovascular complications ($P > 0.05$) but women were more likely to have moderate–severe retinopathy ($P = 0.027$) while men were more likely to have severe neuropathy (defined as greater than two abnormal tests plus symptoms); $P = 0.011$. Logistic regression analysis showed that the use of antiplatelet drugs was associated with a lower risk of microvascular complication in both men and women while diastolic blood pressure above 80 mmHg doubled the risk in men and tripled it in women ($P < 0.05$). Poor glycaemic control in men and total cholesterol above 5.2 mmol/l in men and women were associated with macrovascular complications. Waist circumference below 80 cm was protective from macrovascular complication in women.
Conclusion
Women with T2DM had worse cardiometabolic risk profile while men achieved therapeutic goals less frequently than women. Complications occurred commonly in both sexes but there was significant male predominance as severity of neuropathy worsened and female predominance as severity of retinopathy increased.

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## P265

**A study of the influence of West African ethnicity and gender on β-cell function and insulin sensitivity in essential hypertensives treated with hydrochlorothiazide and hydrochlorothiazide–lisinopril combination**

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Background
We assessed the effects of hydrochlorothiazide given alone and in combination with an ACEI on β-cell function in a negroid population to further explore possible ethnic differences in the effect of antihypertensive drugs on HOMA-IR.
Materials and methods
Eighty newly diagnosed Nigerian essential hypertensive patients were assigned to receive either hydrochlorothiazide 25 mg daily or both hydrochlorothiazide and lisinopril (25/10 mg daily) in an open label study for a period of 12 weeks. The treatment groups were well matched in clinical and demographic baseline features. Changes in HOMA-IR from baseline to end of study (week 12), fasting plasma glucose, potassium, serum insulin, and blood pressure over the same period were also evaluated.
Results
After 12 weeks, there was no statistically significant difference in delta HOMA-IR between the two groups; Blood pressure reduction was similar in both groups; mean reduction in systolic blood pressure was $17.8 \pm 10.8$ mmHg vs $18.1 \pm 15.3$ mmHg and diastolic blood pressure was $10.6 \pm 7.0$ mmHg vs $8.8 \pm 12.7$ mmHg ($P > 0.05$) for HCT monotherapy and HCT–lisinopril combination groups respectively.
Conclusions
HCT monotherapy in hypertensive indigenous Nigerians, was not associated with worse metabolic effects when compared with combination therapy using lisinopril, an ACE inhibitor after 12 weeks. Low dose thiazide diuretic as first-line antihypertensive medication may be safe in the short term, further larger and long-term studies are needed to corroborate this finding. The possibility that concurrent ACE inhibitor therapy mitigates and thiazide induced increase in insulin resistance, requires further study as well.

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## P266

**Comparison of surrogate measures of percentage body fat with bioelectric impedance in Nigerians**

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Background
Quantifying the body fat percentage (%BF), its scientific and clinical implications is critical to timely intervention as it has been proven to be a better measure of patient's risk of cardiovascular diseases than the BMI. Several techniques have been developed to assess BF including imaging, DEXA, bioelectric impedance analysis (BIA), and underwater weighing. The use of anthropometric indices to derive %BF provides an inexpensive surrogate. There are very few studies comparing this surrogate with direct measurements of %BF in Nigeria.
Objective
To determine the relationship between %BF determined with BIA and anthropometric indices.
Methodology
The study was a cross sectional study carried out on 129 (73 females and 56 males) apparently healthy subjects. BIA was used to determine the %BF using the standard protocol. Anthropometric measurements were taken and BMI was derived from the raw data. Duremberg formula, Rope and Choke equation were used to predict %BF from anthropometric indices. Anthropometric indices and %BF obtained were expressed as means and S.D.S. The estimates of %BF by BIA and the anthropometric indices were compared using Student's *t*-test and correlation test between the various %BF determined. $P$ value $< 0.05$ was regarded as significant and $r > 0.800$ considered very strong.
Results
One hundred and twenty nine subjects comprising 73 females and 56 males were studied. Mean age was $27.91 + 6.9$ years. The mean BMI was $23.11 + 4.97$ kg/m$^2$ for females and $24.22 + 3.94$ kg/m$^2$ for males. The mean %BF composition obtained by BIA was $31.79 + 9.53\%$, $20.67 + 8.04\%$, Duremberg formula $28.35 + 6.79\%$, $19.84 + 5.74\%$, and Rope and Choke equation $29.94 + 9.73\%$, $29.93 + 8.03\%$ for females and males respectively. There is a strong correlation between the %BF obtained using BIA and the anthropometric indices, $r = 0.832$ and 0.814 for females and 0.887 and 0.832 for males.
Conclusion
There is a strong correlation between BF estimation using BIA and the anthropometric indices. These surrogate measures can be used reliably to estimate %BF and hence determine the cardiovascular risk.

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## P267

**Evidence of foetal growth restriction in South-Asian foetuses of GDM mothers?**

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Research question
Are there ethnic differences in foetal growth patterns in GDM?
Methods
Retrospective data collection was undertaken for all women with GDM between 2008 and 2012 at University Hospital Coventry. Foetal biometric data was obtained at 28, 32, and 36 weeks gestation for 125 South-Asian (SA) and 142 WC.
Results
Compared to WC, SA had had lower BMI ($28.5 \pm 6.1$ kg/m$^2$ vs $32.2 \pm 7.4$ kg/m$^2$), height (159, IQR: 155–163 cm vs 165, IQR: 160–169 cm), less likely to smoke (1.3% vs 17.5%), had lighter babies ($3226.5 \pm 588$ g vs $3419.4 \pm 630$ g) despite higher FPG ($5.3 \pm 0.7$ vs $5.1 \pm 0.76$) and 2 hPG ($8.1 \pm 2.1$ vs $7.6 \pm 1.9$) mmol/l ($P < 0.01$ for all). At 28 weeks, abdominal circumference (AC) for SA and WC were similar ($243.4 \pm 18$ vs $247 \pm 16$ vs $248.3 \pm 23$, $P = 0.19$). At 32 and 36 weeks, AC of SA was lower than WC despite adjustment for maternal BMI, height, glucose values at OGTT and offspring sex ($P = 0.03$ and $P = 0.007$). Head circumference (HC) was similar in both at all gestations. Both HC:AC ratio and femur length:AC ratio was higher in SA at 28 and 36 weeks after full adjustment ($P < 0.02$).
Conclusions
Despite smaller overall size, SA showed evidence of abdominal adiposity in early foetal life. With progressing gestation there was a pattern of growth restriction with sparing of HC and lower AC compared to WC, signifying a possible adverse intra-uterine.

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## P268

**Prediabetes incidence and risk of developing cardiovascular disease in women with polycystic ovary syndrome**

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Objective
In this study we analysed the prediabetes incidence in women with polycystic ovary syndrome (PCOS), as well as prediabetes and cardiovascular disease (CVD) risk factors in women with PCOS.
Methods
This study included 148 women with PCOS, with no type 2 diabetes mellitus (T2DM) and CVD present at the baseline. We determined a comprehensive panel of biochemical parameters, including lipid profile, glucose and insulin levels during oral glucose tolerance test, levels of C-reactive protein (CRP), steroids, 25-hydroxyvitamin D (25OHD), lipid accumulation product (LAP), prolactin, TSH, and parathyroid hormone.
Results
Prediabetes was present in 18% of PCOS women and it progressed to T2DM in 3% of the cases during the three-years follow-up period. The incident prediabetes was noted in 32% or 4.7/1000 person/year. It was more common in PCOS patients with high BMI as compared to women with normal weight ($P=0.007$). There was a significant association of prediabetes incidence with levels of CRP ($P<0.001$), LAP ($P=0.001$), 25OHD ($P=0.016$) as well as insulin resistance index ($P<0.001$). The prediabetes incidence was higher among participants whose baseline levels of CRP, HOMA-IR, and LAP were in the highest versus the lowest tertile ($P<0.001$). The lowest and middle tertile of 25OHD were associated with prediabetes incidence ($P<0.05$). The results appear to show that CRP ($P<0.05$) and LAP ($P<0.01$) are the most important predictors of cardiovascular risk in PCOS.
Conclusion
Our results demonstrated the high incidence of prediabetes in PCOS women, its strong association with BMI, insulin resistance, markers of inflammation, and LAP, as well as its inverse association with serum 25OHD concentration. The evaluation of the prediabetes risk factors showed that the insulin resistance is more important than other predictors, while the most important predictors of CVD risk in PCOS women were CRP and LAP.

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## P269

**Improving glycaemic control in T1DM: the Fife Insulin and Food Education for Diabetes (FIFE Diabetes) structured education programme**

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Background
The 2013 Scottish Diabetes Survey demonstrated that only 22% of individuals with type 1 diabetes have optimal glycaemic control, defined as a HbA1c $<58$ mmol/mol. The 2014 Diabetes Improvement Plan in Scotland prioritised, 'improving the care and outcomes of all people living with type 1 diabetes'. Scotland's Diabetes Education Advisory Group has now been established. Accordingly, the Fife Insulin and Food Education for Diabetes (FIFE Diabetes) structured education programme was developed.
Methods
Since 2013, patients with poor control were provided with an information leaflet in clinic and invited to register. FIFE Diabetes is an education course for adults with T1DM using multiple injection or basal bolus insulin regimes. Aims include learning interactively about carbohydrate counting and insulin dose adjustment, improving knowledge and encouraging self-management to improve control and reduce complications. Three courses annually are led by diabetes specialist nurses and dieticians over 4 consecutive weeks. Participants are required to attend all sessions, keep a diary, have serial HbA1c measurements and complete Problem Area In Diabetes (PAID) and Symptom Awareness of Hypoglycaemia questionnaires. The paired *t*-test, McNemar's and Fisher's exact tests were used.
Results
Forty-eight patients have participated to date, with median age 41 at enrolment, and mean baseline HbA1c 79 mmol/mol. Mean HbA1c at 12 months was 71.4 mmol/mol, a 9.62% reduction (two-tailed *P* value 0.0221). The number of participants reporting significant distress and emotional burnout on the PAID tool decreased, but this did not reach statistical significance. No significant difference in hypoglycemia symptom awareness was observed between baseline and 6 months.
Discussion
Our structured education programme demonstrated significant improvements in glycaemic control in this adult cohort with T1DM. A more sensitive tool for assessing hypoglycaemia symptom awareness will be trialled. Further analyses will enable us to better predict patients most likely to benefit, improving selection to the programme.

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## P270

**Intra-Individual correlation between flow mediated dilation and reactive hyperaemia peripheral arterial tonometry in PCOS**

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Background
Endothelial damage is one of the early signs of cardiovascular disorders. Assessing endothelial function in women with polycystic ovary syndrome (PCOS) has shown evidence of cardiovascular disease in the absence of clinically evident disorder. Flow mediated dilation (FMD) and reactive hyperaemia peripheral arterial tonometry (RH-PAT) are the frequently used non-invasive techniques for assessing endothelial function. Both the techniques have been used to assess endothelial function in PCOS patients. However there is limited data on the two techniques being used simultaneously in the same individual and recent research suggests the underlying physiological mechanisms may differ as the two techniques look at different set of vessels.
Methods
FMD and RH-PAT were performed simultaneously on thirty apparently healthy normotensive women (15 PCOS and 15 controls, with a mean age of $31.5\pm7.5$ and $32\pm7.8$ years respectively), who underwent 5 min of suprasystolic cuff-induced ischemia followed by post-ischemic measurements.
Results
There were no differences in endothelial function measurements between PCOS and control groups for either FMD ($6.9\pm3.1\%$ vs $5.7\pm3.1\%$ ($P$ value $=0.14$) and reactive hyperaemic index (RHI) ($2.0\pm0.7$ vs $2.2\pm0.7$ ($P$ value $=0.51$) respectively). There was also no association between FMD and RHI ($r=0.326$, $P$ value $=0.079$).
Conclusion
The endothelial function assessed by the two techniques FMD and RH-PAT does not differ in PCOS. Both the techniques have been shown to correlate well with coronary endothelial function and can be used alternatively in PCOS. RH-PAT is advantageous as it is easy to set-up, is non-user dependent and can be used to identify individuals at risk of developing cardiovascular disease at an early stage.

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## P271

**Evaluation of hypoglycaemia unawareness in individuals with type 1 diabetes mellitus using continuous glucose monitoring in a tertiary care centre**

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Background
Intensive glycemic control forms the benchmark of management of type 1 diabetes mellitus (T1DM) and is limited by the risk of hypoglycaemia. Repeated episodes of hypoglycaemia can lead to development of hypoglycaemia unawareness and a sixfold increase in deaths in those experiencing severe hypoglycaemia. Severe hypoglycaemia occurs in 35–42% patients with T1DM. However, there is lack of data from our population and hence we aimed to objectively estimate the prevalence of hypoglycaemia unawareness in subjects with T1DM utilising continuous glucose monitoring (CGM) device.

Methods
The Modified Clarke's Questionnaire was administered to 124 subjects with T1DM (18–50 years). Of these, 40 subjects (31.7%) with documented severe hypoglycaemia, or hypoglycaemia unawareness underwent a 72 h CGM study using the Medtronic-ipro2 Device (MiniMed, Sylmar, CA, USA). Subjects also self monitored blood glucose with a glucometer (eight times a day: pre and post meal blood glucose, 1200 and 1500 h and whenever symptomatic). Data was obtained using ipro2 Software.
Results
The mean age of the subjects was 25.2 years (18–42) with a 3:2 male:female ratio. CGM documented 144 hypoglycaemic episodes in 32 subjects (25%) with 4.5 episodes/subject and more than 50% of the episodes were nocturnal. In comparison to SMBG which revealed only 83 episodes, CGM identified 42% more hypoglycaemic episodes. The mean duration of diabetes was longer in subjects with hypoglycaemic unawareness (11.7 years vs 7.6 years). The mean HbA1c of subjects with hypoglycaemia unawareness was lower (7.6%) than those without hypoglycaemia unawareness (7.78%) and partial unawareness (8.4%).
Conclusion
Hypoglycaemia unawareness is significant problem in T1DM and CGM forms an essential tool for objective assessment of hypoglycaemia unawareness.

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## P272

**Postnatal screening in patients with gestational diabetes**

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Introduction
Approximately 700 000 women give birth in England and Wales each year with up to 5% of these women having either pre-existing diabetes or gestational diabetes (GDM). Women who have diabetes during pregnancy, it is estimated that approximately 87% have GDM. It is well recognised that early diagnosis of diabetes aids in timely intervention to reduce long-term complications.
Objectives
To assess the screening for diabetes mellitus in post-delivery women diagnosed with GDM as per NICE guidelines 2015 part 1.6.
Methodology
The diagnosis of GDM was made with results of oral glucose tolerance test: fasting blood glucose (FBS) $\geq$6.1 mmol/l and 2 h blood sugar $\geq$7.8 mmol/l. 91 patients were identified with GDM between 1st January 2014 and 2nd January 2015 based on records provided by the biochemistry laboratory and diabetes centre. We checked results of these patients for FBS performed between 6 and 13 weeks post-delivery (audit standard 1). For those who did not have the test done, we checked their results for HbA1c/FBS between 13 and 20 weeks post pregnancy (audit standard 2). Exclusion: pre-existing diabetes.
Results
27% undertook a FBS test between 6 and 13 weeks. 7 and 17% of remaining 66 patients undertook FBS and HbA1c respectively 13–20 weeks post-delivery.
Conclusion
27% adhered to NICE standard 1. 16.5% adhered to NICE standard 2. 56.5% had no screening. None of the patients had OGTT after delivery showing 100% compliance.
Recommendations
Diabetes team will hand deliver blood test request forms to patients in the clinic and encourage them to be tested 6–13 weeks post-delivery. Enhance awareness in primary care by arranging audit presentations by diabetes specialist nurses at GP practices. Reminder alerts can be placed on electronic patient records with GP consensus. We aim to re-audit in 18 months to re-evaluate the effectiveness of implemented methods.

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## P273

**Prevalence of vitamin D deficiency and its association with diabetes in a South-Asian population**

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Introduction
Sri Lanka has been experiencing rapid urbanization, with ~30% of the population residing in urban areas. We report the age and sex-specific prevalence of dysglycaemia and vitamin D (VitD) status, along with the association between the two in an urban community in Sri Lanka.
Methodology
Using a stratified random sampling method, 369 subjects (116 men and 253 women) aged 18 years and above, were tested for 25OH VitD3, 75 g OGTT, and HbA1c. Demographic, anthropometric, educational, and social details were recorded using a standard proforma.
Results
The age and sex adjusted prevalence of VitD deficiency (<20 ng/ml) was 57.2% and VitD insufficiency (20–30 ng/ml) was 31%. The cumulative prevalence of VitD deficiency and insufficiency was 88.2%. Age and sex adjusted overall prevalence of diabetes was 26.9% and pre-diabetes was 32.3%. The cumulative prevalence of diabetes and pre-diabetes was 59.2%. Although, not statistically significant, the highest prevalence of VitD deficiency was found in the young adults of 18–40 years (64.2%). Females had a significantly higher prevalence of VitD deficiency at 63.7% ($P<0.000$). There was no significant difference in the different income groups. However, people of Sinhalese ethnicity had a higher prevalence of VitD deficiency (62%). Bivariate analysis using ANOVA *t*-test, to detect correlation between socio-demographic factors and VitD status found statistically significant association with female sex and VitD deficiency ($P<0.01$) Age and income status showed no statistical correlation with the VitD status. VitD status did not show a statistically significant correlation to type 2 diabetes mellitus or pre-diabetes ($P=0.977$ and $P=0.972$).
Conclusion
This study shows a high prevalence of dysglycaemia, VitD deficiency/insufficiency in urban Sri Lanka. Dysglycaemia was seen in half the population, with a large pool of subjects with pre-diabetes. Only 11.8% of the population was VitD replete. Females had a significantly higher prevalence of VitD deficiency. We could not detect a statistically significant correlation between VitD deficiency and dysglycaemia.

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## P274

**What the mouth has to say about diabetes in a Nigerian Tertiary Hospital**

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Introduction
The mouth is said to be the body's mirror. Diabetes can be associated with the development of various types of disorders within the oral cavity of which periodontal disease is about the commonest and may indeed be the first manifestation of diabetes.
Aim
To evaluate the oral health of individuals with diabetes at Federal Medical Centre, Abeokuta, Nigeria.
Methodology
Descriptive study carried out between January and December 2014. The findings during the annual dental examination in each patient with diabetes were documented alongside their HbA1c levels. The World Health Organization Community Periodontal Index was used to assess the periodontal health status.
Results
103 DM patients comprising of 45 males (43.6%) and 58 females (56.4%) participated in the study. Age ranged between 18 and 89 years with mean age of $57.14 \pm 13.06$ years. Duration of diabetes was between 1 month and 30 years with a mean duration of 6 years while mean HbA1c was 66.1 mmol/mol with a range of 22.4–130.6 mmol/mol. 15.5% had healthy periodontium while 84.5% had various periodontal disorders. 66% of those with periodontal disease had HbA1c >53 mmol/mol. 56.3% had mild gingivitis, 40.8% had moderate gingivitis while 2.9% had severe gingivitis. Of these, only 30.1% had HbA1c >53 mmol/mol. 39.8% had missing teeth; 29.1% had between one and four teeth missing, while 10.7% had between five and 15 teeth missing. There was significant association between diabetes duration and missing teeth, $P=0.002$.
Discussion
Oral lesions in diabetes are associated with glycaemic control and disease duration. There was a strong association between duration of diabetes and number of missing teeth in this study.

Conclusion
Good oral health is now one of the accepted strategies in achieving glycaemic control in diabetes. Annual oral examinations are therefore of great value.

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## P275

**Anthropometric indexes correlate of body fat percentage among Nigerians**

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Background
Obesity is significant risk factor for cardiovascular diseases and type 2 diabetes mellitus. Many anthropometric indexes are now being used in clinical practice to identify and determine the prevalence of obesity.
Aim
This study determined the relationship between common anthropometric indexes and percentage body fat (%BF).
Methodology
Ninety-four volunteer subjects participated in this cross sectional hospital based study which was carried out at our endocrine outpatients' clinic. Anthropometric parameters of all subjects were measured using the standard protocol. %BF of each subject was determined by bioelectrical impedance analysis (BIA) method according to the standard protocol with the aid of Omron BIA machine.
Results
Of the participants, 61.7% were females and 38.3% were males, with their mean age being $62.9 \pm 12.2$ years. Their mean BMI, waist circumference (WC), and waist:hip ratio (WHR) were $25.5 \pm 5.3$ kg/m$^2$, $94.5 \pm 13.0$ cm, and $0.95 \pm 0.07$ respectively. The mean %BF was $33.2 \pm 10.8$%. There was a significant and a strong positive correlation between %BF and BMI ($r=0.79$, $P<0.001$), WC ($r=0.71$, $P<0.001$). However, the %BF had a weak correlation with WHR ($r=0.13$, $P=0.200$). Meanwhile, a stronger relationship was demonstrated between %BF and WHR among men than women.
Conclusion
BMI and WC are better surrogate markers of obesity than WHR among adult Nigerians most especially for women. Therefore, routine use of WHR in our clinical practice to assess obesity should be less emphasised.

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## P276

**Lipid profile in apparently healthy Nigerian adults**

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Background
The lipid profile of healthy adults in northern Nigeria is scarcely reported in literature. Yet, hospital records show increasing frequency of dyslipidemia-related illnesses like CVA, MI, and peripheral vascular diseases in practice. We aim to report the pattern of lipid abnormalities in a rural population in Kano, Nigeria.
Methods
A population screening of apparently healthy adults resident in sub urban community in Kano was undertaken over 5 months period (November 2014–April 2015). Anthropometric indices, BP measurements, fasting plasma lipids, and fasting plasma glucose were performed. Data analyzed using SPSS version 16.
Results
A total of 1024 adults with a mean age of 41.85 (S.D. 17.62) years (age range of 18–94 years) were screened. The mean BMI, WC, and FPG were 23.265 (S.D. 4.578), 84.11 cm (S.D. 12.82296), 4.7 mmol/l (1.2) respectively. Mean WHR, weight, and height of the subjects was 0.88097 (S.D. 0.06867), 60.97 kg (12.82), and 1.619 M (0.8318) respectively. Dyslipidemia was found in 37.9% of subjects with the predominant abnormality being low HDL cholesterol 29.3%, followed by elevated LDL cholesterol 19.73%. The proportion of patients with abnormal Fasting plasma glucose was 1.78%, IFG 1.07%, and DM range 3.9%. A significant proportion of the subjects were not knowledgeable about the relevance of periodic routine medical examination and dangers of abnormal lipids.
Conclusion
A substantial proportion of adults in rural Nigeria has abnormal lipid patterns and is unaware of their health status. We recommend public enlightenment on health education to stem the tide of NCDs and improve health-seeking behavior in our population.

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## P277

**Overweight and obesity in a rural Nigerian adult population**

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Objective
To determine the prevalence of overweight and obesity and it's associated risk factors in a rural population in Kano, northwestern Nigeria.
Materials and methods
Thousand and twenty four men and women aged 18 years and above, who reside in Kumbotso ward in Kano, northwestern Nigeria were enrolled into the study using a multistage random sampling technique over a 5 months period (November 2014–April 2015). Anthropometric indices, height, weight, waist circumference (WC), and hip circumference were measured BMI, and waist-to-hip ratio (WHR) were then calculated. BP measurements, fasting plasma lipids, and fasting plasma glucose were performed. Data analyzed using SPSS version 16.
Results
A total of 1024 adults with a mean age of 41.85 (17.62) years with age range of (18–94 years) were enrolled in the study. The mean BMI, WC, and FPG were 23.265 (S.D. 4.578), 84.11 cm (S.D. 12.82296), and 4.7 mmol/l (1.2) respectively. The overall crude prevalence rates of overweight and obesity were 19.73 and 8.8% respectively. In men, 21.96% were either overweight or obese, while in women 33.06% were either overweight or obese. We observed the highest prevalence rates of overweight and obesity in the middle age group. 60.45% of the population had normal BMI (69.69 and 54.05% in men and women respectively). HDL and total cholesterol increased with increasing BMI but there was no increase in triglycerides levels with increase in BMI.
Conclusion
The prevalence of overweight and obesity is high in rural Nigerian population. It is higher in women than men. Public campaign is necessary to curb the spread of the menace especially the benefits of adopting a healthy lifestyle.

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## P278

**Comparison of glycaemic control in patients with type-1 diabetes mellitus on CSII therapy with different basal rates**

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Background
There are very few studies comparing glycaemic control in patients with different basal rates on Continuous Subcutaneous Insulin Infusion therapy (CSII), so the ideal number of basal rates for a patient is not clear.
Objective
To compare the glycaemic control between patients with different basal rates.
Methods
Data were collected from hospital's database and by contacting patients via phone.
Results
75 patients were evaluated. 46 (61.3%) were female; all had type-1 DM except for two (one had type-2 DM and the other had DM after pancreatic disease). Patients were divided in two groups based on those using $<5$ basal rates and on $\geq 5$ basal rates over 24 h. There were 33 patients in the group on $<5$ basal rates, 63% were female. Mean age was $42.7 \pm 10.3$ (mean $\pm$ S.D.) years with BMI of $25.9 \pm 3.4$ kg/m$^2$. Duration of DM was $19.3 \pm 11.0$ years and on CSII for $5.5$ years $\pm 3.4$ years. 30% patients had impaired awareness of hypoglycaemia, 51.5% used temporary (basal) rates and 72.7% used bolus calculator. There were 42 patients in the group on $\geq 5$ basal rates, 54.5% were female. Mean age was $38.7 \pm 9.3$ years with BMI of $25.9 \pm 4.6$ kg/m$^2$. Duration of DM was $19.3 \pm 9.5$ years and of CSII was $4.9 \pm 2.9$ years. 27.2% patients had impaired awareness of hypoglycaemia. 63.2% used temporary (basal) rates and 63.6% used bolus calculator. In both groups, similar number of patients (69.6%) experienced at least one episode of hypoglycaemia on average per week. Mean HbA1c in those on $<5$ basal rates was $7.8 \pm 0.8$% ($61.7 \pm 9$ mmol/mol) vs $8.08 \pm 0.7$% ($64.8 \pm 7.7$ mmol/mol) in those on $\geq 5$ basal rates-$P$ value $=0.16$.

Conclusion
In our study there was no difference in glycaemic control between the patients on fewer ($<5$) or more ($\geq 5$) basal rates. The characteristics of both groups were similar so advice on the optimal number of basal rates for a patient appears to vary from individual to individual.

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## P279

**Effects of gypenoside on pancreatic beta cell function and insulin secretion**

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Diabetes Mellitus is a chronic disease characterised by hyperglycaemia due to peripheral insulin resistance and/or insulin deficiency. This may be due to a decline in viable pancreatic β-cells number or β-cell dysfunction leading to impaired nutrient-induced insulin secretion. The most probable causes of these β-cell effects are exposure to elevated levels of inflammatory cytokines, glucose and free fatty acids. There are many synthetic and herbal drugs available for treatment of diabetes. Gypenosides are saponins extracted from the plant Gynostemma pentaphyllum, used in traditional Chinese medicine as an anti-diabetic and anti-obesity drug. Previous studies in animals showed stimulatory effects of this herbal compound on insulin secretion, although its mechanism of action is not yet fully understood. The present study aims to investigate the cytoprotective and insulin stimulatory effects of Gypenoside using the clonal insulin-secreting BRIN-BD11 cell line. Cells viability was determined by MTT following exposure to gypenoside and various cytotoxic agents for 24–96 h. Gypenoside provided significant cytoprotective effect against palmitate and peroxide-induced cytotoxicity. Gypenoside effects on insulin release under basal and high glucose concentrations were determined over 1 h and measured by ELISA. Gypenoside (100 μg/ml) enhanced insulin secretion by 4.4-fold ($P<0.001$) at 1.1 mM glucose and 3-fold ($P<0.001$) at 16.7 mM glucose. This was similar to secretion in the presence of 10 mM L-alanine with increases in insulin secretion of five and three fold at basal and high glucose, respectively. These findings indicate that gypenoside may enhance insulin secretion and protect against oxidative stress and lipotoxicity and may be useful in treatment of type 2 diabetes.

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## P280

**GPR142 plays a critical role in Tryptophan-induced insulin and incretin secretion in mice**

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Nutrient sensors mediating the stimulatory effects of food-derived carbohydrates and lipids on incretin and insulin secretion have been widely studied. However, mechanisms underlying amino acid regulation of those processes remain poorly understood. GPR142 was recently reported to be highly expressed in pancreatic beta cells and selectively activated by L-Tryptophan (L-Trp). Moreover, synthetic GPR142 agonists were demonstrated to stimulate glucose-dependent insulin secretion and improve glucose tolerance in vivo. In addition to pancreatic islets, GPR142 is also expressed in the gastrointestinal tract. However, whether GPR142 is involved in regulation of incretin release from this tissue and required for sensing of naturally occurring amino acids remains unclear. In this study, we have confirmed that L-Trp potentiates glucose-dependent insulin secretion from pancreatic islets and improves glucose tolerance in vivo, and these effects were blunted in GPR142 knockout mice (KO). Moreover, we found that oral dosing of L-Trp or the literature GPR142 agonist compound A (CpdA) increased plasma levels of incretin hormones, such as GIP and GLP-1, and these effects were absent in KO mice. Noteworthy, meal-induced elevation in plasma incretins and insulin was also reduced in KO mice. Together, our data indicate that GPR142 acts as a nutrient sensor and is critically required for L-Trp to stimulate release of insulin and incretins. Thus, GPR142 agonists may represent a novel class of therapeutic agents that leverages amino acid sensing pathways for the treatment of type 2 diabetes.

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## P281

**Inborn errors of metabolism and the endocrinologist**

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Introduction
Patients with life-threatening inborn errors of metabolism are usually managed at specialist care centres. These patients are living longer and some of them have migrated to areas far from their specialist care centres. We present two such cases.
Case 1
A 37 year old female with Ornithine Transcarbamylase Deficiency (OTCD), well-managed at a specialist care centre since childhood, relocated a few times before settling down in our area. OTCD is an x-linked disorder resulting in the inability to convert ammonia to urea as part of the urea cycle. She is at risk of lift-threatening hyperammonaemia if she gets infections or other stressful conditions. She has had several admissions and has an individualised care pathway for emergency treatment and also for peri-operative management. She is on a low-protein diet and is prescribed long-term nitrogen scavenger medications, which are not well documented in the British National Formulary for adults. She has regular outpatient follow-up with open-access for urgent plasma ammonia estimation if required.
Case 2
A patient with medium-chain acyl-CoA dehydrogenase deficiency (MCADD) well-managed at a specialist care centre since childhood is under our care because he lives too far from his specialist centre. MCADD is an autosomal recessive disorder resulting in the inability to breakdown medium-chain fatty acids to provide energy during periods of low-calorie intake (infections, fasting or vomiting). He is at risk of encephalopathy and sudden death from the accumulation of toxic fatty acids and hypoglycaemia. He has an individualised care pathway for emergency treatment and has regular follow-up in the outpatient clinic.
Conclusion
Training concerning inborn errors of metabolism needs to be incorporated into endocrinology specialist training schemes as more secondary care centres are caring for these patients. The importance of seeking advice from specialist centres and from the Inherited Metabolic Disease Groups cannot be overemphasised.

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## P282

**An unusual case of fish odour syndrome – tabloid paper helped in diagnosis?**

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Introduction
Trimethylaminuria or Fish Odour syndrome is a rare genetic metabolic disorder. It can cause significant suffering to affected individuals due to social isolation and stigma. It is under recognised and ignored.
The case
A 60 years old lady was referred by her GP. The referral – 'She feels she has a problem with personal hygiene and is aware of an odour from her skin, her breath and urine.' This lady has been suffering from this condition almost as long as she can care to remember. She has spent nearly all her money on toiletries and deodorants. Because the problem was so bad, she has had to isolate herself from her near and dear ones, from friends. Consequently, she has been suffering from depression. Her other medical condition included hypertension. Her father, she said, also had problems of malodour very similar to her. She has accepted this as part of her existence until she came across an article in one of newspaper about the existence of this condition. She went to her GP, and was referred. Clinical examination did not reveal any significant abnormality. On the day of her clinic visit, she did apply significant amount of deodorants to mask the odour.
Results
Twenty four hour urine for Trimethylamine (TMA), TMA-n-oxide, TMA/TMA-n-oxide; Trimethylamine (TMA) – 77.6 μmol/mmol creat (2.5–10.8); TMA-n-oxide 91.6 μmol/mmol creat (17.0–147.0); TMA/TMA-n-Oxide 0.85. These results are compatible with Primary or Secondary Trimethylaminuria. Primary Trimethylaminuria – a deficiency of hepatic TMA oxidation. Secondary Trimethylaminuria–enterobacterial overgrowth.
Discussion
Based on above, it appears that this lady has been suffering from Trimethylaminuria. Given the family history, it is most likely primary. The differentiation from primary and secondary could be done by DNA mutation analysis (TMAU1/FMO3 gene test). This condition is largely under recognised and has contributed to this lady's suffering for a long time. Treatment: She has been treated with 2 weeks of amoxicillin.

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## P283

### eGFR using CKD- EPI, MDRD and COCKCROFT – GAULT and glycaemic control among type 2 diabetics in an out-patient clinic in Nigeria

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Introduction
Diabetic nephropathy remains a major cause of morbidity and mortality for persons with diabetes mellitus. Globally, most patients with diabetes are in developing countries that do not have the resources or health infrastructure to provide universal renal replacement therapy. The ADA recommends that estimated glomerular filtration rate (eGFR) be calculated using serum creatinine for diabetics at least once a year.
Objectives
To determine the correlation between glycated haemoglobin and eGFR by CKD-EPI among Type 2 diabetics. To assess validity and reliability of CKD- EPI, MDRD and Cockcroft- Gault equations in diagnosing diabetic nephropathy.
Methods
A cross sectional study was carried out in the Endocrine outpatient clinic in DELSUTH, a tertiary hospital in Delta State, Nigeria. 150 patients were selected. Inclusion criteria included type 2 diabetics seen in the clinics in the last 24 months who consented in the study. Exclusion criteria included known CKD patients, very ill patients. Each patient's biodata was taken and samples were taken for FBS, HbA1c, PCV and creatinine. eGFR was calculated using the MDRD, CKD-EPI and Cockroft- Gault formulae.
Results
Hundred and fifty patients participated in the study. 79 (52.7%) were females and 71 (47.3%) males. 69 (46%) of them were in the 50–59 year- age- group. The average duration of time passed since diagnosis of DM was 8 years. There is a negative correlation between the mean HbA1c ($8.34 \pm 2.59$) and eGFR CKD-EPI ($86.95 \pm 24.69$) with correlation coefficient $= 0.13$. CKD- EPI has a sensitivity of 80%, specificity 62.9%, accuracy of 64%. MDRD has a sensitivity of 90%, specificity of 57.9%, accuracy of 60%. Cockroft- Gault has a sensitivity of 90%, specificity 44.3%, accuracy 47.3%.
Conclusion
eGFR increases as glycaemic control improves in type 2 diabetics. CKD- EPI has the highest accuracy though it also appears to have the lowest sensitivity.

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## P284

### Correlates of dysglycaemia and implications for diabetes care in Calabar, Nigeria

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Background
Besides differences in the overall prevalence between IGT and IFG, there is now clear evidence of differences in phenotype between the two categories. The most consistent and statistically significant difference is that IFG is commoner in men than women in virtually all age groups, typically being 1.5–3 times higher, but up to seven or eight times higher in Europeans aged 50–70 years. Conversely, the prevalence of IGT is higher in women than men in all age groups except over the age of 60 in Asian populations.
Objective
To determine the relationship between age, sex and the development of dysglycaemia in Calabar.
Methods
The study was a cross sectional survey of a representative sample of Calabar metropolis comprising 645 males (56.9%) and 489 females (43.1%) aged between 15 and 79 years. A multistage sampling method was applied to select participants for the study. Anthropometric data was obtained and an oral glucose tolerance test (OGTT) was performed on all participants following which participants were categorized as normal glucose tolerance (NGT), IFG, IGT and diabetes mellitus (DM). Anthropometric indices were expressed as mean (S.D.). The categorisation was done using American Diabetes Association (ADA) classification (2003) and the result in percentages.
Results
The proportion males and females with IFG (56.7% males, 50.6% females), IGT (46.3% males, 44.2% females) and Diabetes Mellitus (64.7% males, 60.9% females) was highest in the middle age group. The prevalence of various forms of dysglycaemia was significantly higher in males than females; IFG (9.3% vs 8.2%), IGT (21.1% vs 17.6%) and DM (7.9% vs 4.9%).
Conclusion
Age and sex differences are important risk factors for dysglycaemia and the tendency for prevalence of IGT to increase in all age groups may have implications for diabetes care.

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## P285

### Prevalence and risk factors for obesity among HIV patients in Kano, north western Nigeria

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Background
Weight gain is a recognised effect of HAART which occur through restoration of the body's immune function and lipodystrophy. Weight gain could have a negative impact on the medical and social wellbeing of the persons affected. Most HIV studies in Nigeria tend to concentrate on the effect of HAART on viral suppression and improvement in quality of life neglecting other consequences of the disease and treatment. We aimed to determine the prevalence of obesity among HIV patients, and to compare between HAART-exposed and HAART-naïve participants.
Methods
HIV positive patients attending the Aminu Kano Teaching Hospital HIV clinic were evaluated. Their anthropometric indices were determined and BMI calculated using the Quetelet's equation.
Results
A total of 300 HIV seropositive patients (150 HAART exposed and 150 HAART naïve) were assessed. The mean $\pm$ S.D. age of the study participants was $34.8 \pm 9.9$ years with a male:female ratio of 1:2. Mean $\pm$ S.D. BMI of the HAART-exposed and HAART-naïve participants was $24.0 \pm 6.0$ kg/m$^2$ and $22.0 \pm 5.6$ kg/m$^2$ respectively, ($P = 0.001$). The prevalence of obesity among all the participants is 11.7%. Among the HAART exposed participants, 23 (15.3%) were obese, 25 (16.7%) overweight, 83 (55.3%) normal weight and 19 (12.7%) underweight. In the HAART-naïve group, 12 (8.0%) were obese, 21 (14.0%) overweight, 71 (47.3%) normal weight and 46 (30.7%) underweight. Exposure to HAART was found to be significantly associated with the development of obesity, $P = 0.04$. Other factors that were found to be associated with obesity include longer duration of HIV infection, raised CD4 cell count, increased waist circumference and family history of diabetes mellitus ($P < 0.05$). Abdominal obesity from lipodystrophy appeared to be an independent predictor of obesity.
Conclusion
Prolonged HIV infection and HAART-exposure are associated with increase in BMI and development of obesity which could have both negative social and cardio-metabolic consequences on the patients affected.

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## P286

### Relationship between three indices of central obesity and fasting plasma glucose

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Objectives
Obesity is a risk factor for Type 2 diabetes mellitus. This study evaluated the relationship between central obesity and fasting plasma glucose (FPG).
Materials and methods
A cross-sectional study involving five hundred and twenty one (521) adult participants (134 males and 387 females) of a community health survey in Sagamu, Isara and Ode-Remo, Nigeria. The waist circumference (WC), waist-to-hip ratio (WHR) and waist-to-height ratio (WHtR) of the participants were

determined. Central obesity was defined as (1) WC ≧94 and ≧80 cm in men and women respectively; (2) WHR ≧0.90 and ≧0.85 in men and women respectively; (3) WHtR ≧0.50 in both men and women. FBG was measured in the morning after an overnight fast and was determined by the glucose oxidase method. Data were analysed with SPSS version 20.
Results
Compared with men, women were significantly older (50.1 ± 15.3 years vs 44.2 ± 16.9 years, $P<0.001$), and had higher mean WC (84.6 ± 15.2 cm vs 79.2 ± 12.4 cm, $P<0.001$), WHR (0.88 ± 0.09 vs 0.91 ± 0.61 $P<0.01$) and WHtR (0.54 ± 0.09 vs 0.48 ± 0.07, $P<0.001$). There was no significant difference in the mean FPG in men (83.25 ± 16.54 mg%) and women (84.13 ± 22.29 mg %), $P>0.05$. In men, there was a positive but weak correlation between FPG and central obesity (WC, 0.333, $P<0.001$; WHR, 0.180, $P=0.038$; WHtR, 0.282, $P=0.001$). There was insignificant correlation between FPG and central obesity in women. The mean FPG of obese men were significantly higher than those who were not obese (94.62 ± 25.86 mg% vs 81.14 ± 13.29 mg%, $P<0.05$ with WC; 86.56 ± 20.09 mg% vs 80.04 ± 11.42 mg%, $P<0.05$ with WHR; 89.23 ± 20.34 mg% vs 79.92 ± 12.92 mg%, $P<0.01$ with WHtR). The mean FPG of obese women was insignificantly higher than those who were not obese.
Conclusions
There was positive correlation between central obesity and FPG. Men with central obesity had higher FPG compared with men who were not obese.

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## P287

**How well do front line healthcare professionals understand type 2 diabetes?**

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The in-hospital pharmacological management of patients with diabetes has recently been identified by the National Diabetes Inpatient Audit as a key safety issue. Over 30% of inpatient drug charts surveyed in the 2013 audit had at least 1 diabetes medication error in the preceding week. Patients with a diabetes medication error on their drug chart were also twice as likely to have one or more hypoglycaemic episodes during their hospital stay.
A paper by Browne *et al*. in 2000 revealed important gaps in health professionals' and patients' knowledge of oral hypoglycaemic agents. New classes of anti-hyperglycaemic agents have since made the management of type 2 diabetes increasingly complex.
Our study evaluated the current knowledge of oral hypoglycaemic agents and insulin amongst healthcare professionals and final year medical students in a regional teaching hospital. 57 junior doctors, 19 staff nurses and 19 medical students were assessed using an anonymous questionnaire.
Results revealed poor knowledge of the mechanisms of action, timing of administration, main side effects and cautions of traditional hypoglycaemic agents amongst both healthcare professionals and students. Under 33% of respondents, for example, knew the main cautions of common sulphonylureas. Knowledge of newer hypoglycaemic agents such as GLP1 agonist, DPPV inhibitors and SGLT2 inhibitors was significantly worse than older classes (eg biguanides and sulphonylureas). Additionally, there were gaps in knowledge relating to the management of insulin sliding scales and of the pharmacology of insulin.
Unawareness of issues central to the safe prescribing and administration of diabetes medication has implications for both patient safety and patient education. This study highlights the need for greater emphasis on teaching healthcare professionals about management of type 2 diabetes at a both an undergraduate level and in the workplace.

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## P288

**Accuracy and reliability of glucose meters used at diabetes clinic of Oauthc Ile Ife**

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Background
Self-monitoring of blood glucose (SMBG), an important tool in diabetes management help patients achieve and maintain target glycaemic levels hence reducing complications. SMBG is usually done with glucose meters which are affordable, portable and easy to use. Significant variations sometimes observed in glucose meter readings necessitated this study. We evaluated the accuracy of glucose meters used routinely in our unit using ISO 15197 guideline.
Methods
Three glucose meters were evaluated: On-CallPlus (ACCON Biotech), Accu-chek (Roche), and Easymax (EPS BioTechnology Corp). Random glucose meter readings of 49 diabetic on routine clinic visit were compared to a simultaneously conducted standard laboratory measurement using glucose oxidase method. Data obtained were analysed using Statistical Packages for Social Sciences.
Results
The glucose meters, Accuchek, OncallPlus, Easymax had 45.5, 27.3 and 18.2% of samples it read as >75 mg/dl within the target range of ±15 mg/dl of the reference instrument. None met the ISO 15197 target of 95%. While for all samples the reference instrument read as >75 mg/dl; 57.9, 28.9 and 7.9% of Accuchek, Easymax and Oncallplus readings respectively were within ±20% accuracy. Accuchek had the highest accuracy of 27.3% for samples <75 mg/dl within the target range of ±10 mg/dl and ±5 mg/dl to the reference instrument. Pearson correlation analysis of meter and laboratory readings were Accuchek (.980), Easymax (.983) and OnCallPlus (.971) respectively. Accuchek had the least mean absolute deviation from standard test results at 20% and thus was most accurate while OnCallPlus was least accurate at 50%.
Conclusion
The three glucose meters tested varied in their accuracy and consistency when compared to standard laboratory procedure. This should be borne in mind when interpreting test results and selecting self-monitoring tools.

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## P289

**Behavioural risk factors for diabetes mellitus: a study of secondary school staff in a community in south west Nigeria**

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Introduction
The prevalence of type 2 Diabetes is increasing worldwide. It is associated with significant morbidity and mortality, hence the need for early identification of risk factors. This study therefore aimed to determine the overall knowledge of the participants about Diabetes Mellitus and to assess its risk factors.
Method
A cross sectional survey of 118 staff of three secondary schools in Owo was conducted using a structured self-administered questionnaire. Weight, height, waist circumference, hip circumference, blood pressure and fasting or random blood sugar measurements were done using standard methods. Data was analysed with SPSS version 21.0. Descriptive statistics were done. Associations were explored with the chi square test and Pearson correlation at 5% level of significance.
Results
Mean age of participant is 43 + 8 years, 59.3% were female, 66.9% had a good knowledge of Diabetes Mellitus. In all, 13.6% smoke cigarette, 35.6% drink alcohol, 49.2% does not add at least a spoonful of vegetable to their meals, 27.1% does not engage in physical exercise while 78.0% do not eat fruits at least once a day. About 75% of the respondents had at least one risky behaviour. Type 2 DM was found in 2.8% of the participants, 18.5% were hypertensive, 22.0% were Obese while 25.4% had abdominal obesity. More male respondents 22.9% had self-perceived risk of DM ($P=0.001$), 40.0% of female respondents had abdominal obesity ($P<0.001$). Correlation ($r=0.347$) exist between Waist/Hip ratio and random blood sugar, ($P=0.012$).
Conclusion
Despite good knowledge of DM, many had at least one risky behaviour. Intensifying efforts on educating the general population on the risk factors for DM and lifestyle modification is important.

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## P290

**Influence of deuterium depleted water on indicators of prooxidant-antioxidant and detoxifying systems in experimental diabetes**

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Studying the effect of deuterium depleted water (DDW) on isotope (D/H) composition and condition of an antioxidant-prooxidant plasma and lyophilized tissues of internal organs (liver, kidney) balance appropriate indicators in rats were compared: in group 1 ($n=15$) consumed mineralized water (150 ppm), which by a single intraperitoneal injection of alloxan (at a dose of 17 mg/100 g body weight) was established experimental model of diabetes; group 2 ($n=15$) consumed mineralized water (40 ppm) for 30 days before creation of a similar model of experimental diabetes. On day 5 after modeling of alloxan diabetes in rats of both groups was observed increase in blood glucose level in 2.2 times, increase in the activity of enzymes (aspartate aminotransferase, alanine aminotransferase), characterizing cytolytic processes, increasing concentrations of creatinine, bilirubin and urea.

It was found that in group 2 on 45 day drinking 40 ppm water deuterium content in the blood plasma was reduced to $99.7 \pm 0.4$ ppm ($P<0.05$), which was on 34.1% lower in comparison with the group 1 ($P<0.05$). In addition, the integral index of the functioning of the low molecular unit prooxidant-antioxidant blood system (COMBer – patent number 2236008 RU) in group 1 was 17.9% higher than in group 2 ($P<0,05$), indicating that the perspective of DDW using at the complex correction of metabolic disorders in the antioxidant system, observed during the development of diabetes.

Also observed a decrease in the concentration of endogenous toxic substances in the blood of rats from group 2, which was confirmed by lower (on 27.1%, $P<0.05$) integral index values of endogenous intoxication in group 2 (94.2% hypercatabolism) compared with the group 1 (129.3% hypercatabolism), that indicating on increased functional activity of detoxifying system and increasing of nonspecific organism resistance when DDW administered in the rats diet.

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## P291

**Renal function in type 2 diabetes patients at presentation in a tertiary hospital in Nigeria – a preliminary report**

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Introduction

The prevalence of type 2 diabetes is increasing worldwide. It is associated with significant morbidity and mortality, with majority of the patients presenting for the first time with complications. We set out to determine the renal function of type 2 DM patients presenting in our facility.

Method

A cross sectional study involving 50 type 2 diabetes patients. At presentation to the DM clinic, these were interviewed using a structured questionnaire to obtain the demographic data and clinical history. Anthropometric parameters, blood pressure, fasting blood glucose, glycated haemoglobin and serum creatinine were all assessed. Data were analyzed with SPSS version 21.0. Descriptive statistics were done. Associations were explored with the $\chi^2$-test and Pearson correlation at 5% level of significance.

Results

The mean age of subjects is $56.4+14$ years, 58% of them were females. The average duration of Diabetes was $6.5+5.7$ years. The mean systolic and diastolic blood pressures were $131.7+21.2$ and $81.7+10.5$ mmHg respectively. The average glycated haemoglobin was $9.2+2.4$%. Of the patients seen, 30.3% were in CKD stage 1, Stages 2, 3 and 4 were 32.6, 30.7 and 7% respectively. None was in stage 5 (ESKD). The HbA1c negatively correlated with the CKD stage but it was not statistically significant ($P=0.78$, $r=-0.056$).

Conclusion

Patients in this series have declining renal function with worsening glycated Hb at first presentation. Early nephrology referral is indicated in patients in our environment.

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## P292

**Bloods glucose monitoring in diabetic inpatients – hyperglycaemia**

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Background

Hypoglycaemia is considered a medical emergency and is treated as such. However, our experience as junior doctors has shown us that hyperglycaemia, despite being equally damaging to patients' long term prognosis, can often be left to 'run high'. This could be due to a number of factors, surrounding how often blood sugars (BM) are checked and how they are managed. We audited three standards set out in local guidelines for BM monitoring in diabetic patients, focussing on hyperglycaemia.

Method

The audit involved monitoring diabetic patients on three general medical wards over a 2 week period. 24 diabetic patients were admitted, four T1DM and 20 T2DM. We retrospectively audited the BMs for each diabetic patient over the previous 24 h against three standards: 100% of diabetic patients should have their BMs checked four times a day (QDS); T1DM should have their ketones checked in 100% of hyperglycaemic episodes (defined as BM $>16$ mmol/l); 100% of hyperglycaemic episodes should be treated.

Results

Across the three wards QDS BMs were achieved only 71% of diabetic inpatients; in T1DM with hyperglycaemic episodes, only 73% had their ketones checked; only 35% of hyperglycaemic episodes were treated; of these 35%, there was a significant prescribing variation.

Conclusions

Poorly controlled BMs are responsible for extensive inpatient morbidity across all disciplines. Maintaining healthy BMs will optimise patients' recovery and expedite their discharge. The above standards should be achieved 100% of the time. We have presented the results of this audit at our local audit meeting and to nursing staff. Subsequently, the BM chart has been improved to prompt QDS BMs, checking of ketones and treating hyperglycaemia. We also recommended standardisation of fast acting insulin prescription for the treatment of hyperglycaemia. We will be re-auditing next week and the results will be presented.

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## P293

**Case report of a type 2 DM patient with vitamin B12 deficiency anaemia**

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A 54 year-old businesswoman who has been diabetic for 6 years and had a history of PUD. She was referred following complaints of increasing hyperpigmentation of her palms, feet and old scars. She also had a boil on her back, significant weight loss, poor appetite and a slight non-productive cough.

She was transfused with two pints of blood a month earlier at a private hospital when her PCV was found low. She is not hypertensive. Her drug history included metformin, glimepiride, rabeprazole, antacid and hematinics.

On examination, she was obese (BMI 33.8 kg/m$^2$), pale, afebrile, anicteric and had no pedal oedema. Her pulse rate was 80 beats/min, regular and of normal volume and the blood pressure was normal. Apart from the hyperpigmentation of her palms and feet, other systems were normal. Her HbA1c 8%, eGFR was 36.1 ml/min, Serum Vitamin B12 was 109.4 pg·ml (211–946), Blood count showed pancytopenia – Hb 6 g/dl, MCV 97.8 fL, MCH 2.6 pg, MCHC 33.3 g/dl, WBC $3.34\times10$^9/l, RBC $1.84\times10$^9/l, Platelets 137x10^9/l. Her bone marrow study showed megaloblastic changes in the marrow and peripheral film. A chest radiograph showed no lesion. HIV1 & 2 serology were negative. Her TFT, ACTH and cortisol were normal. Investigations to exclude pernicious anaemia and celiac disease could not be done for financial reasons.

She was treated as a case of vitamin B12 deficiency anaemia probably metformin-induced with intramuscular hydroxycobalamin 1000 µg weekly for 6 weeks, then monthly for 6 months. She presently takes daily oral B12 and is healthy. Her repeat laboratory workup showed HbA1c 6%, Hb 11.2 g/dl, normal MCV and MCH, WBC, RBC and platelet counts. Serum B12 302.8 pg/ml and eGFR of 109.1 ml/min.

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## P294

**Relationship between anthropometric indices and insulin resistance in Nigerians with type 2 diabetes mellitus**

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Background/aims
Insulin resistance is predominant in type 2 diabetes mellitus (DM) patients. Obesity is a risk factor for insulin resistance. This study aims to determine the relationship between indices of nutriture and insulin resistance in type 2 DM patients.
Methods
This is a cross-sectional study of type 2 DM patients attending the Diabetes Clinic, Nnamdi Azikiwe University Teaching Hospital, Nnewi, Anambra State, Nigeria and evaluated over a 6 week period. Variables studied include age, BMI, waist circumference (WC), waist-to-hip ratio (WHR), HbA1c and Homeostasis Model Assessment for Insulin Resistance (HOMA-IR) score. Statistical analysis was done using Statistical Package for the Social Sciences (SPSS) version 20.Data were expressed as mean (median) and percentages. Continuous variables were compared with the Mann-Whitney *U* test. Spearman rank correlation coefficient (rs) was used to test association between continuous variables while linear regression models were used to predict HOMA-IR scores from indices of nutriture. $P<0.05$ defined statistical significance.
Results
Seventy-two patients with complete data participated in the study (33 (45.8%) males and 39 (54.2%) females). Mean age of patients was 57.0 (58.0) years though females were significantly older than the males ($P=0.000$). BMI ($P=0.002$), WC (p=0.09), WHR ($P=0.348$), HbA1c ($P=0.932$) and HOMA-IR ($P=0.109$) were higher in males. Waist-to-hip ratio correlated strongly with HOMA-IR (rs=0.284, $P=0.016$) while BMI correlated weakly with HOMA-IR (rs=0.053, $P=0.658$). Only WHR significantly predicted HOMA-IR ($P=0.004$).
Conclusion
Waist-to-hip ratio correlated strongest and significantly predicted insulin resistance in type 2 DM Nigerians.

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## P295

**Assessment of the relationship between obesity indices and lipid parameters among Nigerians with hypertension**

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Background
Both obesity and presence of certain patterns of lipid abnormalities has consistently been identified as a strong determinants of cardiovascular health. In resource limited populations, it is important to ascertain methods of reducing resources utilisation by identifying simple measures to cardiovascular risk. Considering the relative ease of measuring anthropometries (BMI, waist circumference-WC, and waist-hip ratio-WHR) data, we sought to determine if any measure of anthropometric measures could be used to identify persons with hypertension who have lipid abnormalities associated with atherogenicity.
Methods
This is a cross-sectional study in which 414 participants newly diagnosed with essential hypertension formed the study population. Demographic and anthropometric data including weight, height, waist and hip circumferences were obtained. Fasting serum lipids including total cholesterol, HDL-C and triglycerides (TG) were measured. LDL-C was calculated by Frieldewald formula. Statistical analysis was done to determine the relationship between anthropometric indices and lipid profile levels.
Results
The study population consisted of 124 male and 290 female subjects with a mean age of 66±16.95 years (range, 30–100 years). The female subjects were older than the male subjects ($P=0.020$). Eighty five percent, 58.5% and 30.7% of the study population had abnormal waist circumference (WC), abnormal waist-hip ratio (WHR) and BMI $>25$ kg/m$^2$ respectively. Decreased HDL-C (70.1%) was the commonest lipid abnormality found followed by elevated LDL (6.0%). None of the anthropometric indices independently predicted abnormal lipid levels; older age and female sex independently predicted occurrence of at least one serum lipid abnormality.
Conclusion
None of the measures of obesity independently predicted abnormal lipid levels in newly diagnosed hypertension. Female gender, older age and systolic blood pressure were independently associated with abnormal serum lipids.

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## P296

**Hyperuricaemia and peripheral arterial disease in patients with diabetes mellitus**

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Introduction
The objective of this Report is to evaluate for a possible association between serum uric acid levels and PAD in persons with diabetes mellitus.
Methods
This was a cross sectional Study carried out in 232 DM patients, Anthropometric indices and ankle brachial pressure indices were documented. Biochemical analyses were carried out for lipid parameters, glycosylated haemoglobin and uric acid.
Results
The mean age and age range of the study subjects were 61.1 (10.6) years and 28–87 years respectively. A total of 92 patients (39.7%) had PAD (ABI of $\leq 0.9$). Hyperuricaemia was found to be a possible predictor of PAD (OR 0.51, 95% CI 0.27–0.98, $P=0.004$). There was however a positive and significant correlation between duration of DM ($r=+0$; 14, $P=0.02$), glycosylated haemoglobin ($r=+0.13$, $P=0.04$) and ankle brachial pressure indices.
Conclusion
Elevated uric acid level is a potential risk factor for PAD in persons with DM.

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## P297

**Similia similibus curantur: using DPP-IV inhibitor to treat reactive hypoglycaemia**

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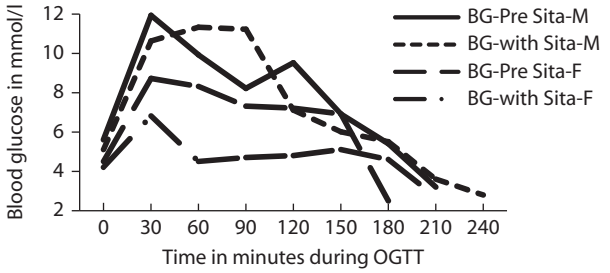
Introduction
Reactive hypoglycaemia (RH) is a complex problem, with no definite treatment apart from lifestyle changes.
Methods
We used Sitagliptin in two patients with RH, who had tried lifestyle changes for at least 2 years but without much effect. A gentleman with initial BMI of 31.4 kg/m$^2$ and another lady of BMI 24.1 kg/m$^2$ was treated with Sitagliptin 100 and 50 mg daily, in view of their weight and thereby volume of distribution, with informed consent about this unlicensed indication.



Results
Within a month, the incidence of hypoglycaemia had gone down to none and in 6 months' time, the gentleman had lost 11.1 kg, while the lady's weight remained stable. OGTT performed before and 6 months after treatment with Sitagliptin taken in the morning of the test showed reduction in glycaemic excursions and delay in the onset of hypoglycaemia in both these patients.
Conclusion
DPP-IV inhibitors by their property of glucose mediated insulin release and slowing of gastrointestinal motion, can be useful adjuncts to patients with RH. Further studies on larger number of patients need to be done.

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## P298

**Clinical profile of persons with diabetes attending an endocrinology clinic in a tertiary hospital in rural Nigeria**

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Background
Diabetes mellitus (DM) is a leading cause of morbidity and mortality globally. Its prevalence is increasing worldwide due to the transition phase. The present prevalence of DM is projected to double in the year 2035.
Materials and methods
This is an ongoing prospective observational study. Subjects were persons who had been diagnosed of DM using the World Health Organization (WHO) criteria, and had been on treatment for at least six months. Standardised questionnaires were administered, biodata, and clinical profile of subjects were obtained.
Results
A total of 92 subjects have so far been recruited. Most of the subjects have T2DM (89.1%). The mean (S.D.) for age was 56.52 (15.8) years. 50% were males. The mean fasting blood sugar (FBS) for 63 subjects who had their FBS completed was 155.60 (75.3) mg/dl with a range of (61–389) mg/dl. Subjects who had abnormal BMI were 67%. Metformin was the most commonly used drug with 66.3% of subjects on it. The percentages of those on glibenclamide, glimepiride, insulin, and vidagliptin were 19.6, 26.1, 37.0, and 1.1% respectively. An optimal glycemic control; was achieved in 30.1% of the subjects (FBS $<$110 mg/dl). Eighty-three subjects had completed data for duration of diabetes with a mean of 4.9 (4.7) years.
Conclusion
Management of diabetes constitute a great challenge in a rural setting Several factors such as poor compliance contribute to this problem. Routine education of patients is therefore advocated.

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# Pituitary

## P299

**A role for $^{11}$C-methionine PET/CT–MRI in the management of *de novo* and residual acromegaly**

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Background
Although MRI remains the investigation of choice for pituitary imaging, it does not provide information about the 'functionality' of lesions (e.g. residual adenoma vs post-surgical scar tissue), and cannot reliably identify all microadenomas. These limitations are of particular relevance in acromegaly where clinical and biochemical evidence of disease activity mandates (further) treatment.
Methods
We hypothesised that i) imaging with the PET ligand $^{11}$C-methionine, which is taken up at sites of peptide/protein synthesis, would permit more reliable identification of functioning pituitary adenoma in patients with acromegaly and ii) co-registration of PET–CT with SPGR/volume MRI would yield more accurate anatomical localisation of the site(s) of $^{11}$C-methionine uptake.
Results
Thirty patients with acromegaly were scanned in our centre using this technique between 2011 and 2015. All patients had indeterminate MRI appearances with respect to primary or residual/recurrent adenomas. $^{11}$C-methionine PET co-registered with SPGR/volume MRI provided additional information to inform management in all but one patient. More specifically, we found that $^{11}$C-methionine PET: i) reliably identifies the primary culprit lesion (e.g. when two separate candidate lesions are present on MRI); ii) defines surgical targets in patients who have been deemed not to be surgical candidates based on MRI appearance (e.g. partially empty sella); iii) delineates targets amenable to repeat surgery or targeted radiotherapy in post-op patients with persistent disease but inconclusive MRI findings (e.g. residual adenoma vs post-operative change/scar tissue); and iv) identifies areas of residual adenoma after failed radiotherapy or gamma knife therapy.
Conclusions
To our knowledge, this is the largest series of patients with acromegaly evaluated with $^{11}$C-methionine PET/CT–MRI. We have found that this technique offers significant advantages in different clinical scenarios when MRI is inconclusive, thus confirming its potential to contribute to better care and outcomes in patients with acromegaly.

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## P300

**The effect of AIP on AHR transcriptional activity: implications for *AIP* mutations pathogenicity**

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Background
*AIP* mutations cause sporadic and familial pituitary adenomas, but establishing the pathogenic role of missense *AIP* variants with unknown significance is difficult. The AIP interaction partner AHR – a xenobiotic-activated transcription factor – regulates transcription of xenobiotic-metabolising enzymes, mediates xenobiotic toxicity, and has been implicated in tumorigenesis.
Aim
To describe the effect of *AIP* dosage and mutations on AHR-dependent transcription and use it to assess *AIP* variant pathogenicity.
Materials and methods
*Aip* was knocked-down (KD) by *Aip*-siRNA transfection or *Aip*-shRNA lentiviral transduction in the GH3 rat somatotrophinoma cell line. WT or mutant human-AIP were over-expressed by transfection. AIP protein levels were assessed by immunoblotting. Expression levels of *Aip*, *Ahr*, and *Cyp1a1* (an *AHR* target gene) were measured by RT-qPCR. AHR-dependent transcription was stimulated with an endogenous ligand, 6-formylindolo[3,2-b]carbazole (FICZ).
Results
Immunoblotting confirmed endogenous *Aip* KD and efficient expression of transfected human-*AIP* unaffected by RNA-interference. siRNA *Aip*-KD caused a significant reduction of *Ahr* and *Cyp1a1* mRNA levels, in both FICZ and vehicle-treated cells. In stable *Aip*-KD GH3 cells, reduced *Cyp1a1* expression was rescued by WT-AIP transfection, but not by the pathogenic p.R304* truncation mutant. The p.C238Y and p.R271W pathogenic missense *AIP* variants did not rescue *Cyp1a1* expression, while the likely pathogenic (based on clinical data) p.R304Q and non-pathogenic p.R16H displayed an intermediate rescue.
Conclusions
AIP deficiency through KD or mutation is associated with reduced AHR expression and transcriptional activity. The effect of *AIP* variants on AHR-dependent transcription is a potential measure of mutation pathogenicity.

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## P301

### Investigation of the invasive phenotype of AIP-mutated pituitary adenomas

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Background
Heterozygous germline *AIP* mutations can lead to young-onset invasive GH-secreting adenomas. There are no data available to explain the proliferative and invasive nature of *AIP*-mutation positive somatotrophinomas.
Methods
Cell viability (MTS assay), invasion (single cell fluorescence invasion assay) and migration assays (Boyden chambers) were used to further characterise the phenotype of AIP-silenced GH3 cells. Affymetrix gene expression-profiling was performed on AIP-silenced GH3 cells vs. scrambled control. Results were validated by RT-qPCR and Western blotting.
Results
Lentiviral AIP shRNA-silenced cells proliferated more quickly than controls by 48 hours (25% increase in absorption in MTS assay, $P<0.0001$). In the RNA profiling study the *AIP*-knockdown differential expressed gene list was enriched for transcripts encoding proteins regulating actin cytoskeleton-associated activities (e.g. CDC42, WASL, ARPC2/3, WASP), and thus cell migration and invasion. The small Rho GTPase CDC42 was up-regulated at the RNA (↑43%, $P=0.0152$) and protein level (↑57%, $P=0.0156$). GH3 cells were unable to migrate on a plain plastic surface. However, unlike NT shRNA transduced controls, *AIP*-silenced GH3 cells were able to migrate in response to collagen I and FCS-containing medium, in Boyden chambers coated with collagen IV. Collagen IV is known to be abundant in the pituitary capsule. AIP-silenced GH3 cells showed threefold increased adherence ($P=0.029$) and twofold increased invasion ($P<0.0001$) through type IV collagen matrix.
Conclusion
CDC42 is a small rho GTPase, which is upregulated in various cancers and is associated with lung cancer metastasis. We propose that AIP silencing leads to CDC42 upregulation and that this results in signalling through actin cytoskeleton pathways, promoting filopodia and invadopodia formation. Further, we suggest that the predilection for adherence, migration across and invasion through type IV collagen matrix is a factor in the increased invasiveness of AIP mutated tumours. CDC42 may represent a druggable target for treatment of atypical adenomas.

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## P302

### Menin regulates the expression of miR-15a, which is downregulated and inversely correlates with cyclin D1 expression in mouse *Men1*-associated pituitary tumours

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Multiple Endocrine Neoplasia type 1 (MEN1) is an autosomal dominant disorder characterised by the combined occurrence of parathyroid, pituitary and pancreatic islet tumours, and is due to mutations of the MEN1 gene, which encodes the tumour suppressor protein menin. MicroRNAs (miRNA) are non-coding single stranded RNAs that post-transcriptionally regulate gene expression. Alterations in miRNA expression, including downregulation of two miRNAs, miR-15a and miR-16-1, have been reported in several tumour types including pituitary adenomas, however their contribution to MEN1-associated tumourigenesis is unknown. In this study we therefore investigated the expression of miR-15a and miR-16-1, in pituitary tumours from a MEN1 mouse model ($Men1^{+/-}$). We confirm that both miR-15a and miR-16-1 are downregulated in $Men1^{+/-}$ pituitary tumours compared to normal, wild-type pituitaries (2.3 and 2.1-fold, respectively; $P<0.003$). Furthermore, we investigated the expression of the miR-15a/miR-16-1 target, Ccnd1, and found *Ccnd1* was significantly upregulated in $Men1^{+/-}$ pituitary tumours, compared to wild-type pituitaries (2.5-fold; $P<0.005$); *Ccnd1* expression was also significantly inversely correlated with the expression of miR-15a and miR-16-1 ($R^2=0.81$ and 0.78, respectively; $P<0.005$). To confirm that cyclin D1 upregulation was a direct result of miR-15a/miR-16-1 loss, we inhibited the activity of miR-15a and miR-16-1 in a human cell-line (HeLa), using antagomirs. Reduction of miR-15a and miR-16-1 significantly increased cyclin D1 expression (3.2 and 3.8-fold, respectively; $P<0.01$); but had no effect on menin expression. To further examine the relationship between miR-15a/miR-16-1 and menin we silenced menin, using siRNA, in HeLa cells. Silencing menin had no effect on miR-16-1 expression but significantly decreased miR-15a expression (twofold, $P<0.008$). Thus, our study demonstrates that expression of the cyclin D1 suppressors, miR-15a and miR-16-1, is decreased in $Men1^{+/-}$ pituitary tumours, and that downregulation of miR-15a could be a direct result of menin loss.

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## P303

### An association between visual morphometric changes and psychiatric outcomes in patients with acromegaly

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Background
Acromegaly (Acro) is associated with adverse psychopathological and personality outcomes despite being in remission for over a decade. We hypothesised that persistently poor psychological outcomes may be associated with morphometric changes experienced by Acro patients.
Methodology
We compared 60 consecutive non-functioning adenoma (NFA) and Acro patients who had undergone pituitary surgery and/or radiation therapy (RT). A gender-specific visual morphometric scale was developed grading patients from 0 (no obvious acromegaly changes) to 6 (advanced acromegaly changes). All patients underwent standardised photographs that were independently graded by several physicians and filled several questionnaires regarding quality of life, psychological and personality outcomes.
Results
A total of 51 Acro and 48 NFA patients completed the study. Mean ages were 58.29 years (Acro) and 60.13 years (NFA) and females constituted 54.8% (Acro) and 52.1% (NFA) (Both $P=$NS). Of acromegaly patients 11 were in remission without medical therapy while 37 were controlled on medical therapy. Mean morphometric scale rating in NFA was 0.41 and Acro was 3.5 ($P<0.001$). Patients with Acro were significantly more likely to have affective lability ($P=0.04$), cognitive dysregulation ($P=0.025$), identity disorder ($P=0.04$), apathy ($P=0.02$), poor self-esteem ($P=0.01$) and dissatisfaction with appearance ($P<0.001$). The was no association between age at diagnosis, gender, surgery, RT, hormonal dysfunction at presentation, medical therapy for acromegaly, GH and IGF-1 at presentation and recent, malignancy and psychological outcomes. However, there was a strong association between advanced morphometric changes and cognitive dysregulation, intimacy problems, restricted expression, satisfaction with personal appearance, personal relationship, self-esteem and appraisal ($P=0.001$ -0.04). Women were more likely to have identity problem ($P=0.009$) and dissatisfaction with appearance ($P=0.03$) while men were more likely to have conduct problem ($P=0.007$) and self-harm ($P=0.04$).
Conclusion
Our data for the first time show a strong gender-specific association between morphometric changes and adverse psychological outcomes.

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## P304

**The founder R304* *AIP* mutation is prevalent in Irish acromegaly and gigantism patients as well as in the general population of Ireland**

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Background
A founder mutated *AIP* allele, R304* was previously identified in several Irish familial isolated pituitary adenoma (FIPA) pedigrees from a small region within Mid Ulster, Northern Ireland, but the allele's general population impact remains unknown.
Aims
To estimate R304* prevalence in the general population and pituitary adenoma (PA) patients and to calculate the allele's time to most recent common ancestor (tMRCA).
Methods
We tested for *AIP* mutations 116 somatotrophinoma patients from the Belfast and Dublin endocrine tertiary referral centres and studied additional R304*-positive pedigrees from our international FIPA database (five Irish/five non-Irish). We genotyped three population samples from Mid Ulster ($n=936$), Greater Belfast ($n=1000$) and Republic of Ireland (ROI, $n=2094$). Based on AIP-centred microsatellite haplotypes, we estimated through coalescent-based simulation the R304* allele's tMRCA and current number of allele carriers.
Results
R304* was very frequent in somatotrophinoma patients (12.6%/6.8% in Belfast/Dublin). General population prevalence estimates were 6/936 (95% CI: 0.0023–0.013; Mid Ulster), 1/1000 (95% CI: 0.000025–0.0055; Greater Belfast) and 0/2094 (95% CI: 0–0·0014; ROI). All 18 Irish pedigrees (two identified through population screening) shared a recent common ancestor, while the non-Irish pedigrees were independent from each other and from the Irish founder. The Irish pedigrees' median tMRCA was 2550 years (95% CI: 1275–5000). Forward simulation predicted 144 allele carriers/generation (95% CI: 30–1725).
Conclusions
R304* has a clinically-relevant prevalence in Ireland, especially in Mid Ulster, due to an approximately 2500-year old founder ancestor. While 40 affected carriers have already been identified, we estimated through forward simulation that 86 PA cases are currently alive (95% CI: 18–1035). Although R304* population screening cannot be currently advocated, our data strongly support the testing of Irish-origin young-onset somatotrophinoma patients. Our study generated increased disease awareness locally, possibly leading to early diagnosis of these typically aggressive AIP-related PAs, crucial to their effective management.

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## P305

***In vitro* effects of Imatinib on somatotrophinoma cell line**

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Introduction
Acromegaly is a neuroendocrine disorder caused by excessive secretion of growth hormone (GH). Current treatment includes surgery, radiotherapy and drugs like somatostatin or dopamine receptor agonist. In spite of combination of therapies cure rate is dismal. There is a quest for new therapeutic targets with optimal efficacy, least side effect without any cost constraints. Recently, a few reports have shown that tyrosine kinase inhibitor (Imatinib) causes growth failure in pediatric chronic myeloid leukemia (CML) cases probably by targeting the GH/IGF-1 axis. There is no study to report the effect of TKI on pituitary adenoma either *in vitro* or *in vivo*. Here we present data on the effect of Imatinib on GH release from primary cultures of human somatotrophinoms.
Materials and methods
Primary culture was performed on tumor samples obtained from 15 acromegaly patients undergoing transsphenoidal surgery. Pure somatotrophinoma population was obtained by cell sorting using GHRHR-FITC labelled antibody. Selected cells were treated with different concentrations of Imatinib (0–10 μM). We studied cell viability (MTT assay), GH release, GH protein expression (immunocytochemistry) and cell morphology with electron microscopy. Similar experiments were performed on rat somatomammotroph GH3 cells (ATCC, USA).
Results
Low concentration (0.5 μM) of Imatinib reduced GH positivity with immunostaining and GH secretion in both human somatotroph primary culture and GH3 cells. Electron microscopy of Imatinib-treated cells exhibited a reduction in GH positive granules. GH inhibition appears at a concentration of 0.5 μM of Imatinib without any added advantage of increasing concentrations. On the other hand, Imatinib did not show any effect on cell viability.
Conclusion
Imatinib inhibits GH secretion in somatotrophinoma cells without affecting cell viability. We provide new insights into use of tyrosine kinase inhibitor- Imatinib as an alternative treatment for GH-secreting pituitary adenomas.

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## P306

**Unique clinical picture in patients with X-linked acrogigantism**

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Introduction
Non-syndromic pituitary gigantism can result from *AIP* mutations and the recently identified Xq26.3 microduplications causing X-LAG.
Patients and methods
DNA samples and clinical data were collected from 151 patients with pituitary gigantism. All samples were tested for *AIP* mutations; *AIP* mutation negative cases (*AIP*neg) were screened for Xq26.3 microduplications.
Results
Xq26.3 microduplications were found in ten female simplex patients (6.6%). Two X-LAG cases we previously clinically described (Igreja Hum. Mut. 2010) were included in Trivellin NEJM 2014 and Daly ERC 2015. Median age at onset (1.6 years (0.9–2.6)) and diagnosis (3.8 years (2.4–6)) was earlier compared to *AIP*pos (onset: 15 years (12.5–15); diagnosis: 16 years (13–20)) and *AIP*neg cases (onset: 15 years (11.2–16); diagnosis: 18 years (14–23)), $P<0.0001$. Mean IGF-1 was $3\times$ULN$\pm1.17$, not different from *AIP*pos ($2.2\pm1.33$) and *AIP*neg cases ($2.6\pm1.37$). 90% of patients had hyperprolactinaemia, as opposed to 23% of *AIP*pos and 31% of *AIP*neg cases ($P<0.005$). Eight patients had macroadenomas, two had pituitary hyperplasia. Five patients were first managed with medical treatment (SSAs and/or dopamine agonists) with poor results. Seven macroadenoma patients had surgery, resulting in remission in three, the other four also received radiotherapy. Among these, remission was achieved in one; two patients are controlled on SSAs, and one on pegvisomant. One patient was treated with intrasellar Yttrium implants and is now controlled with SSAs. One hyperplasia case was treated with total hypophysectomy; the other did not respond to SSAs and her disease is now controlled on pegvisomant. No other tumours or manifestations other than gigantism were found, including the two oldest patients (now aged 29 and 50 years).
Conclusions
The clinical picture in X-LAG is distinct, being characterised by a very young age at onset, high prevalence of affected females and associated hyperprolactinaemia. Treatment of these patients is challenging, although multi-modal treatment can eventually lead to the successful control of GH excess.

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## P307

**Silencing of aryl hydrocarbon receptor protein (AIP) up-regulates the small Rho GTPase, CDC42**

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AIP mutation-positive familial isolated pituitary adenoma is commonly diagnosed in young patients who have a poor prognosis due to large, treatment-resistant tumours. Microarray analysis carried out on *AIP* knockdown pituitary (GH3) cells and control cells, identified *CDC42* as one of the genes that was up-regulated by loss of AIP protein. This small Rho GTPase activates MAPK signalling, suggesting it may contribute to the proliferative phenotype of AIP knockdown cells. Using a specific non-competitive inhibitor of CDC42, ML141, we studied elements of the MAPK-pathway – ERK1/2 (proliferative), JNK and p38 (dependent on context may promote proliferation or apoptosis/senescence) – in stably-transfected GH3 cells.
Method
GH3 cells were transduced with lentiviruses containing either an *AIP* or control shRNA, and stable clones expanded. Cell viability MTS assays and immunoblotting for CDC42 and for MAPK-pathway components (total&pho-spho-ERK1/2, JNK and p38) were quantified in the presence and absence of ML141.
Results
*AIP* silencing significantly elevated CDC42 RNA and protein levels in the stably-transfected cell line (RNA, $P=0.0156$, protein $P=0.0152$ vs non-targeted control). Cell viability was significantly increased and a non-significant rise in the phospho/total ERK1/2 ratio was observed. No change in p38 activation was observed. Based on dose-response curves, 20 μM of ML141 was used to determine the effect of CDC42 inhibition on cell viability and MAPK activation in the AIP-silenced and control cell lines. ML141 reduced cell viability in both ($P<0.0001$). It had no significant effect on ERK1/2 activation but did increase p38 activation: *AIP* knockdown ($19\times$ $P=0.0286$) and non-targeted cells ($13\times$ $P=0.0286$).
Conclusion
Up-regulation of the ERK-pathway could be responsible for the proliferative phenotype seen in AIP-silenced cells. ML141 is associated with an increase in p38 activation, suggesting that p38-dependent apoptosis/senescence might be responsible for the reduced cell viability. ML141 is a potential candidate for *AIP* mutation-positive pituitary adenoma treatment.

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## P308

**Antisense oligomer therapy directed at the GH receptor is associated with reduction in circulating GHBP levels**

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ATL1103 is a second generation antisense 20mer intended to inhibit expression of the GH receptor (GHR) gene. Phosphorothioate and 2′-O-methoxyethyl modifications to nucleotides increase its plasma half-life and affinity for the target RNA to allow post-hybridization RNaseH degradation. We previously reported a phase 2, randomised, open-label, parallel group study of ATL1103 in 26 patients with acromegaly which demonstrated a fall in serum IGF-I of 26% with 200 mg twice weekly ($577\pm198$ vs $411\pm174$ ng/ml, $P<0.0001$) and rise in GH. Once weekly dosing did not result in a significant fall in IGF-I. Here we present the changes in GHBP during this study.
In humans, circulating GHBP is the result of proteolytic cleavage of the extracellular domain of the cell-surface GHR with plasma levels being influenced by GH secretory status, body composition and age. Approximately 50% of circulating GH is bound to GHBP and it is a potential regulator of GH action. It has been suggested that GHBP levels reflect GHR number. GHBP was measured by a modification of a ligand immunofunctional assay.
At baseline, median GHBP were higher in the once weekly cohort than those randomised to twice weekly (1179 (range 386–7637) vs 525 (<69–6434) pmol/l) and the difference persisted throughout the dosing period. ATL1103 resulted in significant decrease in median GHBP in the once and twice weekly cohorts at week 14 of −453.0 (range −2219 to −237 ($P=0.0046$)) and −309.3 (−2392 to 443, $P=0.0186$) pmol/l, respectively.
In conclusion, these data indicate that plasma GHBP levels fall with ATL1103 which is further evidence of the efficacy of ATL1103 and its ability to inhibit GHR gene expression but suggest that dosing can be further optimised. Data from future clinical trials will help to evaluate the relationship between ATL1103 dose, GH levels, GHBP levels and the change in IGF1 with treatment.

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## P309

**Developing a pituitary distress thermometer (PDT) – a means to improve patient quality of life**

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Introduction
Patient distress may be associated with a reduced quality of life (QoL), poor adherence to treatment and lower satisfaction with medical care. Best practice

guidance recommends that distress is assessed at key points within the patient-care pathway. The aim of this study was to develop a pituitary-specific distress assessment tool.
Method
Working with the Pituitary Foundation, a Wellbeing Survey was generated, comprising 36 pituitary-specific items, plus all 40 items from the Oncology Distress Thermometer (ODT). It was posted to all Pituitary Foundation members ($n=2500$), enclosed with Pit Life (the magazine of the Pituitary Foundation).
Results
completed surveys were returned. Respondents' age ranged from 18 to 90 years (mean age $59.13 \pm 14.25$); 60% of participants were female; with hypopituitarism the most commonly reported diagnosis (43%). Multivariate regression modelling was used to determine the symptom clusters associated with the various diagnoses reported by respondents. The final analysis generated a 39–item problem-list for the PDT, comprising 32 symptoms, three practical problems, and four emotional concerns. In terms of symptom clusters per pituitary condition, Cushings disease recorded the largest symptom list (n = 24 symptoms), while both prolactinoma and non-functioning tumour had the smallest lists (n = 4 items). Only one third of ODT items (n = 13) appear on the PDT.
Conclusions
The ODT is already widely and effectively used in oncology services as a structured way to enable patients and healthcare professionals to collaborate on finding options for dealing with some of the common concerns (practical, emotional, physical and psychological) patients experience. Many studies using disease-specific questionnaires have demonstrated distress in patients with pituitary disease, which may not be disclosed or discussed during regular consultations. The PDT offers a potential solution, as well as illustrating the need for the development of disease-specific distress thermometers and greater knowledge about specific symptom clusters as reported by patients.

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## P310

### Mortality of patients with non-functioning pituitary macroadenoma is significantly elevated: systematic analysis of 546 cases in a tertiary referral centre in the UK

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Introduction
Data on the mortality of patients with non-functioning pituitary macroadenoma (NFA) are limited. Aim: To assess the mortality of patients with NFA and predictive factors.
Patients/methods
All patients presenting to our Department with NFA between 1963 and 2011 were studied. Status was recorded as either dead or alive, as of 31 December 2011.
Results
546 patients (333 males) were identified (median age at surgery 58.7 years; range 16.1–94.2). Data on mortality were available for all patients covering a median period of 8 years (range 1 month-48.5 years); 83 patients died (median age 77.8 years; range 36.4–98.3) (causes: cardio/cerebrovascular 33.7%, infections 30.1%, malignancies 28.9%, peri-operatively 1.2%, gastrointestinal haemorrhage 1.2%, suicide 1.2%, unknown 2.4%, old age 1.2%). SMR for total group:3.62 (95% CI:2.90–4.47; $P<0.001$), for those diagnosed before 1990:4.66 (95% CI:2.65–7.63; $P<0.001$) and for those after 1990:3.53 (95% CI:2.77–4.44; $P<0.001$). Clinical follow-up data (until date of death or date the database was frozen) were available for 436 patients [269 males, median age at surgery 58.5 years; range 16.11–94.19), 203/431 with no or intrasellar remnant – 228/431 with extrasellar remnant after surgery, median follow-up 6.9 years (range 1 month-48.5 years), 111/436 with NFA regrowth, 188/436 received radiotherapy adjuvant or for regrowth]. Cox regression analysis (univariate followed by multivariate approach) demonstrated that amongst age at surgery, NFA regrowth, radiotherapy, sex, extent of removal, untreated GH deficiency, untreated FSH/LH deficiency, ACTH deficiency, TSH deficiency and treatment with DDAVP, only age remained an independent significant factor (HR 1.099, 95% CI: 1.073–1.126; $P<0.001$).
Conclusions
This is the first study assessing mortality in a large series of non-selected patients with NFA in the UK. Despite the improvement in the last three decades, mortality remains high. Apart from age, factors related with the management/outcome of the tumour are not independent predictors and pituitary hormone deficits managed with the currently used substitution protocols do not adversely affect mortality in this group of patients.

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## P311

### The burden of AIP mutations in pituitary adenoma patients from the UK

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Introduction
Familial isolated pituitary adenoma (FIPA) and young-onset sporadic pituitary adenoma patients are suggested to be screened for mutations in *AIP*, a gene described in 2006 and amenable to UK testing since 2008.
Methods
affected subjects have been tested in Exeter and Oxford genetic laboratories. Data were collected from 120 FIPA-families and 193 sporadic cases with young-onset disease (<30y) from 49 centres in the UK. The Mann–Whitney U test and $X^2$ were used for statistical analysis.
Results
We have identified 38 *AIP* pos kindreds (16 families and 22 simplex kindreds), representing 77 affected patients. In the 16 families (13.3% of FIPA-families), out of 211 members tested, 121 were carriers and 55 were affected. Of the 193 young simplex patients (44.5% GH-excess), 22 (11.4%) were *AIP*pos and 17 unaffected carrier family members were identified. 96 (60%) *AIP*pos carriers had a founder mutation: p.R304*, 79 subjects and p.F269_H275dup, 17 subjects. In the *AIP*pos FIPA kindreds, those affected were more frequently males (60.7 vs 41.1%, $P<0.001$), had younger-onset disease (19y(15–28) vs 32.5y(24–45), $P<0.001$), and had higher frequency of pituitary apoplexy (12.2 vs 3.8%, $P=0.034$) compared to *AIP*neg FIPA families. There were no differences in tumour size (77.6 vs 72.8% macroadenomas) or extrasellar extension (40 vs 39.8%). Of young sporadic cases, *AIP*pos patients were more frequently males (81.8 vs 46.8%, $P=0.003$), with larger adenomas (100% vs 80.3% macroadenomas, $P=0.043$) and extrasellar extension (100 vs 50%, $P=0.004$), but no differences in age-of-onset (18(13.5–23) vs 20 (15–25)) or pituitary apoplexy. GH&GH/PRL-secreting adenomas were more frequent in *AIP*pos compared to *AIP*neg cases (FIPA: 76.8 vs 31%, $P<0.001$; sporadic: 95.5 vs 37.9%, $P<0.001$).
Conclusion
In the UK population, *AIP* mutations are found in 13.3% of families and 11.4% of young-onset patients. The two founder mutations identified are responsible for over half of the affected and unaffected carrier cases.

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## P312

**ACTH and gonadotrophin deficiency predict mortality in patients treated for non-functioning pituitary adenomas (NFPAs) in the UK and Republic of Ireland: long-term follow-up of 519 patients across two tertiary referral centres**

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Non-functioning pituitary adenomas (NFPAs) are the commonest subtype of pituitary tumour. Surgical resection, accompanied by radiotherapy (RTX) in selected cases, is the treatment of choice for compressive tumours. Long-term health consequences of treatment for NFPAs are unclear. In this retrospective study, we assessed long-term pituitary function and mortality rates in a large NFPA cohort across two tertiary centres in the UK and Ireland.
Case-note review of all patients treated for NFPA in University Hospitals Birmingham and Beaumont Hospital Dublin between 1997 and 2012 was performed. Clinical presentation, treatment strategy and pituitary function at last clinic visit were recorded in each case. Data on mortality was recorded via Clinical Portal in Birmingham and via GP contact in Dublin. Mortality risk was calculated by external Poisson regression for comparison with the general population.
A total of 519 patients were included in the analysis (Birmingham n = 271, Dublin n = 248). Mean duration of follow-up was 8.4 ± 6.3 years. The rate of pituitary irradiation was higher in Birmingham than in Dublin (42.4 vs 27.5%, $p < 0.001$). The rate of panhypopituitarism was significantly higher in RTX-treated compared to RTX-naïve patients (51.1 vs 39.8%, $p < 0.001$). A total of 78 deaths were recorded (15.02%). On external Poisson regression incorporating mortality rates in the background UK and Irish populations, ACTH and gonadotrophin deficiency were associated with increased relative risk of death (OR 2.24, 95% CI 1.14–4.40, $P = 0.01$, and OR 2.47, 95% CI, 1.07–5.70, $P = 0.02$, respectively) after adjustment for RTX, age at diagnosis and attained age. RTX was not independently associated with an increased relative risk of death.
Hypopituitarism may be associated with increased mortality rates in patients treated for NFPA. Specifically, ACTH- and gonadotrophin-deficient patients have increased mortality rates compared to the background population. Further studies are necessary to determine if these associations are directly related to hypopituitarism or to its treatment.

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## P313

**A prospective observational study of the causation and management of SIADH in a tertiary referral hospital**

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Background
SIADH is the most frequent underlying cause of hyponatraemia but is frequently ignored and suboptimally treated.
Aim
To identify the treatment applied in clinical practice for hyponatraemia due to SIADH and to evaluate the effect of fluid deprivation.
Method
A prospective, non-intervention observational study of a sequentially evaluated cohort of hyponatraemic patients during first 48 h after hospitalization from January 1st until May 25th. Patients were identified from the hospital laboratory database on a daily basis. The role of endocrine team was in the differentiation of SIADH from other causes of hyponatraemia, and ascertainment of full diagnostic criteria in SIADH patients. Treatment remained at the discretion of admitting team.
Results
*Patients:* 748 patients with plasma sodium < 130 mmol/l were evaluated. 343 (45.8%) had SIADH according to standard criteria. 232 (67%) had hyponatraemia on admission, and 111 (33%) developed hyponatraemia during hospitalization. pNa at the time of evaluation (m,IQR):129 (126.130) mmol/l, UOsm 470 (345.591) mOsm/Kg, UNa 54 (28.89) mmol/l. 9 Am Cortisol: 457 (387.554) nmol/l. One patient was hypothyroid. *Etiology of SIADH:* CNS ($n = 85$), respiratory (82), cancer (68), postsurgery (29), drug-induced (23), 56 (other/unknown). Therapies during first 48 h after admission: 0.9% saline infusion-121 patients (35%), fluid restriction-49 (14%), furosemide-19 (5%), 3%saline-6 (1.7%), Tolvaptan-4 (1.1%), Demeclocycline-2 (0.5%), multiple therapies in 19 (5.5%) and no treatment in 133 (38%). Plasma sodium at discharge: ($n = 239$) in 141 patients with hyponatraemia (pNa < 135 mmol/l), 47 with pNa < 130 mmol/l. Fluid restriction was not superior than no treatment (discharge mean pNa = 132 (S.D.: 5.5) mmol/l, vs 133.2 (S.D.: 4.4) mmol/l, $P = 0.33$).
Conclusion
Treatment for hyponatraemia was heterogeneous and did not follow recent guidelines (1). First line treatment with fluid restriction was no more effective than no treatment. Routinely used therapies were suboptimal in normalising plasma sodium in hospital prior to discharge. More effective hospital policies for management of SIADH are needed.
Reference
1. Verbalis *et al.* Am J Med 2013.

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## P314

**Biochemical assessment of disease activity in acromegaly; a comparison of single GH, GH day series mean, OGTT nadir and IGF-1 in 51 patients**

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Background
Accurate assessment of GH & IGF1 status in acromegaly is crucial for informing management to minimise excess morbidity/mortality. Expert panels have differed with respect to recommended testing modalities and thresholds – the most recent being the Endocrine Society 2014 guidelines. We evaluated their simplified algorithm, which minimises the need for day-case testing, against other more resource-intensive measures.
Methods
A retrospective analysis was performed of prospectively collected data from a cohort of 51 patients with acromegaly, who received up to 6 months of pre-surgical medical therapy prior to transsphenoidal surgery. Single 0900 h GH and IGF1 levels were recorded at diagnosis, post-medical therapy and post-surgery. OGTT nadir was determined at diagnosis and post-operatively. GH day curve mean (GHDC) was measured at diagnosis and after medical treatment.
Results
A single 0900 h GH value correlated well with GHDC results both at diagnosis ($R^2 = 0.94$, $P < 0.0001$) and following medical treatment ($R^2 = 0.92$, $P < 0.0001$). Three patients were below the suggested target of 1 μg/l on a single GH reading post-medical therapy, but had a GHDC > 1 μg/l; all three had an IGF1 above the upper limit of normal (ULN). Four patients had a 0900 h GH > 1 μg/l, but a GHDC < 1 μg/l and an IGF < ULN. Post-operatively, a single 0900 h GH reading of < 1*** μg/L was observed in 3 patients who failed to suppress to < 0.4 μg/l on an OGTT, although suppression in each case was to < 1 μg/l; two of these had an IGF1 > ULN. Five patients with a 0900 h GH > 1 μg/l suppressed to < 0.4 μg/l on OGTT; IGF1 was < ULN in one of these.
Conclusions
In the majority of patients, a single measurement of GH status together with IGF1 provides an accurate assessment of biochemical control in acromegaly. For the small number of cases in whom 090 h GH and IGF1 are discordant, GHDC (post-medical therapy) and OGTT nadir (post-surgery) remain important adjuncts.

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## P315

**Expression of MGMT in a large series of pituitary adenomas: lactotroph and corticotroph adenomas are more likely to be MGMT-negative than somatotroph and gonadotroph adenomas**

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Background
Lack of expression of MGMT (O-6-methylguanine-DNA methyltransferase) is associated with a better tumour response to temozolomide. There are no systematic studies of MGMT expression in pituitary tumour series.

Aim
We introduced MGMT immunohistochemistry for all pituitary adenoma specimens in 2011. In this study we investigated the immunohistochemical expression of MGMT in a large series of pituitary adenomas, in order to determine (1) the overall frequency of expression (2) the relative frequency of MGMT expression according to tumour type and (3) a relation to the proliferative index Ki67.
Methods
We identified a consecutive series of 233 pituitary pathological specimens between 2011 and 2014. MGMT and Ki67 staining were available for 177 patients. MGMT immunohistochemistry was assessed semi-quantitatively as follows: $<10\%$ negative, 10–50% intermediate, and $>50\%$ strongly positive. Pituitary adenomas with Ki-67 $>3\%$ were defined as atypical. The Fisher's exact test and the $\chi^2$-test were used to analyse the association between the types of tumours and MGMT. Correlations between MGMT and Ki-67 expression was assessed by Spearman's rank correlation analysis.
Results
Among the 177 tumours, 93 (52.5%) were non-functioning (NFPA) and 84 (47.5%) were biochemically active (somatotroph: 32, corticotroph: 24, prolactinoma: 24 and TSHoma: 4). Among NFPAs 81 were gonadotrophs and 12 were immunonegative. MGMT was negative in 17/177 cases (9.6%). Of the negatively stained MGMT tumours, 13/17 (76.47%) were biochemically active and 4/17 NFPAs (23.53%), $P=0.039$. Corticotroph (25%) and lactotroph adenomas (25%) were more often MGMT negative than gonadotroph (3.7%) or somatotroph adenomas (0%). Overall, no correlation between MGMT and Ki67 staining was detected.
Conclusions
MGMT expression shows tissue specificity in pituitary adenomas. Corticotroph and lactotroph tumours are most likely to be MGMT negative, indicating that temozolomide is most likely to be useful in aggressive ACTH and PRL-secreting tumours.

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## P316

**Does basal post-operative early morning cortisol measurement predict HPA axis integrity as assessed by an insulin tolerance test in patients who have undergone pituitary surgery?**

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Background
Correct identification of patients with HPA axis dysfunction following transsphenoidal pituitary surgery is important. Our centre measures post-operative day 5 0900 h serum cortisol (24 h after the last dose of glucocorticoid) to identify the need for glucocorticoid replacement. This is given if post-operative day 5 0900 h cortisol concentration is $<300$ nmol/l, until dynamic pituitary function assessment occurs.
Methods
Data were reviewed for 51 patients undergoing an insulin tolerance test (ITT) $\sim 6$ weeks after pituitary surgery (2010–2015) using a new cortisol assay. The peak cortisol concentration during an ITT was compared to post-operative day 5 0900 h serum cortisol.
Results
Of the 27 patients who passed the ITT (peak cortisol $>450$ nmol/l) 18 had post-operative day 5 0900 h cortisol concentration $<300$ nmol/l. Of the 24 patients who failed the ITT, seven had post-operative day 5 0900 h cortisol concentrations of $>300$ nmol/l. The cortisol concentration cut-off point of 300 nmol/l had 70.8% (95% CI: 48.91–87.38%) diagnostic sensitivity and 33.3% (95% CI: 16.52–54.0%) diagnostic specificity. 100% (85.75–100.00%) sensitivity was achieved by increasing the cut-off to 633 nmol/l. Decreasing the cut-off to 101 nmol/l resulted in 100% (87.23–100.00%) specificity. The ROC curve investigating the predictive ability of post-operative day 5 0900 h cortisol had an AUC of 0.60 (95% CI 0.44–0.76).
Conclusions
Following pituitary surgery, post-operative day 5 0900 h cortisol measurement is effective at identifying patients who will subsequently fail an ITT, and hence, require glucocorticoid replacement. However, the current cut-off of 300 nmol/l falsely reassures some patients they do not need glucocorticoid replacement post-operatively.

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## P317

**Patient experiences of living with acromegaly in the UK**

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61 patients (104 invited participants), within 10 years of active treatment for acromegaly, from five hospitals in the UK (one neurosurgical centre) were interviewed to explore the experience of living with acromegaly, access to information, support and their ability to make decisions about their care. Semi-structured interviews by an independent consultant (60–120 min each) included 34 males, 27 females; 25–85 years old; 85% patients had surgery. 56 (92%) experienced a significant delay from symptoms to diagnosis, but prompt effective secondary care treatment. Communication and coordination of care between endocrine and surgical teams was criticised. Patients felt a lack of psychological and emotional support. Those in contact with a 'specialist' consultant or an endocrine nurse specialist felt most supported. 85% felt they had access to a consultant who understood the condition. Those without contact with an endocrine nurse specialist felt their care might have been compromised as a result. Patients felt unprepared for the life-long effect of their condition. 36 out of 61 were on additional medical treatment and had issues with the ordering, dispensing or administering of the drug. 75% would be willing to travel anywhere in the UK to receive treatment (25% to next available hospital) if not available locally. Although many felt involved in decision-making, few had been offered choice (but would take advice from their consultant). Whilst 87% felt they understood about their condition now, many felt this took a long time with extensive personal research. 67% had been offered written information, 69% had looked on websites, 61% were aware of patient groups but only 20% had joined or sourced information from one and 15% attended a meeting (despite a desire to help others with acromegaly). This is the largest acromegaly qualitative patient experience study undertaken, reflecting the issues of living with acromegaly.

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## P318

**An association between self-drawing, morphometric changes and psychological outcomes in patients with acromegaly vs non-functioning pituitary adenoma**

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We recently developed a visual scale to assess the extent of morphometric changes in Acromegaly (Acro) patients which is strongly predictive of adverse psychological outcomes. We sought to identify if there was any association between the self-drawing test and the visual morphometric scale and if there was any correlation with psychological outcomes.
We compared 60 consecutive non-functioning adenoma (NFA) and Acro patients who had undergone pituitary surgery and/or radiation therapy (RT). Each patient was asked to draw two images: i) an image of what an average healthy body looked like, and ii) an image of what they thought their body looked like. Delta scores for right hand, right foot, height and head width were calculated for each set of drawings as percentage change based on the formula (drawing 2/drawing 1)-1)$\times$100). We compared those scores with the grading on the morphometric scale and investigated whether the correlation could predict adverse psychological outcomes.
A total of 51 Acro and 48 NFA patients completed the study. Mean ages were 58.3 years (Acro) and 60.1 years (NFA) and females constituted 54.8% (Acro) and 52.1% (NFA) (Both $P=$NS). Of acromegaly patients, 11 were in remission without medical therapy while 37 were controlled on medical therapy. The delta values of Acro vs NFA were: 6.69 vs $-1.62$ for height ($P=0.01$), 47.51 vs $-2.70$ for right hand width ($P=0.005$) and 34.11 vs 8.10 for right foot size ($P=0.002$). Although both the self-drawing test and the morphometric grading predicted adverse psychological outcomes, different outcomes were predicted by each test. Both tests predicted cognitive dysregulation, apathy, dissatisfaction with appearance, poor personal relationships and low self-esteem. However, patients with a higher delta in the self-drawing test were significantly more likely to have affective lability, anxiousness, conduct problems, oppositionality, low affiliation and self harm whereas those with high morphometric grading were more likely to have problems with expression and appraisal. When comparing the two there was an overall positive correlation between high morphometric

readings and delta scores with a significant association between width of the right hand on the drawing test and morphometric grading ($P=0.02$).

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## P319

**The accuracy of bilateral inferior petrosal sinus cannulation and usefulness of prolactin adjustment in one Scottish centre**

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Bilateral inferior petrosal sinus sampling (BIPSS) is the gold standard investigation in Cushing's disease for identifying the pituitary as the ACTH source. This technique aims to demonstrate a gradient of central:peripheral ACTH levels of >2:1 in such patients, or >3:1 after CRH stimulation. In patients without significant pituitary MRI abnormalities this facilitates neurosurgical exploration.

The test is limited by difficulties in achieving adequate sinus cannulation in some patients. Sinus to peripheral prolactin ratio is being used in some centres to confirm cannulation and allow for result adjustment, with ratio >1.8 indicating success. The 'prolactin-adjusted' ACTH ratio may also guide adenoma lateralisation.

In our tertiary referral centre, between 2010 and 2015, 15 patients with confirmed ACTH-dependent Cushing's underwent BIPSS with the addition of prolactin measurements from 2013. We aimed to demonstrate whether this modification improved the diagnostic yield by careful review of electronic case records of all fifteen patients.

Overall, 13 patients had definite pituitary Cushing's disease; 12 subsequently confirmed on hypophysectomy and one with empty sella syndrome who underwent adrenalectomies. Two of 15, despite clinical suspicion, had negative results. The first predated prolactin adjustment, however the other had a low prolactin ratio indicating possible procedural failure and a second BIPSS is planned. Curiously, of the five patients who underwent BIPPS with prolactin measurement, only one met criteria for adequate bilateral cannulation. However, in three of the other four there was a clear central:peripheral ACTH gradient despite a central:peripheral prolactin ratio of <1.8.

BIPSS is a reliable test to confirm Cushing's disease; we report an 87% success rate over the past 5 years. However, our data sheds some doubt on the reliability of using prolactin to confirm successful BIPSS, as we cannot easily explain a clear central ACTH excess with an apparently 'non-diagnostic' test. This merits further study in larger series.

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## P320

**Neuronatin emerges in the rat pituitary stem/progenitor cells and terminates its role in the terminally differentiating cells**

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The pituitary gland that synthesises and secretes pituitary hormones is an indispensable endocrine organ. Its development progresses by plural factors. Among them, neuronatin (NNAT), which was discovered in the neonatal mouse brain and is involved in neural development, was later revealed to be an abundantly expressing gene in the pituitary gland, but its role is yet elusive.

We analysed the expression profile of *Nnat* and its localisation in the rat pituitary. Level of *Nnat*-expression was high during the rat embryonic period but remarkably decreased after birth. In embryonic pituitary development, NNAT first appeared in the SOX2-positive stem/progenitor cells and disappeared at the initiation of terminal differentiation into hormone-producing cells. After birth, the number of NNAT positive cells remarkably decreased and most of them co-localized with SOX2 or PIT1, a commitment cell marker. Investigation of localisation in the organelles showed that NNAT widely localises in the mitochondria, peroxisome, lysosome and endoplasmic reticulum, but not in the Golgi.

The present study suggests that NNAT plays a role in stem/progenitor cells and supports progression of cellular differentiation existing in the subcellular organelles.

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## P321

**Current clinical management of acromegaly in the UK; a survey of endocrinologists**

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Background

Acromegaly treatment options include transsphenoidal surgery (TSS), medical management with somatostatin analogues (SSAs), growth hormone (GH) receptor antagonists and dopamine agonists, radiotherapy or a combination of these, depending on symptom nature and severity, tumour size, age and health status. In 2014 the Endocrine Society published guidelines for management of acromegaly.

Methods

To describe current UK practice following guideline publication, between November 2014 and March 2015 we conducted telephone or face-to-face surveys of 21 endocrinologists from 19 UK secondary/tertiary care NHS centres on local management of acromegaly, biochemical control criteria and factors driving treatment decisions.

Results

The estimated number of new patients seen per year ranged between centres from 1 to 20 and the number of existing patients from 11 to 250. Most common referral sources (not mutually exclusive) were other endocrinologists ($n=14$) and general practitioners ($n=12$). All respondents stated that both IGF1 and GH are important for assessing biochemical control; the local biochemical control criteria were GH <1.0 ng/ml ($n=17$) and IGF1 < upper limit of normal ($n=19$). For 12/21 the possible effect on tumour volume is an important criterion for treatment choice. In rating the importance of biochemical vs symptomatic control for treatment decision-making (visual analogue scale: 0=symptomatic control; 100=biochemical control) the median score was 70 (range 22–88); 12/21 considered biochemical control most important. TSS was the most common first-line therapy and medical management (usually SSAs) most common following TSS failure. There was considerable variation in the reported proportion of patients who receive radiotherapy following surgical failure. Although reported as least common, visual failure and cardiomyopathy were most likely to drive management change.

Conclusions

This survey relied upon clinical opinion of a limited number of clinicians; nevertheless, it provides useful insights into current acromegaly management by UK endocrinologists. It highlights variation in practice and some guideline recommendations not yet fully embedded, requiring further exploration.

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## P322

**Traditional cardiac risk factors in a cohort of hypopituitary patients: a preliminary look at the utility of QRISK2 score**

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Introduction

Increased cardiovascular risk in hypopituitary patients was first documented by Rosen in 1990. Subsequent studies confirmed increased prevalence of cardiovascular and cerebrovascular disease in these patients. The exact mechanism for this is unclear. There is no clear consensus on how best to quantify or predict cardiac risk in hypopituitarism. QRISK2 cardiovascular disease risk algorithm provides estimates of 10-year cardiovascular disease (CVD) risk in patients from different ethnic groups in England and Wales, based on information from a large primary care database. NICE recommend 10-year CVD risk at which lipid-lowering treatment should be offered is ≧10%.

Methods

A prospective audit was carried out on a cohort of 86 patients (49 male, 37 female; 25–84 years old) with hypopituitarism, recruited from St Bartholomew's Hospital. Clinical history, aetiology of hypopituitarism and cardiovascular risk factors were recorded, including: ethnicity, age, sex, smoking status, systolic blood pressure, ratio of total serum cholesterol:HDL cholesterol, BMI, T2DM and family history of CVD. QRISK2 scores were calculated, to evaluate the contribution of traditional risk factors.

Results

The median age of the cohort was 58 years. Causes of pituitary disease included 34 pituitary macroadenoma, comprising 15 non-functioning pituitary adenoma, and 13 due to other causes. 47 (54.7%; 31 male, 16 female) out of 86 patients had QRISK2 score ≧10% (defined as high-risk group). Of these, 34 had systolic BP

>120 (29 known hypertensives), 14 had HbA1c >43 mmol/mol, 22 had BMI> 30 and 16 had elevated TC:HDL-C (>4). Mean duration of follow-up since diagnosis of hypopituitarism was 19 years. To date, no history of IHD and only one case of TIA was reported in the high-risk group. By contrast, the low-risk group comprised 18 with systolic BP >120, 3 with HbA1c >43 mmol/mol and 13 with elevated TC:HDL-C.

Discussion

Hypopituitarism is associated with increased traditional cardiovascular risk factors, albeit in a small cohort of patients. These should provide a focus for prevention.

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## P323

**Long term follow up of patients with craniopharyngioma**

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Introduction

Patients with craniopharyngioma are characterised by a high incidence of hypopituitarism, visual failure and hypothalamic dysfunction. Standardised mortality is markedly elevated and controversy exists about optimal treatment.

Aim

We aimed to examine the temporal trends in the treatment of craniopharyngioma at our centre. Also, we sought to examine treatment needs and long-term morbidity in this patient group.

Methods

We performed a retrospective review of all patients with craniopharyngioma currently attending the out-patient clinic at St Bartholomew's Hospital. Data recorded include demographic, clinical, biochemical and radiological variables. Study period for date of diagnosis (1951–2013) was divided into quartiles and trend analysis performed using $\chi^2$ test.

Results

patients (25 male) were identified. Median age at presentation was 30.5 years (range 2–79); majority presented with symptoms from tumour compression. Median clinical follow-up 30 years (range 1–59). 46 patients underwent primary surgical treatment; 30 patients (64%) had surgery via the transcranial route. There was a significant temporal trend towards use of the transsphenoidal approach in the treatment of contemporary cases; $P=0.02$. 15% required repeat surgery for tumour recurrence; median time to recurrence was 3 years. 31 patients underwent post-operative external beam radiotherapy; 77% had immediate radiotherapy following the first operation while the remainder had radiotherapy for tumour recurrence. The majority (45/47) had multiple pituitary hormone deficiencies. 81% had cranial diabetes insipidus. Median current BMI was 30.02 kg/m$^2$; 13% are treated for type 2 diabetes; 23% are treated for hypertension; 30% are treated for hyperlipidaemia. 24 patients (51%) had permanent visual impairment.

Conclusion

Patients with craniopharyngioma suffer from high rates of hypopituitarism, visual loss and an adverse metabolic phenotype. Further follow-up is required to determine the impact of contemporary treatment approaches on long-term morbidity.

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## P324

**Metabolic state in ACTH insufficient and ACTH sufficient patients with hypopituitarism not treated for growth hormone deficiency (GHD): a comparative study**

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Background

Inadequate glucocorticoid (GC) replacement may be associated with over-exposure to GC, which can adversely influence metabolic and cardio-vascular state of hypopituitary growth hormone deficient adult patients (A-GHD). In this study we compared metabolic profile of ACTH insufficient and sufficient patients in A-GHD not treated with GH.

Patients and methods

A cohort of 260 patients (age 48.6±12 years and BMI 28.7±6.6 kg/m$^2$) with hypopituitarism and A-GHD was divided according to ACTH status and analysed for anthropometric and metabolic parameters including lipid status and glucose metabolism. All ACTH insufficient patients were replaced with hydrocortisone (10–20 mg/day in divided doses), and for other hormone deficiencies adequately. None of the patients were treated with GH.

Results

In our cohort prevalence of ACTH insufficiency was 75.4%. ACTH insufficiency was more prevalent in males compared to females (59.7% vs 40.5%, $P<0.05$). Although our patients were overweight with adverse lipid profiles, no differences were found between the two groups in body weight, waist to hip ratio and lipid profile (total, LDL and HDL cholesterol, triglycerides and Lp a). Prevalence of metabolic syndrome defined according to NCEP (3/5) criteria was similar in both groups (33.9% vs 30.7% ACTH suff. vs ACTH insuff. $P>0.05$). Significant differences were found in glucose metabolism characterized by lower glucose leves at baseline (4.5±0.9 vs 4.8±0.8 mmol/l, $P=0.04$) and during oral glucose tolerance test (OGTT 75 g) peak (7.7±2.0 vs 8.6±2.1 mmol/l, $P=0.006$) and area under the curve values (753.3±197.6 vs 851.2±217.8 mmol/l.min $P=0.002$) calculated using trapezoidal rule. However insulin concentrations at baseline and during OGTT as well as HOMA IR were not significantly different between ACTH sufficient and ACTH insufficient GHD hypopituitary patients.

Conclusion

Adverse metabolic profile in patients with hypoptuitarism and GHD is not significantly influenced by ACTH deficiency state if it is replaced with low to moderate doses of hydrocortisone.

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## P325

**Using modified clamp study to replicate insulin stress test**

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Introduction

Insulin stress test (IST) has long been the gold standard for testing the hypothalamus-pituitary–adrenal (HPA) axis. There are concerns about the safety of IST as hypoglycaemia can give rise to various symptoms including seizure and coma although this is rarely seen in clinical practice. Hyperinsulinaemic hypoglycaemic clamp is a common research procedure used to induce hypoglycaemia in a step-wise fashion to study the effect of hypoglycaemia.

Method

We modified the clamp procedure, aiming to reach nadir glucose of just under 2.4 mmol/l using a set insulin infusion protocol and variable rate dextrose infusion. We recruited patient referred from endocrine clinic for HPA axis assessment. Participants underwent both IST and modified clamp study at least 2 weeks apart. The primary outcome is to compare the nadir glucose level between routine IST and modified clamp. We also compared the peak cortisol and growth hormone in both the procedures.

Results

Total of nine patients were recruited, with two patients having HPA axis failure. Data reported as mean±S.D. in IST and modified clamp respectively. In all participants, the nadir glucose was 1.6±0.4 vs 2.0±0.3 mmol/l ($P=0.007$). Peak cortisol was 539±312 vs 531±341 nmol/l ($P=0.815$) and peak growth hormone was 2.4±2.1 vs 4.1±3.2 μg/l ($P=0.031$). In participants with intact HPA axis, the nadir glucose was 1.5±0.4 vs 2±0.3 mmol/L ($P=0.023$), peak cortisol 663±222 vs 656±270 nmol/l ($P=0.878$), and peak growth hormone 2.9±2.1 vs 5.1±2.9 μg/l ($P=0.028$). In participants with HPA axis failure, the nadir glucose was 1.9±0.1 vs 2.2±0 mmol/l ($P=0.09$), peak cortisol 106±9 vs 94± 30 nmol/l ($P=0.570$), and peak growth hormone 0.5±0.3 vs 0.5±0.1 μg/l ($P=0.861$).

Conclusion

Modified clamp might be a safer way to assess the HPA axis with a higher nadir glucose level compared to IST with similar peak cortisol and growth hormone response.

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## P326

**Impaired quality of life in patients with acromegaly despite long-term disease control: results from a longitudinal study**

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Introduction
Patients with acromegaly are frequently left with long-term adverse sequelae. Cross-sectional evaluation of health-related quality of life (HR-QoL) using both generic and specific questionnaires (AcroQoL) has confirmed HR-QoL to be severely impaired in acromegaly. However, long-term HR-QoL outcomes following disease control have been less well-described.
Methods
The disease specific questionnaire AcroQoL and three validated generic questionnaires (the Psychological General Well-Being Schedule (PGWBS), the 36-item Short-Form (SF-36) health survey, and the EuroQol (EQ-5D)) were used to evaluate quality of life in 28 patients with acromegaly. Two sets of responses were obtained from each participant, within a time interval of minimum 5 years.
Results
Twenty-eight patients with acromegaly (male 46.4%, mean age at baseline 57.2 ± 11.5 years) were assessed over a mean 5.68 ± 0.6 years period, between baseline and follow-up responses. All patients had pituitary surgery, 57% required additional cranial radiotherapy and 50% medical treatment. Growth hormone levels at baseline and follow-up were 1.6 ± 2.3 and 0.74 ± 0.73 μg/l respectively ($P = 0.002$), while IGF1 values were 102.4 ± 63 and 105 ± 52% of the upper limit of normal respectively ($P = 0.81$). No difference in the scores of the AcroQoL (65.11 ± 16.3 vs 66.11 ± 19.1; $P = 0.66$), PGWBS (66.29 ± 20.9 vs 64.57 ± 19.4; $P = 0.61$), SF-36 (53.87 ± 22.4% vs 48.96 ± 23.9%; $P = 0.07$), and EQ-5D (0.61 ± 0.32 vs 0.55 ± 0.32; $P = 0.32$) were observed between baseline and follow-up responses. The physical domains of energy/fatigue, vitality, and bodily pain have been consistently the most under-marked areas in all questionnaires, in comparison with the psychological/emotional domains which were not as affected. The domain of physical function, in particular, was found to be significantly worse at follow-up compared with baseline, as assessed by the SF-36 (58.5 ± 24.7% vs 43.1 ± 31.1%; $P = 0.002$).
Conclusions
Patients with acromegaly demonstrate impaired QoL which persists, despite long-term biochemical disease control. Physical limitations due to irreversible complications of acromegaly, such as arthropathy, appear to have the biggest impact on patients' perceived quality of life.

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## P327

**Pituitary incidentaloma: features and outcomes: recent experience at a tertiary centre**

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Background
The prevalence of incidental pituitary adenoma is estimated between 10 and 20% in autopsy and radiological data. Such pituitary incidentalomas are increasingly detected, as access to sensitive imaging modalities improves. We present a review of the recent pituitary incidentaloma referrals at a tertiary centre during 2 years from January 2013.
Methods
All patients with pituitary incidentalomas on brain imaging between 2013 and 2015 were reviewed. The referral source, original indication for imaging, MRI scan findings, visual fields, pituitary function, management decisions, and outcomes were analysed.
Results
Twenty-nine cases, age ranging from 17 to 75 (mean 48), were identified. 18 were females (62%) and 11 males (38%). Neurology accounted for 13 referrals (45%) with headache dominating the indications for MRI scan (10/29; 35%). 10/29 (35%) were macroadenomas (≥10 mm). Surgery was recommended for 5/10 (50%) due to tumour size and involvement of optic apparatus or the cavernous sinus. Four underwent surgery but one patient declined. Immunohistochemistry of the four samples showed mixed GH/ACTH adenoma, gonadotrophinoma, prolactinoma and null-cell adenoma. Three had visual field deficit. Two had at least two pituitary axes affected. 19/29 (65%) were microadenomas (<10 mm), all with normal pituitary function. In one patient, tumour size increased over a year, indicating future need for surgery. Normal pituitary gland was revealed on repeat dedicated pituitary MRI in four cases.
Conclusion
Pituitary incidentaloma is an increasingly common clinical entity. Significant proportion requires surgical intervention over time and it is important that patients are counselled appropriately. In addition, some incidentalomas may be secretory tumours as in our case. On the other hand, false-positive results from non-dedicated brain imaging may cause unnecessary anxiety. Careful evaluation by a pituitary MDT is essential to distinguish clinically significant tumours. A robust pathway including dynamic visual field, pituitary function and dedicated pituitary MRI should be part of such evaluation.

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## P328

**Development of a patient-reported outcome measure for pituitary surgery**

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Background
Healthcare organisations worldwide are making use of patient-reported outcome measures (PROMs) to assess the impact of care received on patient's health-related quality of life.
Aim
A cross-sectional pilot study designed to evaluate the suitability and validity of a proposed pituitary PROM questionnaire pack for patients having pituitary surgery.
Methods
The PROM pack comprised five questionnaires focused on symptoms and psychosocial well-being. Pituitary patients attending the Sussex Pituitary MDT Clinic between December 2013 and April 2014 were invited to take part. Patient demographic and conventional clinical outcome data was collected in parallel.
Results
Twenty of 28 (71%) perioperative patients attending clinic participated; age range 32–77 years (median 54.5), 65% males. Participants were mainly post-operative (65%), with a non-functioning adenoma (65%). Participants did not find the questionnaires distressing and were able to complete them at the clinic. Principal symptoms concerning them were fatigue, libido, and weight. Post-operative participants reported worse biopsychosocial wellbeing than pre-operative participants. Most participants reported good coping mechanisms and social well-being. Participants' SF36 questionnaire data indicated worse quality of life compared to UK norms, although social function was better. HADS data showed similar levels of anxiety and depression to UK norms.
Discussion
The poorer wellbeing reported by post-operative participants may be explained by the recovery period post-operatively, compared to a preoperative state which may have been asymptomatic. These data suggest the timing of administration of PROM questionnaires following pituitary surgery may require elucidation, through collection of longitudinal data in the first instance. Our study, although small, suggests potential for PROM for assessment of pituitary patients.

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## P329

**Management of pituitary apoplexy: the greater Manchester experience**

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Objective
To describe the experience of pituitary apoplexy from a single-centre modern series.
Methods
We retrospectively reviewed the case notes of patients presenting to Salford Royal NHS Foundation Trust between February 2005 and April 2014 with pituitary apoplexy.

Results
A total of 32 cases (20 males) presenting with classical apoplexy were identified, with a mean age at presentation of 54 years (range 22–88 years). 11 patients (34.4%) were managed conservatively and 21 patients surgically. All surgeries were carried out by one of the two pituitary surgeons in our centre. Emergency surgery was carried out in 11 patients. Visual symptoms were present in a higher proportion of patients in the surgical group as compared to the conservative group (87.5% vs 63.6%). The surgical cohort also experienced more severe visual symptoms. The incidence of hypopituitarism at presentation was also higher in the surgical group (88.2% vs 63.6%). All patients in the surgical group showed good improvement or complete resolution of their visual symptoms. The incidence of hypopituitarism was higher in patients who had surgery compared to those managed expectantly (90.5% vs 63.6%). A higher proportion of patients who received emergency surgery had visual symptoms and hypopituitarism at presentation (90.9 and 90.0%) as compared to patients who received elective surgery (80.0 and 75.0%). The Visual recovery and hypopituitarism post procedure was similar in both groups.
Discussion
The incidence of hypopituitarism is high in pituitary apoplexy. In our cohort, patients with more severe symptoms were more likely to undergo surgery. Visual outcomes were good in the surgical group. There was no significant difference in endocrine or visual outcomes between patients who had emergency or elective surgery.

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## P330

**Feedback and GnRH pulse frequency decoding: a mathematical model for GnRH signalling in gonadotrophs**

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Highly regulated pulsatile secretion of the GnRH is essential for reproduction. GnRH pulses act via 7TM receptors to control synthesis and secretion of FSH and LH. GnRH receptors activate a signal-transduction network that includes two parallel pathways mediated by ERK and nuclear factor of activated T cells (NFAT). ERK and NFAT in concert with other effectors mediate transcriptional regulation of *FSHβ* and *LHβ* genes following stimulation by pulsatile GnRH. We present a detailed ordinary differential equation based mathematical model for ERK and NFAT signalling pathways. This differs from an earlier model[1] in that it is parameterised using data obtained from clonal gonadotroph-derived LβT2 cells and includes a negative feedback loop which describes the effects of receptor internalisation on signalling. Our model closely matches the data on the NFAT and ERK nuclear translocation and GnRH receptor internalisation in LβT2 cells. It is well known that the secretion of FHS and LH is maximal at sub-maximal GnRH pulse frequency,[2] but the underlying mechanisms are unclear. We investigate upstream negative feedback as a possiblemechanism for eliciting maximal *LHβ* and *FSHβ* expression at sub-maximal GnRH pulse frequency. We show that for the strength of the negative feedback estimated from the data, and for biologically relevant GnRH pulse frequencies, negative feedback alone cannot explain the experimentally observed non-monotonic GnRH pulse frequency–response relationship. Instead, we suggest that it reflects down-stream adaptive mechanisms and pathway convergence within the network.
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## P331

**Keep calm and give cabergoline: a giant prolactinoma presenting with seizures**

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Background
Prolactinomas are the major subtype (20–30%) of pituitary adenomas. Microprolactinomas ($<1$ cm) are commoner in females, whereas macroprolactinomas ($>1$ cm) occur mainly in men. Giant prolactinomas are rare (2–3% of prolactinomas) – features include size of $\geq 4$ cm, with significant extrasellar extension and prolactin levels of $>1000$ μg/l.
Case report
We report the case of a 35-year-old man with type 2 diabetes, who presented following a generalised seizure. He gave a history of twice-weekly headaches and generalised fatigue, but denied visual disturbance, erectile dysfunction, or loss of libido. MRI showed a $3.2\times3.5\times4.8$ cm intra- and suprasellar mass with cavernous sinus invasion causing significant mass effect on the right temporal lobe. Examination revealed sparse chest hair, bilateral testicular volume of 10 ml, normal genitalia and pubic hair. Visual field tests were normal. Total prolactin was 11 966 000 mU/l with bioactive monomeric prolactin of 128 520 mU/l. FSH was 12.1 IU/l, LH 6.1 IU/l, and testosterone 5.3 nmol/l. IGF1 was 19 nmol/l, GH $<0.10$ μg/l, TSH 2.57 mU/l, $FT_4$ 11 pmol/l, ACTH 7 ng/l, and 0900 h cortisol 574 nmol/l. He was commenced on cabergoline 250 μg twice-weekly and levetiracetam 250 mg twice daily, and referred to neurosurgery. Prolactin levels fell to 991 mU/l within 2 weeks, and continue to decline (271 mU/l at 8 months and 143 mU/l at 11 months). Repeat MRI at 4 months showed decrease in tumour size to $3\times4.2$ cm. The patient has had no further generalised seizures.
Conclusion
Pituitary macroadenomas usually cause complex partial seizures, and most patients have epilepsy prior to diagnosis of a macroadenoma. Uniquely, this patient first presented with a generalised seizure, and visual fields were spared despite cavernous sinus invasion. It also illustrates the importance of checking prolactin when an intracranial mass is found on neuroimaging, to avoid unnecessary neurosurgical intervention. Excellent biochemical response and decrease in tumour size support the view that cabergoline is an effective first-line treatment for giant prolactinomas.

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## P332

**Loperamide-induced hypopituitarism**

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Loperamide is a poorly absorbed opioid μ-receptor agonist that is the most commonly used anti-diarrhoeal medication in the UK. Prescription cost analysis from the Department of Health and Social Care Information Centre reported that 1.79 million prescriptions of the drug were issued in 2014 and it is also freely available 'over the counter'. It is widely believed to be very safe, with constipation as the main side-effect.
A 45-year-old man presented with profound fatigue and erectile dysfunction. Two years previously he had undergone total colectomy with rectal pouch formation for severe ulcerative colitis. To manage diarrhoea, he had been taking loperamide in doses between 40 and 50 mg daily for many months (BNF recommended maximum 16 mg daily). At presentation, he was hypogonadal with a morning serum testosterone of 2.9 nmol/l (normal range 9–25) and a peak cortisol following 250 μg Synacthen of 191 nmol/l. ACTH was 20 ng/l and gonadotrophins were inappropriately normal (LH 3.1 U/l and FSH 2.0 IU/l). Pituitary MRI scan was normal. He commenced hydrocortisone and testosterone replacement and felt much improved. More than a year later, pouch inflammation was treated with antibiotics; diarrhoea improved enough to temporarily reduce his loperamide dose to 4–6 mg daily for 48 h. On this dose, a repeat Synacthen test showed a peak cortisol of 833 nmol/l. However, over subsequent months worsening diarrhoea necessitated an increase in loperamide dose again to 15–20 mg/day, with a corresponding fall in peak cortisol to 453 nmol/l following Synacthen.
We have demonstrated that this man has dose-related hypopituitarism due to loperamide use. This is an important and hitherto unrecognised side-effect of this very commonly used medication. Critically, it occurs in our patient at a dose equivalent to, or just slightly higher than the maximum recommended dose. Clinicians need to be vigilant for adrenal insufficiency during high dose loperamide treatment.

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## P333

**An unusual case of pituitary pathology and the utility of whole body $^{18}$F-FDG PET–CT imaging in identifying extraneural biopsy targets**

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Background
Most cases of hypopituitarism are due to pituitary tumours or their treatment. Surgery is the treatment of choice in cases of pituitary adenomas which account for 90% of sellar and parsellar lesions. We present an unusual case of a non-adenomatous pituitary mass presenting with panhypopituitarism.
Case
A 56-year-old male with background of sickle cell trait and hypertension presented with syncope, lethargy and nausea. He had an 18-month history of headache and recurrent episodes of sinusitis.Clinical examination revealed proximal myopathy and normal visual fields. Pituitary function tests demonstrated an IGF1 of 27.1 nmol/l and partial hypopituitarism $fT_3$ 2.9 pmol/l, $fT_4$ 8.9 pmol/l, TSH $<0.03$, LH $<0.5$ IU/l, FSH $<1$ IU/l, and testosterone 3.4 nmol/l. He was also found to be in acute kidney injury with unremarkable renal ultrasound and negative urinary protein: creatinine ratio with no casts or red blood cells. Autoimmune profile revealed anti-MPO Abs 7.9 U/ml and anti-PR3 Abs 2.4 U/ml. ESR was elevated at 73 mm/hr. Pituitary MRI demonstrated a 16× 10 mm pituitary mass with supra-sellar extension. $^{18}$F-FDG whole body PET–CT was performed to investigate a possible underlying vasculitis or granulomatous process, this revealed FDG avid biapical lung nodules and right cervical lymphadenopathy.
Conclusion
The biochemical and imaging findings coupled with the patient's longstanding history of sinusitis point to a granulomatous/vasculitic cause for his pituitary mass. PET–CT has provided a target for extraneural biopsy, which is less risky and may be of higher diagnostic yield than neural biopsy. On confirmation of the underlying diagnosis we aim to initiate medical treatment of the underlying cause which might enable reduction/resolution of his pituitary mass without the risks of surgery.

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## P334

**Clinically non-functioning pituitary macroadenomas: presenting features and outcomes: recent experience at a tertiary centre**

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Introduction
Non-functioning pituitary macroadenoma (NFMA) can cause considerable morbidity due to pituitary dysfunction and pressure effects. We present recent experience in managing cases diagnosed with NFMA at a single tertiary centre between January 2009 and October 2013.
Results
Of the 63 patients with NFMA, 28 (44%) were females. Age ranged from 22 to 91 (mean 63). Visual disturbance symptoms (35/63; 57%) were the commonest presentation. Headache was the next commonest (21/63; 33%), followed by hypogonadism (11/63; 17%), pituitary apoplexy (8/63; 13%), and cranial nerve palsies (5/63; 8%). Ten patients (16%) were diagnosed incidentally. Objective visual field defects were noted in 35 (57%). Prolactin levels ranged from 25 to 2536 mU/l (mean 394). Six patients (10%) were found hypothyroid at presentation. Pituitary MRI scan showed elevation or compression of optic chiasm in 55 patients (87%).
Forty-nine (78%) underwent endoscopic transsphenoidal hypophysectomy. The indications were large tumour abutting chiasm (19/49; 39%), visual field defects (10/49; 20%), loss of vision (8/49; 16%), headache (6/49; 12%), cranial nerve involvement (3/49; 6%), and further tumour enlargement (3/49; 6%). Post-operative complications included transient diabetes insipidus (5/49; 10%), CSF leak (2/29; 4%), and haemorrhage (1/49; 2%). 35 patients (71%) reported symptom improvement post-operatively. Post-operatively, hormone replacement was required with testosterone (19/49; 39%), thyroxine (15/49; 31%), hydrocortisone (14/49; 29%), and GH (1/49; 2%). Patients with apoplexy had emergency surgery ($n=2$), elective surgery ($n=4$), and conservative management ($n=2$). Five patients had improved vision following surgery, while one patient undergoing surgery after a year had no visual recovery.
Conclusion
NFMAs represent the bulk of patients undergoing pituitary surgery. The clinical presentation can vary widely from asymptomatic incidental finding to catastrophic pituitary apoplexy as seen in this series. Majority of NFMAs require surgery for different indications, although a select subset can be managed conservatively with long-term follow-up.

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## P335

**Pituitary service review in a District General Hospital: a case for nationally agreed guidelines?**

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Background and aims
In 2010 pituitary service improvements were instigated, e.g., creation of local pituitary MDTs/joint pituitary clinics with surgeons. In the absence of national guidelines on post-surgical follow up of pituitary patients there was an evolution of local best practice standards. An audit was conducted in March 2015.
Methods
Twenty-six patients underwent pituitary surgery at local tertiary centre in 2013/14 with endocrine care based at the local District General Hospital (DGH). Standards post-operatively were: Pituitary clinic within 8 weeks; dynamic function tests of an appropriate nature/timing; imaging at 6 months; visual fields between 3 and 6 months; and surgical follow-up within 6 months.
Results
88.5% of patients were reviewed in the pituitary clinic (68% within 8 weeks). 92.3% of patients had an appropriate dynamic test. With regards to the aforementioned timings, 44% of imaging, 19.2% of visual fields (as documented in notes) and 65.4% of surgical clinics met our standards.
Overview
Good practice was seen in patients reviewed in the correct clinic at an appropriate time, both pituitary and surgical. Tests used showed excellent compliance with local standards. Challenges were faced with timing of follow-up actions. Communication from tertiary hospitals was noted to be variable. Record of visual field tests was patchy.
Action points
Dialogue between DGH and tertiary hospitals has been improved by liaising with neurosurgical juniors to ensure all post-op information is transferred electronically in the future. All visual field reports are to be received and filed on an electronic database in the DGH. Repeat audit in 2016.
Points for discussion
Nationally agreed guidelines for the post pituitary surgery follow up would greatly enhance the patient journey. We would suggest a national database for centers to compare their pituitary service on agreed national guidelines post discussion with Society for Endocrinology.

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## P336

**A case of pituitary functional recovery in a patient with Langerhan's cell histiocytosis following chemotherapy with chlorodeoxyadenosine and mercaptopurine**

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Introduction
Langerhans cell histiocytosis (LCH) is a rare multisystem disease. Pituitary manifestations include failure of the anterior and/or posterior pituitary, with diabetes insipidus and gonadotrophine deficiency being most prevalent. We present a case of a female who had pituitary failure yet conceived naturally following chemotherapy.
Case description
A 24-year-old female presented with polydipsia, polyuria, and lethargy. A water deprivation test was diagnostic of cranial diabetes insipidus. MRI showed thickening of the infundibulum and an abnormal pituitary. She initially responded well to DDAVP but following a miscarriage developed progressive anterior pituitary functional failure requiring thyroxine, hydrocortisone, and GH replacement. She had secondary amenorrhoea and did not tolerate HRT or OCP. At age of 36 she developed new and increasing breathlessness, which prompted further investigation. HRCT confirmed progressive diffuse pulmonary shadowing and histology following VATS biopsy was consistent with LCH presumed to be involving the pituitary gland and the lungs. Owing to increasing respiratory dysfunction she was being considered for lung transplantation and the decision was made to treat her with four cycles of chorodeoxyadenosine

(cladribine or 2-CDA) and this was followed with 1 year of 6-mercaptopurine. Prior to chemotherapy she was referred for ovum harvest and possible IVF. Following chemotherapy, her periods returned but in an irregular manner. One year later, she had a LHRH test with LH rose from 3.8 to 23 and FSH from 2.9 to 6.8. She is now 42 years old and has just found out that she is 26 weeks pregnant, something that she never thought possible.
Discussion
The case demonstrates functional pituitary recovery post cladribine and 6-mercaptopuirine in a patient with LCH.

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## P337

**Unusual features in a case of hypophysitis following ipilimumab therapy**

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Endocrinopathies are becoming increasingly recognised with the use of new anticancer drugs. Ipilimumab therapy has recently been associated with hypophysitis. The presentation of hypopituitarism can be non-specific and diagnosis in an oncology setting may be challenging. We describe a 77-year-old man who presented to oncology with a short history of lethargy, nausea, anorexia, and weight loss. He had completed four cycles of ipilimumab as treatment for melanoma 7–9 months earlier. He was dehydrated and hypotensive, and had hyponatraemia (Na 128 mmol/l). He improved with i.v. fluids and was discharged after 6 days. He re-presented 1 week later with vomiting, lethargy, and weakness. The hyponatraemia had worsened (Na 122 mmol/l). He was markedly cortisol deficient, 0900 h cortisol 24 nmol/l, ACTH $<5$ ng/l; pituitary function was otherwise intact (TSH 5.68 mU/l, $fT_4$ 11.7 pmol/l, and testosterone 22 nmol/l), posterior pituitary function was normal, and prolactin was 1984 mU/l. Treatment with hydrocortisone led to an immediate marked improvement. MRI pituitary showed a partially empty sella; the pituitary gland was small, with otherwise normal appearance and homogenous enhancement.
This case of ipilimumab-associated hypopituitarism is unusual in that there was no radiological evidence of hypophysitis, and hypopituitarism was partial, with selective corticotroph damage only. Ipilimumab is a MAB therapy directed at cytotoxic T lymphocyte antigen 4 (CTL4); it is thought that CTL4 is expressed in the anterior pituitary gland, and that this represents a mechanism for the development of hypophysitis. In this case the loss of corticotroph function developed 9 months after first exposure, later than would be expected. It may be that the partially empty sella reflects the end result of hypophysitis. Endocrinologists should be aware that ipilimumab-associated hypophysitis may be delayed in onset and selective in loss of pituitary function; furthermore it may occur without radiological evidence of hypophysitis.

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## P338

**Evolution in acromegalic patients with discordant GH–IGF1 levels during medical treatment**

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Acromegaly control/remission is defined by stringent criteria: normal age-/sex-adjusted IGF1 and random GH (GHr) $<1$ ng/ml or a GH nadir (GHn) during oral glucose tolerance test (OGTT) of $<0.4$ ng/ml. However discordances between GH and IGF1 have been recorded in about 30% of patients. We retrospectively analyzed the evolution in patients with acromegaly and discordant GH–IGF1 levels while being treated with somatostatin analogs (SSA) and/or cabergoline (CAB).
Out of 22 patients with acromegaly treated with SSA and/or CAB in 2011–2015, seven patients (all women, mean age at diagnosis 48 years, range 29–63 years) had discordant normal GH and elevated IGF1 levels (31.8%). One patient had also a transient increased GHr with normal IGF1 during follow-up. All had macroadenomas, 42% were obese, 42% overweight. One patient had diabetes mellitus and four had impaired fasting glucose. Six patients were treated with SSA (two also+CAB), one only with CAB, five had previous pituitary surgery, and two had also radiotherapy. The patients had a mean number of 6.7 evaluations of IGF1 and GH (4–8) during a mean follow-up of 34.5 months (25–42). Mean elevated IGF1 was $1.43\times$ upper limit of normal (1.08–1.83).
A SSA dosage increase and/or CAB addition in six patients normalized both GH and IGF1 in three patients (50%), one patient was not controlled on both GH and IGF1 and in two patients the discordance persisted. In one of these latter patients the tumor had a $>50$% decrease in size; in all the others the tumors had no or up to 25% shrinkage. No significant alterations of glycemia or blood pressure have been recorded.
Conclusion
Management of acromegalic patients with discordant GH–IGF1 values needs to be individualized. Long-term studies on morbidity and mortality in these patients are needed.

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## P339

**Frontal bone recurrent ectopic craniopharyngioma after transfrontal resection: case report**

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Craniopharyngiomas are rare solid or mixed solid-cystic tumours. Although benign histologically, these tumours frequently shorten life and should be considered low-grade malignancies.
We present the case of a 12-year-old boy diagnosed in 2008, at age 5, with a suprasellar tumour of 22/21/20 mm with mixed solid and cystic areas. The tumour was operated twice by left transfrontal approach in 2008 and right transfrontal approach in 2009. The pathology exam revealed an adamantinomatous craniopharyngioma, with a MIB1 of 5%. Postoperatory, the patient developed hypopituitarism: the insulin tolerance test revealed maximum GH: 0.52 ng/ml, maximum cortisol: 0.33 μg/dl, glycaemic nadir: 25 mg/dl, normal prolactinaemia: 15.48 ng/ml, LH: undetectable, FSH: 0.15 mUI/ml, and TSH: 1 mU/l with low $T_4$. He also developed a polyuro-polydipsic syndrome of 6 l/day–insipidus diabetes. The hypopituitarism was well controlled on levothyroxine 50 μg/day, hydrocortisone 15 mg/day (with dose adjustments), and desmopressin 240 μg/day. Even though the patient associated GH deficiency, this did not prejudice his growth (at 12 years and 5 months he had $-0.55$ S.D.).
No imagistic recurrence of the tumour was identified until 2014 when he presented with a left frontal tumefaction with progressive expansion which was operated in 2015 – pathology exam: frontal bone tumour of 30/25 mm with solid and cystic parts, IHC: β-catenin positive, Ki-67 = 10% – ectopic recurrence of the adamantinomatous craniopharyngioma in the left frontal bone, 6 years after the first resection.
Conclusion
We emphasise the importance of preventing iatrogenic tumour implantation. Long-term follow up is mandatory even if the resection is complete and no local recurrence is detected in early check-ups. External radiotherapy should be taken into consideration in such cases.

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## P340

**An unusual case of acromegaly**

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A 57-year-old woman presented with a 4-week history of lethargy, weight loss, polyuria, and polydipsia. Her only past medical history of note was hypertension for which she was taking lisinopril. On admission her initial investigations showed: glucose 22.6 mmol/l, capillary blood ketones Hi and a metabolic acidosis ($H+57.5$ nmol/l and $HCO_3-$ 13.7 mmol/l). She was commenced on the DKA protocol and her acidosis resolved without complication. She was maintained on a variable rate insulin infusion until she was eating and drinking. Her daily intravenous insulin requirement was in excess of 150 units/day. Her HbA1c was 110 mmol/mol and anti-GAD antibodies were strongly positive ($>2000$ units/ml). She was reviewed by the inpatient diabetes team, started regular s.c. insulin and was noted to have clinical features of acromegaly, course facial

features, doughy hands and prognathism. Anterior pituitary function showed a baseline cortisol <40 nmol/l rising to 404 nmol/l after ACTH stimulation, protactin 47 nU/l, $fT_4$ 10 pmol/l, TSH 0.24 mU/ l, and inappropriately low gonadotrophins and oestradiol. Oral glucose tolerance test showed no suppression of GH (2-h value of 2.6 ng/ml). IGF1 was elevated at 603 μg/l. An MRI pituitary confirmed a macroadenoma (17×18×20 mm) abutting the optic chiasm and extending into the right cavernous sinus. Formal visual field assessment was normal. She started replacement hydrocortisone, somatostatin analogues and she is awaiting neurosurgical review.
Acromegaly is often associated with T2DM. Although our patient has T1DM, the suspicion of concurrent insulin resistance arose from a high fasting intravenous insulin requirement. Biochemical interpretation of GH and IGF1 levels can be difficult in poorly controlled diabetes. However, our patient had clinical features consistent with acromegaly and a pituitary mass making the diagnosis unequivocal. This case highlights the need for speciality assessment in all patients presenting with DKA.

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## P341

**How common is ipilimumab-induced hypophysitis leading to cortisol deficiency?**

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A 73-year-old female, fit and well otherwise, was not any regular medications, was diagnosed with choroidal melanoma in 2010, and enucleated, subsequently had DTIC for multiple metastasis in 2014. She also received ipilimumab for 3 months as second line treatment for metastases. Presented to neurosurgeons with cold intolerance and dizzy spells, random cortisol was <30, started on dexamethasone 1 mg twice daily. Also had a low TSH of 0.71 with low free $T_4$. Short Synacthen test showed baseline cortisol of 18 and 30 min value was 96. Dexamethasone was stopped and started on hydrocortisone 20 mg in the morning and 10 mg in the afternoon. Had normal gonadal profile, IGF1, TFT on repeat blood test. MRI pituitary did not show any focal pituitary lesion. The objective of this case report is to review the literature and propose any further evidence of ipilimumab-induced hypophysitis, treatment and follow up. Hypophysitis is a common side effect in patients with malignant melanoma treated with monoclonal antibodies such as ipilimumab as evidence from the following cohort study in Massachusetts General Hospital. Hypophysitis diagnosed in 17 patients (11%). Male gender ($P=0.02$) and older age ($P=0.005$), but not the cumulative dose of Ipi, were risk factors for IH. All patients with IH had anterior hypopituitarism (none had diabetes insipidus). Hypopituitarism was persistent in most individuals (76%). Hormone deficiencies improved except corticotroph function. MRI brain does not necessarily show changes in all patients. Physicians should be aware of IIH and diagnose early in order to prevent fatal complications such as adrenal insufficiency. Clinicians involved in case study are: Drs Sid McNulty, Fareha Bawa, and Dhanya Kalathil.

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## P342

**TSH-secreting pituitary adenoma with negative TSH staining**

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A 31-year-old woman presented with oligomenorrhoea and a raised prolactin. Pituitary scan revealed a 1.8 cm pituitary mass with compression of the optic chiasm. TSH was normal at 3.66 mIU/l with a raised $FT_4$ of 25.8 pmol/l. The rest of pituitary function was normal. Visual fields demonstrated a bitemporal upper quadrantanopia. A diagnosis of TSH-secreting pituitary adenoma was suspected because of the high normal TSH in the presence of a high $FT_4$. She underwent transsphenoidal surgery which resulted in good decompression of the visual pathway. Histology showed a non-functioning pituitary adenoma with negative staining for all pituitary hormones including TSH. Post-op pituitary function was normal including $FT_4$ of 16.4 pmol/l and TSH of 0.77 mIU/l and pituitary scan showed a small residual tumour. Soon after surgery the patient defaulted from follow up. She was re-referred after three years with headache, visual disturbance, and weight loss. $FT_4$ was elevated at 30.3 pmol/l and TSH was 2.6 mIU/l. Repeat MRI showed an increase in the size of the pituitary tumour. Visual fields and alpha subunit were normal. There had been a gradual rise in $FT_4$ and TSH since surgery and given the increase in tumour size along with biochemical changes suggesting a TSHoma, it was decided to give the patient a trial of somatostatin analogue therapy. Response to therapy was excellent with $FT_4$ and TSH reducing to 12.4 pmol/l and 0.7 mIU/l and significant tumour shrinkage confirming a diagnosis of TSHoma.
TSHomas are rare pituitary tumours characterized by high levels of circulating free thyroid hormones in the presence of non-suppressed TSH. Surgery is the usual first line of therapy. In this case the histology failed to confirm the diagnosis. Subsequent clinical course and positive biochemical and radiological response to somatostatin analogue therapy was suggestive of a TSHoma.

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## P343

**An unusual cause of headache in pregnancy**

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A 35-year-old lady was admitted at 24 weeks gestation with a 3–4 weeks history of persistent severe, left sided headaches associated with vomiting but not with visual disturbance. She had a prior history of migraines. However, her current headaches were very different in character. On examination her Glasgow Coma score was 15. There was no neck stiffness or photophobia. Visual fields and fundoscopy was normal. She was haemodynamically stable. Magnetic resonance imaging (MRI) and venogram (MRV) of her brain were performed to exclude a venous sinus thrombosis. MRI revealed an enlarged sella turcica containing a macroadenoma with a fluid-fluid level, with signal characteristics consistent with subacute haemorrhage within the pituitary gland. She was reviewed by the endocrinology team and baseline pituitary function tests were performed: TSH 0.50 mIU/l (0.35–4.10), free $T_4$ 10.26 pmol/l (9.63–17.0), ACTH 20.7 ng/l, 0900 h cortisol 214 nmol/l, random GH 0.118 μg/l (0–0.8), and IGF1 19 nmol/l (16.1–39.8). Oestradiol was 26 963 pmol/l. In view of her low 0900 h cortisol she was commenced on hydrocortisone replacement. Formal Humphrey's perimetry was normal. Her case was discussed at the regional pituitary MDT. She did not require neurosurgical intervention but was transferred over to a tertiary care centre and jointly managed by the endocrinologists and obstetric team. She had an uneventful pregnancy and a healthy baby was delivered at 39 weeks gestation by caesarean section. She has ongoing endocrinology follow up and remains on hydrocortisone. Pituitary apoplexy is a medical emergency caused by haemorrhage and/or infarction of the pituitary gland. Pregnancy is a risk factor so this diagnosis should be considered in any pregnant woman presenting with acute severe headache or significant change in headache phenotype. Corticosteroid replacement may be life-saving. Neurosurgical intervention should be considered in cases with persistent visual field defects or deteriorating conscious level.

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## P344

**The aggressive clinical course of silent corticotroph pituitary adenomas: a case series**

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Silent corticoptroph adenomas (SCA) are pituitary tumours positive on immunohistochemical staining for ACTH but without clinical evidence of hypercortisolism. They account for 1.1–6% of surgically removed pituitary adenomas. Most tumours are macroadenomas with suprasellar extension present in 87–100% of the cases. They present with mass effects and this is in contrast to Cushing's disease, which is mostly attributed to microadenomas. Reports suggest that these tumours may behave in a more aggressive way then other pituitary adenomas. In some cases hypercortisolaemia may be observed later in the course of the disease. There is a high rate of tumour recurrence and in one series it was reported to be about 33%. Recurrence has been reported to be more frequent in patients treated with adjuvant radiotherapy. Pathological indices (increased mitotic range and Ki-67) do not predict recurrence or malignant transformation of the tumours. Surgery remains the main therapeutic approach and repeated neurosurgical intervention may be required due to recurrence.
We describe the clinical course, imaging, and histology of four such cases who presented as large non-functioning pituitary macroadenomas with visual field defects, with no clinical or biochemical features of hypercortisolaemia.

This case series highlight the importance of early neurosurgical input, recognition of histology and regular surveillance for recurrence. Management and follow-up protocols should be planned taking into account their potential aggressive behaviour, particularly upon recurrence. The development of florid pituitary Cushing's syndrome and local recurrence followed by metastatic disease (occasionally outside the central nervous system) has been reported.

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## P345

**Intrasellar meningioma**

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Meningiomas account for about 1% of sellar masses; they can mimic macroadenomas. Although majority are WHO grade 1 tumours, these are technically challenging due to high vascularity and often present with visual disturbance. Certain radiological features might help to suspect sellar/suprasellar meningiomas. We present two cases sellar meningioma.

Case 1

A 48-year-old-female with presumed diagnosis of non-functioning pituitary macroadenoma (NFPA) with mild hyperprolactinaemia was referred with hypopituitarism (on hydrocortisone, GH, and HRT). Repeat contrast MRI in 2013 suggested a possibility of sellar meningioma due to intense contrast enhancement, encroachment onto planum sphenoidale with associated bulging of roof of sphenoid sinus. Later she underwent successful transsphenoidal removal of the mass after developing subtle visual field defect. Histology confirmed grade 1 meningioma.

Case 2

A 62-year-old lady came with history of head injury 6 months ago when she had a fall and since then she had noticed a lump on her head. MRI and CT scan brain confirmed the presence of an interosseous and falcine lesion encroaching the surrounding brain area. She had elective excision of tumour. Histology showed a meningioma attached to dura without evidence of atypia or brain invasion. The bony part of specimen is undergoing decalcification.

Conclusion

Sellar meningiomas can be suspected preoperatively based on MRI signal characteristics, dural tail and evidence of hyperostosis on CT. Compared to pituitary adenomas there is increased risk of bleeding during surgery and hypopituitarism postoperatively.

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## P346

**Silent corticotroph adenoma presenting with severe visual loss**

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A 51-year-old man presented to an optometrist with a 3-week history of visual impairment following a minor headache. He was found to have reduced visual acuity 6/24 in both eyes and a dense bitemporal hemianopia and was referred urgently to Musgrove Park Emergency Department. MRI brain showed a cystic bleed from a pituitary macroadenoma, 30 mm in diameter, with compression of the optic chiasm. He had no clinical features of Cushing's or other hormone excess or deficiency and thyroid function, prolactin, FSH/LH, testosterone, 0900 h cortisol (672 nmol/l), IGF1, and electrolytes were normal. He underwent an urgent transphenoidal resection performed in Queen's Square, London and had an excellent recovery in vision and an intact pituitary axis postoperatively. The histology showed focal weak positivity to ACTH with ~20% of cell expression and a Ki-67 < 3%. He had a normal overnight dexamethasone suppression test (cortisol 25 nmol/l) consistent with diagnosis of silent corticotroph adenoma (SCA); the acute presentation likely secondary to subacute apoplexy due to pituitary haemorrhage. He will be followed up via the pituitary MDT with serial MRIs and pituitary hormone testing. SCAs show positive immunohistochemical staining for ACTH but are not associated with clinical or laboratory features of hypercortisolaemia and account for 1–6% of all surgically removed pituitary adenomas. At presentation most are macroadenomas with suprasellar extension; hence symptoms of mass effect are usually the first signs. It is not understood why SCAs do not cause cortisol excess but is thought to be due to molecular differences and altered cellular processing. A low Ki-67 index implies the tumour is less invasive but it has not been shown to predict recurrence. SCAs are no more likely to recur than null cell tumours but if they do they are typically more aggressive and so close follow-up is required.

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# Reproduction

## P347

**Analysis of the human foetal gonadal proteome at 13–14 weeks of gestation**

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Introduction

The human masculinisation programming window (8–14 weeks of gestation) sees testis-derived androgen drive the foetus towards a male phenotype. However, there are few systematic studies of human foetal gonad development.

Aim

To conduct a pilot analysis of the foetal gonadal proteome at the end of the masculinisation programming window (13–14 weeks of gestation).

Methods

Twenty-eight electively terminated foetuses (13–14 weeks of gestation: REC04/S0802/21) were divided into four groups ($n = 7$/group) based on foetal sex and maternal smoking status. Protein (and RNA) were extracted from whole gonads and 2D difference in-gel electrophoresis (DIGE) and SameSpots Software were used to analyse the proteomes. Proteins were identified in 18 consistently altered protein spots by LC–MS/MS and followed up by qPCR (same 28 foetuses plus 44 foetuses reported in the literature).

Results

There were no significant differences between foetal or maternal ages and morphometrics (except testes heavier than ovaries; $P < 0.05$). Of 448 protein spots included in the study, 147 were significantly ($P < 0.05$) different between groups (1.2- to 13.7-fold). Of these 55 were over-expressed in males, 76 in females and maternal smoking induced or abolished sex differences in 16 and 32 respectively. SERPINB9, KRT8, and KRT85 were higher in females; VIM, ECHS1, FDXR, VCL, EZR, HSPD1, IDH1, PRDX4, CYP11A1, PRDX3, TPI, GDI, UCHL1, IDI1, and GSTA1 higher in males. Transcript levels encoding these proteins were also significantly different between sexes. 14 proteins significantly associated in a network of drug metabolism and endocrine system development/function. Protein differences suggest increased cell proliferation, steroid synthesis/metabolism, and oxidative stress in the testis, consistent with known differences in testis and ovary developmental biology.

Conclusions

Proteomic analysis of abundant proteins showed that 29% protein spots have sex-specific expression. In-depth proteomic analysis of proteomes is likely to reveal further insight into sex-specific mechanisms and pathways in each type of human foetal gonad.

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## P348

**Development of a single-injection non-surgical sterilant via modification of measles virus pseudotyped particles**

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Feral cat numbers in the UK are thought to number around one million and the Dogs Trust estimates it dealt with just over 110 000 stray and abandoned dogs in 2013. This scenario is common across the world and in the developing world in

particular the control of feral populations is an ongoing challenge as well as a huge welfare issue. The only options currently available are expensive surgical neutering, or euthanasia of otherwise healthy animals. There is an urgent need to develop new approaches to deal with this growing issue. To address this issue and reduce these numbers we are developing a single-dose, non-surgical sterilant for cats and dogs as an alternative to surgical neutering and euthanasia.
To achieve this goal we have designed a targeting strategy based upon the inclusion of bespoke peptide sequences into the measles virus hemagglutinin envelope protein, permitting specific targeting of testicular Sertoli cells (essential for sperm development). Combining this cell-specific targeting approach with delivery of miRNAs against key genes, we demonstrate that a single injection leads to persistent knock-down of gene expression required for sperm production, resulting in infertility which is maintained for many months. This proof of concept study highlights the potential of this novel approach, which is now being taken forward for preclinical trials.

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## P349

**Decoding gonadotrophin receptor signalling via spatial regulation of the LH receptor**

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The LH receptor (LHR) is essential for mediating multiple functions in reproduction and pregnancy. LHR belongs to the superfamily of G-protein-coupled receptors (GPCRs) that impacts nearly every aspect of human physiology and pathophysiology. Membrane trafficking is a critical mechanism for cells to decode complex signalling networks into specific downstream responses, including the signalling pathways activated by GPCRs. A detailed molecular description of GPCR trafficking is indispensable for our basic understanding of cellular regulation and how aberrant signalling can result in disease.
We have recently identified a novel endosomal compartment critical for the sorting and signalling of distinct GPCRs, we term the very early endosome (VEE). Using our model GPCR for the VEE, LHR, we provide evidence for an unprecedented role for the adaptor protein APPL1 in regulated sorting from the VEE driven by LHR signalling. Employing high resolution total internal reflected fluorescence microscopy (TIR-FM), in combination with a pH sensitive GFP-tagged LHR to directly visualise individual LHR recycling events at the plasma membrane, we unveil detailed mechanistic information about LHR recycling and the role of PKA and APPL1 in this process. Altering LHR trafficking fate through the use of chemical inhibitors, siRNA and a conformational biosensor we revealed differential activation of the signalling pathways either from the plasma membrane and/or from VEEs. Interestingly, whilst Gαq/calcium pathway was totally dependent on LHR internalization, two waves of Gαs/cAMP pathway activation occurred, from the plasma membrane and from VEEs.
Overall these findings propose a novel mechanism for the post-endocytic sorting of GPCRs and a system where receptor activity could be reprogrammed by altering location of receptors. Physiologically, this may be a key adaptive mechanism for GPCRs, however, this could be exploited therapeutically in order to provide important insight to perturbed LHR activity under pathophysiological conditions.

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## P350

**IGF1 action in trophoblast involves endocytic and post-endocytic pathways**

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IGF1 plays a central role in placental growth. IGF delivered from maternal circulation binds to the IGF1 receptor (IGF1R) in syncytium resulting in the activation of Akt; inhibition of this pathway affects cytotrophoblast turnover. However, the route(s) by which IGF1 enters placenta and how its signal is delivered into the two trophoblast compartments is unknown. We have utilised quantum dots (QDs) to deliver/track IGF1 binding and internalisation in first trimester placenta (EP) and BeWo cells and investigate the role of endocytosis in IGF1 signalling in trophoblast.
EP (8–12 weeks) explants and BeWo cells were exposed to clathrin or caveolin dependent endocytosis inhibitors – CPMZ (50 μM) or m-β-CD (5 mM) respectively – for 1 h and subsequently treated with QD–IGF1 (50 nM) or IGF1 (20 nM) for 5–30 min. The effect of IGF1 on intracellular signalling was investigated by western blotting and immunocytochemistry.
IGF1 and QD–IGF1 induced phosphorylation of IGF1R and Akt in both EP and BeWo cells. In EP, IGF1R phosphorylation was observed on the syncytial microvillous membrane (5 min), and later in cytotrophoblasts (10–30 min), but pAkt was observed only in cytotrophoblasts (10–30 min). Initially, QDs co-localised with clathrin and early endosomes in the apical syncytioplasm, but co-localisation was lost with deeper penetration of the ligand. In EP, CPMZ but not m-β-CD decreased IGF-stimulated increase of pAkt reflecting absence of caveolin in the syncytial layer. In BeWo cells, QD–IGF co-localised with caveolin and m-β-CD, but not CPMZ, affected Akt activation.
IGF binding at the syncytial surface leads to clathrin-dependent internalisation and remarkably rapid Akt activation in cytotrophoblasts. Unlike villous trophoblasts, BeWo cells use caveolin for IGF internalisation reflecting differences in the cell membrane protein profile of distinct placental models. Further studies are required to identify specific functions of IGF1R/Akt signalling in respective compartments of the EP.

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## P351

**Intracrine androgens enhance decidualisation and modulate expression of human endometrial receptivity genes**

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During the establishment of pregnancy, the endometrium undergoes dynamic remodelling in order to establish a 'receptive' microenvironment. Decidualisation, a key part of this process, is characterised by differentiation of endometrial stromal fibroblasts which secrete factors that regulate implantation and placental development. Recent studies in our laboratory have revealed that decidualisation results in altered expression of enzymes that regulate biosynthesis and metabolism of estrogens. These results have prompted us to propose a role for local endometrial steroid biosynthesis in the establishment of a receptive endometrium. In the current study we have investigated whether tissue specific synthesis of androgens might also play a regulatory role in the endometrium during decidualisation.
Primary human endometrial stromal cells were isolated from endometrial biopsies collected from women during the proliferative phase of the cycle ($n=20$). *In vitro* decidualisation was induced by treatment with progesterone and cAMP. The expression of androgen biosynthetic enzymes and putative androgen-regulated receptivity markers was assessed by qPCR and western blot. Concentrations of IGFBP1, SPP1, testosterone and dihydrotestosterone (DHT) were determined by ELISA.
We found that decidualisation was associated with biosynthesis of androgens. Time-dependent changes in the expression of androgen biosynthetic enzymes *AKR1C3* and *SRD5A1* were detected by qPCR and Western blot. Decidualisation was associated with secretion of testosterone and DHT ($n=8$, $P<0.001$). When AR was inhibited by co-treatment with the antiandrogen flutamide a significant reduction in secretion of the decidualisation marker IGFBP1 ($n=8$, $P<0.01$) was detected. Flutamide also altered expression of putative androgen-regulated receptivity markers; osteopontin (*SPP1;* $n=8$, $P<0.01$), monoamine oxidase (MAOA; $n=8$, $P<0.01$), and endothelin receptor type B (EDNRB; $n=8$, $P<0.001$).
These data suggest intracrine biosynthesis of androgens plays a key role in decidualisation and endometrial receptivity. We speculate that efficient decidualisation is dependent upon formation of a unique steroid microenvironment that 'fine tunes' the endometrium in preparation for pregnancy.

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## P352

**I.v. and s.c. infusions of kisspeptin-54 stimulate gonadotrophin release similarly in healthy women**

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Background
Kisspeptin stimulates hypothalamic GnRH secretion resulting in gonadotrophin release and has the potential as a future therapeutic for infertility. Previous studies have observed that kisspeptin increases LH and to a lesser degree FSH when administered to healthy women, which may limit its therapeutic potential. However, studies in women with hypothalamic amenorrhoea show that i.v. infusions of kisspeptin-54 stimulated both LH and FSH equally. Chronic s.c. infusion of kisspeptin via a pump (similar to an insulin pump) may be an ideal route of administration in the future. However, there has been no direct comparison between the routes of administration in humans.
Aim
To compare the effects of i.v. and s.c. administration of kisspeptin-54 on gonadotrophin secretion in healthy females.
Design and patients
A prospective, single-blinded placebo-controlled study. Healthy women ($n=4$) received a single 8-h infusion of saline or kisspeptin-54 0.1, 0.3, or 1.0 nmol/kg per h (i.v. and s.c.) in the early follicular phase of consecutive menstrual cycles in random order. Gonadotrophins and oestradiol were measured every 10 min during the infusions.
Results
All doses of kisspeptin-54 administered both i.v. and s.c. increased LH (mean change AUC LH in h/IU per l: $-317\pm731$, vehicle; $360\pm178$, i.v. 0.1 nmol/kg per h; $412\pm446$, s.c. 0.1 nmol/kg per h; $4130\pm1508$, i.v. 0.3 nmol/kg per h; $1251\pm598$, s.c. 0.3 nmol/kg per h; $3014\pm1879$, i.v. 1.0 nmol/kg per h; and $2184\pm474$, s.c. 1.0 nmol/kg per h). There was no significant difference between i.v. and s.c. administration on gonadotrophin levels.
Conclusion
We report that i.v. and s.c. administration of kisspeptin-54 at the same dose has no significant difference on gonadotrophin release. This has important implications for the development of kisspeptin to be given subcutaneously at home as a potential future therapeutic for patients with infertility. Further studies are required to establish the optimum dose and duration of treatment.

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## P353

**Assessment of reproductive parameters and oxidative stress following treatment with alcohol and/or cannabinol in male Wistar rats**

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The male gonads have been exposed to various degrees of toxicants over the years and these substances affect fertility success rate. The present study investigates the effects of alcohol and/or cannabinol administration on reproductive parameters and biochemical profile in male Wistar rats. Twenty-five Wistar male rats were divided into five groups of five rats each. Group 1 served as the control, group 2 was administered methanol, 5 mg/kg BW (vehicle for cannabinol), group 3 was treated with alcohol (3 g/kg BW as 25% v/v), group 4 was administered cannabinol (10 mg/kg BW), and group 5 was treated with alcohol (3 g/kg BW as 25% v/v) plus cannabinol (10 mg/kg BW) for a period of 8 weeks via oral administration. Ethical approval was obtained from National Institute for Pharmaceutical Research and Development (NIPRD) and the experiment conducted was in conformance with guidelines for experimental procedures as set forth in the Declaration of Helsinki on the Guiding Principals in the care and Use of Animals. At the end of the experiment, blood was collected via the retro-orbital sinus and allowed to clot to obtain serum for hormonal assay (FSH, LH, and testosterone). The animals were anesthetized and dissected; the testes together with the epididymides were harvested and the caudal epididymis was removed for sperm characteristics (motility, count). The lipid peroxidation was estimated on the concentration of thio-babituric acid reactive product, malondialdehyde (MDA), the testes of both the control and the treated groups were fixed for histological examination. There were significant reductions ($P<0.05$) in sperm profile, serum levels of gonadotrophins (FSH and LH) and testosterone in groups treated with alcohol and/or cannabinol in comparison with the control group; a significant increase ($P<0.05$) in MDA was observed in the alcohol and cannabinol treated rats when compared with the control. Alcohol and/or cannabinol altered reproductive function is associated with reduction in hormone profile and increased in the MDA level. The testicular histology showed reduction in spermatogonia and incomplete maturation Thus, the results of the present study depict that administration of alcohol and/or cannabinol affects reproductive parameters through oxidative stress as measured by MDA and altered hormone profile in experimental rats.

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## P354

**Increased expression of circulating miRNA-93 in women with polycystic ovary syndrome may represent a novel, non-invasive biomarker for diagnosis**

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Introduction
MicroRNAs (miRNA) are a novel class of small noncoding single-stranded RNA molecules 18–24 nucleotides long that regulate gene expression at the post-transcriptional level. There is increasing evidence of their importance in polycystic ovary syndrome (PCOS), with their differential expression depending on obesity. There is recent evidence that miRNA-93 and mi-RNA-223 may have a role to play in the modulation of insulin resistance, through GLUT4 modulation in adipose tissue that is inherent in this condition.
Objective
To determine if miRNA-93 and miRNA-223 are differentially expressed in the circulation of women with PCOS compared to age matched women without PCOS and to correlate these miRNAs to the metabolic indices found in PCOS.
Design
A case–control study comparing women with PCOS ($n=25$) to age ($32.8\pm7.7$ years vs $32.1\pm9.0$ years) and weight ($76.0\pm18.8$ kg vs $77.4\pm16.3$ kg) matched controls ($n=24$) without PCOS. miRNA-93 and miRNA-223 were determined by total RNA RT.
Results
Women with PCOS had significantly higher insulin, HOMA-β and testosterone levels compared to control subjects. Both miRNA-93 and miRNA-223 were significantly increased relative to the control group ($P<0.016$ and $P<0.019$ respectively). In both the control group and the PCOS group there was no correlation of either miRNA-93 or miRNA-223 with any of the parameters including insulin, HOMA-IR, HOMA-β, or testosterone levels. The area under the receiver operator characteristic (ROC) curve (AUC) for miR-223 and miR-93 was 0.66 and 0.72 respectively, suggesting miR-93 is a more efficient biomarker than miR-223 for the diagnosis of PCOS. The combination of the two miRNAs together, tested using multiple logistic regression analysis, did not improve the diagnostic potential of miR-93 alone (AIC miR-93+miR-223, 64.868 and AIC miR-93, 63.51).
Conclusions
Circulating miRNA-93 and miRNA-223 were higher in women with PCOS compared to age and weight matched controls independent of insulin resistance and testosterone levels, and miR-93 may represent a novel diagnostic biomarker for PCOS.

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## P355

**Vitamin D regulates extravillous trophoblast migration by inhibiting sphingosine-1-phosphate (S1P) signalling via S1P receptor 2**

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Failure of trophoblast invasion and remodelling of maternal blood vessels leads to the pregnancy complication pre-eclampsia (PE). Metabolomic profiling of placentas from such pregnancies has identified deranged sphingolipid metabolism as one of the pathways altered in PE. In other systems, the bioactive sphingolipid, sphingosine-1-phosphate (S1P) controls cell migration therefore this study aimed to determine its effect on extravillous trophoblast (EVT) function.

S1P (50 nM–10 μM) attenuated migration of the EVT cell lines, Swan-71 and SGHPL-4 ($P<0.05$). QPCR and immunolocalisation demonstrated that these cells express S1P receptors 1–3. However S1PR2 was responsible for mediating S1P's inhibitory effect as the specific S1PR2 inhibitor, JTE-013 (100 nM) abolished S1P-attenuated migration ($P<0.05$) whereas treatment with the S1R1/3 inhibitor, FTY720 (100 nM), had no effect. S1PR2 can associate with the G proteins Gα12/13, Gαq, or Gαi, however analysis of Swan-71 cell migration and actin cytoskeleton in the presence of S1P ± the Rho kinase inhibitor, Y-27632 (10 μM; $n=6$) suggests preferential activation of Gα12/13. Recent studies of osteoclast suggest that S1PR2 is regulated by vitamin D thus we investigated whether vitamin D affects S1P signalling in trophoblast. QPCR analysis revealed a significant reduction (fourfold decrease; $P<0.05$) in S1PR2, but not R1 and R3, expression after treatment with 10 mM $1,25(OH)_2D_3$ for 48 or 72 h. Moreover, S1P did not inhibit the migration of Swan-71 cells exposed to 10 nM $1,25(OH)_2D_3$ ($P<0.05$).

This study demonstrates that although EVT express three S1P receptor isoforms, S1P predominantly signals through S1PR2/Gα12/13 to activate Rho and actin stress fibre formation and thereby acts as potent inhibitor of EVT migration. Importantly, expression of S1PR2, and therefore S1P function, can be down-regulated by vitamin D. Our data suggest that vitamin D deficiency, which is known to be associated with PE, may contribute to the impaired trophoblast migration that underlies this condition.

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## P356

**Visceral fat drives 5α-reductase activity independent of BMI in women with polycystic ovarian syndrome**

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Context

Androgen excess, obesity and hyperinsulinaemia are the cardinal features of polycystic ovarian syndrome (PCOS). While several studies have addressed the relationship between androgen excess and hyperinsulinaemia, the link between androgen excess and fat distribution remains largely undefined. Recent work has highlighted the importance of adipose tissue as an organ of androgen activation. Here, we evaluated the relationship between visceral fat and androgen excess in women with PCOS.

Patients and methods

Seventeen women with PCOS were compared with 17 healthy volunteers matched for sex, age, and BMI. All subjects underwent BMI measurement and body composition assessment by dual-energy X-ray absorptiometry and assessment of their androgen status by measurement in serum and 24-h urine utilising mass spectrometry-based assays. A Pearson's product-moment correlation coefficient was computed to assess the relationship between visceral adiposity and androgen generation.

Results

Women with PCOS, in comparison to healthy controls, had significantly higher visceral fat mass (944 ± 593 g vs 406.1 ± 378.9 g, $P<0.01$). Across the groups, there was a positive correlation between visceral fat mass and two urinary steroid metabolite ratios reflective of 5α-reductase activity, androstenedione/etiocholanolone (An/Et) ($r=0.455$, $n=32$, $P=0.009$), and 5α-tetrahydrocortisol/tetrahydrocortisol (5αTHF/THF) ($r=0.443$, $n=32$, $P=0.011$). 5α-reductase activity was significantly higher in PCOS compared to healthy controls (An/Et ratio 1.4 ± 0.6 vs 1.1 ± 0.3 and 5αTHF/THF 1.2 ± 0.6 vs 0.8 ± 0.3), and correlated significantly with visceral fat.

Conclusion

Our results lead us to hypothesise that visceral adiposity may drive androgen activation in PCOS by upregulation of 5α-reductase activity, which in turn results in further fat accumulation. Weight management is therefore likely to be a highly useful tool in ameliorating androgen excess in obese women with PCOS.

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## P357

**Impact of male cholestasis on the sperm epigenome and consequences for the health of the offspring**

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Introduction

Accumulating evidence has shown that not only maternal health during pregnancy, but also the paternal metabolic status at the time of conception have an impact on the subsequent health of the offspring. Cholestatic liver diseases are metabolic conditions characterised by increased circulating serum bile acid and lipid levels. A previous study has shown that long-term cholestasis results in destruction of the blood–testis barrier and germ cell apoptosis. We hypothesise that paternal cholestasis alters intratesticular homeostasis and impacts the sperm epigenome, resulting in altered disease susceptibility in the offspring.

Methods

A 7–9-week-old male mice were fed a RM3 normal chow (NC) diet or RM3 diet supplemented with 0.5% cholic acid (CA diet) for 10 weeks. At completion of feeding, the effects of cholestasis on sperm and testes were studied. Sperm DNA damage, global DNA methylation and hydroxymethylation, and sperm microRNA content were assessed. A separate cohort of male mice was fed a NC diet ± supplementation with 0.5% CA for 10 weeks and mated to age-matched NC-fed females. Females were allowed to deliver and litters were standardised to five to six pups at day 1.

Results

CA feeding for 10 weeks resulted in a significant increase in sperm DNA damage ($n=5$–6, $P$ value $\leq 0.05$). Concomitantly, increased mRNA expression of the apoptotic marker FasL in the testes ($n=5$–6, $P$ value $\leq 0.05$) was observed. After 10 weeks of CA feeding, global sperm DNA methylation and hydroxymethylation showed a trend for decreased 5-mC% and 5-hmC% DNA content ($n=5$–6). Altered sperm miR-34c_1 content was also observed ($n=5$–6). Ongoing studies investigate the impact of paternal cholestasis on the phenotype of the offspring.

Conclusions

Male cholestasis was associated with DNA damage in the sperm, accompanied by increased apoptosis in the testes. Global sperm DNA methylation, hydroxymethylation, and microRNA content were affected by male cholestasis.

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## P358

**Isoform-specific knockdown of PKC isoforms reveals PKCβ2 is required for epidermal growth factor stimulated expression of the prostaglandin synthase COX2 in human myometrium**

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Intrauterine prostaglandin (PG) synthesis and release are key components of human labour at term and pre-term, resulting in synchronised membrane rupture, cervical dilation and myometrial contractility. A key mediator of uterine PG production is the highly inducible enzyme cyclooxygenase 2 (COX2 or PTGS2). Epidermal growth factor (EGF) is released by the foetus during pregnancy and amniotic fluid levels rise rapidly during late gestation, moreover EGF stimulates expression of COX2 in myometrial and amnion cells. We have previously demonstrated using pharmacological inhibitors that both ERK1/2 (PD-184352) and PKC (bisindolylmaleimide I) are required to mediate the effect of EGF on COX2 expression in human myometrium, and now extend these studies by determining the involvement of distinct PKC isozymes. Isoform-specific siRNAs were used to decrease expression of PKC proteins in cultured myometrial cells; preliminary data reveal that PKCβ2 siRNA ablates EGF-stimulated COX2 expression measured by immunoblotting, while PKCα siRNA significantly enhances COX2 expression. Taken together, our results suggest that PKCβ2 and PKCα adopt opposing roles in the regulation of COX2 expression, and highlight

the limitation of using pharmacological kinase inhibitors that target multiple isoforms. These studies are being extended to include PKC isoforms from conventional, novel and atypical families, providing us with comprehensive insights into regulation of the *COX2* gene by PKC and the involvement of PKC in myometrial EGF action. The findings contribute to our understanding of the complex regulation of PG synthesis at labour.

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## P359

### Information transfer in GnRH signalling: ERK-mediated feedback loops control hormone sensing

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Single cell measurements of signalling proteins typically reveal high cell–cell variability raising questions about how reliably individual cells sense their environment in order to make decisions. Information theoretic approaches can be used to explore such sensing, treating cell signalling pathways as 'noisy' communication channels. Mutual information (MI) can be calculated between system inputs and outputs as a statistical measure of the reliability of sensing (Voliotis *et al*. *PNAS* **111** E326, 2014). GnRH acts via $G_{q/11}$-coupled seven-transmembrane receptors to stimulate ERK, but information transfer has not previously been quantified for these (or other) hormone receptors. Here we do so using automated fluorescence microscopy to quantify dual-phosphorylated (pp)ERK in HeLa cells transduced with recombinant adenovirus to express the GnRH receptor. Population-averaged data showed concentration-dependent responses to GnRH that were rapid (maximal at 5 min) and transient (near basal at 60–360 min). Individual cell measures were used to calculate MI, which showed a similar time course for information transfer; I(ppERK;GnRH) increased to $>0.6$ bits at 5 min with a gradual reduction to $<0.2$ bits by 60–360 min. We found that MI is controlled by the relative strength of distinct feedback loops. Reducing fast negative feedback (by expressing catalytically inactive ERK2 alongside siRNA to knock down endogenous ERK1/2) and increasing slow negative feedback (by Egr1-driven expression of dual-specificity phosphatase 5 (DUSP5)) both reduced MI between GnRH concentrations and ppERK responses. Information transfer was also reduced when protein synthesis was blocked to prevent GnRH from increasing DUSP expression. MI values were always $<1$, implying that information transfer through these pathways is insufficient for an individual cell to unambiguously distinguish between two states of its environment. Thus, by quantifying information transfer we find that individual cells are unreliable sensors of GnRH concentration and that this is influenced by fast and slow ERK-mediated negative feedback.

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## P360

### Whole exome sequencing in congenital hypogonadotropic hypogonadism

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Congenital hypogonadotropic hypogonadism (CHH (MIM161110)) due to GnRH deficiency is a rare genetic disorder (affects $\sim$1/30 000) characterised by abnormal pubertal development and infertility. Over 60% cases have anosmia (Kallmann syndrome) and some exhibit additional phenotypes. CHH is a genetically heterogeneous developmental disease. Most cases present sporadically, although familial forms (AD, AR, and X-linked) with incomplete penetrance and variable expressivity occur. Research suggests it is emerging as a digenic or oligogenic disease, rather than a monogenic trait. Genetic testing for this condition has hitherto been costly, time-consuming and incomplete, with mutations identified in $<30\%$.

In this study, we investigated three simplex cases (2M with partial anosmia, 1F) who presented with delayed puberty and hypogonadism due to isolated GnRH deficiency. Whole exome sequencing (WES) was performed on each patient using the Illumina HiSeq2500 platform and the Agilent SureSelect Human All Exon v5 Kit. Following alignment to the human genome, an in-house pipeline applied a virtual panel to restrict the genes to be analysed based on the clinical presentation (22 *CHH* genes). Variants were filtered to identity potential pathogenic mutations, which were subsequently confirmed by Sanger sequencing.

Results

Seven variants in four *CHH* genes (*FGFR1*, *GNRHR*, *HS6ST1* and *IL17RD*) were identified. One case had four separate variants in total. Variants in these genes are recognised to result in deficient GnRH activity, confirming that a Kallmann phenotype can result from mutations in *CHH* genes other than *KAL1*.

Conclusion

We demonstrate that WES, with analysis limited to relevant genes, can successfully and efficiently confirm a molecular diagnosis in a genetically and phenotypically complex disease, such as CHH. Further studies will increase our understanding of the functional consequence of gene variants, to help inform prognosis, enable family studies and timely intervention. In the future it may inform choice of optimal treatment.

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## P361

### Implantation and pregnancy outcome of rats fed with low and high salt diet

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Minerals and micronutrients deficiency have been reported to impair reproductive function. We investigated the influence of low salt diet and high salt diet on implantation and pregnancy in Sprague-Dawley rats. One hundred and forty-four rats were acclimatized and divided into three groups consisting of control/normal salt diet (0.3% salt), low salt diet (0.14% salt) and high salt diet (8% salt). All the rats were fed with respective diets for 6 weeks. The oestrous cycle was then monitored daily and on the evening of proestrus male rats were introduced for mating. Mating was confirmed by the presence of sperm cells in the smears the following day and was taken as day one of pregnancy. Implantation studies were carried out on days 6 and 8 of pregnancy while foetal parameters were ascertained on day 19 of pregnancy and at term. Levels of progesterone, oestradiol, prostaglandin E2, nitric oxide (NO) and cyclic guanosine monophosphate (cGMP), were measured on days 6, 8 and 19 of pregnancy. The results showed decreased implantation sites on days 6 and 8 in high salt fed rats, decreased birth weight in low salt fed rats and increased placental to birth weight ratio in the high salt fed rats when compared with control. A significant decrease in PGE2 level was found on day 8 of pregnancy in the high salt fed rats, while progesterone: oestradiol ratio was decreased on days 6 and 8 of pregnancy in both low salt and high salt fed rats. Also, NO and cGMP levels were decreased on day 19 of pregnancy in the low salt fed rats when compared with control ($P<0.05$). The results showed that a shift in salt homeostasis either to a high or a low salt level causes hormonal imbalance, impaired implantation and pregnancy outcome.

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## P362

### Vitamin D metabolic profiling across pregnancy

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Vitamin D-deficiency during pregnancy has been associated with increased complications of pregnancy including a high risk of pre-eclampsia (PET). Current analysis of vitamin D 'status' is based exclusively on analysis of maternal serum 25-hydroxyvitamin $D_3$ ($25(OH)D_3$), the circulating precursor form of vitamin D. We hypothesised that comprehensive profiling of vitamin D metabolites may provide a more accurate determination of vitamin D function in pregnancy. A liquid chromatography-tandem mass spectrometry (LC-MS/MS) method was developed to separate multiple vitamin D metabolites from serum samples obtained from: normal pregnant women (NP) at 1st trimester ($n=22$) and 3rd trimester ($n=21$); women with PET ($n=21$); non-pregnant female controls ($n=20$). Data (see table, Mean $\pm$ S.E.M.) demonstrated that active ($1\alpha,25(OH)_2D_3$, $P<0.001$), catabolic ($24,25(OH)_2D_3$, $P<0.05$) and inactive (Epi-$25(OH)D_3$, $P<0.01$) are increased in serum from 3rd trimester and PET pregnancies relative to NP women. Epi-$25(OH)D_3$ was elevated in all women across trimesters ($P<0.01$). By contrast, conventionally measured $25(OH)D_3$ showed no significant change in any of the groups. Ratio of $24,25(OH)_2D_3$/1-$\alpha,25(OH)_2D_3$ was $100.8\pm2$ in normal 3rd trimester, and $258.9\pm39$ in PET

($P<0.05$). Ratio of Epi-25(OH)$D_3$/1α,25(OH)$_2D_3$ was 77.4±7 in normal 3rd trimester, and 172.3±29 in PET ($P<0.01$). These data, suggests a shift towards catabolic pathway of vitamin D metabolism in PET. The albumin (decreases) and DBP (increases) levels inversely correlated with gestation (Table 1).

**Table 1**

| | 25(OH)$D_3$ (nM) | Epi-25(OH)$D_3$ (nM) | Iα,25(OH)$_2D_3$ (pM) | 24,25(OH)$D_3$ (nM) | DBP (μm) | Albumin (μm) |
|---|---|---|---|---|---|---|
| Non-pregnant | 35.18±3.8 | 5.04±0.4 | 42.04±4.1 | 4.66±0.95 | 1.69±0.21 | 557.04±28.86 |
| 1st trimester | 34.7±4.34 | 7.98±0.37 | 52.36±5.17 | 2.84±0.65 | 2.32±0.25 | 554.43±25.85 |
| 3rd Trimester | 44.61±5.04 | 7.64±0.41 | 108.34±6.67 | 10.6±2.12 | 2.48±0.31 | 330.06±21.07 |
| PET | 38.45±5.94 | 9.49±0.76 | 77.84±9.27 | 15.96±2.56 | 2.38±0.31 | 409.26±20.15 |

Measurement of multiple vitamin D metabolites and metabolites ratios may improve the interpretation of vitamin D status in pregnancy, possibly as an additional marker of adverse pregnancy events such as PET.

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## P363

### Vitamin D and foetomaternal immunity: effects on uterine natural killer cells

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Vitamin D deficiency is prevalent in pregnant women. Active vitamin D ($1,25(OH)_2D_3$) exerts important non-classical immune-regulatory effects, and the maternal placenta (decidua) is a potential target for this. $CD56^+$ -uterine natural killer (uNK) cells are the most prominent cell type in the decidua during early pregnancy. Given their critical role in foetal implantation and placentation, we hypothesised that uNK cells are a pivotal immunomodulatory target for vitamin D in the placenta. $CD56^+$ uNK were isolated from 1st trimester decidua tissue (uNKs)and matched maternal peripheral blood natural killer (pNKs) and stimulated with IL2, IL15, and IL12 in the presence and absence of $1,25(OH)_2D_3$ (10 nM). At 24 h, RNA encoding 1-α-hydroxylase (CYP27B1), 24-hydroxylase (CYP24A1), the vitamin D receptor (VDR) and IFN-γ were measured by qPCR and protein expression by flow cytometry. At baseline, both uNK and pNK cells expressed an intracrine vitamin D metabolic system, characterised by CYP27B1, CYP24A1 and VDR. The functionality of this system was demonstrated by both NK cell types as expression of deactivating CYP24A1 (~44- and 85-fold respectively) decreased following $1,25(OH)_2D_3$ treatment. In response to cytokines vitamin D activating CYP27B1 and VDR were increased in both NKs, but there was no significant change in CYP24A1. Thus stimulation appears to enhance uNK and pNK responsiveness to intracrine vitamin D. However, in studies of cytokine-induced NK immune function, $1,25(OH)_2D_3$ only suppressed inflammatory IFN-γ RNA expression by uNK cells (seven fold) suggesting differential vitamin D sensitivity in uNKs and pNKs. These data show for the first time that uNK cells have a functional vitamin D system, and may be more sensitive to $1,25(OH)_2D_3$ than their peripheral blood counterparts.

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## P364

### Use of an animal model to identify the origin and validity of the testicular dysgenesis syndrome hypothesis in humans

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From human epidemiological and related studies, there is strong (indirect) evidence that common male reproductive disorders that manifest at birth (cryptorchidism, hypospadias) or in adulthood (low sperm count, low testosterone, primary hypogonadism) may have a common origin in foetal life due to impaired androgen (testosterone) production or action; the so-called testicular dysgenesis syndrome (TDS) hypothesis. Whilst the foetal origin of cryptorchidism and hypospadias is self-evident, it is not an obvious explanation for adult onset disorders, other than for testicular germ cell cancer (TGCC). Consequently, the TDS hypothesis remains largely untested and unproven. If the TDS hypothesis was proved correct, it would refocus research effort towards identifying causal factors acting via the pregnant mother that might be preventable.

We have developed an animal model of TDS based on foetal exposure to the environmental chemical dibutyl phthalate (DBP), which causes impairment of foetal testosterone production in males. Here we use a refined version of the DBP rat model that included different time-windows of foetal exposure, such as only during the masculinisation programming window (MPW), in order to rigorously test the TDS hypothesis and to establish whether or not TDS disorders originated only within the MPW. Our results provide robust validation of the TDS hypothesis. They show that, irrespective of the endpoint evaluated (four TDS disorders, dysgenesis, anogenital distance), or the age when assessed (end of foetal life, adulthood), only disruption of testosterone production during the MPW results in TDS disorders, with all endpoints being intrinsically inter-related. In contrast, equal/greater DBP-induced suppression of testosterone production immediately after the MPW (late gestation) has no discernible effect on TDS disorders and associated endpoints. These findings have considerable human health and research implications.

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## P365

### Investigation into the effects of glucocorticoids in a mouse model of induced menstruation

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Introduction
Heavy menstrual bleeding (HMB) affects over 1 million women in the UK. At menses, progesterone ($P_4$) withdrawal drives inflammation, tissue break-down and repair of the endometrium. Glucocorticoids are mediators of inflammation and angiogenesis. Our previous studies have demonstrated that differential endometrial expression of the glucocorticoid-metabolising enzymes, 11β-HSD-1 and -2, may play a role in HMB. We hypothesise that aberrant local endometrial glucocorticoid production perturbs vascular and inflammatory mediators necessary to resolve menstruation. Our aim here was to elucidate these mechanisms using an established mouse model of menstruation in mice genetically deficient in 11β-HSD-1 or -2.
Methods and results
Mice lacking either 11β-HSD-1 (*Hsd11b1*$^{-/-}$) or 11β-HSD-2 (*Hsd11b2*$^{-/-}$) and C57Bl/6 WT controls ($n\leq4$/group) underwent induced menstruation. Uteri were collected 8 and 24 h following $P_4$ withdrawal. Tissue sections were histologically examined and graded for endometrial repair stage. Endometrial repair was significantly increased in *Hsd11b1*$^{-/-}$ mice 24 h following $P_4$ withdrawal ($P<0.05$). Immunohistochemistry for CD31, an endothelial marker, revealed no difference between genotypes. RT-qPCR showed levels of *Tsp1* mRNA, an angiostatic mediator, were significantly decreased in *Hsd11b1*$^{-/-}$ mice 24 h after $P_4$ withdrawal ($P<0.05$), while *Cox2* mRNA levels were decreased at both 8 and 24 h ($P<0.05$). Stereological analysis of tissues immunostained for the neutrophil marker Ly6G revealed a significant increase in neutrophil abundance in the basal endometrium of *Hsd11b2*$^{-/-}$ mice.
Conclusions
These findings suggest that local glucocorticoid tone contributes to rapidity of endometrial repair following menstruation. Alterations in glucocorticoid tone leads to vascular and inflammatory changes. Studies using a mouse model have the potential to contribute to our understanding of how aberrant local tissue glucocorticoid signalling may play a role in HMB. Further studies are now warranted.

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## P366

### Structured education programme to improve cardiovascular risk in women with polycystic ovary syndrome: SUCCESS-RCT improved physical activity and illness perception

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Background
Structured education programmes (SEP) have proved effective in changing illness perception and increasing physical activity (PA) in those with or at risk of diabetes. The SUCCESS–RCT was designed to test such a programme in women with polycystic ovary syndrome (PCOS).
Methods
This was a single centre, randomised controlled trial in overweight and obese women with PCOS aimed at increasing their walking activity by 2000 steps/day after 12 months following a single 7 hours SEP. Secondary outcomes were any improvement in PA pattern, glycaemic indices, cardiovascular risk factors, health related quality of life (PCOS Questionnaire = PCOQ12) and their understanding of the disease (Brief Illness Perception Questionnaire). Per-protocol analysis and T-tests were performed and reported as mean difference (MD) with 95% confidence interval (CI).
Results
162 women (66% White) were recruited; mean age 33.3 (SD 7.5; range 19 to 50) years. Six women withdrew before education and 65 out of 78 (83%) attended the SEP, 106 [52 Education arm, 54 control] attended the 6 months (6M) follow up and 100 [48 Education arm] attended the 12M visit. At 6M, step counts (MD 1097 [175, 2008] steps/day) and moderate to vigorous PA (MD 8.7 [1.3, 16.9] minutes) were significantly higher in the education than the control arm. At 12M, the groups showed no difference in PA but the education had improved participants' perception of their condition, in terms of overall control as well as understanding their disease with less anxiety in regard to their weight (PCOQ12). Although all of the biometric measurements tended towards improvement with SEP in the intervention arm none reached the statistical significance for example; MD for body mass index was -0.6 kg/m$^2$, $P=0.051$.
Conclusion
A single structured education empowered patients and improved their PA pattern at 6M follow-up. Although the primary outcome was not achieved but re-enforcement such as media based reminders or face to face short course follow-up educations after 6 month, might have further improved these outcomes. This type of intervention has proved cost-effective in other chronic conditions.

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## P367

**Characterisation of a GnRH-type signalling system in an echinoderm**

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Gonadotropin-releasing hormone (GnRH) regulates secretion of the reproductive hormones luteinising hormone (LH) and follicle-stimulating hormone (FSH) in mammals. Homologs of GnRH have been identified in protostomian and deuterostomian invertebrates, and functional studies suggest an ancient role in regulation of reproductive physiology. The aim of this study was to characterise for the first time a GnRH-type signalling system in an echinoderm, using the starfish *Asterias rubens* as a model experimental system. This is of interest because starfish and other echinoderms i). are deuterostomian invertebrates and therefore they can provide unique insights on the evolution of neuropeptide systems and ii). have a pentaradial body plan without a brain, providing a unique neuroanatomical context for analysis of neuropeptide function. Cloning and sequencing of an *A. rubens* GnRH-type precursor (ArGnRHP) revealed that it contains a single copy of the putative neuropeptide pQIHYKNPGWGPGamide (ArGnRH). The existence of this peptide in extracts of nerve cords from *A. rubens* was confirmed by mass spectrometry. Analysis of the expression of ArGnRHP in *A. rubens* using mRNA *in situ* hybridisation revealed that it is restricted to cells in both the ectoneural and hyponeural regions of the radial nerve cords and circumoral nerve ring. A cDNA encoding an *A. rubens* GnRH-type receptor (ArGnRHR) was cloned and sequenced and pharmacological characterisation of this receptor revealed that ArGnRH is its cognate ligand. Furthermore, phylogenetic analysis revealed that this receptor is indeed an orthologue of vertebrate GnRH receptors. Characterisation of the GnRH-type signalling system in *A. rubens* provides a basis for investigation of its physiological roles in starfish. Discovery of the physiological roles of the ArGnRH in starfish may provide important new insights into the evolution of GnRH neuropeptide systems in the animal kingdom.

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## P368

**Low-dose gonadotrophin therapy for induction of ovulation: comparison of results in women with PCOS or hypothalamic amenorrhoea**

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We have reviewed the outcome of a low-dose step-up regimen for induction of ovulation in clomiphene-unresponsive women with PCOS and in women with hypothalamic hypogonadism (HH), treated at a single centre. Data from 366 women with PCOS and 80 with HH (1165 records) were entered into a FileMakerPro database, refined and constrained to allow stratification and analysis of relevant data. 85% of cycles were ovulatory in both PCOS and HH. PCOS patients were more likely to have cycles characterised by single follicle development than were women with HH (57% vs 42%, $p=0.0003$) whereas cycles cancelled because of multiple follicle development were more common in women with HH (37% v 19%, $p<0.0001$). Women with HH needed a higher threshold (maximum daily) dose of FSH than PCOS women to achieve ovulation (median dose 132iu/day v 75iu/day, $p<0.0001$) but the cumulative conception rate was higher in HH than in PCOS (65% v 49%, $p=0.0007$). The prevalence of multiple pregnancies was low in both groups (PCOS 0.01% cycles, 4% of pregnancies; HH 0.04% cycles, 5% of pregnancies). In summary, despite the need for higher doses of FSH and an increased risk of multiple follicle development in women with HH, a low-dose gonadotrophin regimen is equally effective in women with PCOS and HH in terms of ovulation rate and low frequency of multiple pregnancies. The lower pregnancy rate in women with PCOS is likely to reflect the fact that these women, by definition, had received previous treatment for induction of ovulation (and some had ovulated without conceiving and were more likely to have other, confounding causes of subfertility) whereas those with HH had not previously received treatment.

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## P369

**Characterisation of bile acid pathways in steroidogenic tissues**

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Background
Bile acids (BAs) are end-products of cholesterol catabolism, which act as signalling molecules to regulate glucose, lipid and energy metabolism. BAs activate several receptors including the ligand sensitive transcription factor Farnesoid X receptor (FXR) and the membrane G-protein coupled receptor, TGR5. Besides the organs physiologically in contact with BAs, like the gut and liver, BA-receptors are also expressed in cholesterol-rich steroidogenic tissues, such as the testes, ovaries and adrenal glands where they regulate steroidogenesis and affect fertility. Currently there is no definitive evidence that BAs exist as potential endogenous ligands in these tissues. Here we undertake a comparative analysis of BA-receptors, transporters and enzymes with ratios of BA species all necessary for functional BA pathways in steroidogenic tissues.
Methods
Steroidogenic tissues (testicular, ovarian and adrenal) and liver (control) from 12-week-old C57BL/6 mice were used to study BAs in parallel with gene expression studies. Untargeted ultra-performance liquid chromatography tandem mass-spectrometry (UPLC/MS) characterised the ratios of key BA species. Relative expression of BA-activated receptors, transporters and enzymes was assessed using quantitative RT-PCR (RT-qPCR).
Results
UPLC/MS demonstrated the presence of physiologically relevant concentrations of BA species in testes, ovaries and adrenal glands. Taurocholic (TCA) and cholic acid (CA) were found in all steroidogenic tissues. Interestingly, Deoxycholic acid (DCA), which can be cytotoxic, was found in reproductive tissues. RT-qPCR confirmed Fxr expression, which can be activated by CA and DCA to a lesser degree and Tgr5 which is activated by conjugated BAs. Furthermore, BA transporters (Mrp3, Mrp4, Bsep) and enzymes, including the rate-limiting enzyme of BA synthesis Cyp7a1, were expressed.
Conclusion
Here we systematically compare BAs in steroidogenic tissues and confirm expression of BA sensors and homeostasis genes, indicating that BAs may act as ligands in these tissues. Functional activation of BA pathways in steroidogenic tissue is the subject of future work.

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## P370

**Food, drink and medical plants and their molecules can affect female hormones and reproductive functions**

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The aim of our studies was to examine the effect of some food, drink and medical plants and their molecules on female ovarian endocrine and other functions *in vitro* and *in vivo*. We analysed the effects of plant (green tea, rooibos, ginkgo, flaxseed, chia, yukka) extracts and plant substances (green tea polyphenols, green tea epigallocatechin-3-gallate, curcumin, resveratrol, diadzein and diosgenin) on the release of hormones and markers of proliferation, apoptosis of cultured porcine and rabbit ovarian cells and the rabbit reproductive indexes. It was demonstrated, that green tea, rooibos, ginkgo, flaxseed and chia extracts were able to suppress ovarian cell functions: inhibit the accumulation of proliferation-related peptide (PCNA), promote the expression of apoptosis-associated peptide (Bax) and alter (mainly down-regulated) progesterone, testosterone and leptin release in porcine and rabbit ovarian cells. Furthermore, they prevented the response of these cells to the action of the upstream hormonal regulators (FSH, LH and IGF-I). Pure polyphenols, epigallocatechin-3-gallate, curcumin, resveratrol, and diadzein (but not diosgenin) had similar effects suggesting that the biological activity of plants could be due to the presence of these molecules. Yukka ad curcumin expressed an opposite, stimulatory action on ovarian cell functions. Furthermore, feeding with yukka and curcumin (but not with green tea) promoted rabbit ovarian functions in-vivo (altered ovarian hormones release, increased ovulation rate, fecundity and pups viability). It is concluded, that molecules of some plants widely used for preparation of food, drinks and folk medicine could have direct influence on ovarian functions whose could be due to the alteration in ovarian hormones release and their response to hormonal regulators.

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## P371

**Regulation of Cyp17a1 expression in mouse ovarian theca cells in vivo and in vitro**

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Polycystic ovary syndrome (PCOS) is one of the most common endocrine disorders in reproductive-aged women. Its most typical form is the association of hyperandrogenism with chronic anovulation. Theca cells (TC) of large antral and ovulatory follicles are the main source of ovarian androgens and express P450c17α, encoded by *Cyp17a1*, which converts progesterone to 17α-hydroxyprogesterone and androstenedione. It is known that *Cyp17a1* expression in TC from PCOS women is up-regulated by the hypersecretion of LH. However, the pathophysiology of PCOS still remains unclear.

To investigate the regulation of *Cyp17a1* by LH in TC, 3-month-old female C57BL/6J mice were treated with PMSG and hCG, and ovaries were collected at 0, 4, 8, 12, 16, 20, 24 and 48h after hCG. TC isolated from 21–25-day-old mice were cultured in the RPMI culture medium with or without LH (1, 10, 100 or 1000 ng/ml) and collected at 0, 4, 8, 12, 24 and 48h. To examine the effects of E2, TC were cultured in RPMI with or without LH (100 ng/ml) plus ethanol or E2 ($10^{-9}$M) and collected 48h later. Total RNA was isolated from ovaries or TC, and real-time RT-PCR was performed.

*In vivo*, the expression of *Cyp17a1, Hsd17b1,* and *Cyp19* mRNA was reduced at 8h after hCG compared with 0h and it remained decreased through 48h. In contrast, the expression of *Cyp17a1* mRNA in TC was increased at 48h after the addition of LH and a dose-dependent response was not shown *in vitro*. E2 ($10^{-9}$M) had no effect on an increase of *Cyp17a1* expression. These results suggest that *Cyp17a1* may be differently regulated between *in vivo* and *in vitro*, and the granulosa cells may contribute to the regulation of *Cyp17a1 in vivo*.

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## P372

**Maternal insulin-like growth factor-1 and transforming growth factor beta-1 levels during pregnancy in rats fed with low and high salt**

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Low birth weight has been reported in the offspring of rats fed with low salt diet. This study estimated the levels of maternal growth factors of pregnant rats fed with low salt and high salt diet. Seventy-two female Sprague–Dawley rats were acclimatized and divided into three groups consisting of control/normal salt diet (0.3% salt), low salt diet (0.14% salt) and high salt diet (8% salt). Rats were fed for six weeks and the cycles of the rats were observed in the 6th week for the introduction of male rats to female rats on the evening of proestrus for mating. The presence of sperm cells in the smears the following day confirmed mating and was taken as day one of pregnancy. Implantation studies were carried out on days 6 and 8 of pregnancy while fetal parameters were ascertained on day 19 of pregnancy and at term. Transforming growth factor β-1 (TGFβ-1) and Insulin like growth factor -1 (IGF-1) levels were measured on days 6, 8 and 19 of pregnancy. The results showed that implantation sites were decreased on days 6 and 8 in high salt fed rats. Litter size was decreased in both low and high salt diet fed rats, while birth weight was decreased in low salt fed rats and placental to birth weight ratio was increased in the high salt fed rats compared with control. There was a significant decrease in IGF-1 levels on days 8 and 19 of pregnancy in low salt fed rats while TGFβ-1 levels were decreased on days 6, 8 and 19 of pregnancy in low salt fed rats. Findings suggest that reduced fetal development and low birth weight in low salt fed rats is associated with reduced levels of IGF-1 and TGFβ-1 during pregnancy.

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## P373

**Identification of novel transcription factors that may regulate transcription of the equine chorionic gonadotrophin beta subunit**

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Equine Chorionic Gonadotrophin (eCG) is a heterodimeric glycoprotein produced by terminally differentiated, bi/multi-nucleate trophoblast cells in the placenta of horses and humans. It is responsible for the maintenance of early pregnancy via rescue of the corpus luteum and subsequently promotion of progesterone production. The beta subunit of eCG is expressed at levels ten-fold lower than that of the alpha subunit and confers the glycoproteins specificity to the placenta. Very little is known about the regulation of eCGβ. The aim of this study was to identify novel transcription factors that may regulate *eCGβ* expression in the chorionic girdle.

Conceptuses were obtained via non-surgical uterine lavage from mares between days 27–34 of pregnancy, dissected into various tissue components and snap frozen. An Agilent 44K microarray was performed using RNA extracted from Chorionic Girdle and Chorion (control) samples from pregnancy days 27, 30, 31 and 34 (n = 5 at each timepoint) and analysed using Genespring (Agilent) and Ingenuity Pathway Analysis (Qiagen) software. Predicted transcription factor binding sites were identified using Match.

Microarray analysis determined that 127 transcription factors were differentially regulated (fc > 2) at day 31 compared to day 27 and were specific to the Chorionic Girdle ($P < 0.05$). Nine of these transcription factors had predicted binding sites in a 2500bp length of the *eCGβ* promoter (core sequence match of 100%) and were also significantly correlated with *eCGβ* expression. These are *CREB1* (FC = 2.37, $R^2 = 0.59$), *ELF4* (FC = 5.18, $R^2 = 0.62$), *CREB3L4* (FC = 2.19, $R^2 = 0.71$), *ELF5* (FC = 22.44, $R^2 = 0.82$), *NKX2-5* (FC = 2.43, $R^2 = 0.86$), *SRF* (FC = 2.36, $R^2 = 0.78$), *SREBF1* (FC = 2.29, $R^2 = 0.65$), *HNF4G* (FC = 2.26, $R^2 = -0.54$) and *STAT5A* (FC = 2.51, $R^2 = 0.62$). Current studies are investigating the capacity of these transcription factors to bind to the *eCGβ* promoter.

A greater understanding of the transcriptional regulation of eCG in the equine placenta will offer an insight into the maintenance of early pregnancy and where miss-regulation may occur in early pregnancy loss, which occurs in 15% of equine pregnancies.

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## P374

**A rare form genetic abnormality in Turner syndrome**

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Turner Syndrome (TS) is defined as the combination of characteristic physical features in phenotypic females and complete or partial absence of the second sex chromosome. Short stature is a constant clinical finding in patients with TS. We report the case of a 18 year old female with TS and normal stature. Primary amenorrhea was the reason for the first clinical presentation. Laboratory evaluation showed hypergonadotropic hypogonadism, low oestrogens and testosterone. A pelvic ultrasound showed the presence of uterus. A G-band chromosomal analysis on peripheral blood lymphocytes was used to perform the karyotype. The classical karyotype was 45,X, der(13)t(13;Y)(q10,q10). Y chromosome material was identified by FISH using a Y centromeric probe. Array CGH was performed with normal male genome as reference and revealed the full deletion of SRY region in the presence of otherwise complete Y chromosome: arrYp11.31(2 565 871–2871,490×0, 3 025 075). From our knowledge this is the first report of a Turner syndrome female due to SRY gene complete deletion and translocation of Y chromosome on autosome.

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## P375

**Long-term monitoring of hypogonadal men receiving intramuscular testosterone replacement: a retrospective audit**

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Background
Intramuscular long acting testosterone injections are widely used, convenient form of androgen replacement in patients with hypogonadism.
Aims
To evaluate the long term effects of parenteral long acting testosterone replacement on patients commenced on treatment in years 2006–2014 and adherence to monitoring undertaken by primary care after discharge from specialist service.
Method
We reviewed the results of 64 patients (mean age 58.5 years). Indications for starting testosterone treatment were both primary and secondary hypogonadism. Patients were followed up for an average of 4.33 years (between 3 months and 9 years). Total follow up time was 277.2 patient-years. 53 patients continued with treatment, seven patients discontinued the treatment and four patients died during the follow up period time. We followed the changes of the following blood tests: alanine transaminase (ALT), aspartate transaminase (AST), total cholesterol (TC), haematocrit (HCT), haemoglobin (Hb), prostate-specific antigen (PSA) and testosterone. Adherence to the advised monitoring intervals of these parameters was reviewed.
Results
Over the follow up period time the mean changes in the results were as follows: ALT +1.05 IU/l (+5%), TC -0.41 mmol/l (-8.1%), HCT +0.030 (+7.0%), Hb +7.06 g/l (+4.9%), PSA +0.91 μg/l (+96%), testosterone +19.2 nmol/l (+206%). Adherence to the monitoring intervals advised by our specialist service was: ALT 78.9%, HCT and Hb 80.3%, PSA 52.2% and testosterone 66.3%.
Conclusions
Long acting parenteral testosterone replacement in our group of patients resulted in insignificant changes in liver function tests, haematocrit and haemoglobin. We observed a rise in PSA levels, although the mean value remained in the normal range. Reduction of TC levels was noticed and testosterone replacement is reported in the literature to have no adverse effects on lipid profiles. Adherence to the advised monitoring intervals, especially for PSA and testosterone levels needs further attention.

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## P376

**Prevalence and correlates of premature ejaculation among Nigerians in a tertiary health centre: a preliminary cross-sectional study**

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Background
Premature ejaculation (PE) is believed to be one of the most common male sexual disorders across all age groups. However its prevalence and risk factors has been rarely investigated among Nigerians. Hence, the study determined the prevalence and risk factors for erectile dysfunction (ED) especially PE among this populace.
Subjects and methods
This cross-sectional survey was conducted on seventy-three (73) males aged 18-75years who were sexually active recruited from the medical out patients' clinic (MOPC) of a tertiary health institution. The following instruments were used in the survey: International index of erectile function (IIEF)-5, premature ejaculation diagnostic tool (PEDT) and erection hardness score (EHS) questionnaires. Subjects with hypertension, diabetes, cancer, those with chronic illnesses and those on erection-enhancing agents were excluded.
Results
The mean age of the subjects was 40.15±9.58 years, and all participants identified themselves as heterosexual. The average frequency of sexual intercourse was 7.0±5.49 (median-5.0, IQR-7.0) times/month. This frequency decreased with age (10.0±4.32 in ≤25, 8.28±5.79 in the 26–35years, 6.35±5.11 in the 36-45 years, 5.50±5.11 in the 46-55years, $P=0.004$). The prevalence of erectile dysfunction was 60%, although majority had mild to moderate ED. Premature ejaculation, as assessed by PEDT was present in 24.7% of the participants. It was most prevalent in those younger than 30 years, and decreased with age. We found age-dependent decrease in EHS and frequency of sexual activities. PE was not related to occupation, income, educational level; it was significantly related to age ($P=0.002$).
Conclusion
There is high prevalence of both erectile dysfunction and premature ejaculation among these Nigerians and premature ejaculation was only related to younger age. Hence, physicians need to be trained to detect, diagnose, and manage PE as well as ED.

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## P377

**Effects of oral, injectable, and implant hormonal contraception on serum levels of iron, ferritin, copper, ceruloplasmin and vitamin E**

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Recently there has been reports that the administration of hormonal contraceptives alter the metabolic processes and micronutrients levels. Meanwhile some of these micronutrients are cofactors and or coenzymes of enzymes and are involved in important metabolic pathways. We compared the influence of three contraceptive methods (oral contraceptive, injectables and implants) on serum levels of Iron, ferritin, copper, ceruloplasmin and Vitamin E, using standard methods. Blood samples were collected from women aged 15–49 years attending Family planning clinics within Lagos metropolis and obstetrics and gynaecology department of the Lagos University teaching Hospital, idi-araba, Lgaos, 50 implant users, 100 injectable users, 100 oral contraceptive users were compared with 50 age matched controls. Results found that iron ferritin, copper, ceruloplasmin and vitamin E were significantly higher $P<0.05$ in all the hormonal contraceptive users compared with control and baseline. Changes in tissue or serum levels or bioavailability of these micronutrients might play a significant role in health risk and might be involved in the pathogenesis of some disorders.

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## P378

**Importance of social support and implications of gender reassignment in congenital adrenal hyperplasia due to 3-β hydroxysteroid deficiency**

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Introduction
3-β-hydroxysteroid dehydrogenase (3BHSD) deficiency is a rare genetic disorder of steroid biosynthesis that results in decreased production of all three groups of adrenal steroids, which include mineralocorticoids, glucocorticoids, and sex steroids.
Case presentation
An infant was born with ambiguous genitalia in 1975 to a conservative Muslim family and was registered as male. The child was hospitalised with addisonian crisis on the eleventh day of life. Baby's genotype was XY and was diagnosed as having 3β-HSD deficiency following a year of investigations due to the rarity of the condition. Prevailing medial expertise in this area in combination with inadequate growth of male sexual structures led to a decision to reregister and raise this child as a female at 18 months. There were difficulties in accepting this for the child's conservative family especially as there was no involvement of the patient or of a wider multidisciplinary team. After a consensus was reached with the family the child underwent bilateral orchidectomy, phallidectomy with vulval plastic procedure (opened urogenital sinus to vaginal introitus) and initiation of Oestradiol supplementation at ages of 4, 10 and 12 respectively.
Current issues
The subject is now 45 years, she has overcome many serious infections and adrenal crises and is relatively healthy. However there is huge psychological burden in the form of being shy and reserved resulting in increasing social isolation, along with non-compliance to treatment. She continues to live with elderly parents and has a difficult and uncertain future.
Dilemma
Would this individual have done better if raised as a male?
Management
MDT meeting organised between adult Endocrinologist, Psychiatrist and Family to encourage and support her in the community in addition to medical management.
Discussion and conclusion
We believe that this lady's psychological issues were at least partly related to the management approach adopted at the time of the diagnosis. This deviated from current practice of deferring final gender assignment (surgery, hormone replacement) until the child approaches the age of puberty. This also facilitates the participation of the child in informed decision-making. It is also crucial to involve multidisciplinary team especially psychosocial professionals early with regular follow up.

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## P379

**Testicular regression syndrome and severe psychiatric disorder – a rare association preventing the optimal management of the endocrine condition**

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Introduction
Testicular regression syndrome (TRS) or vanishing testis syndrome is a rare condition defined as the absence of testicular tissue in a genetic and phenotypic male. Rudimentary accessory structures can be present; in that case, the removal of all remnants is recommended.
Case report
Male patient, 39 years old, had initially been evaluated at the age of 8 for persistent bilateral cryptorchidism. The medical documents are lacking except for a brief description of the laparoscopical surgical exploration of the abdomen and inguinal area, with no testicular or accessory tissue being found. The patient received parenteral androgen replacement only for a few months in the pubertal period. At the age of 14 a schizoaffective disorder (paranoid schizophrenia) was diagnosed. The psychiatrist recommended neuroleptics and strongly advised against the androgen replacement which was stopped and not resumed ever since. At the time of the referral to our department the patient was 39 years old. He had no subjective complaints. The clinical examination revealed a 182 cm-tall phenotypical male with eunuchoid habitus, absent facial and truncal hair. No testes were palpable in the undeveloped scrotum or the inguinal canal. Bilateral gynecomastia was present. The psychical status was stable under neuroleptics. After the endocrine evaluation, severe primary hypogonadism was diagnosed. The antimullerian hormone serum level was undetectable. The karyotype was 46, XY. The bone mass was unaffected. Topical androgen replacement was recommended but it aggravated the psychotic aggressive features so the psychiatrist team taking care of the patient contraindicated it.
Conclusion
We present the case of a patient with testicular regression syndrome, with no surgically evident wolffian rudiments, associating from early adolescence a severe psychiatric pathology with aggressive tendencies which constantly precluded adequate testosterone replacement until the current age of 42.

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## P380

**Self medication in transpeople is not associated with deterioration in cardiovascular risk factors but is associated with reduced vitamin D levels and antidepressant use**

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Objective
This is a prospective audit looking at cardiovascular parameters in transpeople comparing those who have self medicated verses those who have not.
Methods
Patients attending a workshop for new patients were questioned about cardiovascular risk factors including diabetes hypertension and smoking status. Measurements were made of lipid profile, glucose, blood pressure, waist, height and weight measurement. Those that were self medicating (SM) were compared to those that were not (control).
Results
Seventy nine transwomen and 30 transmen. 48.1% of transwomen were self medicating and 14.7% of transmen were self medicating. For SM transwomen baseline oestradiol was high at $254.6 \pm 39.1$ vs control $128.4 \pm 17.9$ pmol/l. Total cholesterol nor triglyceride were different. HDL rose from $1.13 \pm 0.06$ to SM $2.1 \pm 0.08$ mmol/l ($P < 0.01$). For SM transmen there is an increase in testosterone from $1.07 \pm 0.08$ to $27.48 \pm 11.2$ nmol/l. Total cholesterol remained stable but HDL fell (control $1.97 \pm 0.53$ vs SM $1.1 \pm 0.19$ mmol/l $P < 0.01$). In both groups there were no significant differences in height, weight, blood pressure or BMI either on or off hormonal therapy. In SM transwomen vitamin D was lower, (SM $52.9 \pm 24.0$ to $40.97 \pm 20.1$ nmol/l $P$ 0.034). In transmen however hormonal therapy was associated with an increased vitamin D (SM $50.8 \pm 25.3$ to $58 \pm 20.4$ nmol/l $P > 0.05$). Self-medication was also associated with antidepressant prescription in transwomen but not transmen (2.4% vs 15.8%, $\chi^2$ 4.353 $P$ 0.037).
Conclusion
Self-medication does not appear to have a dramatic impact on cardiovascular risk factors in both trans men or trans women apart from the known effects of sex steroid therapy on HDL. There does appear to be an association between antidepressant prescription and self-medicating in transwomen. It is also of note that vitamin D deficiency is associated with self medication in transwomen. Tiredness associated with vitamin D deficiency may contribute to the symptoms of depression in these individuals.

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## P381

**A pragmatic review of patient satisfaction and testosterone replacement therapy**

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Introduction
In addition to safety and efficacy, patient satisfaction with treatment is potentially a key factor in maintaining therapy adherence. Testosterone replacement therapy (TRT) is provided using a variety of products with different features and routes of administration. These differing product characteristics may impact on patient satisfaction and adherence with a concomitant impact on well-being and health-related quality of life. The aim of this study was to explore patient satisfaction with TRT and the underlying factors.

Methods
A pragmatic literature search was carried out to identify studies on TRT for men with testosterone deficiency reporting patient satisfaction. The search was conducted in MEDLINE, and the Cochrane databases (Systematic Reviews, Central Register of Controlled Trials). The search was restricted to adult men (aged >18) with a primary diagnosis of hypogonadism/testosterone deficiency.
Results
The searches identified 682 records. Following deduplication, 397 records were assessed for relevance of which 53 were eligible for full review. A final total of eight articles was included. The results in terms of patient satisfaction were highly varied. Two studies noted no differences in patient satisfaction between injections and gels, or between those two modalities and implants. Patients were more satisfied with their current compared to previous (unspecified) TRT (two studies), as well as newer gel formulations (two studies). The factors underlying patient satisfaction included doctors' recommendation, ease of use, efficacy, symptom improvement and convenience.
Conclusion
There is a significant degree of heterogeneity in patient satisfaction with TRT, which appears largely due to factors that are specific to the chosen TRT and individual patient preferences. Further work is required to determine whether specific features of TRT are instrumental in patient satisfaction. The results of this study should help inform patient interactions with their healthcare providers regarding the choice of TRT.

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## P382

**Spontaneous resolution of hypothalamic amenorrhea post diagnostic GnRH test**

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Introduction/background
Hypothalamic Amenorrhea (HA), or stress-induced anovulation, is one of the most common causes of secondary amenorrhea (1,2) and accounts for the reproductive dysfunction seen in under nutrition, excessive exercise, sever emotional stress and chronic disease. From a teleological standpoint, in the face of nutritional or physical stress, it is adaptive for an organism to allocate energy resources for its own survival rather than the costly process of reproduction and therefore HA is a physiologic response to environmental, physical or emotional stressors. In this case we report a 37y.o otherwise healthy mother, whose longstanding postpartum secondary amenorrhea resolved post gonadotropin releasing hormone(GnRH) stimulation test done for diagnostic purposes.
Aims
To test gonadotropic cell ability of secreting LH and FSH under the stimulating action of GnRH.
Materials and methods
A standard GnRH test was used, after all other underlying conditions likely causing primary or secondary amenorrhea were excluded via biochemical parameters and imaging methods (Pituitary MRI, abdo-pelvic U/S scan). Furthermore, the patient was on no medication.
Results
Pre GnRH administration, 08:30am blood tests were performed, indicating euthyroidism (TSH:0,94mU/L), normoprolactinaemia (PRL:121mU/L), normocortisolaemia (Cort:595 nmol/L) and normoandrogenism (Testo:0,4 nmol/L, DHEAS:5,7 nmol/L). MRI of the pituitary and adrenal-ovarian U/S scans previously performed, were also unremarkable. Pre GnRH administration, gonadotropins were (LH: <1IU/L, FSH:3IU/L) while 30 min after, (LH:10 and FSH:9IU/L) and 60 min after (LH:14IU/L and FSH:18IU/L) indicating a reverse response pattern, non characteristic of a female adult. A few days after the test, her menstruations returned and remained regular since then, after an absence of four years.
Conclusion
To the extent of our knowledge, there are very few recorded cases of spontaneous resolution of a hypothalamic amenorrhea after a GnRH test. This necessitates for further research in this disorder's complex pathophysiological mechanisms in order to allow for early diagnosis and better patient outcomes.
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# Steroids

## P383

**Adrenocortical function in glucocorticoid receptor deficient mice**

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Introduction
Humans with glucocorticoid receptor (GR) deficiency and global heterozygous GR knockout (GR+/−) show compensatory activation of the hypothalamic-pituitary-adrenal axis, resulting in salt-sensitive hypertension due to increased mineralocorticoid activity. Previous studies suggest renal mechanisms, including changes in cell proliferation, gene expression and electrolyte transport, may contribute to this phenotype but underlying adaptive adrenal responses have yet to be investigated.
Methods
Bromodeoxyuridine (BrdU) infusion was used to label proliferating cells and apoptosis was assessed by TUNEL staining. Cell size and cell proliferation were quantified in fixed adrenal sections from GR+/− mice and wild-type littermate (WT) controls using Image J software. Zonal expression of aldosterone synthase (AS; Cyp11b2) and 11-beta hydroxylase (11β-OH; Cyp11b1) was assessed using immunofluorescence.
Results
Compared to WT adrenals, the outer zona fasciculata (OZF) cells of GR+/− adrenals were larger ($P=0.0001$), medulla cells were smaller ($P=0.025$) and zona glomerulosa (ZG) cells appeared unaffected. Irrespective of genotype, BrdU+ve nuclei were more abundant in the outer cortex. However, the adrenal cortex of GR+/− mice ($P<0.05$) had fewer BrdU-labelled cells and the distribution of labelled cells was shifted markedly ($P<0.001$) away from the ZG towards the medulla. Dual immunofluorescent staining for BrdU and either AS (expressed only in ZG cells) or 11β-OH (expressed only in ZF cells) showed that, in GR+/− adrenals, AS may be increased but not in association with proliferating cells. Nearly all BrdU+ve cells expressed 11β-OH. Tunel staining showed no difference in apoptosis between genotypes.
Conclusion
Impaired negative feedback of the hypothalamo-pituitary-adrenal axis in GR+/− mice causes ACTH-dependent hypertrophy but not hyperplasia of the glucocorticoid-synthesizing zona fasciculata cells of the adrenal cortex. Although adrenal aldosterone synthesis may be increased in GR+/− mice, it is not yet clear that this is sufficient to explain mineralocorticoid-dependent hypertension. The distribution of BrdU labelled nuclei may indicate altered adrenocortical cell turnover.

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## P384

**TNFα regulates steroid sulphatase activity in healthy and malignant tissue**

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Steroid sulphatase (STS) is the primary enzyme for desulphating steroids from their inactive to their active forms. Principal substrates include steroid precursors oestrone-sulphate and dehydroepiandrosterone sulfate. Alterations in STS activity can directly affect local concentrations of oestradiol, testosterone and dihydrotestosterone; steroids that are frequently dysregulated in disease. Despite the importance of STS activity on steroid synthesis, little is known about its regulation. *In vitro* studies on breast and prostate cancer have indicated that TNFα may in part regulate STS activity. To investigate how inflammation regulates STS, we examined TNFα effects *in vitro* and *in vivo*. Colorectal cancer (CRC) cells were treated with TNFα and STS activity measured using $^{3H}E_1S$ desulphation. *In vivo*, STS activity was measured by the same method in multiple tissues of TNF-Tg mice, which globally overexpress TNFα, and compared findings to age matched WT control animals. Tissues analysed included liver, heart, kidney, spleen, lung, bowel, skin and omental fat. Consistent with other findings, TNFα treatment increased STS activity in CRC cells, suggesting this response occurs in multiple cancer types. The glycosylation inhibitor tunicamycin significantly ($P<0.01$) inhibited TNFα-induced STS activity rise, suggesting a post-translational modification is important for activity. STS activity was generally increased in most organs of TNF-Tg mice when compared to WT controls, with significant rises seen in liver (2.7–3.6 nmol/mg per h, $P=0.0052$), spleen (406–715 pmol/mg per h, $P=0.006$) and large bowel (312–365 pmol/mg per h $P=0.0002$). Here we demonstrate both *in vitro* and *in vivo* that TNFα

regulates STS activity in multiple tissue types. This increase in STS activity is not just a phenomenon seen in malignancy, but instead may be a normal physiological response to inflammation. In the acute setting this could be a homeostatic mechanism, however the effects of chronic increases in STS activity remains unknown.

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## P385

**Genome-wide binding analysis of glucocorticoid receptors in the rat hippocampus in response to corticosterone and stress**

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Glucocorticoids act via the glucocorticoid receptor (GR). Upon ligand binding, GR translocates to the nucleus and binds directly to glucocorticoid responsive elements (GREs) to regulate transcriptional output. Glucocorticoid secretion increases in response to stress to affect transcriptional output within specific areas of the brain including the hippocampus (HC). Therefore, here we assess changes in genome-wide GR chromatin binding profiles in the rat HC in response to a glucocorticoid rise, with or without an acute stress in order to identify significant motifs at the sites of GR binding. Male ADX Sprague Dawley rats were sacrificed after a 30 min glucocorticoid infusion with/without a 30 min restraint stress and HC dissected for chromatin immunoprecipitation assays (ChIP) using a GR antibody cocktail. GR binding sites and DNA motifs were identified, including the previously characterised Per1 regulatory sites. Novel sites were identified proximal to Bdnf, SGK1 and Camk2a. Interestingly, there was no significant difference in peak tag density between basal and stress groups. HOMER analysis identified motifs for GRE, NF1 and NeuroD1 as top recurring motifs at 53, 62 and 18% respectively. The motifs identified in this study represent targets for further study. NeuroD1 in particular is thought to be involved in learning and memory. Therefore NeuroD1 is an interesting target for future analysis of GC-regulated HC function. BDNF and Camk2a mRNA levels have previously been described as regulated in response to stress. Interestingly our findings have shown no change at the point of GR binding to these GREs and little change to the genome-wide chromatin binding profile in response to acute stress compared to increased glucocorticoids in the absence of a concomitant stressor. These findings suggests that glucocorticoid dependent stress regulation in the HC is primarily regulated by the glucocorticoid rise, and not further modulated other stress associated factors in our ADX rat model.

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## P386

**Impact of 5α-dihydrotestosterone (DHT) on glandular epithelial proliferation in the mouse uterus**

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The uterus is an androgen-responsive organ; androgen receptors (AR) are expressed in human endometrial stromal fibroblasts and can regulate proliferation and survival of this cell type. Although chronic androgen exposure results in atrophy of human endometrium, previous studies have suggested that it may have trophic effects in the rodent uterus. We hypothesised that androgens may have different impacts on endometrial function in the presence or absence of oestrogens. In the current study we investigated the effects of the non-aromatisable androgen DHT (alone and in combination with 17β-oestradiol, $E_2$) using both short-term (24 h) and long-term (7 days) dosing regimes. Eight-week old C57BL/6 female mice were ovariectomised; 7 days later they were given a s.c. SILASTIC implant (empty control or $E_2$). After a further 7 days (day 14) they received either 1× or 7× daily s.c. DHT (0.2 mg/mouse). Mice were culled 24 h after the last DHT injection; 2 h prior to death they received a single intraperitoneal injection of BrdU. Uteri were collected and processed for immunohistochemistry and gene expression analysis (qRT-PCR). Treatment with $E_2$ alone induced an increase in uterine weight and endometrial surface area; treatment with DHT alone induced a similar effect after 7 days of treatment. Treatment with DHT alone altered gene expression in glandular epithelial cells so that they became AR+ which was accompanied by increased cell proliferation (cyclinD1+/BrdU+/MKi67+). Gene expression analysis identified significant changes in mRNA concentrations of *Igf1, MKi67, Wee1* and *Rb1* with $E_2$ either blunting or masking the DHT response.
In conclusion, in mice the impact of androgens on endometrial tissue is dependent upon whether oestrogens are present. It is notable that metestrus is characterised by low oestrogens/androgens ratio. We speculate that androgens may also be important in regulating endometrial tissue remodelling when oestrogens are low at the time of menses.

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## P387

**Plasma corticosteroid-binding globulin: biomarker of inflammation onset and severity in a rat arthritis model**

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Background
Corticosteroid-binding globulin (CBG) plays a critical role in regulating glucocorticoid bioavailability. During inflammation, plasma CBG behaves as an acute phase "negative" protein due to the down-regulation of hepatic *SerpinA6* (*Cbg)* expression and proteolytic cleavage of CBG. CBG contains a protease-cleavage domain (RCL) that promotes the release of CBG-bound steroids at sites of inflammation. Using an established adjuvant induced arthritis (AA) model of rheumatoid arthritis, we have previously demonstrated that on day 16 post injection – the peak of inflammation – CBG levels decrease to ∼50% of baseline values in Sprague Dawley (SD) rats that develop severe arthritis.
Objective
Characterise plasma CBG levels in a rat AA model, determining (a) when changes in CBG levels occur and (b) what causes these changes.
Methods and results
Charles River SD rats were administered complete Freud's adjuvant (0.6 mg/animal) or saline intradermally at the tail base, separated into two groups ($n=12$ each) and alternatively sampled (plasma) every other day until day 16 post injection. Overall, the magnitude of decline in CBG matched the severity of inflammation. In severely inflamed rats, significant decreases in CBG levels occurred 2-3 days preceding any clinical manifestation of inflammation. Decreases in CBG levels coincided with the appearance of a proteolytic product in plasma that is consistent with RCL cleavage. Moreover, in severely arthritic rats, separation of the intact CBG from the RCL-cleaved CBG demonstrated that the cleaved CBG had no steroid-binding activity.
Conclusions
In rats that become severely arthritic, decreases in CBG levels occur prior to clinical signs of arthritis, suggesting CBG may be a useful biomarker of arthritis onset and severity. In addition, the RCL-cleaved CBG in plasma lacks high-affinity steroid-binding activity. Dynamic changes in the levels and function of plasma CBG during inflammation likely modulates the tissue availability of corticosterone, effecting the inflammatory reaction and healing process.

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## P388

**A novel animal model to explore the whole-organism response to 21-hydroxylase deficiency**

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Congenital adrenal hyperplasia (CAH) is one of the most common inherited disorders. The majority of CAH cases are due to 21-hydroxylase deficiency (21-OHD) caused by mutations in CYP21A2. Because of the profound impact of steroids on physiology and gene expression, the imbalances in steroid hormones resulting from 21-OHD are supposedly leading to a whole-organism response on transcriptome and metabolome level. The systemic consequences of severe 21-OHD during early development and adult life remain poorly understood. This gap in knowledge is due to a lack of suitable animal models, as *Cyp21a2* knock-out mice are not viable. Therefore, we have developed a zebrafish model for 21-OHD. A single 21-hydroxylase gene is annotated in the zebrafish genome based on sequence homology, *cyp21a2* (ENSDARG00000037550). Our *in silico* analysis of the Cyp21a2 protein sequence suggests a sufficient degree of similarity for the usage of zebrafish *cyp21a2* to model human 21-OHD *in vivo*: The majority of residues corresponding to CAH causing mutations are conserved and the

predicted impact on protein structure caused by known CAH mutations is similar. In contrast to control transfected cells, zebrafish Cyp21a2 expressing COS7 cells convert 17-hydroxyprogesterone into 11-deoxycortisol (measured by LC/MSMS) at a similar rate as human CYP21A2, confirming the correct annotation of *cyp21a2* as zebrafish 21-OH. In addition, we determined the spatio-temporal expression patterns of *cyp21a2* by RNA- *in situ* hybridisation and RT-PCR throughout early development (first five days) and in adult tissues. Early c*yp21a2* expression is restricted to the interrenal gland (zebrafish adrenal counterpart) and the brain, where it is also expressed in adults. To further explore the *in vivo* consequences of Cyp21a2 deficiency we created several *cyp21a2* null-allele zebrafish lines employing a TALEN (transcription activator-like effector nuclease) genomic engineering strategy. This new *in vivo* model will provide novel insights into the whole organism response and pathophysiology of 21-hydroxylase deficiency.

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## P389

### Regulation of glucocorticoid receptor action by ARID subunits of the SWI/SNF complex

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AT-rich interacting domain (ARID) subunits can interact with the glucocorticoid receptor (GR) and therefore may be essential for gene transcription dependent on receptor signalling. Glucocorticoids are steroid hormones important for regulating a variety of physiological processes through the binding and activation of GR. GR associates with numerous co-regulators, such as chromatin remodelling factors, to mediate gene induction or repression. One chromatin remodelling complex which interacts with GR is the ATPase driven SWItch/Sucrose NonFermentable (SWI/SNF) complex. ARID1a is an essential subunit of the SWI/SNF complex thought to be responsible for interacting with GR and recruitment of the complex to DNA. ARID1a mutations have been discovered in a wide range of human carcinomas including ovarian, uterine, liver, lung, breast, pancreatic, renal and colon cancers. A subset of GR dependent genes require the SWI/SNF remodelling complex for regulation, therefore absence of the functional ARID1a protein may impair GR signalling pathways. We hypothesise that inactivating ARID mutations could interfere with GR dependent gene regulation potentially through disrupting GR binding to target sequences. We have used two human cell lines, HeLa cells which possess the endogenous ARID1a and SKOV3 cells which contain an inactivating ARID1a mutation, to assess the impact of the absence of the functional ARID1a on GR dependent transcription. Using chromatin immunoprecipitation (ChIP) assays in the SKOV3 cells compared to HeLa cells, we show a lack of GR binding at the Per1 binding site known to require chromatin remodelling. We further demonstrate a delay in the transcriptional induction of GR dependent genes, including Per1, in response to dexamethasone treatment in the SKOV3 cells in comparison to HeLa cells. The findings from this study strongly implicate ARID1A in regulating temporal dynamics of GR transcriptional dynamics, and this is being further assessed by gene knockdown and functional interference studies.

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## P390

### Alpha-MSH secretion from a gastro-intestinal stromal tumour leading to ACTH-independent Cushing's syndrome

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A 51 year old woman presented with severe Cushing's syndrome. In addition to a typically Cushingoid appearance she demonstrated increased cutaneous pigmentation in her face and upper chest. Biochemical investigation confirmed elevated serum cortisol levels with loss of circadian variation, and failure to suppress with low dose dexamethasone (0.5 mg 6 hourly for 48 hours). Serum ACTH levels were undetectable. Cross-sectional imaging revealed bilateral macronodular adrenal hyperplasia (R = 5 cm and L = 2 cm diameter) and a mass lesion arising from the lesser curvature of the stomach consistent with a gastro-intestinal stromal tumour (GIST). The larger right adrenal was excised with the GIST, and post-operative cortisol levels were below 50 nmol/l. Immunohisto-chemical staining for c-Kit and DOG-1 confirmed the gastric lesion to be a GIST, and a somatic mutation in PDGFRA (pAsp842Val) was identified through Sanger sequencing. Cell supernatant from a primary culture of the GIST stimulated cortisol release from the adrenocortical cell line NCI-H295R and dispersed human adrenal cells. GIST immunostaining was negative for ACTH, but positive for α-MSH. α-MSH was identified in the GIST culture supernatant using sandwich ELISA. RT-PCR demonstrated GIST expression of POMC processing enzymes proprotein convertases 1/3 and 2. The right adrenal contained multiple large pigmented nodules: this was identified as melanin using a series of histological stains. The pattern of pigmentation correlated with immunostaining for 11-beta hydroxylase (CYP11B1), melanocortin 1 receptor (MC1R), and the melanosome marker HMB-45. This suggested that α-MSH binding to MC1R was driving cortisol production, and both skin and adrenal pigmentation. The ability of α-MSH to stimulate cortisol release in vitrowas confirmed by incubating dispersed human adrenal cells with the peptide. This is the first recorded case of Cushing's syndrome as a result of alpha-MSH secretion alone, and the first as a result of any hormone secreted from a GIST.

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## P391

### Involvement of the TORC isoforms in adrenal responsiveness to basal and stress-induced HPA activity

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ACTH signalling at the adrenal zona fasciculata induces rapid synthesis of glucocorticoids, accompanied by rapid transcription of genes encoding steroidogenic enzymes, including the labile, rate-limiting protein, StAR protein. ACTH induction of StAR transcription has been demonstrated to be predominantly CREB-dependent, and this mechanism is regulated by both CREB phosphorylation and its binding to the CREB co-activator TORC (transducer of regulated CREB activity, also known as CREB regulated transcription coactivator (CRTC)). Here we show that CREB phosphorylation, and nuclear localisation of the TORC2 and TORC3 isoforms follows ACTH treatment in adrenocortical Y1-BS1 and ATC7-L cells, and this precedes a rapid increase in star transcription (StAR hnRNA). Furthermore, by using chromatin immunoprecipitation we demonstrate that both isoforms bind the endogenous StAR promoter by 30 min after ACTH treatment in ATC7-L cells. In order to further establish the roles of these two isoforms during different types of HPA activity in the adrenal, we used two rat *in vivo* models; firstly, i.v. injection of a low dose of ACTH was used to mimic an ACTH ultradian pulse; secondly, rats were subjected to an immune stressor through an i.v. LPS challenge. Following both treatments, adrenals were harvested at various time-points and processed for RNA and protein measurements. As expected, we found rapid increases in both CREB phosphorylation and StAR hnRNA in both the i.v. pulse and LPS experiment. Interestingly, we found differential activation of the two TORC isoforms, with only significant TORC3 nuclear localisation induced by the i.v. ACTH ultradian pulse, whilst both TORC2 and TORC3 were induced by the immune stressor. These data demonstrate differing sensitivities of TORC2 and TORC3 to ACTH signalling, suggesting different roles for each in their mediation of ACTH-induced StAR transcription.

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## P392

### Exploring metabolomic changes due to cortisol deficiency in early development using the ferredoxin (fdx1b) null-allele zebrafish

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Steroid hormones are important regulators of many physiological processes. The steroid precursor pregnenolone is converted through several enzymatic steps into all types of steroids, including the stress hormone cortisol. Mitochondrial steroidogenic cytochrome P450 (CYP) enzymes crucially relying on electron transfer from the redox partner ferredoxin (FDX1) are involved in key steps of the cortisol biosynthesis pathway.

Cortisol is well-known regulator of glucose metabolism; however, only little is known about the impact of cortisol deficiency on metabolic pathways during embryonic development. Zebrafish represents a vertebrate model using cortisol as main glucocorticoid hormone. Thus, it lends itself as a whole organism model to study the impact of cortisol deficiency on metabolism during development. By genomic engineering we have generated a mutant fdx1b null-allele zebrafish line. Fdx1b represents the zebrafish equivalent of human FDX1. fdx1b deficient embryos appear darker due to a failure in their Visual Background Adaptation (VBA) behaviour, a glucocorticoid mediated pigmentation response in teleosts. Being in line with this observation, hyperpigmentation due to cortisol deficiency is also observed in humans with primary adrenal insufficiency. Interestingly, hyperpigmentation in the fdx1b mutants can be rescued after glucocorticoid replacement with dexamethasone suggesting dysregulation of the stress axis after fdx1b disruption. Indeed, in fdx1b mutant larvae *pomc* is significantly increased and cortisol synthesis and signalling is significantly impaired. Moreover, fdx1b mutant larvae have a blunted cortisol response to stress. Metabolomic analysis by nuclear magnetic resonance (NMR) spectroscopy reveals severe changes on the global metabolome in fdx1b null-alleles impairing metabolites also linked with human pathogenesis.

In conclusion, the fdx1b mutant line is a promising *in vivo* model to explore the pathophysiologic impact of glucocorticoid deficiency on energy metabolism relevant to early development and potentially adult life.

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## P393

**The effects of chronic administration of the synthetic glucocorticoid dexamethasone on memory and learning in the rat**

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Dexamethasone (DEX) is a widely used synthetic glucocorticoid whose effects are mediated via glucocorticoid receptor (GRs). Synthetic glucocorticoids are widely used in the clinical setting, due to their potent anti-inflammatory actions. However they are associated with numerous systemic side-effects and may also promote cognitive dysfunction, including impaired memory and learning processes in both patients and animal models.

Discrete pulses of endogenous GR ligands corticosterone (rats) or cortisol (humans) promote pulsatile activation of GR, with GR activity returning to baseline levels during the interpulse interval (within 60 min). In cell lines, prolonged GR activation occurs following a single pulse of DEX. However the duration of DEX-induced GR activation *in vivo* is unreported. Therefore, we have used Western blotting of GR in purified nuclear extracts obtained from discrete brain regions from adult male Sprague–Dawley rats following single SC DEX injections. We found DEX promoted significantly prolonged GR activation times compared to endogenous ligand in brain regions associated with memory and learning processes including the hippocampus, amygdala, and perirhinal cortex.

We next assessed the effects of a sub-chronic DEX treatment paradigm, using rats injected twice-daily over 5 days. *In situ* hybridization histochemistry was used to measure treatment-related changes in mRNA for CRH in the PVN, and GR and MR within the hippocampus. *CRH* mRNA was significantly downregulated after treatment with 1 mg/kg DEX, but not 500 μg/kg. These findings strongly support chronic central GR activation resulting from the 1 mg/kg treatment. Interestingly, no changes in *GR* mRNA levels were observed within the hippocampus with either dose, whilst significant upregulation of *MR* mRNA was measured in the CA2 and CA3 subfields in response to 1 mg/kg DEX.

Since we have shown significantly prolonged GR activation in memory-and-learning dependent regions *in vivo* following DEX treatment, this is likely to contribute to mechanisms which may underlie DEX-induced cognitive deficits.

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## P394

**Synthetic glucocorticoid (GC) treatment induces prolonged activation of GC receptors in discrete brain regions and impairs memory and learning processes in the rat**

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Chronic treatment with the synthetic glucocorticoid (GC) prednisolone has been reported in association with many detrimental health effects. In addition to well-documented adverse metabolic effects, there is evidence for memory impairments in these patients. GCs are known to have effects on memory, either enhancing or impairing dependant upon timing of exposure. Cell experiments have shown that synthetic GCs such as methyl-prednisolone (MPL) cause an alteration in timing of glucocorticoid receptor (GR) activation. In contrast to the rapid pulsatile GR activation associated with natural GC hormones corticosterone and cortisol, synthetic GCs including MPL induce a prolonged GR activation profile. Therefore we have now investigated the action of MPL in vivo. We report that a single subcutaneous injection of MPL into adrenalectomised rats induced prolonged GR activity in discrete brain regions vital to learning and memory, including the hippocampus, prefrontal and perirhinal cortices. Three-day treatment in adrenal-intact rats (1 mg/ml MPL in drinking water) supressed endogenous corticosterone secretion, induced significant GR activation during the circadian peak and loss of circadian GR nadir consistent with prolonged MPL-induced GR activation. Interestingly, radiotelemetry data showed a significant dysregulation of circadian activity rhythms in the MPL treated rats. To test if MPL treatment resulted in impaired hippocampal or perirhinal-dependent memory, rats were tested in object location and object recognition tasks. MPL treated rats were able to discriminate between novel and familiar in the object recognition and object location tests following a 1 h delay. However at a 6 h delay, MPL treated rats were not able to discriminate. Therefore, we conclude that chronic MPL treatment impairs both perirhinal-dependent recognition memory and hippocampal-dependent spatial memory following a 6 h delay, but not a 1 h delay. Our experimental model may provide a robust method for determining the mechanisms underlying memory deficits in patients treated with prednisolone and other synthetic GCs.

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## P395

**Vitamin D2 vs vitamin D3: effects of total and free 25-hydroxyvitamin D on immune cells *in vivo***

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Vitamin D metabolites such as 25-hydroxyvitamin D (25D) circulate bound primarily to vitamin D binding protein (DBP). However, for most extra-renal tissues 25D uptake is independent of DBP, even though the 'free' 25D fraction is very small. DBP has a lower binding affinity for 25D2 compared to 25D3. We hypothesized that this would increase serum free 25D2, with possible variations in vitamin D function. Mice were placed on diets containing equal amounts (1000 IU/kg) of vitamin D2 or D3 at week 3 of age. At week 8 mice fed D2 diet had only 25D2 in circulation (26.6 ng/ml ± 1.9), and mice fed D3 mice had only 25D3 (28.3 ng/ml ± 2.0). By contrast, measured 'free' 25D was significantly higher in D2 animals (16.8 pg/ml ± 0.65 vs 8.4 pg/ml ± 0.63, $P < 0.001$). Parathyroid hormone showed no significant difference between D2 and D3 mice (193 pg/ml ± 9.0 vs 196 pg/ml ± 6.2). However, analysis of spleens from week 8 D2 and D3 mice showed that in female mice on D2 there was increased mRNA expression of the vitamin D-activation enzyme Cyp27b1 (2.35-fold ± 0.45), the monocyte-macrophages marker CD11b (1.77-fold ± 0.45), and the osteoclastogenesis precursor RANKL (1.80-fold ± 0.37) relative to female D3 mice. Conversely, another monocyte marker, CD14, showed decreased mRNA expression (0.39-fold ± 0.19) in D2 vs D3 mice. Flow cytometry revealed a significant increase in total CD45+ monocyte-macrophage populations in spleens from D2 females (38.04%) compared to D3 females ($P = 0.0173$). Conversely natural killer cells were reduced in D2 mice (39.25%) compared to D3 mice ($P = 0.0054$). There was no difference in T cell or B cell markers between D2 and D3 mice. These data suggest that free 25D (25D2 > 25D3) has extra skeletal effects that include effects upon immune cell development.

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## P396

**Model systems to define the role of AKR1D1 in metabolic liver disease**

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Non-alcoholic fatty liver disease is the hepatic manifestation of the global epidemic of metabolic disease. It is tightly associated with obesity and type 2 diabetes, yet the precise mechanisms that drive its aetiology are not fully defined. Steroid hormones, including glucocorticoids and sex steroids, regulate metabolic phenotype, and in addition, bile acids have recently been identified as potent metabolic regulators. AKR1D1 (5β-reductase), is predominantly expressed in the liver, and is a crucial regulator of steroid hormone clearance as well as bile acid synthesis. Its role in pathogenesis of metabolic disease has not been examined. We have therefore developed systems to define the enzymology of human AKR1D1 in cell free assays and to determine the impact of manipulation of AKR1D1 expression and activity in human hepatocyte models. B21 Rosetta bacteria cells were transformed with an AKR1D1 construct (pNIC-CTHF+ AKR1D1) and recombinant protein extracted and purified. A high throughput assay was developed to determine AKR1D1 activity, substrate specificity and enzyme kinetics. Furthermore, AKR1D1 activity was inhibited by Finasteride (selective 5αR2 inhibitor), but not Dutasteride (non-selective 5αR inhibitor). AKR1D1 mRNA expression was characterised in four different hepatoma cell lines (Hep3b, HepG2, C3A, and Huh7.0) as well as primary cultures of human hepatocytes. In addition, HepG2 cells were differentiated using an established protocol (including 1% DMSO treatment), and gene expression analyzed after 7, 14, and 21 days. Over-expression and siRNA knock down of AKR1D1 in HepG2 cells were also performed. AKR1D1 was highly expressed in human hepatoma cell lines and expression decreased across differentiation, to levels that were similar to those seen in primary cultures of human hepatocytes. Successful over-expression and knock down of AKR1D1 were confirmed in HepG2 cells using real-time PCR. Importantly, changes in gene expression were paralleled by functional activity as measured by progesterone clearance. We have characterised human AKR1D1 in cell-free systems and in established liver cell models. We have successfully manipulated AKR1D1 expression and activity that will serve as the platform for future studies to define its role in the regulation of metabolic phenotype within the liver.

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## P397

**Molecular and immunohistochemical analysis of aldosterone producing adenomas**

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Primary aldosteronism (PA) accounts for the largest proportion of cases of secondary hypertension worldwide. The majority of PA cases are a result of a unilateral aldosterone-producing adenoma (APA). The pathogenesis of APAs, the most curable form of hypertension, has been the focus of worldwide clinical interest, and is associated with mutations in four genes: *KCNJ5*, *ATP1A1*, *ATP2B3*, and *CACNA1D*. Investigation into these mutations may lead to earlier detection, critical for a chronic condition in which the likelihood of a complete cure decreases with time.

Analysis of APA tissue often reveals a zona fasciculata (ZF)-like phenotype, based on cellular morphology, despite the zona glomerulosa (ZG)-restricted expression of CYP11B2, the enzyme required for aldosterone production, in normal tissue. Recently, it was demonstrated that ZG-like adenomas preferentially harbour mutations in *ATP1A1* and *CACNA1D*, but *ATP2B3* mutations were not identified[1]. We collected samples from 36 patients. RT-PCR and Sanger sequencing identified mutations in *KCNJ5* and *ATP2B3*, and these adenomas were stained with CYP11B1 and CYP11B2 antibodies to identify cellular phenotypes.

We confirmed that *KCNJ5*-mutant APAs are more common in women and are generally of a larger size than APAs with mutations in *ATP1A1* and *ATP2B3*. Interestingly, our population of patients has a significantly greater prevalence of mutations in the *ATP2B3* gene, 8.3%, and no mutations in *ATP1A1*, compared to the worldwide prevalences of 1.7 and 5.3% respectively.[2] Results from immunostaining indicate a complex relationship between genetic mutation and cell phenotype; one *ATP2B3* mutation was *CYP11B1* negative whereas one was positive, and a similar pattern for adenomas harbouring *KCNJ5* mutations was observed, supporting the observations of others.[2] We hope further investigations will lead to a genotype–phenotype correlation, particularly regarding gender differences and adenoma size, and ultimately improved treatment options.

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## P398

**Discrimination of adrenocortical carcinoma from other adrenal lesions: use of a new 13 steroid serum panel based on LC–MS/MS**

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Adrenocortical carcinoma (ACC) is a rare malignancy, but accounts for up to 11% of adrenal masses investigated in referral centres. Diagnosis remains a challenge. Up to two thirds are biochemically inactive, resulting from *de facto* enzyme deficiencies in the steroid hormone biosynthetic pathways, as shown by urine steroid profiling by gas chromatography-mass spectrometry. Increased metabolites of pathway intermediates in ACC discriminate it from benign adrenal lesions and provide markers for follow up. Serum assays for most intermediates (e.g. 17-hydroxypregnenolone) are unavailable, due to low demand or lack of immunoassay specificity. Serum steroid analysis by liquid chromatography–tandem mass spectrometry (LC–MS/MS) is increasingly replacing immunoassay, especially for those most subject to cross-reaction. We have developed an LC–MS/MS method for measurement of testosterone, progesterone, androstenedione, DHEAS, pregnenolone, 11-deoxycorticosterone, corticosterone, 17-hydroxypregnenolone, 17-hydroxyprogesterone, 11-deoxycortisol, 21-deoxycortisol, cortisol, and cortisone in serum. We investigated its utility in discriminating ACC (six cases) from 24 non-ACC adrenal lesions (11 phaeochromocytoma, three cortisol-producing and three aldosterone-producing adenomas, and seven lesions demonstrating no hormonal excess) and 61 healthy controls. Samples containing internal standards were prepared for LC–MS/MS by sequential protein precipitation and liquid–liquid extraction. Steroids were resolved on a reverse-phase C18 column with the MS operated in positive APCI ionisation mode. In the ACC cases, between four and 10 steroids were increased (mean = 6), whilst in the non-ACC group up to two steroids were increased. 11-Deoxycortisol was markedly increased in all ACC cases, whilst increases were also seen for androstenedione (five cases), 17-hydroxyprogesterone (four cases) and pregnenolone, 17-hydroxypregnenolone, 11-deoxycorticosterone, and DHEAS (three cases each). The cortisol:11-deoxycortisol ratio best discriminated between ACC (mean = 14.9), non-ACC (335.9), and healthy controls (324.9, $P = 0.003$). In conclusion, serum steroid panelling by LC–MS offers a promising diagnostic test for ACC by combining the measurement of steroid hormones and their precursors in a single analysis.

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## P399

**Glucocorticoid-induced lipolysis across human ageing and the relationship to fat mass and androgens**

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Background

Excess glucocorticoid (GC) exposure is associated with an adverse body composition and metabolic profile characterised by increasing fat mass, muscle atrophy, insulin resistance, fatty liver, and dyslipidaemia culminating in increased cardiovascular risk. GCs are widely prescribed with increasing age where their effects can have deleterious effects.

Objectives
To investigate the impact of GC exposure on subcutaneous adipose lipid metabolism across healthy human ageing and to investigate associations with phenotypic and hormonal markers of ageing.
Setting
NIHR–Wellcome Trust Clinical Research Facility.
Participants
Human volunteers aged 20–81 years ($n=134$; 77 women and 57 men). A subsection ($n=72$) had adipose microdialysis analysis performed.
Methods
Day attendance for baseline observations, DEXA, 24-h urine collection for GC/MS analysis**,** measurement of prednisolone generation from prednisone by LC/MS and subcutaneous adipose tissue microdialysis for 4 h following 10 mg oral prednisone.
Results
Subcutaneous adipose microdialysis glucose (1.9-fold, IQR 1.4–2.7), lactate (4.6-fold, IQR 3.2–8.2), pyruvate (4.0-fold, IQR 2.2–9.1), and glycerol (2.8-fold, IQR 1.9–4.4) were increased in response to prednisone. There was a positive correlation between glycerol (AUC; a lipolysis marker) and age ($\rho=0.31$, $P=0.04$), with no associations with other measured adipose metabolites. Glycerol also correlated positively with total fat ($\rho=0.29$, $P=0.04$). These correlations were more marked in men (total fat $\rho=0.64$, $P=0.01$ and % body fat $\rho=0.78$, $P=0.0009$). There were significant correlations between glycerol and urine (DHEA/THE + THF + 5aTHF) ($\rho=-0.29$, $P=0.04$) and PT/(THE + THF + 5aTHF) ($\rho=$-0.40, $P=0.005$). In women, correlations were also seen with (DHEA/THE + THF + 5aTHF) ($\rho=-0.38$, $P=0.02$) and PT/(THE + THF + 5aTHF) ($\rho=-0.43$, $P=0.01$), along with 16a-OH DHEA ($\rho=-0.35$, $P=0.04$). Glycerol also correlated negatively with serum DHEAS ($\rho=$-0.38, $P=0.03$).
Conclusion
GC-induced adipose tissue lipolysis is increased with age, which is likely a function of increased fat mass. The metabolite ratio associations raise the possibility that DHEA exerts an anti-GC effect in adipose tissue. Further interventional studies are required to determine the impact of androgens on lipid metabolism.

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## P400

**Rapid equilibriation of cortisol between the free and total plasma pools**

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Background
Using a deuterated tracer (D4-cortisol) to measure cortisol regeneration from cortisone *in vivo* we have quantified tissue-specific 11βHSD1 activity in health and disease, and acute regulation eg by insulin. These studies relied on tracer enrichment in the total plasma cortisol pool, assuming rapid equilibration between free and protein-bound pools. Slower equilibration would result in underestimation of enzyme activity, and has implications for the contribution of protein-bound cortisol to glucocorticoid action. Here, we investigated cortisol turnover in the free compared with the total cortisol pool at steady state and after acute up-regulation of 11βHSD1 by a high carbohydrate meal.
Methods
As reported (*J Clin Endocrinol Metab* 2014 **99** 160), 8 healthy men were infused with D4-cortisol before consuming blinded liquid meals containing minimal calories (placebo), high protein or high carbohydrate in random order. Plasma samples were obtained every 15 min for 3 h after the meals. Free steroids were separated using equilibrium dialysis. Deuterated and endogenous cortisol concentrations were quantified using LC–MS/MS. To achieve the sensitivity necessary multiple plasma samples were pooled from each subject across steady state and with each meal type. Data are mean ± S.E.M.
Results
At steady state, dilution of D4-cortisol by endogenous cortisol and D3 cortisol were similar in the free vs total pools, yielding similar rates of appearance (Ra) of cortisol and D3-cortisol (Ra cortisol 59.0 ± 7.2 nmol/min vs 54.9 ± 6.6 nmol/min and Ra D3 cortisol 20.1 ± 1.1 nmol/min vs 19.4 ± 1.0 nmol/min). With acute perturbation, similar up-regulation of Ra cortisol (after protein or carbohydrate) and Ra D3-cortisol (after carbohydrate) was observed using enrichments measured in the free and total plasma cortisol pools.
Conclusion
Rapid equilibration occurs between free and bound cortisol pools in plasma, and measurement of cortisol turnover in the total pool does not underestimate 11βHSD1 activity.

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## P401

**Do androgens lead to increased erythropoiesis in women with congenital adrenal hyperplasia?**

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Background
In men there is a strong relationship between androgens and erythropoiesis; however this has not been shown in women. Patients with congenital adrenal hyperplasia (CAH) if uncontrolled may have high androgen levels and if over treated with glucocorticoids low androgen levels. Therefore CAH provides a potential model to examine the relationship between androgens and erythropoiesis in women.
Aim
To investigate the relationship between androgens and erythropoiesis in women with CAH.
Methodology
A retrospective analysis of data from two cohorts of CAH patients. Androgen levels and blood counts performed on the same day were collected. Cohort 1: 27 women and cohort 2: 53 women with CAH.
Results
Mean age; cohort 1, 35.5 ± 13.2 years and cohort 2, 30.8 ± 11.3. There was a positive correlation of testosterone, androstenedione and 17-OH progesterone (17-OHP) levels with haemoglobin (Hb) and haematocrit (Hct) in both cohorts (Table 1).

**Table 1** Correlations of Hb and Hct with androgens and 17-OHP in women.

| | 17-OHP | | Androstenedione | | Testosterone | |
|---|---|---|---|---|---|---|
| | Cohort 1 | Cohort 2 | Cohort 1 | Cohort 2 | Cohort 1 | Cohort 2 |
| Hb | $r=0.420$ ($P=0.0069$) | $r=0.319$ (0.02) | $r=0.413$ ($P=0.0012$) | $r=0.445$ ($P=0.001$) | $r=0.444$ ($P=0.0028$) | $r=0.470$ ($P=0.0001$) |
| Hct | $r=0.356$ ($P=0.024$) | $r=0.274$ ($P=0.047$) | $r=0.435$ ($P=0.0006$) | $r=0.398$ ($P=0.003$) | $r=0.367$ ($P=0.0154$) | $r=0.449$ ($P=0.001$) |

Conclusions
There is a positive correlation between adrenal androgens and erythropoiesis in women with CAH, which was seen across two different cohorts. Suboptimal control of androgens in this group of patients may increase the risk of either polycythaemia or anaemia. Haemoglobin and haematocrit may be used as additional biomarkers of disease control in women with CAH.

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## P402

**Bioinformatic analysis of microRNAs associated with aldosterone secretion**

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Many cases of hypertension are associated with inappropriately high levels of aldosterone secretion and it has been proposed that microRNAs play a role in this dysregulation. Previously, we showed that microRNAs expressed within the adrenal cortex significantly repress aldosterone production. Furthermore, stimulation of aldosterone secretion by three different means in the H295R cell line – the most commonly-used *in vitro* model of the human adrenal cortex – resulted in consistent changes to the microRNA profile of these cells. These profiles were subjected to bioinformatic analysis in order to predict which biological pathways are targeted by these differentially-expressed. Here we present some of those results.
Analysis centred on microRNA derived from H295R cells ($n=3$/group) incubated for 24 h with either 100 nM angiotensin II (AngII), 10 mM dbcAMP (simulating the effects of ACTH), or 20 mM potassium chloride (KCl). Stimulation of aldosterone synthase (*CYP11B2*) mRNA levels were confirmed for each treatment. Full microRNA profiles were generated using microarray. Six microRNAs were consistently downregulated across all three treatment types: three are co-transcribed from a cluster on the X chromosome, while two others

also come from a single cluster located on chromosome 13; the sixth is transcribed singly from chromosome 14.
Ingenuity Pathway Analysis (IPA) software was used to predict transcripts targeted by these six microRNAs. Only one was predicted to target components of the steroidogenic pathway directly. Further analysis focusing on validated interactions between these microRNAs and specific transcript targets identified effects across a diverse range of biological pathways. These include cell cycle regulation, insulin receptor signalling, and Wnt/β-catenin signalling.
These data suggest that differing methods of aldosterone stimulation result in common effects on specific microRNA levels. Bioinformatic analysis identifies plausible targets for these microRNAs and will aid in the direction of future *in vitro* studies.

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## P403

**Immunogenicity in AAD patients treated with depot tetracosactide**

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ACTH is a 39 amino acids polypeptide which stimulates adrenocortical steroid production. The N-terminal segment of ACTH(1–24) is biologically active and the C-terminal is considered to have greater antigenicity. In one previous ('RoSA') and one current ('RADS2') clinical trial synthetic ACTH (zinc tetracosactide; depot synacthen) was administered to autoimmune Addison's disease (AAD) patients to stimulate adrenocortical regeneration. 4/13 RoSA patients developed allergic cutaneous reactions following administration of depot Synacthen.
An ELISA was used to detect reactivity to ACTH(1–39), ACTH(1–24), ACTH(1–13), and ACTH(18–39) peptides anchored to solid phase. Binding activity was measured in triplicate sera samples in 18 AAD trial participants and a control cohort of 100 A + E attenders. To determine seropositivity, the highest relative absorbance level of the control cohort was used as an arbitrary threshold. No control sera showed reactivity to full-length ACTH(1–39) or to the ACTH (1–13) (αMSH) peptide. 3/18 (17%) trial participants showed reactivity to full-length ACTH(1–39), with an additional 2/18 (11%) showing reactivity to the ACTH(1–13) (αMSH) component in isolation. 2/18 (11%) AAD patients demonstrated reactivity to 1–24 post-treatment with depot tetracosactide, with steadily rising absorbance readings during the treatment course. Quantitatively, patient reactivity to tetracosactide depot (ACTH(1–24)-Zn) was higher at baseline than in controls ($P = 0.0002$), but reactivity against soluble ACTH(1–24) was less in both groups ($P = 0.03$). In those patients who had received concurrent rituximab therapy, no reactivity to depot tetracosactide was demonstrated.
A proportion of AAD patients receiving repeated doses of depot tetracosactide develop anti-ACTH antibodies, with varied reactivity to constituent portions of ACTH. Rising antibody concentrations during treatment may explain adverse effects in patients and resistance to long-term tetracosactide therapy. Furthermore, hypersensitivity to zinc phosphate in the tetracosactide depot preparation is likely to play a cumulative role in the mounting antibody response to ACTH (1–24).

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## P404

**Unveiling the complexity of the undifferentiated zone in the human adrenal cortex**

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Background
The human adrenal cortex is composed of different zones, namely the zona glomerulosa (ZG), zona fasciculata (ZF), zona reticularis, and the recently proposed undifferentiated zone (UZ). The adrenal cortex synthesises and secretes steroids, mainly aldosterone and cortisol, both responsible for essential physiological and metabolic functions. Adrenal cortex disorders can be life threatening and current treatment involves life-long steroid replacement, which is not ideal.
Aim
We aim at identifying markers to distinguish (and eventually isolate) cells from the different zones, with a specific focus on the subcapsular region.
Methods
Following ethical approval, adrenal glands were collected from patients undergoing adrenalectomy, snap–frozen in liquid nitrogen and cut using a cryostat. We tested a wide array of antibodies to antigens known to have a zonal expression in murine studies. Non-radioactive *in situ* hybridization was also employed.
Results
We demonstrated that the human ZU is a more complex environment than we had anticipated. Delta-like homologue-1 (DLK1) seems to be a good marker of the ZU; however we found clusters of non-steroidogenic cells in the subcapsular region that are DLK1 negative and we are currently searching for specific markers.
Conclusion
The detailed characterisation of the subcapsular region in the human adrenal will provide invaluable information of steroidogenic differentiation processes and tissue homeostasis. It will also aid in the isolation of cells from the different zones using laser capture microdissection followed by important downstream applications, such as a transcriptome comparative analysis.

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## P405

**Cytosolic innate immune receptor RIG-I amplifies glucocorticoid action within lung epithelial cells**

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Glucocorticoids (GC) influence immune responses, and exogenous GC are widely used clinically for their potent anti-inflammatory effects. Endogenous GC action is modulated by pre-receptor GC metabolising enzyme 11β-hydroxysteroid dehydrogenase (11b-HSD). 11b-HSD1 predominantly reactivates GCs, amplifying their action. Recent work has shown that 11b-HSD1 modulates immune and inflammatory response. However how innate immune system affect GC metabolism within cells remains largely unknown. Retinoic-acid-inducible gene I (RIG-I) is a cytosolic receptor that sense RNA viruses, such as influenza, producing cytokines and type 1 interferons. The aim of this study was to evaluate how RIG-I activation affect GC metabolism in human lung epithelial cells.
Methods
5′-Triphosphate modified RNA (3pRNA), the ligand for RIG-I, was transfected by lipofection in human lung epithelial A549 cells. Cells were cultured for 24 h in the presence or absence of 1 μM cortisol following 3pRNA treatment. Cells were harvested. RNA was reverse transcribed and quantified by real-time PCR.
Results
Treatment of 3pRNA increased 11b-HSD1 and steroidogenic enzyme *CYP11A1* mRNA levels ($P < 0.01$), which were significantly increased by cortisol treatment. Interestingly combination treatment of 3pRNA and cortisol synergistically increased 11b-HSD1 mRNA levels ($P < 0.01$). Pulmonary surfactant protein B (SP-B) mRNA levels were significantly increased by 3pRNA treatment. Cortisol also increased SP-B and SP-D mRNA levels ($P < 0.01$). 3pRNA and cortisol treatments additively increased SP-B mRNA levels ($P < 0.01$), showing similar pattern to 11b-HSD1 induction.
Conclusion
Innate immune receptor RIG-I may contribute to the amplification of GC action and could be important in the generation of pulmonary surfactant within lung cells. Further investigation may address the role of 11b-HSD1 during infection.

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## P406

**Quantitative analysis of an adrenal steroid profile, canrenone, and mifepristone in plasma by triple quadrupole mass spectrometry**

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Canrenone and mifepristone (RU486) are respectively mineralocorticoid receptor (MR) and glucocorticoid receptor (GR) antagonists commonly used to block or displace steroid for clinical pharmacological or research purposes. The aim of this study was to develop and validate a sensitive, quantitative assay for the combined analysis of glucocorticoids (cortisol, cortisone, corticosterone, and 11-dehydrocorticosterone), mineralocorticoids (aldosterone), canrenoate, and mifepristone. HPLC mass spectrometric method development was carried out on an ABI 5500

QTrap triple quadrupole mass spectrometer with an Acquity UPLC System. Under mixed mode electrospray ionisation and collisional activation, the following parent–product transitions were monitored; *m/z* cortisol: 363→121; cortisone: 361→77; corticosterone: 347→91; 11-dehydrocorticosterone: 345→121; aldosterone: 359→331; canrenone: 341→91, and RU486: 430→134. Ions for corresponding deuterated internal standards were incorporated. Analytes eluted between 2 and 6 min. Optimal separation was achieved using water (0.1% formic acid)/acetonitrile (0.1% formic acid) at 70:30, 0.5 ml/min on a Waters Sunfire C18 HPLC column (3.5 μm; 150×2.1 mm) at 30 °C. A gradient rising to 90% acetonitrile was applied, with a total run time of 9 min.
Satisfactory recoveries of all analytes (mean 83.56% (relative S.D. 11.6%)) were achieved following liquid–liquid extraction of 100 μl plasma with chloroform (1:10). Intra-assay validation parameters for quantitative analysis were assessed with three replicates at low, medium, and high concentration. The limit of quantitation was ∼0.1 ng for endogenous steroids and ∼5 ng for the drugs with mean precision of 9.4%. The assay was linear ($r^2 \sim 1$) over a range of concentrations (0.1–100 ng).
This assay is suitable to detect expected endogenous steroid levels and drug doses in one aliquot of ∼200 μl of plasma and will allow rapid simultaneous profiling of circulating adrenal hormones.

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## P407

**Conventional vs modified release hydrocortisone in mitotane treated patients with adrenocortical cancer**

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Background
Mitotane is a strong inducer of hepatic CYP3A4 activity (cortisol metabolism) and increases cortisol-binding globulin (CBG). High hydrocortisone dosages are necessary in patients with adrenocortical cancer (ACC) on mitotane treatment. The newly modified release hydrocortisone has not been used in mitotane-treated ACC patients yet.
Aim
To compare cortisol (serum and saliva), calculated free serum cortisol and ACTH levels in ACC patients on mitotane treatment with conventional and modified release hydrocortisone.
Design
Nine patients with ACC on mitotane treatment and on stable daily hydrocortisone dose (60 mg) were included. Patients were taking 1 day conventional hydrocortisone (40–20–0 mg) and the next day modified-release hydrocortisone (40–20–0 mg).
Methods
Cortisol and ACTH were measured by chemiluminescent enzyme immunoassay, and CBG by ELISA and RIA. Salivary cortisol was analysed by electro-chemiluminescence immunoassay.
Results
Serum cortisol levels varied widely in the supraphysiological range (20–140 μg/dl) after conventional hydrocortisone intake, and showed a more homogenous pattern after modified release hydrocortisone intake. Most of the patients showed suppressed ACTH levels with no differences between the two hydrocortisone preparations. CBG levels were 0.87–3.0 times above the sex-specific upper-limit of the normal range. Calculated free cortisol levels showed more pronounced peaks after tablet intake of conventional hydrocortisone, whereas peaks were blunted after intake of modified release hydrocortisone. Calculated mean free cortisol level showed no peak after modified release hydrocortisone tablet intake resulting in a continuous free cortisol level between 6 and 12 nmol/l. After 40 mg of conventional hydrocortisone intake the mean free cortisol level peaked at 45 nmol/l, and after 20 mg of conventional hydrocortisone intake at 17 nmol/l.
Conclusions
Modified release hydrocortisone results in smoother total and free cortisol levels, but is lacking a morning free cortisol peak. In contrast conventional hydrocortisone provided a similar free cortisol peak as seen in Addison's patients on conventional hydrocortisone therapy on a 10–10 mg dose regimen.

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## P408

**Effects of diet and gender on postprandial salivary glucocorticoid levels and bile acid excretion in healthy volunteers**

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The synthesis and metabolism of bile acids and steroid hormones are conflicted at several levels. We investigated potential interactions using a feeding paradigm to stimulate bile acid secretion and salivary steroid measurements to monitor post-prandial changes.
The study followed a randomised cross over design; both cortisol and cortisone were measured by specific ELISAs to assess effects on 11 beta-hydroxysteroid dehydrogenase activity (11βHSD). At midday, groups of male and female volunteers ($n=8$ each, aged 18–26 years; BMI = 22.77 ± 3.58 kg/m$^2$ and systolic/diastolic BP were 122.9 ± 7.7/70.5 ± 8.1 mmHg) were fed isocaloric low carbohydrate/high fat or high carbohydrate/low fat meals with uniform content of protein. On a third occasion samples were collected with *ad libitum* feeding. On each test day, saliva was obtained immediately before feeding, 15, 30, and 90 min later and also early that morning and later in the evening**.** Urine samples over 24 h were collected.
Salivary cortisol and cortisone values showed expected diurnal variation. Female compared to male values were similar for cortisol, lower for cortisone and urinary bile acids levels and higher for cortisol:cortisone ratios ($P<0.005$). Post-prandial cortisol increases were more marked for females after a high fat meal than a low fat meal whereas in males, postprandial cortisone was greater after a high fat than a low fat meal ($P<0.05$). High and low fat meals did not affect urinary bile acid levels but, in males, urinary levels of bile acids correlated positively with the salivary cortisol:cortisone ($r=0.64$; $P<0.001$). Neither cortisol nor cortisone correlated with bile acid levels in females or males.
Salivary steroid measurements are a useful non-invasive method of assessing effects of food intake. We observed sex-specific effects of high and low fat diets on cortisol and cortisone levels. Contemporaneous measurements of circulating bile acids are needed to assess whether food-induced changes in bile secretion influence post-prandial steroid levels. Sex-specific differences in bile metabolism may affect 11 βHSD1 activity.

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## P409

**Shocking? A systematic review of adrenal insufficiency in adults on oral steroids**

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Background
One percent of the adult population are, at any one time, prescribed oral glucocorticoids (GC). GCs are known to be associated with hypothalamic–pituitary–adrenal axis suppression. However, there remains uncertainty regarding: i) the prevalence of GC-induced adrenal insufficiency (AI); ii) the effects of GC dose and duration; and iii) the time course of adrenal recovery and how GCs should be withdrawn. We undertook a systematic literature review to address these questions.
Methods
Searches were performed in MEDLINE and Web of Science in November 2014. Eligible papers studied adult patients with an indication for long-term GCs, exposure to oral GCs, and adrenal function tests. Screening was performed in duplicate and additional articles identified through citation screening. Three categories each for increasing dose, duration, and cumulative dose were assessed.
Results
From 645 screened papers, 42 met the inclusion criteria (14 randomised controlled trials and 28 observational studies). The prevalence of AI ranged from 0 to 100%. When examined within exposure categories, the prevalence still ranged from 0 to 100%, with medians of 33–43%. Only exposure <5 mg prednisolone equivalent dose/day had reduced AI (range 0–36%, median 15%). There was evidence of persisting adrenal suppression 1–3 years after GC cessation. Only five studies reported weaning of GCs and these were too heterogeneous in study design to draw useful conclusions.
Conclusions
Significant variation exists in the reported prevalence of AI after oral GC therapy, irrespective of exposure category. There is evidence, albeit limited, that even low

doses can suppress adrenal function, and some patients may have AI after several years of cessation. We suggest clinicians be vigilant for AI with all doses and durations of oral GC therapy. The evidence base supporting current practice, particularly with regards to withdrawal of steroids, is scant. There is imperative need for large-scale prospective studies to guide future practice.

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## P410

**Hormone replacement therapy with prednisolone in adrenal insufficiency patients: data from the European Adrenal Insufficiency Registry (EuAIR)**

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Introduction
Prednisolone is the standard treatment for most inflammatory conditions. However, it is also used in hormone replacement therapy in adrenal insufficiency (AI) due to historical reasons or due to its longer half-life and once daily application. Recent data showed that 5 mg daily prednisolone promotes loss in bone mineral density compared to 20 mg of hydrocortisone in patients with AI questioning the 4:1 conversion rate. Data is scarce on prednisolone treated AI patients. Therefore we analyzed the European Adrenal Insufficiency Registry (EuAIR).
Design
EuAIR with 19 centres across Germany, The Netherlands, Sweden, and the UK started enrolling patients with AI in August 2012. Patients on hydrocortisone or dexamethasone were excluded from this analysis as were patients with congenital adrenal hyperplasia. An individual patient could appear in more than one category for dose or frequency, but each patient is represented only once within a particular category.
Results
Up to November 2014, 64 patients (62.5% females) on replacement therapy with prednisolone were registered in EuAIR (26 primary AI and 38 secondary AI) . The overall mean age of patients was $58.3 \pm 16.7$, the mean duration of disease $24.0 \pm 12.9$ years. 14.1% of patients received $<4$ mg, 14.1% 4–$<5$ mg, 65.6% 5–$<6$ mg, and 21.8% $>6$ mg prednisolone/day. 73.4% received prednisolone once daily, 29.7% twice, and 1.6% trice daily. Mean BMI was $27.0 \pm 3.7$ kg/m$^2$; 45.3% of patients were overweight and 20.3% obese. Approximately a third of patients had a diagnosis of hypertension (31.3%) and dyslipidemia (35.9%). Systolic and diastolic blood pressure, and LDL-cholesterol, HDL-cholesterol and triglyceride levels were also analysed.
Conclusions
This is the largest cohort of prednisolone treated AI patients reported so far. A considerable proportion seems to be treated with too high daily doses. In addition, in real life, a substantial proportion of patients use prednisolone more frequently than once daily.

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## P411

**The effect of adrenalectomy on cardiovascular morbidity in adrenal tumor patients with subclinical Cushing's syndrome: systematic review and meta-analysis**

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Introduction
Patients with subclinical Cushing's syndrome (SCS) present with increased cardiovascular morbidity and mortality, however, the beneficial effect of adrenalectomy on cardiovascular risk factors is uncertain.
Objective
Systematic meta-analysis to determine the effect of adrenalectomy vs conservative follow-up on cardiovascular risk factors in patients with adrenal tumors and SCS.
Methods
We searched 6 databases through July 2014. Pairs of independent reviewers selected studies and appraised the risk of bias. We included and extracted studies with at least five SCS patients with adrenal tumors undergoing adrenalectomy where outcomes of interest were measured before and after surgery. Outcomes of interest included hypertension, diabetes, dyslipidemia, and obesity. In majority of studies, improvement of an outcome was defined as postsurgical decrease in dose, number or discontinuation of medications used to treat the comorbidity.
Results
Definition of SCS was heterogeneous among the 23 included studies (526 SCS patients). In eight studies, SCS patients undergoing adrenalectomy ($n=116$) were compared with SCS patients followed conservatively ($n=128$). In comparison with follow up, adrenalectomy had a positive effect on improvement in hypertension (RR 11.3 (4.2–30.2)), diabetes mellitus (RR 5.0 (1.74–14.4)), dyslipidemia (RR 3.0 (1.0–8.9)), and obesity (RR 3.4 (0.95–12.1)). When compared with follow up, patients with adrenalectomy had a systolic blood pressure decrease of 12.5 (6.5–18.6) mmHg, diastolic blood pressure decrease of 9.3 (3.5–15) mmHg and fasting glucose decrease of 23.4 (8.3–38.5) mg/dl. The quality of evidence was low due to increased risk of bias, heterogeneity in both SCS and outcome definitions, as well as variable time of postsurgical assessment.
Conclusion
Low quality evidence from the included studies suggests improvement of cardiovascular factors in patients with SCS undergoing adrenalectomy. This is derived from short-term imprecise evidence warranting low confidence. However, our conclusions represent a summary of the best available evidence. Prospective studies are required to confirm these findings.

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## P412

**DHEA reduces the palmitate-induced apoptosis in L6 myotubes**

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Introduction
DHEA and its sulfated form (DHEAS) are the most abundant steroid in humans, produced mainly by the adrenal cortex, converted to androgens and estrogens in peripheral tissues by tissue-specific steroidogenic enzymes. There are experimental evidences indicating DHEA anti-obesity, anti-inflammatory, and anti-oxidative effects in cell lines, animal models, and human.
Aim
The aim of this study was analyse the protective effect of DHEA in the skeletal muscle cell line, L6 myotubes, treated with palmitate toward the AKT, mTORC1/p70S6k, and ATF4/GADD34 pathways.
Methods
Differenciated L6 myotubes were incubated with DHEA 10 or 100 nM for 36 h plus 0.5 mM palmitate incubation for the last 12 h. The samples were used for typical immunoblotting with antibodies against the mentioned proteins above, and morphological analysis of cell death. Data are represented as mean $\pm$ S.E.M. Statistical analyses were performed using one-way ANOVA ($P<0.05$).
Results
DHEA (10 nM) was able to increase the protein level of AKT ($\sim$10%), mTORC1 ($\sim$36%), p70S6K ($\sim$80%), ATF4 by 30%, and GADD34 by 30% compared to control ($P<0.05$). Furthermore, 100 nM DHEA induced enhanced of mTORC1 to 160%, ATF4 to 120%, and GADD34 to 130% compared to control ($P<0.05$). The 12 h incubation with palmitate induced reduction of all analyzed proteins except the p70S6K to $\sim$30% compared to control ($P<0.05$). Despite no effect of DHEA in the protein expression change due to palmitate, the 10 nM DHEA was able to improve the survival of the cells incubated with palmitate to an extent of 10% apoptosis, similar to controls.
Conclusion
The physiological DHEA concentration was able to increase the expression of proteins involved in the both protein synthesis and UPR pathways. It was also able to have a protective effect upon lipotoxicity.

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## P413

**Profiling of multiple vitamin D metabolites in a healthy human cohort by high-throughput liquid chromatography–tandem mass spectrometry analysis**

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Although a complex metabolic pathway for vitamin D exists, serum measurement of inactive 25-hydroxyvitamin D3 (25OHD3) continues to be the most common determinant of vitamin D 'status'. However, several other metabolites contribute to the physiological role of vitamin D, notably the active form 1α,25-dihydroxyvitamin D (1α,25(OH)2D3), inactive 3-epi-25OHD3 and chiral 23R and 24R,25(OH)2D3 metabolites. Quantification of these additional metabolites could provide a more accurate assessment of vitamin D status, particularly when comparing active/inactive metabolite ratios. The aim of this study was to develop and apply a liquid chromatography–tandem mass spectrometry (LC–MS/MS) method to analyse multiple vitamin D metabolites in a cohort of human serum samples obtained as part of a study on hormones and ageing ($n=116$; age range = 20–74). A Waters AQCUITY UPLC, using a Lux cellulose chiral column, coupled to a Xevo TQ-S mass spectrometer was used for separation and quantitation. Serum samples were prepared by supportive liquid–liquid extraction (SLE). Quantifiable measurement of the following metabolites where achieved using 0.2 ml serum: 25OHD3; 3-epi-25OHD3; 24R,25(OH)2D3; 1α,25(OH)2D3; 25OHD2. Concentrations of 25OHD3 ranged between 2.166 and 42.706 ng/ml. 3-Epi-25OHD3 and 24R,25(OH)2D3 concentrations ranged between 0.445–5.977 ng/ml and 0.084–9.514 respectively. Both 3-epi-25OHD3 and 24R,25(OH)2D3 correlated with increased 25OHD3 ($r=0.714$ and 0.883 respectively). It was possible to quantify 1α,25(OH)2D3 in 40% of samples measured. Close correlation was observed for 25OHD3 and 24R,25(OH)2D3 ($r=0.714$, $P<0.001$), and 25OHD3 and 3-epi-25OHD3 ($r=0.883$, $P<0.001$). However, correlation was not observed between 25OHD3 and 1α25(OH)2D3 ($r=0.276$, $P=0.032$), and 24R,25(OH)2D3 and 1α25(OH)2D3 ($r=0.297$, $P=0.023$). No differences in metabolite levels where observed between age groups, although some seasonal alterations in serum vitamin D was observed.
The high-throughput LC–MS/MS method developed provides a detailed profile of vitamin D metabolites, enabling a more comprehensive analysis of vitamin D function. This approach may be particularly informative for studies of vitamin D and human disease.

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## P414

**Support from clinical audit data for liberal minimum 0900 h cortisol cut-off to avoid short Synacthen tests**

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Background
Short Synacthen tests (SST) are inconvenient and expensive, especially since the cost of tetracosactide recently increased to over £45/ampule. Historic literature on the minimum 9 am cortisol required to avoid a SST ranges from 243 to 500 nmol/l and individual endocrinologist practice varies greatly.
Methods
In an audit of all 182 SST's from 1 year at our institution, either a previous 0900 h cortisol was recorded or the basal cortisol was measured in the SST. The 30 min stimulated response was evaluated and the documented clinical interpretation noted.
Results
Of 79 patients with a previous 0900 h cortisol level, 71 measured above our laboratory's normal 0900 h lower limit of 119 nmol/l. 66 of these had a stimulated cortisol above 500 nmol/l and were interpreted as normal. The remaining five patients all had a stimulated cortisol above 400 nmol/l, but only one was judged to require commencement of hydrocortisone and two were already taking hydrocortisone. Of 103 patients without a previous 0900 h cortisol, 95 had a basal cortisol in the SST above 119 nmol/l. 91 of these had a stimulated cortisol above 500 nmol/l. The remaining four patients all had a borderline response above 400 nmol/l, but only one was judged clinically to require steroid replacement. In summary, of 166 patients with basal or 0900 h cortisol above 119 nmol/l, only two were judged to require commencement of hydrocortisone – therefore the SST did not change management in 99% of cases.
Discussion
Defining normality in measured adrenal function is notoriously difficult. Borderline SST's are rarely acted upon, and it is usually doubtful if these patients require steroid replacement. It therefore does not seem clinically relevant to detect these cases. We therefore argue that most SST's could be avoided by measuring a 0900 h cortisol level if it is within the local laboratory true 0900 h normal range.

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## P415

**Lineage conversion of human cells to an adrenocortical phenotype: a new technology to study the adrenal gland**

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The adrenal cortex is the primary site of steroid synthesis, producing glucocorticoids under the control of the hypothalamic–pituitary axis and mineralocorticoids under the control of the renin–angiotensin system.
Adrenal insufficiency, which can be life threatening, is cause by a number of adrenal disorders, and lifelong management of these patients with exogenous steroids can be challenging. No drug suitably mimics the diurnal pattern of cortisol noted in healthy individuals, and objective variables to measure replacement quality are lacking. Our long-term goal is to develop novel personalized and curative treatments that use stem cells to treat the many progressive and debilitating conditions affecting the adrenal cortex. The potential to produce and expand disease- and patient-specific cells may also revolutionize our understanding of the underlying pathophysiology of adrenal disorders, paving the way for the identification of novel therapeutic targets.
Steroidogenic factor 1 (SF1) is a transcription factor essential for both adrenal and gonadal development. SF1 not only binds to responsive elements in the promoter region of steroidogenic genes to positively regulate their transcription, but can be considered a true effector of cell fate as it starts a genetic program driving embryonic mesenchymal cells towards a steroidogenic phenotype/lineage; its absolute requirement for steroidogenesis has been recently demonstrated *in vivo*. Also, the capacity to impose an SF1-dependent steroidogenic-like gene expression program in a variety of murine cells (embryonic fibroblasts, adipose stromal fraction, bone marrow stroma, ES cells, and IPSCs) has been verified by several groups.
By forcing the expression of SF1, but not of other transcription factors involved in adrenal development, we demonstrate the ability of human fibroblasts, blood-derived late outgrowth endothelial progenitor cells (L-EPCs), and urine-derived stem cells (USCs) to lineage convert to steroidogenic-like cells, as assessed by changes in cell morphology, gene expression, activation of adrenal-specific signaling pathways, and hormonal output.

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## P416

**Steroid-dependent patients with multiple co-morbidities are more vulnerable to adrenal crisis**

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Steroid-dependent patients have a lifelong dependency on replacement therapy and a lifelong vulnerability to sudden death from under treated adrenal crisis. Yet evidence about the adequacy of medical response to adrenal crisis within the UK is largely anecdotal.
We invited members of the main UK support groups representing steroid-dependent patients to complete an online questionnaire identifying the frequency, causes and location of their adrenal crises (episodes needing injected steroids and/or IV fluids). Respondents were asked to describe the nature and timeliness of their medical treatment and to provide demographic information that explored predisposing factors. 1042 patients belonging to the UK support groups for pituitary conditions, Addison's, endocrine cancer and congenital adrenal hypoplasia gave responses.
Patients with comorbidities reported more frequent crisis episodes. Asthma and diabetes were the co-morbidities that acted to destabilize steroid-dependence most strongly. Those patients whose fluid balance is medication-dependent – primary adrenal insufficiency and diabetes insipidus – were less stable and more vulnerable to adrenal crisis, than those with secondary adrenal insufficiency and intact fluid homeostasis.

Vomiting was the overwhelming factor triggering crisis episodes, reported in around 80% of cases. The most common location, at 70%, was the home. 9% reported they were already a hospital inpatient and their adrenal crisis was iatrogenic – that is, triggered by insufficient steroid medication during surgical recovery or post-labour.
For their most recent crisis, over one-third either gave themselves an initial injection of 100 mg hydrocortisone IM, or received this from a partner, parent, child, friend or neighbour. Two-thirds of all respondents were happy with the quality of the medical treatment they received for their most recent adrenal emergency. The largest factor influencing satisfaction levels was the timeliness of the medical response. Less than two-thirds thought they had received prompt medical treatment.
Good patient education and readiness to self-treat remain important for the steroid-dependent patient, as delays in the medical response can be predicted for roughly one-third of patients experiencing adrenal crisis.

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## P417

### Comorbidities are the norm for steroid-dependent patients and predispose to adrenal crisis

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Steroid-dependence is a life-long condition with a risk of premature mortality from undertreated adrenal crisis, hospital omission of steroids, or running out of maintenance drugs at home. We invited members of the main UK support groups representing steroid-dependent patients to complete an online questionnaire about their experiences of adrenal crisis. Respondents ($n=1042$) were asked to provide demographic information that explored predisposing factors, including comorbidities and length of repeat prescription for their essential steroid drugs.
Paediatric and geriatric patients were more likely to be on the minimum length 28 day repeat, while adults of working age were more likely to receive an extended length repeat from 2 to 6 months. Comorbidities, which are recognised to increase vulnerability to adrenal crisis, were the norm for patients steroid-dependent from any cause. 23% of autoimmune Addison's patients and 7% of pituitary patients reported no additional conditions. 57% of CAH replies, mostly from parents of a paediatric patient, reported no additional conditions; 16% identified asthma.
Hypothyroidism was the most common comorbidity, reported by 44–58% of patients with autoimmune Addison's or a pituitary condition. Growth hormone dependency was reported by 53% of pituitary patients. Diabetes insipidus, which predisposes to adrenal crisis due to instability of the fluid balance, was reported by 33% of pituitary patients. Asthma, another condition predisposing to adrenal crisis, was reported by 12% of pituitary, 13% of autoimmune Addison's and 27% of patients whose adrenals had been surgically removed. Osteoarthritis was reported by 15% of pituitary and 16% of autoimmune Addison's patients. Pituitary patients reported more mobility problems (14%) than those with Addison's (8%). 19% of those whose adrenal had been surgically removed reported both mobility and cardiac problems.
These findings emphasise the vulnerability of steroid-dependent patients to adrenal crisis and the importance of good patient education about self-management of their multiple drug dependencies.

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## P418

### Lack of awareness contributes to delayed diagnosis and inappropriate management in men with low testosterone: findings from a UK study of men diagnosed with hypogonadism

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Introduction
Hypogonadism is prevalent in older men and associated with various comorbidities. Despite this, it is underdiagnosed and undertreated.
Aim
To identify factors associated with underdiagnosis and undertreatment of male hypogonadism.
Methods
Quantitative, questionnaire-based, online survey conducted in the UK among men aged ≧40 years with hypogonadism confirmed by blood test ($n=101$, from $N=3\,871$ screened respondents). All information was self-reported. The survey assessed medical history, treatment-seeking and treatment.
Results
Respondents had been diagnosed with low testosterone for a mean of 4.1 years. Prior to diagnosis, they most commonly experienced decreased libido (65%), tiredness/fatigue (64%), erectile dysfunction (ED; 59%), and listlessness/no energy (48%). Two-thirds of respondents experienced symptoms for up to 2 years before seeking advice. The main reasons for this were a belief that it was not a serious problem (49%), it was 'part of life' (46%) or a normal consequence of ageing (44%), and/or embarrassment (41%). The key driver for treatment-seeking was a sexual health problem (ED: 31%, decreased libido: 13%). Only 16% of men suspected they had hypogonadism prior to testing, based on discussions with their partner/spouse (38%), internet research (31%), or reading about it in a magazine (25%). Over half of men (53%) reported that it was their GP who recommended a testosterone test. A fifth of men had to wait 2–4 weeks for this, following their initial consultation. Reasons men gave for this delay included their GP pursuing an alternative diagnosis and lack of available facilities. Importantly, almost one-quarter of men aged ≧40 years, and 40% of those aged ≧60 years, never received treatment for their hypogonadism.
Conclusions
Low testosterone is under-recognised, and misperceptions and embarrassment can discourage men from seeking help. A substantial proportion of men are untreated following a correct diagnosis, highlighting the need for improved awareness and management of this condition.

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## P419

### Heterogeneity in the clinical management of adrenal insufficiency – data from EU-AIR

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Introduction
Glucocorticoid (GC) replacement therapy is administered to patients with adrenal insufficiency (AI) in multiple daily doses; however, there are no consensus guidelines on the optimal regimen.
Methods
The European Adrenal Insufficiency Registry (EU-AIR) is a multinational, multicentre, observational study sponsored by Shire, initiated to monitor the long-term safety of GC replacement in patients with chronic AI. We analysed baseline data to gain insight into the current clinical management of primary (PAI) and secondary AI (SAI). Patients receiving modified-release hydrocortisone and those with congenital adrenal hyperplasia were excluded from this analysis.
Results
As of 13 May 2015, 1166 patients (364 (31.2%) with PAI, 801 (68.7%) with SAI, 1 (0.1%) with unknown aetiology) were enrolled in EU-AIR. Overall, 87.4% of patients were receiving hydrocortisone (HC), 5.1% prednisolone and 4.0% cortisone acetate. For patients receiving HC, daily doses ranged from 5–>45 mg. Most patients (42.2%) were receiving 20–<25 mg/day; 22.9% of patients were receiving 15–< 20 mg/day, and 27.8% were receiving ≧25 mg/day. Patients with PAI and SAI were receiving average HC doses of 23.4 and 19.6 mg/day, respectively. Most patients with PAI were receiving HC three times daily (BID, 38.8%; TID, 53.7%) whereas most patients with SAI were receiving HC twice daily (BID, 52.9%; TID, 39.2%). Overall, HC was taken QD, BID, TID and QID by 5.5, 48.7, 43.6% and 2.1% of patients, respectively. There were 25 different regimens used to deliver a daily dose of 20 mg HC.
Conclusions
There is significant heterogeneity in the clinical management of AI. This likely reflects individualization of therapy in the absence of consensus guidelines.

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## P420

**Phaeochromocytoma related reversible Takotsubo cardiomyopathy**

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Stress related cardiomyopathy or Takotsubo LV dysfunction has been described in phaeochromocytoma related cardiomyopathy.
16 y female, admitted in A&E on 23 June 2013 with severe epigastric pain, vomiting and dyspnoea. History of episodes of palpitations and exertional syncopal attacks for 2 years, for which she had been investigated. Her symptoms were attributed to panic attacks. There was no known family history of Endocrinopathy. On admission, BP was 184/117 with heart rate 140/min, cold peripheries, R/R 26/min and tender epigastrium. ECG showed sinus tachycardia with T wave changes. Chest X-ray showed mild pulmonary edema. She became more dyspnoeic and referred to ITU for management of possible Phaeo crisis with alpha blockade, then transferred to ward on Phenoxybenzamine 20 + 30 mg and Propranolol LA 160 mg. Echo revealed known bicuspid aortic valve with mild to moderate impaired LV function on 24th June 2013. CT Abdomen /pelvis revealed 7.0×5.9 cm left adrenal mass with area of necrosis and normal right adrenal. MRI abdomen right adrenal mass 1.9×2.8×1.9 cm and left 6.9×5.0×5.2 cm, heterogeneous with areas of central necrosis MIBG scan showed bilateral phaeochromocytoma, left adrenal with weak uptake and right adrenal with tense uptake. Echocardiography repeated in 4 weeks showed normal LV function.
Considering the possibility of MEN 2, she was screened with thyroid ultrasound, calcium and calcitonin levels which were all normal. She underwent uncomplicated bilateral laproscopic adrenalectomy, commenced on replacement therapy with hydrocortisone 10/5/5 and fludrocortisone 50 µg. She was reviewed by clinical geneticist. Sequence analysis of RET, VHL, SDHB, SDHC, SDHD, SDHAF2, TMEM127 and MAX genes did not detect pathogenic mutation. Repeat MRI neck, thorax abdomen and pelvis did not show any evidence of recurrent disease, no evidence of malignancy.
This is uncommon presentation of phaeochromocytoma in young patient with normal genetics and impaired cardiac function which was reversed with treatment. Acute medical physicians need to be aware of this well described clinical presentation of phaeochromocytoma.

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## P421

**Iatrogenic Cushing's syndrome secondary to combined oral contraceptive pill in a patient with congenital adrenal hyperplasia**

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Introduction
Congenital Adrenal Hyperplasia (CAH) is a rare genetic disorder characterised by deficiency of cortisol and/or mineralocorticoid hormones with over production of sex steroids. 21-hydroxylase deficiency is the commonest cause of CAH accounting for 95% of cases. Severe form of classic CAH occurs in 1 in 15 000 live births.
Case report
A 30 year old women with CAH diagnosed at birth was on replacement with hydrocortisone and fludrocortisone. She was investigated for ongoing diarrhoea by the gastroenterologist and was subsequently diagnosed with Irritable Bowel Syndrome (IBS). She was then started on buscopan and codeine phosphate for symptom relief. However during her menstrual period her abdominal symptoms were not sufficiently controlled. She was thus commenced on a Combined Oral Contraceptive Pill (COCP). Within a year of initiation she developed cushingoid features, became hypertensive and gained 18 kg in weight. The COCP was then changed to a progesterone only pill which resulted in improvement of her cushingoid features. She lost 7 kg in weight and her blood pressure improved within 6 months.
Discussion
Cortisol is the main glucocorticoid hormone, the majority of which is circulated bound to Cortisol Binding Globulin (CBG). Only about 5% of the circulating cortisol is free. Free cortisol is filtered and excreted through the kidneys. Medications such as oestrogen increases CBG. This leads to an increase in total circulating cortisol and a reduction in renal excretion. The net clinical effect is the development of cushingoid features.
Conclusion
This case illustrates that apart from over-replacement with corticosteroid, iatrogenic Cushing's syndrome may also develop secondary to drug interactions resulting in increased CBG. Clinicians should be wary of starting such drugs in patients on steroids.

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## P422

**Bilateral adrenal haemorrhage in a patient with anti-phospholipid syndrome**

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A 38 year old woman was admitted with three day history of right sided chest pain, right upper abdominal pain and vomiting. She has anti-phospholipid syndrome (APS) treated with long term lower molecular weight heparin (Dalteparin) for inferior vena cava thrombosis. On presentation, she was hemodynamically stable with haemoglobin of 154 g/l, CRP 170 mg/l, white cell count $15\times10^9$/l, platelet of $90\times10^9$/l and sodium of 136 mmol/l. CXR showed consolidation in the right middle and left lower lung zone. CT scanning demonstrated right adrenal haemorrhage, a normal left adrenal and chronic IVC thrombosis. Treatment with intravenous fluids and antibiotics was started. Four-days later, the patient became hypotensive and hyponatremic (sodium 124 mmol/l) with undetectable random cortisol. Her haemoglobin dropped to 91 g/l, CRP increased to 314 mg/l and platelet dropped to $28\times10^9$/l. A repeat CT scan showed bilateral adrenal haemorrhages. The diagnosis of acute adrenal insufficiency secondary to bilateral adrenal haemorrhages was made. Treatment included intravenous fluids, antibiotics and hydrocortisone (100 mg bolus and infusion at 4 mg/kg over 24 h). Hydrocortisone was later converted to methylprednisolone temporarily treat her thrombocytopenia. The patient's condition improved steadily in both clinical and biochemical parameters- CRP 206, WCC $13.2\times10^9$/l, haemoglobin 104 g/l, sodium 136 mmol/l platelets $38\times10^9$/l. She was later converted to oral hydrocortisone and fludrocortisone. MR adrenal 2 months later showed no change in hematoma size. Bilateral adrenal haemorrhage is a rarely recognised complication of APS. Adrenal insufficiency can be the first presentation of APS. Risk factors for adrenal haemorrhage are; primary APS, venous thromboembolism, thrombocytopenia, anti-coagulation, sepsis and disseminated intravascular coagulopathy. Recovery is very rare and life-long corticosteroid and fludrocortisone replacement is needed in the majority of patients. Our case suggests that patients with unilateral adrenal haemorrhage are at high risk of evolving to bilateral haemorrhage and subsequent crises.

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## P423

**Transient adrenal insufficiency following acute bilateral adrenal haemorrhage**

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A 61-year-old gentleman was referred to the Endocrine team with acute bilateral adrenal haemorrhage. He had undergone a successful left popliteal embolectomy for critical limb ischaemia a week ago following which he was commenced on anticoagulation therapy. He presented to the Emergency Department with acute severe abdominal pain and low grade pyrexia of 37.6 C. He was haemodynamically stable. His International Normalised Ratio was noted to be elevated at 4.7. An urgent abdominal CT arranged for suspected bowel ischaemia demonstrated new adrenal masses with surrounding fat stranding consistent with bilateral adrenal haemorrhage. He was promptly commenced on intravenous hydrocortisone.
A subsequent 0900 h cortisol (47 nmol/l) off hydrocortisone confirmed adrenal insufficiency. Following discharge, he described good energy levels despite not being fully compliant with hydrocortisone replacement. A short synacthen test 5 months post-event demonstrated partial recovery of adrenal function – baseline cortisol 370 nmol/l, 30 min value 436 nmol/l. He was weaned off regular hydrocortisone with advice to take hydrocortisone only during intercurrent illness. He remained well off hydrocortisone and a further short synacthen test at 10 months confirmed full recovery of adrenal function – baseline 410 nmol/l, 30 min 592 nmol/l.
Acute bilateral adrenal haemorrhage is a potentially life-threatening condition where early diagnosis and prompt initiation of hydrocortisone replacement greatly affects the outcome. Due to its non-specific clinical manifestations, treatment is often delayed as diagnosis is frequently made following abdominal imaging, or in some cases, post-mortem. It is therefore crucial that this is given consideration in acutely unwell patients, especially in the presence of predisposing factors such as recent surgery or anticoagulation. Our case demonstrates that it is beneficial to reassess the hypothalamic-pituitary-adrenal axis following recovery as this may prevent unnecessary lifelong steroid replacement and its associated complications.

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## P424

### X-linked adrenoleukodystrophy – how to improve identification in the Addison's population

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Objective
X-linked adrenoleukodystrophy (X-ALD) is a rare, sometimes devastating disorder caused by-mainly inherited- mutations in the ABCD1 gene. Cerebral X-ALD can be prevented or modified if diagnosed presymptomatically. Mutation can lead to very long chain fatty acid (VLCFA) accumulation in adrenocortical cells, which can cause antibody negative primary Addison's disease (ANPAD). There are recommendations that all males affected by ANPAD should have VLCFA analysis. We aimed to identify whether all male patients with ANPAD received VLCFA testing.
Methods
All male patients with negative anti-adrenal antibodies testing and a subsequent diagnosis of ANPAD at Addenbrooke's Hospital 2005–2014 were identified by i) Assessing all requests for adrenal antibodies, ii) Excluding all who tested positive, iii) Identifying those diagnosed with AD, iv) Excluding patients with secondary AD or with a clear cause for primary AD, e.g. adrenal haemorrhage, v) Checking whether those remaining had undergone VLCFA testing on the Addenbrooke's results reporting system.
Results
252/269 male patients tested negative for adrenal antibodies. 30/252 had been diagnosed with AD, of which 21 had ANPAD. 16/21 of the ANPAD cases were of unknown aetiology, only 8/16 underwent VLCFA analysis and none were found to have ALD.
Conclusions
There was a 50% failure rate for screening ANPAD patients with VLCFA in our centre. ALD-Life believes the figure is likely to be similar elsewhere. VLCFA is an economical, highly sensitive and specific test for detecting males carrying ABCD1 mutations and hence having family members at risk of cerebral ALD. We propose that the Society for Endocrinology should alert their members to offer VLCFA testing to all males with ANPAD.

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## P425

### Primary testicular lymphoma with bilateral adrenal masses and adrenal insufficiency

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Introduction
Primary testicular lymphoma is very rare and constitutes about 2% of all extra nodal lymphomas, mostly in men above the age of 60. Bilateral adrenal masses could be due to metastatic disease, congenital adrenal hyperplasia, bilateral macro-nodular hyperplasia, adrenal adenomas, lymphomas, infiltrative diseases, amyloidosis and infections like tuberculosis. Metastasis occurs most commonly from lung, bowel, breast and pancreatic cancer. Metastasis from lymphomas is far less common. We report a case of primary testicular lymphoma with bilateral adrenal metastases.
Case report
A 71 year old man presented with right sided testicular swelling and underwent a right radical orchidectomy. Histology showed diffuse large cell B cell lymphoma. A subsequent staging CT scan revealed bilateral adrenal masses with no significant lymphadenopathy. There was marked FDG uptake in both adrenals on PET-CT, in keeping with FDG avid disease. His 24-hour urinary cortisol was 31 nmol (normal range 100–379 nmol). Plasma metanephrines were within normal range. He complained of excessive tiredness and ongoing weight loss, and had clinical evidence of orthostatic hypotension. His serum electrolytes were within normal range but a short synacthen test confirmed adrenal insufficiency with a baseline cortisol of 142 nmol/l and 141 nmol/l at 30 min. His symptoms markedly improved on hydrocortisone replacement. He underwent a CT guided biopsy of the right adrenal gland which confirmed diffuse large B cell lymphoma on histology, likely metastatic from his previous testicular lymphoma. He was then followed up by the haematologist for chemotherapy and radiotherapy.
Conclusion
Patient with bilateral adrenal masses, especially in the context of underlying malignant disease should be investigated for hormone excess and deficiency before any further intervention is undertaken. Biochemical investigation to rule out adrenal insufficiency is recommended to prevent adrenal crisis during invasive or operative interventions.

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## P426

### Adrenal insufficiency from bilateral adrenal haemorrhage in a post-operative patient on warfarin

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Introduction
We present a case which highlights the non-specific nature of the presenting symptoms of adrenal failure due to bilateral adrenal haemorrhage.
Case report
A 75-year-old gentleman had an uncomplicated right total hip replacement for osteoarthritis and his warfarin for atrial fibrillation was restarted on the third post-operative day before discharge. A week later, he was admitted with watery diarrhoea and vomiting. On examination, he was clinically dehydrated, blood pressure 96/64 mmHg and mild peri-umbilical tenderness. Laboratory tests revealed; sodium 130 mmol/l (134–145), potassium 5.6 mmol/l (3.5–5.3), urea 14.8 mmol/l (baseline 6) and creatinine 171 μmol/l (baseline 80). INR was 4.6. He was adequately resuscitated, managed as likely gastroenteritis and discharged when he stabilised. He was then readmitted another two weeks later, dehydrated and hypotensive with potassium of 7.6 mmol/l and sodium 132 mmol/l. His potassium was adequately treated but as the gentleman had persisting symptomatic hypotension he then had a short synacthen test which revealed baseline serum cortisol 62 nmol/l and post synacthen cortisol 63 nmol/l, confirming a diagnosis of adrenal insufficiency. His antibodies were negative and an adrenal MRI scan confirmed bilateral adrenal haemorrhage. He was then started on appropriate steroid replacement and fludrocortisone. His warfarin however was never discontinued.
Discussion
Adrenal failure due to bilateral adrenal haemorrhage is very rare and its pathophysiology is poorly understood. It may be of a multifactorial aetiology. Several pathophysiological changes during the intraoperative and postoperative period make the adrenal gland susceptible to adrenal haemorrhage. These may include intra-operative hypotension, vascular engorgement and stasis which cause an increase in adrenal venous pressure. The diagnosis of adrenal insufficiency secondary to adrenal haemorrhage is often delayed because of the nonspecific nature of the clinical presentation and low index of clinical suspicion. There is a need for clinicians to be aware of this potentially life-threatening but readily treatable condition.

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## P427

### Cushing's syndrome secondary to bilateral functioning adrenocortical adenoma

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**Abstract**
Sixty seven years old lady was admitted with profound weakness and weight loss for 3–4 months. She has background of Primary hyperparathyroidism, HTN and Osteoporosis.
Examination revealed BP of 175/90 mmHg, slightly plethoric with proximal myopathy and bruising. CTTAP bilateral axillary and para aortic lymphadenopathy and right adrenal adenoma 32×26 mm. CT guided

**Table 1**

| Investigations | Patients values (normal values) |
|---|---|
| 24 h urinary free cortisol (1st) | 429 (50–300 nmol/24 h) |
| 24 h urinary free cortisol (2nd) | 306 (50–300 nmol/24 h) |
| Plasma ACTH (1st) | 6 (0.0–40 ng/l) |
| Plasma ACTH (2nd) | <5 (0.0–40 ng/l) |
| 24 h urinary Metadrenaline | 0.58 (0.0–2.0 μmol/24 h) |
| 24 h urinary Normetadrenaline | 0.78 (0.0-4.3 μmol/24 h) |
| Plasma aldosterone concentration | 71 (40–310 ng/l) |
| Plasma renin activity | 2.8 (3–40 mU/l) |
| Dehyroepiandrosterone sulphate | 5.8 (0.26–6.68 μmol/l) |
| Androstenedione | 21.2 (0.0–3.5 nmol/l) |

**Table 2** Results of Adrenal vein sampling

| | Peripheral | Right adrenal vein | Left adrenal vein | A:U Ratio |
|---|---|---|---|---|
| Baseline cortisol | 475 | 2559 | 740 | 3.45 (>2) |
| Baseline aldosterone | 146 | 3000 | 999 | |
| 15 min post ACTH | | | | |
| Cortisol | 719 | 17 500 | 10 743 | 1.62 (<2) |
| Aldosterone | 260 | 14 540 | 10 570 | |
| 25 min post ACTH | | | | |
| Cortisol | 826 | 17 500 | 17 500 | 1.0 (<2) |
| Aldosterone | 285 | 13 160 | 14 970 | |

biopsy which showed CLL. She was worked up for hypercortisolism (Table 1).
She had overnight 1 mg DST which showed cortisol levels of 156 nmol/l. Her 48 h DST results were 793–158 nmol/l. She had MRI adrenal which revealed right adrenal of 3.3 cm and left adrenal of 1.4 cm with normal MRI pituitary. DEXA Scan showed osteoporosis. Octreotide Scan no uptake in both adrenals. Hormonal dynamic studies revealed bilateral autonomous secretion of cortisol (Table 2). She underwent laparoscopic bilateral adrenalectomies, commenced replacement therapy with hydrocortisone (HCT) and fludrocortisone postoperatively. Histopathology showed nodular proliferation of predominantly lipid containing cells suggestive of bilateral adrenal cortical adenomata.
Bilateral functioning adrenal adenomas are very rare. Only 25 cases are reported since 1977. Abnormal adrenal expression of receptors for various hormones can lead to ACTH independent bilateral macro nodular hyperplasia (AIMAH). These are multiple nodules, not encapsulated with hypertrophic adjacent areas. However cortisol producing adrenal adenomas are usually single, unilateral and encapsulated, usually associated with suppressed ACTH levels to atrophy adjacent non nodular area.

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## P428

**Complications of CAH in pregnancy**

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Case
A 32 year old with known classical 21 hydroxylase deficiency was planning her first pregnancy. She had a history of clitoral reduction and vaginal reconstructive surgery as a baby. Pre-conception control on prednisone and fludrocortisone was good: 17(OH) progesterone 8.3 nmol/l, androstenedione 9.8 nmol/l (0.7–10.8), testosterone 3.0 nmol/l (0.2–3). EUA showed a small vaginal opening and she was given vaginal dilators. She conceived successfully and was counselled about the potential virilising effects on a female foetus. She declined amniocentesis, but a detailed scan suggested a male foetus. Throughout pregnancy growth scans were appropriate. In the 3rd trimester, vaginal examination revealed a narrow canal, and the possibility of caesarean section was discussed. At 29 weeks she was admitted with a chest infection, treated with antibiotics and IV hydrocortisone, making a good recovery. The obstetric anaesthetics and neonatologists were informed about her with a plan for increased steroids at delivery. At 36 weeks she presented after missing a routine scan and was found to have intrauterine foetal death. Chorionic villous biopsy was performed and she had caesarean delivery of a growth restricted stillborn male.
Discussion
Classical CAH in pregnancy may have serious complications. Maternal complications include increased risk of miscarriage, pre-eclampsia, gestational diabetes mellitus and caesarean section. The foetus is at risk of growth restriction and, if female, virilising effects. Exogenous steroids used to treat CAH are safe in pregnancy because they are inactivated by placental 11-BHSD, protecting the foetus against adrenal suppression. The literature is sparse on outcomes of pregnancy in women with CAH due to low fertility rates. This is attributed to amenorrhea, anovulation, polycystic ovarian syndrome and psychosexual problems following corrective surgery. Management requires increased surveillance and a multidisciplinary approach to ensure best outcomes for mother and baby.

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## P429

**Liquorice induced hypermineralocorticoidism – a case report from Midyorkshire Hospitals NHS trust**

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Background
Excessive ingestion of Liquorice produces a state of apparent mineralocorticoid excess may result in sodium and water retention, hypertension, hypokalemia and suppression of the renin-aldosterone system. Yorkshire is famous for Liquorice confectionaries. In this paper we discuss about a patient with persistent hypokalemia secondary to excessive liquorice.
Case report
Fifty years old lady referred by GP with one year history of hypertension on Ramipril and fluctuating potassium levels (between 2.4 and 3.7 mmol/l). PMH of Migraine and recurrent UTIs. O/A: BP 202/118, Ramipril replaced with Amlodipine & Spironolactone. BP 140/80 mmHg on discharge with normal potassium & renin/aldosterone ratio. She got readmitted to MAU twice for IV potassium replacement. History was reviewed when she was referred to Endocrine clinic. She was asymptomatic with low potassium, stable weight and no symptoms of diabetes. She doesn't smoke and drinks alcohol occasionally. She worked as Children centre manager. On further questioning, interestingly she did admit that she used to eat significant amount of liquorice (Pontefract cakes) although she stopped this recently (4 months ago) which normalised her potassium & BP.O/E: PR:80/min regular, BP 130/78 mmHg, no cushingoid features. Systemic examination was unremarkable. We repeated her serum potassium which was 4.2 mmol/l. This is a classic case of liquorice induced hypokalemia. We suggested to the patient that she doesn't need to give up liquorice altogether, perhaps they should be eaten intermittently to avoid recurrence. Subsequently she was discharged from clinic. In this case, we would like to reiterate the importance of obtaining the relevant basic history, which prevents further expensive investigations and admissions.
Conclusion
Appropriate history taking remains the most fundamental aspect of clinical medicine which is fading away in this busy modern medicine. Physicians should also look into patient's demography which would have clinched the diagnosis straight away in this case.

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## P430

**Autoimmune polyendocrine syndrome type 4**

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Autoimmune Polyendocrine Syndromes (APS) are heterogenous organ-specific autoimmune disorders affecting multiple endocrine glands, although non-endocrine organs can also be affected. We describe a 58 year old gentleman with a background of type 1 diabetes mellitus (T1DM), Addison's disease and primary autoimmune hypothyroidism, who presented with a two day history of vomiting. He had no associated diarrhoea, abdominal pain, or fever and there was no history of recent travel, contact with unwell persons or consumption of unusual foods. He subsequently developed a sore throat and hoarse voice and was investigated with a gastroscopy, which revealed extensive candida infection of the vocal cords and oesophagus. APS are categorized into four types, based on the combination of endocrine glands that are affected. APS type 1, characterised by hypoparathyroidism, mucocutaneous candidiasis and Addison's disease, is frequently seen in childhood. For a more common APS type 2 to be diagnosed, Addison's disease together with autoimmune thyroiditis and/or together with diabetes mellitus type 1 must be present. The third type of autoimmune polyendocrine syndrome APS type 3 involves the same disorder of endocrine glands as type 2 but usually without any defect of adrenal cortex. If the autoimmune polyendocrinopathies do not fulfil the criteria of APS 1 to 3, the disease may be categorized as autoimmune polyendocrine syndrome type 4. Although our patient had features most consistent with autoimmune polyendocrine syndrome type 2, he presented with mucocutanous candidiasis as well. This combination leads us to a diagnosis of APS type 4. This case highlights the importance of being vigilant about new components of a syndrome developing at different times though a patient's life.

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## P431

**A severe case of ectopic ACTH presenting with Cushing's syndrome with hypokalaemia, hypomagnesemia, hypophosphatemia, hyponatraemia and hypocalcaemia**

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We present a case of 79 year old lady who presented to hospital with symptoms of polyuria and polydipsia requiring admission for level 3 care. She reported no bowel symptoms or alcohol usage. Her past medical history showed that she suffered from T2DM, Epilepsy, Asthma, Hypertension and Chronic Kidney disease. Her biochemistry revealed a combination of Hyperglycaemia, Hypokalaemia, Hypomagnesemia, Hypophosphatemia, Hyponatremia and Hypocalcaemia. Her medications revealed no causes of above biochemical abnormality (i-e loop and thiazide diuretics, aminoglycosides, ciclosporin, cisplatin) but her ACE inhibitor was stopped. On exmaination there were no systemic features of Cushingoid habitus. Her blood gas revealed metabolic alkalosis and she was treated with Potassium replacement and Spironolactone with limited improvement in intensive care.

Further advised was sought from the Endocrinology team and her cortisol/ACTH levels were elevated (i-e 3160 (5–23 μg/dl) and 386 (10 and 60 pg/ml)). It was treated with oral spironolactone. Her Urine revealed a high urine osmolarity with a 24 h urine Na of 326 mmol/l and urine K 268 mmol/l. Low Dose Dexamethasoen Suppression test (LDDST) showed lack of suppression of cortisol (Table 1)

She was initiated on Metyrapone on basis of abnormal DST. Her CT Chest, Abdomen and Pelvis revealed no apparent source of ectopic ACTH but bilaterally enlarged adrenal glands. Unfortunately further imaging and histology was not possible because of multiple epsiodes of sepsis including a HDU admission.

Conclusion

Hypokalemia, Hypomagnesemia, Hypophosphatemia, Hyponatremia and Hypocalcemia as first presentation of Ectopic ACTH production is rare. Other common causes like alcoholism, Cisplatin therapy, Diuretic use etc have been defined in Literature. This case highlights that this rare diagnosis should be considered in similar clinical presentation and early treatment should take priority over complete work up in severe case of Ectopic ACTH presenting with Cushing's syndrome. A management plan with high dosage Spironolactone, Metyrapone and Ketaconazole might be advisable as part of the decision making process. There is a need for future studies to improve the evidence base in the similar area.

| | Cortisol (μg/dl) | ACTH (pg/ml) |
|---|---|---|
| Baseline | 3730 | 313 |
| 48 h | 3157 | 194 |

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# Thyroid

## P432

**Peripheral blood microRNA markers in patients with papillary thyroid cancer**

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Monitoring patients with thyroid cancer for recurrent disease relies heavily on measuring serum thyroglobulin (Tg). Tg cannot be assessed reliably in the presence of anti-thyroglobulin antibodies for analytical reasons, this being the case in about a third of patients. There has been recent interest in microRNA profiling of fine needle aspiration biopsies of thyroid nodules, with encouraging results. The objective of this pilot was to explore microRNAs in peripheral blood of patients with papillary thyroid cancer (PTC). Sera from ninety patients with PTC were studied. They were divided into three equal groups based on clinical, radiological, biochemical (serum Tg) and cytological/histological assessments: i) recurrent/metastatic thyroid cancer, ii) athyreotic with no evidence of recurrent thyroid cancer, iii) known thyroid remnant, no evidence of recurrent thyroid cancer. Real-time PCR panel analysis of microRNAs was performed. Each RNA sample was reverse transcribed (RT) into cDNA and run on the miRCURY LNATM Universal RT miRNA PCR Human panel I and II. Eighteen microRNAs of potential interest emerged ($P<0.05$). hsa-miR-484 was associated with the highest statistical significance ($P<0.001$) for discriminating between patients with metastatic/recurrent PTC and the other two groups. hsa-miR-484 has not been previously described as a marker of thyroid cancer, although it is implicated in breast, ovarian, pancreatic cancer and malignant melanoma. Serum levels of three other microRNAs previously reported to be differentially expressed in thyroid cancer tissue (miR-122-5p, miR-885-5p, miR-17-5p), were also found to be associated with recurrent/metastatic thyroid cancer. This pilot has identified potential microRNAs in peripheral blood of patients with PTC that could potentially be used as markers for recurrent/metastatic disease. Further larger studies are required to confirm these findings.

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## P433

**Effect of hypothyroidism on pancreatic β-cell mass and circulating insulin concentration in the ovine foetus**

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Thyroid hormones are important regulators of fetal growth, although their mechanism of action remains unclear. This study investigated the effect of hypothyroidism on pancreatic β-cell development in foetal sheep.

All procedures were carried out under the UK Animals (Scientific Procedures) Act 1986. Under general anaesthesia between 105 and 110 days of gestation (days; term ~145 days), one twin fetus was thyroidectomised (TX), while the other was sham-operated as a control ($n=19$ ewes). After maternal euthanasia, umbilical arterial blood was taken from both foetuses at either 129 or 143 days ($n=38$). After foetal euthanasia, foetuses were weighed, measured and tissues collected. The whole fetal pancreas was weighed, fixed in 4% paraformaldehyde and embedded in paraffin wax. Pancreatic β-cells were identified immunohistochemically with an insulin antibody and their mass was calculated using the Cavalieri estimator (Visiopharm, Denmark). Plasma insulin, triiodothyronine ($T_3$) and thyroxine ($T_4$) concentrations were measured by ELISA or RIA. Data (mean ± S.E.M.) were assessed by two-way ANOVA followed by Tukey's *post hoc* test.

In TX foetuses, plasma $T_3$ and $T_4$ concentrations were at the lower limit of assay detection ($T_3$, 6.7 pg/ml; $T_4$, 7.6 ng/ml) at both ages. There were no differences in absolute body or pancreas weight between TX and sham foetuses at either age. At 143 days, limb lengths, lungs, heart, stomach and small intestine of TX foetuses were growth retarded, while the kidneys and perirenal adipose tissue were significantly enlarged, compared with sham foetuses ($P<0.05$).

Plasma insulin levels were significantly higher in TX foetuses at both 129 day (sham: 92 ± 60 ng/l; TX: 129 ± 33 ng/l; $P<0.05$) and 143 days (sham: 56 ± 10 ng/l; TX: 168 ± 24 ng/l; $P<0.05$). Relative β-cell mass to body weight was significantly greater in TX foetuses at both 129 days (sham: 47 ± 4 mg/kg; TX: 79 ± 6 mg/kg; $P<0.05$) and 143 days (sham: 46 ± 7 mg/kg; TX: 75 ± 10 mg/kg; $P<0.05$). The results indicate that the thyroid hormone have an important role in the growth and development of foetal pancreatic islets.

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## P434

**Thyroid hormones and mitochondrial development in skeletal muscle of foetal sheep near term**

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Thyroid hormones increase foetal and adult metabolic rates, and, in adult tissues, increase mitochondrial biogenesis. Foetal tri-iodothyronine ($T_3$) concentrations rise towards term in preparation for the increased postnatal energy demands but whether they affect mitochondrial development remains unknown. This study examined mitochondrial development in skeletal muscle of thyroid hormone deficient sheep foetuses near term.

At 105–110 days (d) of gestation (term ~d145), 11 twin-bearing pregnant ewes were anaesthetised for foetal thyroidectomy (TX) and sham operation of the pairs.

Foetal plasma and muscle samples were collected at d129 ($n=6$) or d143 ($n=5$) after maternal and foetal euthanasia. Citrate synthase (CS) activity was measured spectrophotometrically as an index of mitochondrial density, whilst abundance of electron transport chain (ETC) complexes I-IV and ATP-synthase was determined by western blotting to assess mitochondrial function, in foetal biceps femoris muscle. Plasma $T_3$ was measured by radioimmunoassay. Data were analysed by two-way ANOVA with Tukey's *post-hoc* test.
At both ages, muscle CS activity was less in TX than control foetuses (Table). Foetal TX prevented the normal gestational increase in CS activity (Table). Combining all data, muscle CS activity correlated positively with foetal plasma $T_3$ ($r=0.749$, $n=22$, $P<0.0001$). Relative to control muscle, complexes I and IV abundance was lower at d129, while ATP-synthase and all ETC complexes, except complex II, were less at d143 in TX muscle. However, when normalised to CS activity, muscle protein abundance of ATP-synthase was higher in TX than controls, at both ages (Table 1).

| | d129 | | d143 | |
|---|---|---|---|---|
| | Control | TX | Control | TX |
| CS activity μmol/min per mg protein | 0.169 ± 0.016 | 0.069 ± 0.007* | 0.439 ± 0.036† | 0.108 ± 0.008*† |
| ATP-synthase/ CS activity | 7.25 ± 0.66 | 13.68 ± 1.35* | 3.95 ± 0.46 | 9.19 ± 0.53*† |

Data are mean ± S.E.M. *$P<0.05$, TX vs controls at same age or †$P<0.05$ d143 vs d129 with same treatment.

Therefore, thyroid hormones are essential for the normal developmental increase in mitochondrial density and oxidative capacity in foetal skeletal muscle towards term.

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## P435

**The natural history of subclinical hyperthyroidism due to Graves' disease**

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Background
There is little information regarding the natural history of subclinical hyperthyroidism (SH) due to Graves' disease (GD). The objectives of this study were to assess the natural course of individuals with SH due to GD over a prolonged period. We also investigated the factors contributing to the progression or remission in these patients.
Methods
A prospective analysis of patients with SH due to GD between 2007 and 2013 who had at least 12 months of follow-up was performed. SH was diagnosed if serum TSH was below the laboratory reference range (0.4 – 4.0 mU/l) and when free thyroid hormones were normal. GD was confirmed by either a raised TSH receptor antibody level (TRAb) or uniform uptake on Technetium scan. Data regarding demographics, clinical and biochemical parameters were also collected.
Results
Forty-four patients (89% female, 16% current smokers and 5% with active Graves' orbitopathy) were diagnosed with SH due to GD. Over the follow-up period (mean 30 months), approximately one third (34%) of the cohort progressed to overt hyperthyroidism, one third (34%) normalised their thyroid function, slightly less than one third (30%) remained in the SH state while one person became hypothyroid. Multivariate regression analysis showed that older age and positive anti-TPO antibody status had a positive association with risk of progression to overt hyperthyroidism with Hazard Ratios of 1.05 (95% CI 1.01–1.08, $P<0.01$) and 10.15 (1.83–56.23, $P<0.01$), independent of other risk factors including current smoking, TRAb levels at diagnosis or gender.
Conclusions
A third each of patients with SH due to GD progress, normalise or remain in the SH state. Older people and those with positive anti-TPO antibodies have a higher risk of progression of the disease. This seminal data needs to be verified and confirmed in larger cohorts and over longer period of follow-up.

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## P436

**Epitopes, specificity, functional effects, and IgG subclasses of anti-calcium-sensing receptor autoantibodies in patients with autoimmune polyendocrine syndrome type 1**

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Context
Autoimmune polyendocrine syndrome type 1 (APS1) is characterised by multiple autoimmune endocrinopathies and results from mutations in the autoimmune regulator (*AIRE*) gene. Approximately 80% of patients present with hypoparathyroidism which is suggested to result from autoimmune responses against the parathyroid glands. The calcium-sensing receptor (CaSR), which plays a pivotal role in maintaining calcium homeostasis by sensing blood calcium levels and regulating release of parathyroid hormone, has been identified as a parathyroid autoantibody target in APS1.
Objective
The aim of the study was to characterise APS1 patient anti-CaSR autoantibodies in relation to their epitopes, specificity, IgG subclass, and effects upon CaSR function.
Methods
Phage-display; ELISA; and bioassays.
Results
Anti-CaSR autoantibody binding sites (epitopes) were identified between amino acids 41–69, 114–126, 171–195, and 260–340 in the extracellular domain of the receptor. Absorption experiments confirmed the specificity of anti-CaSR autoantibodies in recognising their respective epitope. Anti-CaSR autoantibodies were analysed for their ability to increase both $Ca^{2+}$-dependent ERK phosphorylation and inositol phosphate accumulation in HEK293 cells expressing the CaSR. The results indicated that two APS1 patients had anti-CaSR-activating autoantibodies, suggesting that although the majority of APS1 patients do not have anti-CaSR-stimulating autoantibodies, there may be a small minority of patients in whom the hypoparathyroid state is the result of functional suppression of the parathyroid glands. Autoantibodies against CaSR epitopes 41–69, 171–195, and 260–340 were exclusively of the IgG1 subclass. Autoantibody responses against CaSR epitope 114–126 were predominantly of the IgG1 with a minority of the IgG3 subclass.
Conclusion
The study provides a detailed analysis of the characteristics of anti-CaSR autoantibodies in APS1 patients, although further investigations are required to determine the exact role played by the autoimmune response against the CaSR in the pathogenesis of APS1-associated hypoparathyroidism.

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## P437

**Reliability of thyroid ultrasounds in assessment of thyroid nodules**

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Aim
The BTA guidelines published recently suggested an uniform U1–U5 classification of thyroid nodules on ultrasound (US) and organising management and follow-up based on these grades. The aim of our retrospective analysis was to assess the reliability and usefulness of US in assessment of thyroid nodules, in comparison with post-operative histological diagnosis.
Methods
All patients who underwent thyroid surgery for thyroid nodules were assessed, with a review of pre-operative US over the last 2 years.The post-operative histology results were verified and confirmed by histopathologist. US images were reviewed and graded by radiologists, after being blinded for histology and previous radiological reports.
Results
Five hundred and ninty one cases were analysed. Based on the availability of the actual US images to report on grading of nodules, 88 patients were included in the study. 27/88 had malignancy proven on post-op histology. 45 patients were graded U2 and 43 were graded U3 or above.
Of the U2 nodules ($n=45$): based on histology: 36 were benign on histology (FNA was Thy1 or Thy2 in all these nodules); further four were follicular adenoma; five were malignant (two were incidental micropapillary carcinomas and three were overt malignant nodules) – FNA was Thy1 or Thy2 in all these nodules.

Of the U3-U5 nodules ($n=43$): based on histology: 18 were benign (ten were U3 and the rest U4); 10/18 had FNA being Thy1 or Thy2 as well; three were follicular adenomas; 22 were malignant nodules (five had Thy1 or Thy2 on FNA). The sensitivity and specificity of U3–U5 to diagnose malignancy was 82% and 66%; the same for U3–U5+ FNA being Thy3 or above was 93 and 80%.
Conclusions
i) A small but significant proportion (11%) of patients with U2 may still have malignancy and hence clinical correlation is required, ii) 42% of patients with U3–U5 could actually have a benign nodule, iii) inter-rater variability could be high among US reports and adequate expertise should be mandatory, and iv) concurrent use of FNA and US could improve sensitivity and specificity further.

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## P438

**Subclinical and overt hypothyroidism in pregnancy: what happens next?**

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Recent guidelines indicate that pregnant women with subclinical hypothyroidism (SCH, TSH above the pregnancy-related reference range where available, or $\geqq 2.5$ mU/l) should be treated with thyroxine. We aimed to determine the frequency of overt hypothyroidism (OH; TSH $>5$ mU/l) and SCH (TSH $>2.5$ but $<5$ mU/l) in pregnancy, and to examine how many are diagnosed with thyroid problems after pregnancy. Four thousand three hundred women were recruited between 2007 and 2010. Plasma samples were obtained at gestational weeks 12–14. Delivery information was obtained from hospital databases. Thyroid stimulating hormone (TSH) and free thyroxine (fT4) levels were analysed in 2014 using clinically validated platforms (Roche). In women with TSH $>2.5$ mmol/l hospital records were used to identify more recent thyroid problems. Eighty-six women were known to have pre-existing thyroid disease and were not included in the analysis. Fifty-five women (1.3%) were identified as having OH. Of these one was diagnosed during pregnancy, 12 have been diagnosed since pregnancy, and one diagnosed with Grave's disease. Another three women have had elevated TSH but have no formal diagnosis, five have had TFTs in the normal range, and 33 have had no TFTs checked. 396 women (9.4%) were identified as having SCH. Of these none were diagnosed during pregnancy, 14 have since been diagnosed with hypothyroidism, two have been diagnosed with Grave's disease, four developed post-partum thyroiditis. 21 women have had elevated TSH but have no formal diagnosis. 179 have since had TFTs within the normal range. In this unselected obstetric cohort 9% of pregnant women have SCH, and according to recent guidelines these women should be treated with thyroxine. Of them, only 5% have subsequently been diagnosed with thyroid disorders, compared to 25% of women with OH. Screening of TFTs in all pregnant women, and associated treatment of SCH would have major implications for costing and design of obstetric services.

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## P439

**Soy protein with isoflavones impairs thyroid function**

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In patients with subclinical hypothyroidism, soy protein and isoflavone combination has been shown to increase the risk of developing overt hypothyroidism; however, it is unclear if soy affects thyroid function in those without existing thyroid compromise.
Materials and methods
Two double blind randomised trials were undertaken in which thyroid function was performed routinely at the beginning and end as a secondary end point. In both trials soy protein 30g with and without 66mg of soy isoflavones were used (preparation 1); soy protein alone that was isoflavone free (less than 300 parts per billion following serial ethanol washing)(preparation 2) was used for a period of 12 weeks. Study 1: 200 women within two years of the onset of the menopause to determine the safety of soy on markers of bone turnover as the primary end point. Study 2: 200 men with T2DM with a total testosterone level $\leq 12$ nmol/l with normal gonadotrophins with the primary end point of testosterone change. Here the secondary thyroid endpoints are presented.
Results
All patients had normal thyroid function at baseline in both the studies. In post menopausal women there was a significant increase in TSH ($1.58\pm0.93$ vs $2.57\pm1.19$ mU/l; $P$ value $<0.01$) and reduction of free thyroxine ($1.08\pm0.17$ vs $0.92\pm0.17$ ng/dl; $P$ value 0.02) after preparation 1 after 3 months compared to baseline. In men with type 2 diabetes there was a significant increase in TSH ($1.82\pm0.10$ vs $3.28\pm0.11$ mU/l; $P$ value $<0.01$) and reduction in $fT_4$ ($0.98\pm0.02$ vs $0.86\pm0.02$ ng/dl; $P$ value $<0.01$) with preparation 1 but not after preparation 2 after 3 months compared to baseline. None of the patients developed subclinical or overt hypothyroidism during this period. There were no changes in $fT_3$ after either preparation 1 or preparation 2. There was no significant change in TSH or thyroxine after supplementation of preparation 2.
Conclusions
There was a significant increase in TSH and reduction in free $T_4$ only after soy protein with isoflavones suggesting a direct effect on the thyroid that is unlikely to be clinically significant unless thyroid compromise is already present. We have previously shown that isoflavone alone has no effect on thyroid function suggesting that the isoflavones in the soy protein matrix is active component.

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## P440

**Increased rehospitalisation rate and cardiovascular morbidity in inpatients with hyperthyroidism – a matched case–control study**

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Background
Hyperthyroidism often runs an indolent course and may be undiagnosed for prolonged periods. Most patients are treated in the outpatient setting and the effects of hyperthyroidism on hospitalised patients are poorly studied. We set out to determine the prevalence of hyperthyroidism in a large cohort of inpatients and evaluated their comorbidities, reasons for hospitalisation and rehospitalisation rates.
Methods
A case-control study was conducted using a computerised system of admissions ($n=279\,162$) to a large tertiary centre between 2007 and 2011. Hyperthyroid patients were identified based on ICD-10 coding and/or administration of thionamides and matched by age, gender and year of the first admission with the general hospital population.
Results
Six hundred and seventy three subjects (0.5% of total hospitalised cohort) were hyperthyroid, which was newly diagnosed in 92. Hyperthyroid inpatients were more frequently re-admitted (mean 3.07 admissions during study period in cases vs 2.22 in controls; $P<0.001$) and their total (25.4 vs 15.7 days during 5-year period; $P<0.001$) and mean length of hospital stays (8.7 vs 7.0 days per stay; $P=0.003$) were significantly longer. Hyperthyroid patients were more frequently admitted with cardiovascular disorders (CVD) compared with controls (37.4% vs 26%; $P<0.001$) while proportions of admissions for respiratory, nervous and digestive causes were not different. Similarly CVD were more frequently recorded in hyperthyroid subjects (67.0% vs 57.1% in controls; $P=0.02$). Hypertension (37.6%), atrial fibrillation (29.0%), ischaemic heart disease (18.6%) and heart failure (12.6%) were the most common CVD comorbidities in hyperthyroid patients; AF and heart failure were recorded twice as often in the study subjects compared with controls (OR: 2.09 and 1.85; $P<0.001$).
Conclusions
We demonstrate that inpatients with hyperthyroidism are at significantly increased risk of re-hospitalisation and morbidity from cardiovascular diseases. The identification of these patients and institution of appropriate management and follow-up plans is likely to alleviate this significant health and financial burden.

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## P441

**Small fibre dysfunction in hypothyroidism – a prospective study using methods of small fibre function and structure**

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Introduction
Hypothyroidism (HypoT)-related polyneuropathy is reported to range between 42 to 72%. However, there is paucity of information regarding the prevalence and clinical course of small fibre neuropathy in HypoT. This prospective study examines small fibre function (SFF) – using the Laser doppler imager flare ($LDI_{FLARE}$) technique – and structure (SFS) using *in-vitro* corneal confocal microscopy (IVCCM) in a cohort of HypoT subjects before and after Levothyroxine($LT_4$) treatment and compares the outcomes with a cohort of age-matched healthy controls (HC).
Material and methods
Twenty patients with HypoT (TSH $\geqq$35 μ/l) – 15 primary and 5 post-radioiodine – along with 20 HC were assessed at baseline for SFF and SFS whilst large fibre neuropathy was assessed by determining sural nerve conduction velocity (SNCV) and amplitude (SNAP). After optimal replacement of HypoT patients with $LT_4$ (target TSH 1.5–4 μ/l) for at least 6 months, all subjects including HC's were re-evaluated to determine change in both small and large fibre modalities.
Results
At baseline, compared to HC, both $LDI_{FLARE}$ ($\pm$SD) (6.74$\pm$1.20 vs 8.90$\pm$1.75 $cm^2$; $P=0.0002$) and IVCCM (50.77$\pm$6.54 vs 58.32$\pm$6.54 no/$mm^2$; $P=0.002$) were significantly reduced in HypoT. Neither SNCV nor SNAP were different compared with HC ($P=0.10$ and $P=0.05$ respectively). Following $LT_4$ treatment, both $LDI_{FLARE}$ (7.72$\pm$1.12vs.6.74$\pm$1.20 $cm^2$; $P=<0.0001$) and IVCCM (54.43$\pm$5.70 vs 50.77$\pm$6.54 no/$mm^2$; $P=0.02$) improved significantly whilst both SNCV and SNAP showed no significant change. Interestingly, both SFF and SFS despite improving in HypoT remained significantly reduced compared to HC ($P=0.008$ and $P=0.01$ respectively) at the end of the study period.
Conclusions
Our study shows that compared to HC, both SFF and SFS are significantly reduced in untreated HypoT. Following optimal $LT_4$ replacement, both modalities improved significantly but compared to HC remained low. The latter perhaps suggests that using TSH as the sole biomarker to monitor replacement strategies may not be adequate to improve small fibre dysfunction. Further studies are required to explore this hypothesis.

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## P442

**A cross-sectional survey to determine iodine status of school girls living in Northern Ireland**

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Iodine deficiency is the most common cause of preventable mental impairment worldwide. Recent evidence suggests the re-emergence of mild iodine deficiency in the UK. A recent multicentre survey in the UK reported that 68% of school girls were iodine deficient with the lowest levels seen in Northern Ireland (NI). Unlike many countries, the UK does not have a salt or food iodination program. World Health Organisation defines deficiency as follows: mild 50–99 μg/l; moderate 20–49 μg/l; severe $<$20 μg/l. A cross-sectional survey of 264 schoolgirls, aged 14–15 years of age, was carried out in Belfast and Derry/Londonderry as the initial part of an Island of Ireland wide survey (seven centres). These are the two largest cities in NI and both located on the coast. Participants were surveyed in spring and winter months to look for seasonal differences. Urinary iodine levels were measured from morning spot urine samples using a standardised Sandell-Kolthoff colorimetry method. Median urinary iodine level was 119.1 μg/l (IQR 78.3–166.3). Ninety participants had mild deficiency (34%) while 14 had moderate deficiency (5.3%) and none surveyed had severe deficiency. There was no significant difference in urinary iodine level between spring and winter seasons and no difference between the two cities. In this survey 39% of schoolgirls were iodine deficient. These results are in keeping with the previous UK data and completion of the study in the other five geographical areas will enable a clearer understanding of the extent of this public health issue. Changing farming techniques and dietary habits may account for this re-emergence.

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## P443

**Thyroid abnormalities during anti-PD1 cancer immunotherapy**

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Recent Phase 3 studies have demonstrated the clinical utility of immunotherapy with inhibitors of PD1 in cancers, including melanoma and non-small cell lung cancer. Autoimmune side effects are common. Both hypothyroidism and hyperthyroidism have been observed in up to 16% of patients. We report for the first time the endocrine evaluation of patients with anti-PD1 induced thyroid dysfunction.
Methods
All patients treated in published trials of anti-PD1 therapies for malignant melanoma in our institution were identified. Thyroid function at baseline and during treatment was recorded. Data about investigation and treatment of thyroid abnormalities was obtained from the electronic patient record.
Results
Sixty-four patients with melanoma were treated with nivolumab or pembrolizumab, either alone or in combination trials. one patient was excluded due to pre-existing hypopituitarism. five patients developed overt hypothyroidism during treatment requiring thyroxine replacement. Two had a symptomatic thyrotoxic phase preceding the hypothyroidism. Both of these patients had technetium uptake scans with markedly reduced uptake in keeping with a thyroiditis and one had thyroid antibodies tested which were positive. One required beta blockers but neither received anti-thyroid drugs. A further six patients developed a TSH of $<$0.3 mU/l but did not go on to develop hypothyroidism. Only one of these had an elevated $T_4$. Six patients had elevated TSH less than 2$\times$ ULN not requiring thyroxine. Mean time to first abnormal TSH was 38 days.
Conclusions
These results confirm the significant rate of thyroid abnormalities in patients treated with anti-PD1 agents and show that a thyrotoxic phase can occur before hypothyroidism develops. Uptake scans confirm this to be a thyroiditis; presumed autoimmune in origin. Sub-clinical hyperthyroidism is also common and can resolve without subsequent hypothyroidism. These results will inform endocrinologists managing the side effects of these important new cancer therapies.

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## P444

**The effect of isoflavone-free soy protein supplementation on thyroid status and cardiovascular risk markers in patients with subclinical hypothyroidism: a randomised double blind crossover study**

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Context
Soy phytoestrogens are suggested to have an adverse effect on thyroid function but the contribution to this by soy protein alone when free from isoflavones is unknown.
Objective
The primary aim was to determine the effect of isoflavone free soy protein supplementation on thyroid function, with a secondary aim of assessing the effects on cardiovascular risk indices in patients with subclinical hypothyroidism.
Design and setting
This was a randomised, double-blind, crossover study in a tertiary care setting.
Participants
Eighty patients with subclinical (compensated) hypothyroidism participated in the study.
Intervention
Patients were randomly assigned to either isolated soy (isoflavone free) protein (SP) or casein protein (CP) supplementation for 8 weeks, washed out for 8 weeks and then crossed over for a further 8 week period.
Main outcome measures
The primary outcome was a change in serum free thyroxine, with secondary outcome measures of progression to overt hypothyroidism, blood pressure, insulin resistance, fasting lipids, and high sensitivity C-reactive protein (hsCRP).

Results
Mean percentage change from baseline showed a significant decrease in free thyroxine (−18.1% vs +4.3%, $P<0.01$) with SP, but no patient developed overt hypothyroidism. There were significant decreases in fasting glucose (−22.7% vs +2.3%, $P<0.01$), insulin resistance (−3.4% vs +25.0%, $P=0.02$), total cholesterol (−13.7% vs +2.5%, $P<0.01$), triglycerides (−64.6% vs +14.1%, $P<0.01$) and hsCRP (−71.5% vs +23.6%, $P<0.01$) in the SP group compared to the CP group. Blood pressure, LDL and HDL remained unchanged in both groups.
Conclusion
Isoflavone free soy protein decreased serum free thyroxine in these patients with subclinical hypothyroidism though the decrease was likely not clinically significant. However, it did reduce the cardiovascular risk indices of fasting glucose, insulin resistance, total cholesterol, triglycerides and hsCRP compared to casein protein.

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## P445

**A cross sectional survey of dietary iodine intake in pregnant women living in Northern Ireland**

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Adequate iodine intake during pregnancy is required for the production of thyroid hormones and brain development in the foetus. Recent evidence has suggested re-emergence of mild iodine deficiency in the UK but there are few studies that have specifically looked at iodine intake in pregnant women. Current World Health Organisation recommendation is for 250 mcg/day intake of iodine in pregnancy and the following are good sources of dietary iodine: 1 pint milk (~140–220 mcg); one egg (~20 mcg) and 100 g white fish (~115 mcg). A cross-sectional survey was carried out to assess iodine intake amongst pregnant women (n=241) living in Northern Ireland (NI). Iodine intake was estimated using an iodine specific food frequency questionnaire (FFQ) during each trimester. Twelve women also completed a 4-day food diary during the first trimester. Sixty six per cent of women in the first trimester consumed (≤0.5 pint (280 ml) milk per day although milk consumption increased by the third trimester ($P<0.05$). Egg consumption did not change significantly through pregnancy (15% none; 22% 1 egg/week). White fish intake was low with 111/218 (51%) eating fish never or ≤1/month. Only 4/218 (1.7%) consumed white fish >1/week. In the first trimester 146/215 (68%) women were taking an iodine containing supplement. Mean daily iodine intake from the 4 day food diaries was 108 μg (SD 42 μg). The results suggest that pregnant women living in Northern Ireland have low intake of foods known to be rich sources of iodine. Diet alone does not appear to be adequate to reach the recommended daily intake. Only 68% of women took an iodine containing supplement during the early stages of pregnancy. The UK has no food iodination programme and so public health messaging along with early ante-natal education is key to improving dietary iodine intake.

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## P446

**Is one benign thyroid FNA cytology sufficient to out rule malignancy? A university teaching hospital experience**

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Introduction
Fine needle aspiration cytology (FNAC) is a valuable and cost-effective pre-operative investigation for thyroid nodules. The British thyroid association (BTA) previously recommended two non-neoplastic results 3–6 months apart in aspirates to exclude neoplasia[1]. However, in recently updated BTA guidelines, repeat aspirates were recommended when there are clinical or ultrasound suspicions[2].
Aim
The aim of our study was to investigate whether one multidisciplinary benign FNA cytology can sufficiently out rule malignancy in all cases.
Methods
The cytological diagnoses of all thyroid FNA biopsies performed during the 5 years period (2008–2012) were retrieved retrospectively from the pathology laboratory information system.
Results
Five hundred and sixty seven thyroid FNAs were performed on 433 patients between 2008 and 2012. 424 (74.8%) showed benign cytology (Thy2). 41% of these cases ($n=123$) had a repeat FNA in 3–6 month according to previous BTA guidelines. Of initial Thy2 cytology, 108 (87.8%) remained Thy2 on follow-up aspirates, 3 (2.4%) showed Thy3 features, and 10 (8.1%) were non diagnostic (Thy1). 40 cases with initial Thy2 cytology underwent thyroidectomy for either an intermediate (Thy3) result on follow-up aspirate or due to compressive symptoms. Benign thyroid disease was confirmed in 34 (85%) cases and in the remainder six cases (15%) the histology revealed follicular adenoma. No malignant cases were found on final histology.
Conclusion
One multidisciplinary benign FNA aspirate is sufficient to out rule malignancy in most cases of Thy2 cytology. A repeat FNA should only be performed if there are remaining clinical and/or radiological concerns.
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## P447

**The value of second fine needle aspiration cytology tests when investigating benign thyroid nodules (Thy2/Thy2c)**

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Although the vast majority of thyroid nodules are benign, evaluation by ultrasound-guided fine needle aspiration (FNA) biopsy is usually necessary to exclude underlying malignancy. Partially in recognition of reported variability in FNA sensitivity and specificity, the 2007 British Thyroid Association (BTA) guidelines recommended two benign results 3–6 months apart to exclude neoplasia. The 2014 BTA guidance states that a second FNA is not required for nodules with benign Thy2 cytology and ultrasound characteristics unless there is strong clinical evidence to suggest malignancy. We report a retrospective analysis of patients from two large teaching hospitals who had repeat ultrasound-guided FNA biopsy between 2009 and 2014 following an initial report of Thy2/Thy2c. Radiology and cytology findings for first, second and any subsequent investigations were reviewed. Of the 162 patients with a first FNA of Thy2/2c, 25 repeat biopsies were non-diagnostic (Thy1/1c), and 130 patients again had benign Thy2/2c cytology, although 14 of these repeat biopsies were taken from the opposite side; one of these patients was later diagnosed with an incidental follicular variant of papillary thyroid cancer, another with Hurthle cell adenoma. Seven of the 162 patients with a first FNA of Thy2/2c were found to have Thy3 cytology or higher (3 Thy3a, 2 Thy3, 1 Thy4 and 1 Thy5) on repeat biopsy and progressed to thyroid surgery, where four were diagnosed with thyroid carcinoma. There was no strong clinical evidence to suggest malignancy in any of these patients, and initial ultrasound characteristics were also unremarkable. These results suggest that a second ultrasound-guided FNA biopsy may be necessary to avoid incorrect exclusion of neoplasia in patients initially found to have benign cytology, unless it is proven beyond doubt that by doing so we do not alter clinical outcomes in these generally indolent tumours.

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## P448

**Management of amiodarone induced thyrotoxicosis within the United Kingdom: is it time for a consensus guideline? A single centre retrospective review**

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Introduction
Amiodarone induced thyrotoxicosis (AIT) remains a diagnostic and therapeutic challenge. Broadly, AIT is classified as type 1 (underlying latent thyroid disorder) or type 2 (destructive thyroiditis). Despite being an on-going clinical conundrum,

there is no U.K. wide guidance on management of AIT. We report a retrospective review of recent cases treated within our department as AIT.
Methods
Data was collected for all patients referred to our tertiary endocrine unit with suspected AIT during 2010 to 2014. The review primarily focussed on duration of Amiodarone therapy, type of AIT, thyroid peroxidase antibody status and time to remission of AIT. A total of thirty nine patients were referred to our unit with AIT, within this period. Out of these, six have been excluded so far, due to incomplete data.
Results
A total of 33 patients were included in the review. The average age of patients referred with AIT was 66 years (range 30–81years), predominantly being men (79%). The average duration of Amiodarone therapy prior to diagnosis of AIT was 45 months (range 8–132 months). TPO status was assessed in 82% of patients out of whom 11% were TPO positive. Majority of patients were classed as type 2 AIT (64%) by the treating clinician. Following diagnosis, Amiodarone was discontinued amongst 85% of the patients. In terms of treatment, 30 patients were commenced on Carbimazole (91%) out of which 47% were rendered euthyroid with carbimazole only. Out of 53% that received Carbimazole and Prednisolone therapy, only two were referred for thyroidectomy. Three patients (9%) remitted during 'watch and wait' period. One patient required elective radioiodine in addition to medical treatment. The average time to remission was 2.9 months (range 3 weeks to 6 months).Remission period of AIT was similar in those who discontinued versus who continued Amiodarone therapy (2.8 vs 3 months).
Conclusion
In the absence of clear guidelines for classification and management of both types of AIT, overall practise remains primarily determined by individual clinician's experience. This has been highlighted in surveys undertaken by both the European Thyroid association and within the United Kingdom. Our review further highlights differences in practise amongst endocrinologists in management of AIT. This is particularly true in relation to diagnostic workup as well as management strategies.

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## P449

**Serum thyroid stimulating hormone concentration after withdrawal of thionamides as a predictor of Graves' disease outcome**

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Introduction
Despite high rates of recurrence after anti-thyroid drug (ATD) withdrawal, ATD (mainly Carbmazole) remain the first line of treatment for Graves' disease (GD) in the UK. Limited retrospective observational studies have shown that a low TSH post ATD withdrawal had a positive predictive value of 70% and a negative predictive value of 62% (specificity 85%) for relapse of Graves' disease.
Aim
We wished to determine the relationship between TSH concentration post ATD withdrawal and GD outcome, in this relatively iodine insufficient population of South Wales.
Methods and results
We recruited 100 consecutive subjects with GD, treated with ATD alone or in combination with thyroxine. 27 subjects were excluded for valid reasons (immediate recurrence after ATD withdrawal, lost to follow up, definitive therapy given as primary treatment). Of the 73 subjects analysed 59 were women; 14 men; age range 20–90 years). The median duration of treatment was 13 months. 56 (76%) subjects had ATD alone but 17 (24%) had a block and replacement regime. Following withdrawal of ATD, 43 (59) remained in remission (Rem) but 30 (41%) relapsed (Rel). Thyroid function was tested at a median time of 10 weeks (range 4–24) following withdrawal of treatment. Median TSH concentrations in the Rem and Rel groups were 0.92 vs 0.77 mIU/l respectively ($P=0.1031$) (range 0.01–3.47 (Rem) and 0.04–4.38 mU/l (Rel).
Conclusions
In this group of subjects from a relatively iodine insufficient area of South Wales, serum TSH concentration at a median time 10 weeks after treatment withdrawal, did not predict outcome of GD. This was not consistent with the results of other studies from geographically disparate areas.

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## P450

**Maternal thyroid function in pregnancy and risk of breech presentation**

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Introduction
A breech presentation occurs in 3–5% of all full-term pregnancies and is associated with increased risk of maternal and foetal morbidity. Factors known to increase the risk of breech presentation include prematurity, low birth weight and multiple pregnancies. Recent studies suggest maternal thyroid hormone deficiency in late pregnancy may also be a risk factor. Our study aims to assess if a breech presentation at 36 weeks gestation in a healthy singleton cohort is associated with maternal thyroid hormone deficiency or thyroid autoimmunity.
Methods
Serum blood samples obtained during routine clinical care from 285 women, with no known thyroid disease and a singleton breech or cephalic pregnancy at 36–38 weeks gestation, were analysed for thyroid function tests (TSH and FT4) and TPO antibodies by the local NHS pathology department. Prevalence of subclinical hypothyroidism (defined as serum TSH above 2.5 mIU/l), maternal hypothyroxinaemia (defined by FT4 below the trimester specific reference range and TSH 2.5 mIU/l or less) and thyroid autoimmunity (defined as titre of TPO antibodies $>34$ IU/l) was determined in women with breech and cephalic presentations for comparison.
Results
When comparing breech ($n=147$) with cephalic ($n=138$) presentation there was no difference in maternal age (mean (s.d.)- 30.6 (5.1) vs 31.4 (5.30) years, $P=0.3$), BMI at booking (median(IQR)-23.8 (27–34) vs 25 (28–35) kg/m$^2$, $P=0.09$) or rates (%) of: primiparous pregnancies (43.2 vs 38.7, $P=0.45$), smoking (13.5 vs 6.6, $P=0.06$),or multivitamin use (40.8 vs 39.9). There was no difference in the prevalence of subclinical hypothyroidism (27.2 vs 26.8%, $P=0.94$), maternal hypothyroxinaemia (5.4 vs 2.9%, $P=0.29$) or positive TPO antibodies (6.2 vs 5.1%, $P=0.69$)
Conclusion
A breech presentation at 36 weeks gestation in a healthy singleton cohort is not associated with known markers of maternal hypothyroidism or thyroid autoimmunity.

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## P451

**Ultrasound guided thyroid FNA with cytopathology in-attendance significantly improved accuracy in a university teaching hospital**

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Introduction
Ultrasound (US) is an extremely sensitive examination for thyroid nodules. Fine needle aspiration (FNA) cytology samples taken with ultrasound guidance, increases accuracy and reduces rates of unsatisfactory samples[1]. In addition, the immediate assessment of the sample for adequacy by onsite cytopathology is shown to reduce inadequacy rates by most reports[2].
Aim
The aim of this study was to compare the inadequacy (Thy1) rates between US guided FNA with onsite histopathology and palpation guided (Free-hand) FNAs.
Methods
Retrospective review of all FNAs performed during five years period between 2008 and 2012.Data were collected from the pathology department laboratory and the radiology computerised systems.
Results
Five hundred and sixty seven thyroid FNAs were performed on 433 patients during this period. The cytological diagnoses were as follows: Thy1 (non-diagnostic) in 63 (11.1%) cases, Thy2 (benign) in 424 (74.8%) cases, Thy3 (follicular lesion/neoplasm) in 54 (9.5%) cases, Thy4 (suspicious for malignancy) in 9 (1.6%) and Thy5 (malignant) in 17 (3%) cases. Out of 531 cases were the guidance method was documented, 423 (79.7%) were US guided with onsite cytopathology evaluation and 108 (20.3%) were free-hand. Inadequacy rate (Thy1) was significantly lower in the US guided FNA (37/423, 8.7%) compared to the free-hand aspirates (17/108, 15.7%), ($P<0.05$).

Conclusion
FNAs done under US guidance combined with onsite cytopathology have significantly lower in-adequacy rates compared to free-hand FNA.This method should be used in all cases to improve adequacy and reduce patient distress and cost.
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## P452

**Diagnostic and therapeutic challenges in medullary thyroid carcinoma**
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Medullary thyroid carcinoma (MTC) is an aggressive form of malignancy, virtually incurable except by complete surgical resection. With insidious onset and occasional rare clinical variants, it is often diagnosed late in the course of disease and has a poor prognosis. We aimed to evaluate diagnostic efficiency, treatment modalities and outcome in patients admitted to our department between 2004 and 2015, diagnosed with MTC, with emphasis on rare clinical presentations. We identified 28 patients (12 men, 16 women), aged 49.7±16.1 years: seven with MEN2A syndrome, two with familial MTC and 19 with sporadic MTC. Most patients presented for evaluation of a nodular goiter (57%) or for screening if from a MEN2A kindred (14.28%) but the rest showed signs of extensive disease: dysphagia, dysphonia, spinal compression by bone metastases, chronic diarrhea, ectopic Cushing syndrome or a catecholamine crisis in a MEN2A patient. Diagnostic workup confirmed extensive disease: 19 patients (86.3%) were TNM stages III or IV, and calcitonin was >400 pg/ml in 13 patients (20 of 28 with available data – the remaining were misdiagnosed preoperatively, leading to inadequate initial surgery and the need for repeat interventions in 46.4%). Precise calcitonin measurement is important in diagnosis and follow-up; however, two cases with metastatic disease presented with normal calcitonin levels, suggesting dedifferentiation. Multiple surgeries were followed in persistent disease by adjuvant therapies: chemotherapy, radiotherapy, somatostatin analogues and IFN. In two patients beyond surgical resources, wide metastatic spread required oncologic evaluation. Management of MEN2A patients was complicated by the need for additional parathyroid and adrenal surgery (thyroid surgery could not be performed in a patient with inoperable bilateral infiltrative pheochromocytomas), by patients' reluctance to conform to life-long rigorous follow-up and to undergo thyroid surgery if asymptomatic. At the end of follow-up (79.6±117.4 months) only 37% (ten of 27 patients with available data) had calcitonin <15 pg/ml suggesting cure. Calcitonin testing for selected thyroid nodules might improve diagnostic accuracy and success of surgery.
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## P453

**Less is more: superior efficacy and tolerability of 400 MBq radioactive iodine for management of thyrotoxicosis**
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Background
Radioactive iodine (RAI) therapy is well-established in the management of benign thyroid disorders associated with thyrotoxicosis, but the optimal dose remains controversial. Guidelines recommend 500–800 MBq, though it is thought that Graves' thyrotoxicosis may respond more readily to lower doses (400–600 MBq).
Methods
We studied all local cases receiving RAI over four calendar years (2010–2013; $n=447$). Demographic and clinical data were compiled from case records. Cohort and subgroup analyses of cases receiving an initial dose of ~400 MBq ($n=373$) or ~600 MBq ($n=74$) RAI were conducted using SPSS v21.
Results
Our patient cohort was female-dominated (78.2%) and largely Caucasian (74.4%). The vast majority (89.0%) had received thionamide treatment prior to RAI. We observed 20 cases (4.5%) of RAI-associated acute ophthalmopathy, one case (0.2%) of stroke and no fatalities over the first three months following RAI. The overall cure rate was 91.3%, with 63.5% of cases manifesting permanent iatrogenic hypothyroidism. Among patients receiving an initial dose of ~400 MBq RAI (range 197–420 MBq, mean 398 MBq) the single-dose cure rate was 79.1% and incidence of permanent hypothyroidism was 52.8%. Among patients receiving an initial dose of ~600 MBq RAI (range 550–651 MBq, mean 614 MBq) the single-dose cure rate was 71.7% ($P<0.001$) and incidence of permanent hypothyroidism was 59.5% ($P<0.001$). Graves' disease was equally represented (53.0% vs 55.0%) among patients who were cured after one dose of RAI. Conversely, it was over-represented (71.8%; $P<0.001$) in the group of patients requiring multiple RAI administrations ($n=68$). Overall cure rate in this latter group was 88.2%, with 63.2% of cases developing permanent hypothyroidism.
Conclusions
A RAI dose at the lower end of current recommendations for thyrotoxicosis is more efficacious and better tolerated than higher doses, regardless of underlying pathology. Definitive control of autoimmune thyrotoxicosis is more likely to require multiple RAI administrations.
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## P454

**Incidental thyroid malignancy in Grave's thyrotoxicosis**
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Introduction
Correlation of papillary thyroid cancer and lymphocytic thyroiditis is well documented but the incidence of thyroid malignancies in Graves' disease especially in the absence of nodular thyroid disease is considered to be uncommon.
Method
We conducted a retrospective audit of forty-four patients with established diagnosis of Grave's thyrotoxicosis treated with total thyroidectomy from 2010-2013 (36 months), in a tertiary care centre. We studied the incidence rate of thyroid malignancy. In the study group 66.2% were females, with a mean age 35 compared to 33.85% males with mean age of 40. All patients had TSH< 0.01 mU/l on presentation, and six patients (13%) had associated thyroid eye disease.
Results
All patients had histological confirmation of Graves's thyrotoxicosis. Eight out of 44 (18%) patients (Male $n=3$; females $n=5$) were found to have incidental thyroid malignancy, all of which were confirmed papillary carcinoma with no extrathyroidal or lymph node invasion. patients (37.5%) had known nodular disease before surgery where diagnostic cytology was Thy 2, Thy5 and Thy3a respectively.
Conclusion
Incidental thyroid malignancy in patients known to have Grave's thyrotoxicosis is now increasingly being reported[1], our results support that. Most of them are low-risk papillary thyroid microcarcinoma without lymph node metastasis, lymphovascular and extrathyroidal invasion[1]. The role of autoimmune phenomena in the origin and clinical course of coexisting carcinomas is still controversial.[2]

| | Number | % |
|---|---|---|
| Total number of patients | 44 | |
| Incidence of malignancy | 8 | 18 |
| Malignancy in nodular disease | 3 | 37.5 |
| Malignancy in non nodular disease | 5 | 62.5 |

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## P455

**Management of thyroid cancer: a 5-year retrospective audit**

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Aim
The British Thyroid Association published guidelines on the management of differentiated thyroid cancer and medullary thyroid cancer in 2007. The aim of our audit was to assess compliance with these guidelines.
Methods
Electronic records of patients operated on and followed up in our hospital from January 2009 to December 2013 were reviewed retrospectively.
Results
Forty-nine patients fulfilling these criteria were identified. 61% patients presented with a solitary nodule and 26% had diffuse neck swelling. Only 16% patients had suspicious features on ultrasound. Fine needle aspiration and cytology (FNAC) was performed in 44 out of the 49 cases with 61% being ultrasound-guided. 43% of those who underwent FNAC were diagnosed with Thy3, 7% with Thy4 and 20% with Thy5. Seven out of the ten patients with Thy1 had a repeat FNAC. 90% of cases were discussed at the multi-disciplinary meeting. The mean duration from cytological diagnosis to surgery was 11 weeks; Thy3 and Thy4 cases were operated in less than 8 weeks, apart from one case, which was after 12 weeks. The majority of patients underwent staged total thyroidectomy. Eight patients developed post-operative hypoparathyroidism and seven had nerve palsies. Histological diagnosis was papillary thyroid carcinoma in 76%. Four patients had medullary thyroid cancer and the remainder follicular. Post-operatively 82% of patients in whom radioiodine was indicated received it and 78% of eligible patients had TSH suppression. 60% of patients did not have a baseline thyroglobulin and only 63% had a follow-up thyroglobulin as part of surveillance.
Conclusions
Patients with thyroid cancer were managed in conformity with the guidelines. However, we need to ensure that baseline thyroglobulin and annual follow-up thyroglobulin are checked in all cases of papillary and follicular thyroid carcinoma. We should also aim for improvement in TSH suppression following surgery.

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## P456

**Thyroid eye disease audit – the Wolverhampton experience**

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Thyroid eye disease (TED) affects some 400 000 people in the United Kingdom. Apart from being problematic and cosmetically distressing to patients it can be occasionally sight threatening. The joint TED clinic was first established at the New Cross Hospital in 2010. At this monthly clinic patients are assessed by both an Endocrinology and Ophthalmology consultant. Full orthoptic evaluation is done and the management plan is facilitated by a patient self-assessment questionnaire. An agreed proforma is used to capture all details. Referral criteria include active TED, patients requiring surgical input and pre-radioiodine assessment. 42 consecutive TED clinic attendees were audited over a 1 year period, of which 12 were new to the service. The average number of visits was between 2 and 3. 67% were referred for assessment of active TED. The most common symptom identified was double vision (34%). 69% of attendees were female and the average age of patients was 52. The major ethnic group represented was white British (50%). 17% were current smokers and 93% were hyperthyroid at first presentation. While the majority of patients were managed conservatively, eight patients received steroid therapy, one had botox treatment, one had lid surgery, one was listed for orbital decompression and three were referred for orbital radiotherapy. nine patients received treatment with selenium and eight patients were prescribed prisms. The audit concluded that the Wolverhampton TED service is a useful service for patients with significant eye disease. Such complex patients require early dual specialist input. Patients with active disease showed good response to steroids. There is need for close monitoring because of the risk of relapse and/or inadequate response. The vast majority need supportive measures.

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## P457

**R438H missense mutation related generalised resistance to thyroid hormone (GRTH): a case series report in three generations**

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A family with generalised resistance to thyroid hormone secondary to a missense point mutation in thyroid hormone receptor β (TR-β) gene corresponding to substitution of arginine to histidine at amino acid 438 (R438H) is described in three successive generations.
Case 1
(Index case) A 43 year old lady presented with thyrotoxicosis and was initially treated with carbimazole followed by radio-iodine ablation of the thyroid. Thyroxine was commenced 8 weeks after treatment. Despite thyroxine replacement TSH remained inappropriately raised. The patient was further investigated to exclude an underlying TSH secreting pituitary adenoma or GRTH. CT scan of pituitary revealed gland enlargement suggesting an underlying TSHoma. Pituitary hormone profile was otherwise normal. Further investigations however revealed normal α subunit with preserved TSH response to TRH stimulation suggesting the diagnosis of GRTH confirmed on genetic analysis to be secondary to point mutation R438H in TR-β. Supra-physiological doses of thyroxine led to resolution of pituitary enlargement after 22 months. Family thyroid function test screening detected transmission of GRTH in three successive generations.
Case 2
Daughter of index case also had inappropriately high TSH with high $FT_4$ levels. She didn't require treatment. She was informed of the possibility of transmission of the same mutation to her children and the possibility of temporary hypothyroidism in new born if she became pregnant.
Case 3
A 2 year old child (grand-daughter of the index case) was also shown to have inappropriately raised TSH and $FT_4$. The child is under follow up with the paediatric endocrinologist but has no evidence of stunted growth or mental retardation. This case series report illustrates the importance of family screening to prevent inappropriate therapy following detection of abnormal TFT.

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## P458

**Imaging in subclinical hyperthyroidism: findings from a single centre cohort**

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Background
Subclinical hyperthyroidism provides a challenging condition in clinical endocrinology. The debate about who and when to treat has long been discussed. Despite guidelines describing assessment of such patients, there is surprising little data on imaging in this condition and the role of nuclear medicine or ultrasound scans.
Aim
To review all patients with subclinical hyperthyroidism referred for imaging studies at a district general hospital to ascertain any trends or abnormalities in clinical practice.
Method
All patients attending Royal Wolverhampton Hospital between 2007 and 2013 who had a nuclear medicine thyroid uptake scan or ultrasound of the thyroid and with a diagnosis of subclinical hyperthyroidism were retrospectively analysed. Patient's blood tests were reviewed prior to the scan ensuring biochemically they remained subclinical.
Results
Forty patients were identified, mean age 67.3 years (range 30–91 years). 27 patients had nuclear medicine scans of which 63% (17 patients) had evidence of increased uptake in a focal area. 23 patients had an ultrasound of the thyroid with 15 showing multinodular goitre and five showing a solitary nodule. ten patients

(25%) had both scans with six patients having correlation and indicating functionality. 29 (72.5%) patients had TPO antibodies assessed of which only three were elevated. ten patients (25%) had an undetectable TSH, mean $T_4$ levels were 15.9 pmol/l (10.6–21), mean $T_3$ levels were 4.6 pmol/l (3.2–5.9).
Conclusion
The findings provide an understanding of the characteristics of patients referred for imaging. Nuclear medicine scans provided more clinically beneficial results than ultrasound imaging, though paired imaging was best at guiding treatment. Nuclear medicine scans are important in deciding whether to treat patients with biochemical evidence of subclinical hyperthyroidism and should be part of routine assessment.

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## P459

**Qualitative analysis of ultrasound reports assessing radiological descriptors of thyroid nodules – a retrospective pilot audit**

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Introduction
Thyroid Ultrasound (US) is the recommended first line investigation of suspected thyroid nodules. Specific radiological features, such as micro-calcification, low echogenicity, solid consistency, heterogeneity and ill-defined margins, raise the possibility of underlying malignancy. These findings together with fine needle aspiration cytology guide the management of thyroid nodules. The aim of this audit was to evaluate the quality and adequacy of thyroid US reports with emphasis on the reporting of these pre-defined descriptors.
Methodology
A retrospective audit of thyroid US reports was undertaken at The James Cook University Hospital (JCUH) and University Hospital of North Tees (UHNT) between March 2012 and May 2013. All patients with a solitary thyroid nodule or a dominant nodule within a multi-nodular goitre on US were included. We audited the documentation of these predefined descriptors in each report.
Results
All of the 60 patient reports eligible were included. Documentation of micro-calcification and margins were the only 2 parameters present in $>60\%$ of reports across both sites. The documentation of the other parameters was variable and disparate between sites. Documentation of cervical lymph nodes and geneity was present in $>80\%$ of reports in JCUH but $\leq 50\%$ in UNHT. The halo sign was the least documented parameter across both sites ($<10\%$). Risk stratification was documented in 80% of JCUH reports and 43% of UHNT reports.
Conclusions
A significant proportion of thyroid US reports did not include descriptors of accepted radiological parameters that are essential in guiding further management of thyroid nodules. Reporting of both positive and negative findings was variable between the two sites and was operator dependent. Developing a standardised reporting proforma for thyroid nodules identified at US will help improve both the quality and consistency of reporting of solitary and dominant thyroid nodules.

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## P460

**An audit of hyperthyroidism in pregnancy**

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Overt hyperthyroidism occurs in about 0.1–0.4% of all pregnancies. Propylthiouracil (PTU) is recommended in first trimester due to risk of teratogenicity with Carbimazole (CBZ). CBZ is preferable in rest of the pregnancy and postpartum period due to risk of serious liver disease with PTU. We reviewed our management of hyperthyroidism in pregnancy from 2009 to 2014. Total number of pregnancies $=34$, mean maternal age 32.6 years. Except one all (33/34) were diagnosed pre- pregnancy. One diagnosed in pregnancy is likely gestational thyrotoxicosis in a twin pregnancy. Pre-pregnancy therapy was with Carbimazole (14), PTU (14), radioactive Iodine therapy (4) and surgery (1). PTU was used in first trimester in 70% (19) and 30% (8) were on CBZ. PTU was switched to Carbimazole in only 18% (3/17) in second trimester. 62% were able to come off antithyroid drug during pregnancy and this could explain low rate of conversion back to CBZ. A third of these patients relapsed in the postpartum period. TSHrAb was measured in 67% pregnancies. Growth scans were performed in 29/34 and all were normal. Mean birth weight was 3.1Kg (range 2.27–4.2 kg). There were no cases of neonatal thyrotoxicosis. Three cases of minor birth defects noted, one epispadias, one penile rotation, one skin tag below left nipple and they were exposed to PTU in first trimester. one baby had mild positional talipes. During follow up two babies were diagnosed with developmental delay, received PTU and another baby with bilateral sensorineural hearing loss was exposed to CBZ in first trimester. There are lots limitations in this small retrospective audit and in future we plan to collect data in prospective manner. We need to improve on switching more patients from PTU to CBZ in second trimester and documenting TSHrAb status as missing results could have been measured outside our hospital.

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## P461

**Thyroxine administration: a challenging case**

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Several preparations of thyroxine are available nowadays but is a great challenge for every clinician when the oral and intramuscular administration is failing and has to consider long term intravenous administration. This is a 42 years old lady who had a total thyroidectomy for Graves thyrotoxicosis. She commenced on multiple oral preparations of thyroxine and liothyroxine with no biochemical response. This is not a case of pseudomalabsorption as oral absorption studies of high dose of Thyroxine have shown a flat plasma $T_4$ level. Further gastroenterological investigations failed to reveal any cause of malabsorption. Alternative option was the intramuscular thyroxine. She required twice weekly intramuscular thyroxine to maintain her euthyroid status but in two occasions she has been admitted with severe hypothyroidism complicated by bradycardia and polymorphic ventricular tachycardia. The cardiological complications, the distressing symptoms and the irregular/erratic absorption made this preparation not suitable, as well. Marked increased sensitivity to the effect of catecholamines has been identified as evidenced by the effects of isoprenaline during the Electrophysiology study. The last option in order to achieve effective and controlled administration was the intravenous thyroxine. She is currently on intravenous Levothyroxine in order to maintain a TSH between 10 and 20 mU/l. A subclavian port-a-cath system is currently required which unfortunately in several times has been infected. Being profoundly hypothyroid for some time has made her sympathetic system very sensitive to changes in the level of serum $T_3$. The above TSH range is important in order to prevent significant bradycardia and ventricular tachycardia but also to maintain a reasonable satisfactory thyroid status. This is a rare case of hypothyroidism requiring long term intravenous thyroxine with endocrinological and cardiological interest. There is no similar case previously reported in the literature.

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## P462

**Thyroid deficiency refractory to treatment: is this a case for DOT?**

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Directly Observed Therapy (DOT) is the World Health Organization standard used for tuberculosis treatment, where a trained health worker watches the patient swallow every dose. It can be used for patients receiving doses of Levothyroxine of more than 2 μg/kg with persistently increased TSH levels as they are considered to have thyroid deficiency refractory to treatment. Poor adherence is the most common cause of failure of therapy and if this is suspected, a supervised test may be helpful. We present four patients with hypothyroidism on doses of levothyroxine which varied from 200 mcg to 650 mcg and TSH between 19.4 and 39. They all underwent an observed administration of 1000 μg of levothyroxine with blood tests at baseline and at intervals up to 240 min on day one. Therapy was then continued weekly for four weeks with measurement of TSH and $T_4$ levels. All patients had a peak two-fold increase from baseline of their $T_4$ at 240 min after administration of 1000 μg of levothyroxine. The subsequent results over the course of the 4week supervised dosing showed TSH stabilise within normal limits. The rapid rise in the $T_4$ level after taking the drug essentially ruled out malabsorption of the drug. This simple supervised dosing of levothyroxine can potentially be administered in primary care setting where most patients with hypothyroidism are managed, to evaluate the discrepancy between laboratory results of elevated TSH despite supraphysiological levothyroxine doses before referral for specialist review. If patients continue to show poor compliance then thyroxine administered once weekly under supervision can be offered. Directly Observed Therapy has the advantage of close monitoring that may improve adherence. On the other hand, it moves away from the adherence models of communication with cooperation between patient and provider which could make adherence worse if rigidly applied. Cost of drugs vs resources would need to be evaluated.

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## P463

**Recurring thyroid eye disease: a diagnostic dilemma**

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A 39 year-old man was referred with weight loss and lethargy in 2011. On examination tremor, goitre and mild orbital oedema were present. His past medical history consisted of hypertension (ongoing treatment). Investigations: fT4: 37 pmol/l (9–21), TSH: 0.00 mU/l (0.20–4.50), Anti Thyroid Peroxidase: 242.6 U/ml (0–50) and TRAbs: 11.2 iu/l (0–1.6). Graves' thyrotoxicosis with moderately active eye disease was diagnosed and he started on carbimazole and later thyroxine. Subsequently he developed vertical diplopia with discomfort. He had bilateral orbital swelling and right upward gaze and bilateral abduction were restricted. He eventually achieved a euthyroid state following total thyroidectomy. Despite initial response to intravenous methyl prednisolone his ocular symptoms worsened. MR Orbits showed enlargement of the extra-ocular muscles consistent with active thyroid disease. Orbital radiotherapy was contraindicated due to hypertension and young age and he started oral prednisolone. Rituximab was considered but not given because symptoms improved on prednisolone.
Several months later he developed right ptosis associated with progressive ocular abduction restriction, most troublesome when tired. He was biochemically euthyroid, with undetectable TRAbs and repeat orbital MR scan showed reduction in ocular muscle size. Antiacetylcholine receptor antibodies (AChRAbs) were negative and no other features of myasthenia gravis (MG) were present. In 2015 a trial of pyridostigmine was undertaken and several weeks later his abduction restriction had completely resolved. He was diagnosed with a combination of thyroid eye disease and ocular MG.
Conclusion
There is a known association between autoimmune thyroid disease and MG but their simultaneous occurrence is rare. The absence of AChRAbs does not exclude MG. Diplopia can occur in patients with Graves' disease but diplopia, ptosis and restrictive ocular movements are highly suggestive of MG. The co-existence of the two conditions can present diagnostic difficulty and this case highlights the need for multispecialty input when managing patients with complex thyroid eye disease.

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## P464

**Timing of food intake and L-thyroxine replacement – a cost saving simple change of phrasing in the British National Formula**

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Background
Despite relevant evidence that the absorption and/or action of L-thyroxine is compromised by various factors including the diet, until 2014 the British National Formula (BNF) recommended that L-thyroxine be taken 'preferably before breakfast' only, with no further clarifications. To follow is one of the cases where untimely food intake in hypothyroid patients manifested with severe hypothyroidism due to L-thyroxine malabsorption.
Case
A 79 years old Caribbean male patient with background history of autoimmune hypothyroidism was admitted with poor oral dietary intake and dehydration. TSH on admission was 0.73 mU/l, reflecting adequate replacement with 125 micrograms of L-thyroxine OD. Hospital stay was prolonged for various reasons. Within 2 weeks from admission the patient developed severe, symptomatic hypothyroidism ($fT_4 < 5$ pmol/l, TSH > 100 mU/l). Apparently, L-thyroxine during hospital stay was given at 0800 h, just before breakfast. A simple change in the timing of administration of L-thyroxine to 0700 h, > 30 min before breakfast and isolated from other tablets, rapidly improved both symptoms of hypothyroidism and thyroid function tests within days ($fT_4$: 12.4 and 21.1 pmol/l after 5 and 14 days, respectively), without need for an increase in the replacement dose.
Discussion
In response to the evidence provided, which also included a systematic review of the literature, the BNF has changed the phrasing regarding the correct intake of L-thyroxine to 'preferably 30 min before food, other tablets and caffeine-containing beverages' (implemented March 2014). This simple measure is likely to facilitate more reliable L-thyroxine absorption and reduces swings in thyroid function tests. With a hypothyroidism prevalence of at least 2% in the UK, reducing outpatient and GP appointments by 1 appointment/year only in every 5th patient results in estimated cost savings of £20 000 000 for the NHS.

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## P465

**The value of thyroxine absorption test followed by weekly thyroxine administration in determining the cause of persistent hypothyroidism despite high dose L-thyroxine treatment: a case report**

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Objective
Most hypothyroid patients require an optimal dose of 1.6–1.8 μg/body weight (kg) of L-thyroxine to restore a normal TSH. Poor response to treatment can be due to malabsorption, drugs interaction and poor compliance. We conducted a test to determine the likely cause of persistent hypothyroidism in a *coeliac* patient despite taking supraphysiological doses of L-thyroxine (> 1000 μg OD).
Case report
This is a 41 year old female who developed a post-RAI hypothyroidism, which was difficult to treat. Her TSH levels were persistently elevated (> 30 mU/l) despite taking once daily dose of 1000–1400 μg L-thyroxine and admission of good compliance. Liothyronine was added on but gave little value. Her other significant medical problems include coeliac disease, diarrhoea-predominant irritable bowel syndrome, previous subarachnoid haemorrhage and asthma.
Method
Stage 1: *Thyroxine absorption test*: Baseline TSH and free $T_4$ were checked (47.17 mU/l and 6.5 pmol/l). A 1000 μg L-thyroxine dose was administered. The $fT_4$ levels were repeated, and they rose to 8.9 pmol/l (60 min), 14.1 (120 min), 14.4 (180 min) and 16.8 (240 min), which suggested a degree of absorption.
Stage 2: *Observed weekly thyroxine administration*. Patient was given 1000 μg of L-thyroxine once/week for 4 weeks. A repeat TFT checked 4 weeks after this protocol demonstrated a huge improvement in her TFT, with TSH 1.65 mU/l and $fT_4$ 13 pmol/l. A repeat thyroxine absorption test at the end of this 4 weeks period confirmed a good degree of absorption.
Conclusion
A thyroxine absorption test followed by weekly thyroxine administration as described by Walker JN *et al*. (*Eur J Endocrinol* 2013; 168:913–7) can determine the underlying cause of poor response to thyroxine treatment. As in this case, we suspected poor compliance and we modified the doses from 1400 μg L-thyroxine OD to 1000 μg twice weekly with good response. Future raise in TSH levels would prompt an observed L-thyroxine administration to improve compliance.

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## P466

**Resistance to thyroid hormone in a family of Bangladeshi extraction**

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Resistance to thyroid hormone is a rare genetic disorder which is usually inherited in an autosomal dominant manner. In this disorder, tissues become resistant to thyroid hormones due to the mutations in thyroid hormone receptor beta gene resulting in compensatory rise in hormone levels. Patients are largely asymptomatic apart from goitre, though some can develop symptoms. Estimated rate of occurrence is in the region of 1 in 40 000 live births.

We describe a case of thyroid hormone resistance in South Asian family of Bangladeshi extraction. Resistance to thyroid hormone is very rarely described in this population. A 31 year old, clinically euthyroid, female with goitre was referred to our unit by ENT surgeon due to abnormal thyroid function test (TFT). Her TFT showed: TSH 1.35 miu/l (normal range 0.4–4 miu/l), raised free $T_4$ 29.2 pmol/l (normal range 12–22 pmol/l) and raised free $T_3$ 7.1 pmol/l (normal range 2.80–7 pmol/l). Other investigations including alpha subunit, Sex hormone binding globulin and anterior pituitary function were all normal, which goes against thyroid stimulating hormone related tumour. Her younger brother, sister and father had similar thyroid test results. As Resistance to thyroid hormone being most likely explanation, she was tested for it. This was confirmed by genetic tests after ruling out assay interference. She was found to be heterozygous for thyroid hormone receptor beta mutation due to single base change at c.1378G > A in exon 10 of THR beta gene resulting in abnormal THRbeta protein (p.Glu460Lys). Her other family members are being approached for offering them genetic testing for resistance to thyroid hormone.

Conclusion

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, TSHoma or thyroid hormone resistance syndrome.

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## P467

**More than meets the eye: a case of longstanding hypothyroidism due to Hashimoto's thyroiditis presenting with unilateral thyroid eye disease**

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Introduction

Unilateral proptosis may be due to thyroid eye disease (TED) or retro-orbital tumours. TED is an autoimmune process affecting orbital and periorbital tissue, and generally occurs in patients with Graves thyrotoxicosis. A small subset of patients with TED (5%) have normal thyroid function, but TED is rarer in hypothyroid patients.

Case report

We report the case of a 57-year-old lady who initially presented to ophthalmology with diplopia. She was diagnosed with hypothyroidism 10 years ago and was on levothyroxine 75 μg daily. She reported no symptoms of hypothyroidism or thyrotoxicosis. Eye examination showed a clinical activity score of 3+, mild proptosis of the right eye (2 mm difference in Hertel exophthalmometer measurements), restricted right eye movements, diplopia and lid retraction. Left eye examination was normal. There were no other clinical signs of thyrotoxicosis. Thyroid function was normal, with TSH 1.81 mU/l, $FT_4$ 20 pmol/l and $FT_3$ 5.2 pmol/l. Anti-TPO antibodies were strongly positive (> 1300 ku/l), but TBII was negative (< 1.0 U/l). Thyroid ultrasound was normal. An MRI of her brain and orbits showed marked enlargement and oedema of both inferior recti and right superior oblique muscle with sparing of tendinous insertions, compatible with a diagnosis of right TED. She remains clinically and biochemically euthyroid at 9 months of follow-up, the latest TSH being 1.95 mU/l and $FT_4$ 19 pmol/l.

Conclusion

TED, although common in Graves thyrotoxicosis, has also been recognised in euthyroid and hypothyroid states. Clinically evident unilateral TED as seen in our longstanding well controlled hypothyroid patient due to Hashimoto's is even rarer. This case supports the idea that opthalmopathy could be associated with any autoimmune thyroid disorder, irrespective of the nature of auto-antibodies present. We would also like to emphasise the importance of excluding retro-orbital tumours before diagnosing TED in patients presenting with unilateral eye disease.

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## P468

**TSH receptor antibodies testing in hypothyroid pregnant women**

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Hypothyroidism is a common endocrine condition and if untreated can have serious consequences in pregnant women. Generally, TSH receptor antibodies (TRAb) is performed in hyperthyroid pregnant women as high titres can cause foetal hyperthyroidism Neonatal thyrotoxicosis develops in 1% of infants born to thyrotoxic mothers due to placental transfer of TRAb.

As part of management of hypothyroid pregnant women, TRAb are checked routinely in our hospital in keeping with our trust guidelines in order to minimise the remote incidence of neonatal thyrotoxicosis. In literature there is no evidence to support this practice as routine guideline. Therefore a retrospective consecutive audit was carried out on diagnosed hypothyroid patients attending antenatal clinic in Queen Elizabeth Hospital, Kings lynn from 01/01/2013 to 31/12/2014. Two practices were looked at.

Results of TRAb performed in hypothyroid pregnant women. Any cases of neonatal thyrotoxicosis in patients with positive TRAb. Out of 74 patients audited, 56.75%patients had TRAb checked. 6.75% patients were positive for TRAb, 50% were negative and 43.24% did not have them checked. There were no cases of neonatal thyrotoxicosis seen in any patient included in this audit.

As a result of this audit we concluded that it is safe not to perform TRAB routinely in hypothyroid pregnant women. It is not only cost effective (one TRAb costs £39.16) but would reduce unnecessary work load and anxiety for patients.

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## P469

**Thyroid dysfunction secondary to antiviral therapy: a simple management protocol**

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Introduction

Interferon and Ribavarin-based Hepatitis C therapy can induce or exacerbate autoimmune thyroid dysfunction with variable clinical presentation. Being female, having prior thyroid dysfunction and raised anti-thyroid peroxidase (anti-TPO) antibodies are risk factors. We have a protocol for managing thyroid dysfunction that occurs during hepatitis C antiviral therapy. We present four cases.

Case 1

A 48 year old man diagnosed with hepatitis C infection was found to have mild hypothyroidism with raised anti-TPO antibody levels before starting antiviral therapy. After starting therapy (Pegylated Interferon alfa-2a and Ribavirin) in August 2011, he developed severe hypothyroidism seven months later. He remains on long-term thyroxine replacement.

Case 2

A 55 year old man diagnosed with hepatitis C infection had a normal thyroid function test (TFT) but raised anti-TPO antibody levels before antiviral treatment. In February 2012 he developed severe hypothyroidism five months after starting antiviral treatment. He remains on long-term thyroxine replacement.

Case 3

A 41 year old woman diagnosed with hepatitis C infection had a normal TFT and normal anti-thyroid peroxidase antibody levels before antiviral treatment. In July 2014 she developed thyrotoxicosis six months after starting antiviral treatment. She developed hypothyroidism two months after that and still has mild hypothyroidism.

Case 4

A 56 year old man diagnosed with hepatitis C infection had a normal TFT before antiviral treatment. In May 2012 he developed thyrotoxicosis four months after starting antiviral treatment. He then developed hypothyroidism which required thyroxine replacement. This resolved after six months and he is no longer on treatment.

Conclusion

Our protocol ensures that patients with hepatitis C infection have their thyroid hormones and anti-TPO antibody levels measured prior to starting antiviral therapy. Thyrotoxicosis is monitored and does not require anti-thyroid treatment. Hypothyroidism is treated if severe or there are symptoms. All patients are followed up after antiviral treatment.

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## P470

**Case report: propylthiouracil-induced ANCA-associated-vasculitis**

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We report a rare case of propylthiouracil (PTU) induced ANCA-associated-vasculitis (AAV). This young lady with recurrent thyrotoxicosis and positive Thyroid peroxidase and TSH receptor antibodies since 2004 was treated with Carbimazole but was changed to PTU 100 mg daily during pregnancy in 2012.
She was admitted to our hospital in January 2015 with sore throat, feeling unwell and neutropenia 1.2 (normal range (NR): 1.6–7.5) $10\times9$/l. Thyroid function (TFT) showed TSH $<0.02$ mU/l, $FT_4$ 16.8 pmol/l and $FT_3$ 9.3 pmol/l. PTU dose was reduced to 50 mg daily. She was readmitted in March 2015 with rash, lethargy and worsening neutropenia (0.95 $10\times9$/l). TFT showed TSH $<0.02$ mU/l and $FT_4$ 13.4 pmol/l. PTU was immediately stopped due to suspected vasculitis. Investigations revealed high Anti-Neutrophilic Cytoplasmic Antibody (ANCA) levels. Antibody to proteinase-3 (PR-3) was 102.2 IU/l (NR: 0–1.9) and myeloperoxidase (MPO) was 49.8 IU/l (NR: 0–3.4). Urine albumin creatinine ratio and Computerised tomogram of chest were reported as normal. She was commenced on prednisolone 1 mg/kg bodyweight. Neutrophils normalised (2.58) within five days of stopping PTU. Her rash improved significantly within 4 weeks and she felt much better. PR-3 and MPO improved rapidly to 55.7 and 47.4 respectively. TFT remain stable (TSH $<0.02$ mU/l, $FT_4$ 17.9 pmol/l and $FT_4$ 5.4 pmol/l). Skin biopsy did not reveal any vasculitis. Her response after withdrawal of PTU and steroid treatment is very typical of PTU induced AAV. She is being monitored regularly with TFT and ANCA levels whilst reducing prednisolone dose slowly. She is considering Radio Iodine treatment for thyrotoxicosis.
Conclusion
This case illustrates a relatively rare case of PTU induced AAV. In this particular case, vasculitis confined only to skin without any other organ involvement. PTU induced AAV usually has good prognosis when compared to primary AAV. Most patients respond well within a few weeks to months after stopping PTU and treatment with steroids.

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## P471

**Odd TFTs: when it does not fit, it probably is not right!**

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Case report
A 29-year old Caucasian male was referred to our Endocrinology Outpatient Clinic following a recent admission after an episode of collapse when he was noted to have abnormal thyroid function tests: $fT_4$ 32.6 (7.5–21.1) pmol/l, TSH 6.41 (0.34–5.6) mU/l. These were repeated and again showed elevated $fT_4$ 26.9 pmol/l and normal TSH 2.29 mU/l. He reported some tremor of both hands but denied palpitations. He was clinically euthyroid but periodically had hypothyroid symptoms with lack of energy. There were no thyroid eye disease and no history of neck pain or swelling. He was otherwise fit and well. His sister had hypothyroidism.
Repeat TFTs at another laboratory using different assay showed similar results, thereby excluding thyroid antibody assay interference. Thyroid peroxidase antibodies were slightly raised at 362 IU/ml whilst thyrotropin-binding inhibiting immunoglobulins were negative. TSH alpha subunit was normal and pituitary MRI scan did not show any pituitary abnormality, suggesting TSHoma unlikely. Further questioning revealed that his sister was diagnosed with hypothyroidism since childhood and had suffered short stature.
Subsequently, samples were sent for thyroid hormone receptor gene defect and he was noted to be heterozygous for an autosomal dominant THRB C.958C> Tp.(Arg320Cys) mutation. He required no treatment with regards to his thyroid status and was referred to the clinical genetics service for family screening.
Discussion
This was the first case of resistance to thyroid hormone (RTH) diagnosed in our service. The case highlights the differential diagnosis of raised free thyroid hormone levels together with non-suppressed TSH secretion, which can be the result of assay interference with heterophile antibodies, TSHoma or thyroid hormone resistance. This inherited syndrome is characterised by reduced tissue responsiveness to thyroid hormone. The clinical presentation is highly variable, ranging from isolated biochemical abnormalities to a spectrum of mixed hypo-hyperthyroid signs and symptoms, even among affected siblings carrying the same mutation. It should be appropriately investigated in order to make the correct diagnosis and avoid unnecessary treatment.

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## P472

**Primary radioactive iodine ablation for TSH secreting adenoma – an uncommon treatment for a rare disease**

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Introduction
Thyrotropin-secreting adenomas are a rare cause of hyperthyroidism. Preferred treatment is pituitary neurosurgery.
Case report
A 35 year old lady was first noted to have abnormal thyroid function when presenting with an AV-nodal re-entry tachycardia in 2011. She had tremors, sweating and irritability. $FT_4$ was 35 pmol/l (10-20 pmol/l) with a non-suppressed TSH level of 7.5 U/l (02–6 U/l). Previous meningococcal meninigitis age 13. No family history thyroid dysfunction. Further tests: negative screen for heterophilic antibodies, elevated SHBG at 198 nmol/l, alpha-1 subunit 3.85. The $\alpha$1SU/TSH ratio was 0.46 supporting the diagnosis of a TSHoma. MRI confirmed a 3mm anterior pituitary enhancing lesion. A TRH stimulation test showed a blunted TSH response with a normal insulin stress test. TSH, $T_4$ and $T_3$ failed to suppress with 100mcg of levothyroxine over 10 days. TRH genetic analysis was negative. She was then referred to the tertiary centre for neurosurgical consideration. She received 3 months of Octreotide LAR with little change in TSH or thyroxine levels. Pre and post octreotride dynamic imaging did not show any pituitary adenoma, therefore surgery was considered inappropriate and she had radioactive iodine for symptom control. Six months post $^{131}$I MRI pituitary showed a 2 mm adenoma but no lesion was evident after 18 months. Post $^{131}$I she became clinically hypothyroid; $FT_4$ 3.0 with TSH 32.1. Stabilising on thyroxine 100 µg she was symptomatically better with $FT_4$ 19.7 and TSH 13.7.
Conclusion
Radioactive iodine is not commonly used for TSHoma but was used in this case as there was failure of response to somatostatin analogues and the lack of identification of a definite pituitary adenoma on dynamic imaging.

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## P473

**An unusual cause of hypercalcaemia**

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Introduction
Hyperparathyroidism is the most common cause of hypercalcemia. We present another endocrine cause for hypercalcemia.
Case report
A 49 year old Afro Caribbean man, previously healthy, was admitted with abdominal pain. He had been complaining of a 2 week history of epigastric pain, loose stools, sweating and shortness of breath. On examination, the patient was unwell, afebrile with epigastric tenderness. Bloods results showed anaemia, Haemoglobin 110 g/l (133–180), neutropenia $1.13\times10^9$/l (2.0–7.5), alanine aminotransferase 73 IU/l (7–56), amylase 821 IU (25–12) and calcium 2.68 nmol/l (normal $<2.60$). He was treated for pancreatitis.
While inpatient, he developed recurrent episodes of chest pain and shortness of breath. Electrocardiogram and serial troponin's were normal. CT excluded pulmonary emboli, however showed an 'incidental' diffuse goitre and prominent pancreatic duct. His calcium increased to 3.2 nmol/l and an endocrine review was requested.
By this stage, patient has become markedly confused, agitated, tachypenoic, with a raised JVP, bilateral ankle oedema with profound proximal myopathy, His TSH was fully suppressed with Free $T_4>103$ pmol/l (10.6–21.0) and Free $T_3$ 38 pmol/l (3.2–5.9). Parathyroid hormone (PTH) was suppressed. Anti thyroid peroxidase antibodies were raised, suggesting Grave's disease. A diagnosis of impending thyrotoxic storm, precipitated by pancreatitis and contrast media (Burch-Wartovsky score of 30) was made.
The patient was started on hydrocortisone, propylthiouracil, cholestyramine, propranalol and potassium iodide. He improved remarkably over the next ten days (Free $T_4$ – 54.1 pmol/l, Free $T_3$ – 7 pmol/l). Serum calcium, neutropenia and liver function tests (LFT's), had normalised. He was switched to carbimazole while awaiting Radioactive Iodine.
Discussion
Hyperthyroidism can manifest in unusual ways such as chest pain, shortness of breath, altered mental status, as demonstrated in this case. Biochemical abnormalities may include anaemia, neutropaenia, hypercalcaemia and deranged LFTs. Our patient presented with hypercalcaemia and pancreatitis, precipitated by thyrotoxicosis. The treatment of his hyperthyroidism was challenging due to thyrotoxic storm, neutropenia and deranged LFT's.

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## P474

**Buying time: a unique case of delayed thyroidectomy post thyroid storm**

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Background
Thyroid storm is a rare but dangerous condition which can lead to multi-organ failure if not treated aggressively. Currently accepted treatment is with anti-thyroid medications to convert patients to a euthyroid state before definitive treatment with thyroidectomy is performed 10–14 days later. We present a case that was managed medically for several months due to a delay in surgery for clinical reasons. At present there are few such cases in the literature.
Case description
A 32-year old female presented to clinic with a 5 week history of tremor, palpitations, weight loss and sweating. She had no past medical history and was 2 weeks *post-partum*. Her pregnancy was uncomplicated apart from pre-eclampsia. On examination she was thyrotoxic and thyroid function tests (TFTs) showed TSH $<0.02$, $T_3>50$, $T_4>100$. She was diagnosed with Graves disease and started on carbimazole. A week later she presented with worsening symptoms and thyroid storm was diagnosed. ECG changes were consistent with an inferior myocardial infarction (troponin rise of 6527). A coronary angiogram showed right coronary artery dissection which was treated medically. An echocardiogram confirmed severe left ventricular diastolic function. She was admitted to intensive care for the treatment of thyroid storm with propylthiouracil, Lugols iodine and hydrocortisone. In contrast with current guidance a multi-disciplinary decision was made to delay thyroidectomy due to the myocardial insult. She was discharged and weekly TFTs over the next few months showed no disease progression. Her total thyroidectomy is planned for June 2015; 7 months post presentation, giving time for the myocardium to settle.
Conclusion
There is no data in the literature regarding the optimum time to perform a thyroidectomy in thyroid storm for patients with recent myocardial damage. This case highlights a unique situation where surgery was delayed and shows that thyroidectomy can be delayed safely in some high risk cases.

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## P475

**A disputable but effective therapy in neutropenia associated with Graves' disease**

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Various haematological abnormalities have been reported to be associated with treatment of hyperthyroidism. The Association between neutropenia and untreated hyperthyroidism has been described although the aetiology is unknown, but thought to be related to autoimmunity.
Case
A 41 year old Nepalese lady presented with a three month history of poor sleep and loss of weight. She was found to be thyrotocxic with $T_4$ of 64 mol/l (10–24), $T_3$ of 20 mol/l (4–7) and TSH $<0.03$ (1.5–5). On further assessments she admitted feeling hot, shaky, having a fast heartbeat and also proximal muscle weakness. Her clinical evaluation was consistent with Graves' disease without opthalmopathy. Laboratory investigation results showed elevated TSH receptor antibodies of 6.5 ($<0.4$) and thyroid uptake scan revealed homogeneous increased uptake confirming Graves' thyrotoxicosis. Her full blood count revealed neutropaenia (count of $0.7\times10^9$/l) with normal cell counts in other cell lines. Haematology advice was taken and initial investigations were arranged. It was decided to commence on Carbimazole with careful close monitoring of Full blood count. Improvement of neutrophil count noted as the thyroid disease was controlled ($T_4$ down to 11.6 mol/l and neutrophil count up to $3\times10^9$/l). All the haematological investigation results were unremarkable except an incidental finding of this patient being a thalassemia trait. Derangement of thyroid functions along with return of neutropenia noted with poor compliance to Carbimazole but improved when back on therapy.
Carbimazole and Propylthiouracil are the initial treatment of anti-thyroid drugs in adults presenting with Graves' disease, however rare adverse effects such as agranulocytosis (neutropaenia) secondary to bone marrow suppression caused by these drugs remains a concern. In this lady, untreated Graves' disease itself was thought to be the reason for neutropaenia and improvement noted once thyroid disorder became under controlled. Therefore anti-thyroid medications as first line therapy can be considered in these situations.

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## P476

**Late stage Hashimoto's or Riedel's? A case report illustrating this diagnostic conundrum**

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Riedel thyroiditis, an uncommon form of chronic thyroiditis in which the thyroid gland is replaced by fibrous tissue, is difficult to differentiate from the fibrous variant of Hashimoto thyroiditis or lymphoma. We report a patient illustrating this. A 54 year old lady with a long standing goitre and 3 year history of stable hypothyroidism was referred for progressive thyroid gland enlargement over several months, dysphagia and an increasing thyroxine requirement. Clinically, she had a diffusely hard but mobile, nodular goitre with retrosternal extension and positive Pemberton's sign. Laboratory investigations showed hypothyroidism (TSH 45.4 mIU/l, free $T_4$ 11.4 pmol/l), raised TPO titre ($>600$ IU/ml) and ESR 57 mm/h. Ultrasound thyroid showed a diffusedly enlarged thyroid, with replacement by markedly inhomogenous hypoechoic masses with lobulated contours, minimal increase in vascularity and few foci of calcifications. Some enlarged neck nodes with loss of fatty hilum. CT scan confirmed a diffusely enlarged heterogenous thyroid with tracheal narrowing (1.1 cm diameter) and level II, III nodes (1.2 cm or less). FNA thyroid was compatible with lymphocytic thyroiditis but with atypical lymphoid cells, also seen on the FNA neck node. A thyroid Tru-Cut biopsy excluded lymphoma but revealed lymphoid aggregates and lymphoplasmacytic infiltrate in a sclerotic background raising the possibility of Riedel's or late stage Hashimoto's thyroiditis. Systemic fibrosis was excluded. At the thyroid multidisciplinary team meeting, the case was discussed, decision made for conservative management of possible Riedel's thyroiditis. The patient was commenced on a tapering course of systemic steroids with optimisation of thyroxine replacement. Within weeks, she improved significantly clinically with normalisation of her thyroid function and ESR. A repeat CT scan showed reduction in size of the thyroid with unchanged appearances of her neck nodes. She remains on low dose steroids that we propose to taper subsequently.

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## P477

**Investigating pyrexia whilst awaiting thyroidectomy for Thy 3, Hurthle cell neoplasia**

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Thyroid abscess is a rare condition because of the thyroid gland anatomical and biochemical nature. We report a rare case of a rapidly developed thyroid abscess in the background of follicular thyroid nodule.
A 77 years old lady admitted for elective right thyroidectomy. She had a history of goiter with normal TFTs. FNA cytology revealed Thy3, Hurthle cell neoplasia. The pre-operation CT scan showed right thyroid large nodule with heterogonous enhancement throughout. She has a background of hypertension, diet controlled diabetes and no other immunosuppressant factors. During the pre-operation assessment the patient became acutely unwell, treated for *E coli* urosepsis and the operation has been delayed. Multiple blood cultures showed no growth. Despite the 7 days of appropriate intravenous treatment the patient remained clinically unwell and septic. Abdominal or pelvic abscess as source of sepsis excluded after a CT scan. A repeat CT neck thorax, 20 days after the first scan, revealed dramatic change of the right thyroid nodule, being highly suspicious for abscess. Percutaneous abscess drainage performed and growth of coliform confirmed after microbiological examination.
In this case, surprisingly there was a delay in development of clinical inflammatory evidence, possibly due to the position of the pre existing nodule and the treatment of the concurrent UTI. Also, the recent reassuring imaging was an additional pitfall in the early diagnosis of such a rare condition. It's vital, thyroid abscess to be highly considered as a source of sepsis especially in cases with predisposing factors (thyroid disease, previous FNA, concurrent infection) even in the absence of clinical evidence. We also, suggest urgent repeat thyroid imaging even if there is available recent imaging excluding inflammatory process.

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## P478

**The roller-coaster ride of thyroid function before, during and after pregnancy**

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Introduction
Hyperthyroidism is a common endocrine disorder in pregnancy, with potentially significant foeto-maternal consequences.
Description of the case
25 year old lady with auto-immune hyperthyroidism (TRAb > 400) was treated with Propranolol and Carbimazole and referred to the Endocrine clinic. She was started on Block and replace therapy. She was soon moved over to Carbimazole as she was contemplating pregnancy. She became hypothyroid in 3 months and anti-thyroid therapy was stopped. In another 2 months she required thyroxine replacement. 3 months later, her TSH started to drop and by 12 months, she was found to be hyperthyroid. 2 months later, she was found to be pregnant and was changed to PTU. During the rest of her pregnancy, her $FT_3$ remained high, $FT_4$ – normal with TSH undetectable, despite treatment with PTU 300 mg bd with regular counselling. She delivered a healthy but hyperthyroid baby ($FT_4$: 64, $FT_3$: 31.3, TSH < 0.001, TRAb > 400) of birthweight 3150 grams at 37 weeks + 1 day of gestation. Mum's TFTs 1 day postnatal were still in the hyperthyroid state, but when checked after 5 weeks, showed features of hypothyroidism with TSH 9.11. Her PTU was then reduced to 100 mg/day, but she became grossly hypothyroid in 2 months, needing Thyroxine replacement. Within further 2 months, her hyperthyroidism flared up again and is currently being managed by block and replace regimen. The infant is now being treated with Carbimazole and is euthyroid.
Discussion
This case brings forward the following points for discussion: The role of anti-TSH receptor antibodies in fluctuation of thyroid status. The relative contribution of placental glucuronidase system in degradation of PTU for the relative lack of its efficacy seen in this case during pregnancy, which returned after delivery. The close monitoring required in such patients during and after pregnancy.

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## P479

**Metastatic differentiated thyroid cancer with undetectable serum thyroglobulin: diagnostic, management and follow-up challenges**

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Background
Serum thyroglobulin (Tg) is a reliable tumor marker in patients with differentiated thyroid carcinoma (DTC). Distant metastases of DTC, particularly in the lung, normally presents with higher levels of Tg, which is useful to follow the disease evolution. We describe a patient with DTC with lung metastases, with undetectable Tg and Tg-antibodies (TgAb).
Clinical case
A 52-year-old woman underwent a subtotal thyroidectomy in 1993 because of a growing nodule. The histology was follicular thyroid carcinoma. Eight years later, multiple lung nodules were identified in a thorax-CT scan. A biopsy of one of these lesions was compatible with thyroid carcinoma metastases. The patient was referred to our hospital. The thyroidectomy was completed and seven radioiodine treatments were administered (total activity of 800 mCi). The tail-end-scan following each treatment showed uptake in the lung metastases, despite the undetectable stimulated-Tg throughout these treatments. During the 13-years follow-up period, the suppressed-Tg has been consistently undetectable and the measurement of TgAb was always negative (tested by different laboratorial methods), despite the progression in size and number (some with ~3 cm) of the known fluorodeoxyglucose (FDG-F18)-avid lung lesions. Currently, the patient is asymptomatic with a progressive metastatic DTC, without biochemical evidence of the disease – undetectable Tg and TgAb (assessed by different methods) as mentioned above.
Discussion
Serum Tg is an excellent tumor marker for DTC, and serial monitoring is valuable for the follow-up of this condition. DTC with systemic dissemination normally has measurable Tg. Undetectable serum Tg in recurrent/metastatic DTC may not necessarily predict neither radioiodine uptake nor adverse prognosis in these cases, and it imposes important diagnostic, management and follow-up challenges. In such uncommon cases, imaging methods (CT, MR and PET) should supplement Tg and TgAb measurements in order to permit a correct follow-up and management strategy.

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## P480

**Thionamide resistant Graves' disease – it's not always poor compliance**

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A 54 years old female with no significant past medical history was referred by her GP with thyrotoxic symptoms which were not improving on Carbimazole 40 mg daily over the last 3 months in spite of good compliance with the drug. Her initial $FT_4$ was 49.0 pmol/l with TSH suppressed to < 0.01 and she was started on Carbimazole 20 mg which a month later was increased to 40 mg/day when there was no improvement in her TFTs. Her TFTs at this point showed $FT_4$ 54.5 pmol/l, $FT_3$ 24.3 pmol/l and a suppressed TSH of < 0.01. She was also commenced on Propranolol 20 mg TDS. A further check later showed worsening of her TFTs at which point she was referred to the Endocrine clinic.
In the clinic review she had a full house of thyrotoxic symptoms with significant weight loss, tremors, palpitations and heat intolerance. She confirmed a good compliance with Carbimazole 40 mg daily along with Propranolol. Her compliance was further strengthened by the finding of Leucopoenia of 3.1 associated with neutropenia of 1.5 on her FBC most likely related to Carbimazole. She had mild diarrhoea but no evidence of any malabsorption syndrome. Her TRAb was strongly positive at 35.4 U/l. The dose of Carbimazole was increased to 60 mg day and propranolol 80 mg TDS but her symptoms and TFTs failed to improve and there was a risk of her going into a thyroid storm. Therefore she was treated with Lugol's iodine which made her Euthyroid before she had a successful total Thyroidectomy as a definitive treatment of her Grave's Disease. The histology of the thyroid specimen was also suggestive of autoimmune thyroid disease.
The commonest cause of treatment failure in Grave's Disease is poor adherence but this case highlights the rare possibility of Drug resistance which physicians need to be aware of. The exact cause of thionamides resistance is unknown but possible mechanisms include drug malabsorption, anti-drug antibodies, rapid drug metabolism, impairment of intrathyroidal drug accumulation or action and predominant elevation of $T_3$ rather than $T_4$ levels.

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## P481

**Recurrent Hashimoto's encephalopathy: a case report of reversible coma and status epilepticus**

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Background
Steroid-responsive encephalopathy associated with autoimmune thyroiditis (SREAT) also known as Hashimoto's encephalopathy (HE), is a rare immune-mediated complication independent of functional status of thyroid, which leads to either stroke-like symptoms, or presents as diffuse progressive symptoms of altered mental status, seizures, and cognitive dysfunction. Here we present a case of SREAT in a female with recurrent episodes.
Case history
A 54-year-old female was brought in by ambulance after a prolonged tonic-clonic generalised seizure, requiring intubation with a Glasgow coma scale (GCS) of 6/15. Her past medical history was significant for autoimmune hyperthyroidism, epilepsy, smoker and alcohol excess. She did have previous ITU admissions with a similar episodes and after ruling out all possible causes of encephalitis; was treated as SREAT (based on EEG, thyroid history and cerebrospinal fluid (CSF) raised protein); with a 5 day course of i.v. methylprednisolone with good response. Her medications included propylthiouracil (PTU) 50mg twice-a-day and levetiracetam 1250 mg twice-a-day.
Investigations on admission
Renal and liver function tests and full blood cout were normal. TSH 0.29, $T_4$ 23, $T_3$ 3.2; normal CSF biochemistrymicrobiology/virology (except CSF protein raised at 0.7 g/l), and normal CT head. Antibodies for other causes of encephalitis were negative. Her Anti-TPO antibodies and TSH-receptor antibodies were both positive.
Treatment
While sedated she continued to be in non-convulsive status. Due to the background of autoimmune thyroiditis/possible SREAT and the above mentioned

investigations, a trial of methylprednisolone 1 g i.v. was given for 5 days. She improved dramatically achieving usual mental status and GCS within 24 h.
Discussion
SREAT or HE is a rare diagnosis but the recognition of this uncommon condition is essential for early diagnosis and treatment.

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## P482

**Hyperthyroidism in an elderly patient intolerant to carbimazole**

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Introduction
We present a case which highlights the complexities of managing hyperthyroidism.
Case report
An 81 year old man complained of irritability, insomnia, diarrhoea, shortness of breath and weight loss. Past medical history includes type II diabetes, atrial fibrillation (AF), hypertension, gallstone pancreatitis requiring cholecystectomy (2005). Clinically, he was in fast AF and right heart failure. On examination he had a tremor; normal sized thyroid. His results showed fully suppressed TSH, Free $T_4$ 70.9 pmol/l (10.6–21.0), Free $T_3$ 20.4 pmol/l (3.2–5.9). His liver functions (LFTs) and full blood count were normal. Thyroid ultrasound excluded goitre and suggested features of thyroiditis. This was consistent with Grave's disease.
Carbimazole was started with some clinical and biochemical improvement (Free $T_4$ 33.5, Free $T_3$ 6.6). Two weeks later patient developed marked prutitis, with deranged LFTs (alkaline phosphatase 274 IU/l (40–120), total billirubin 42 μmol/l (3–22) and ALT 139 IU/l (7–56). Following a normal liver screen, carbimazole induced hepatitis was suspected. Carbimazole was stopped and steroids were commenced. The patient was unable to take cholestyramine. His liver function tests improved mildly and pruritis had resolved, but thyrotoxocosis has deteriorated. One month after omitting carbimazole, the patient had further deteriorated (worsening heart failure and thyrotoxocosis, Free $T_3$ – 21.8, Free $T_4$ – 73.7) and wished to go ahead with thyroidectomy. Potassium iodide was commenced prior to operation; high dose steroids and beta-blockers were optimised. Ten days later the patient underwent successful total thyroidectomy with good recovery and full resolution of symptoms.
Discussion
Hepatotoxicity is a rare complication of thionamide therapy. This case highlights the challenges in management of thyrotoxicosis, particularly in elderly with comorbidities.

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## P483

**Radioiodine therapy in benign thyroid disease**

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Introduction
Radioiodine is a safe and effective treatment for benign thyroid disease. It aims to treat hyperthyroidism and achieve a euthyroid state. Radioiodine is indicated in cases of hyperthyroidism caused by Graves' disease or toxic goitre (solitary toxic adenomas or multi-nodular goitre). In practice there has been concerns about long term safety, worsening of eye disease and weight gain with this treatment.
Aims
To audit outcome, management, complications and follow up of patients treated with radioiodine at a district general hospital, and compare results with the Royal College of Physicians guidelines.
Method
A retrospective review of patients treated with Radioiodine at North Middlesex Hospital between January 2012 and December 2014.
Results
All patients were treated with 555MBq as a fixed dose. We had a total number of 27 patients. Of these, the average age was 49 years (21–88). 23 patients(88%) were on antithyroid therapy prior to radioiodine treatment, 21patients (78%) had their thyroid function checked at ~6 weeks and only 11 patients (40%) had it rechecked at 12 weeks. 23 patients (85%) had ongoing thyroid function monitoring at 1 year. Five patients (18%) failed radioiodine treatment with only one having a further dose within 6 months. Overall outcome 13 patients (48%) became hypothyroid requiring levothyroxine, eight patients (29%) became euthyroid, five patients (18%) remained hyperthyroid and one did not attend follow up. Average time to commence levothyroxine was 4.5 months. 16 patients (59%) gained weight with an average weight gain of 4.9 kg over 12 months. Four patients (14%) had graves eye disease but did not require prophylactic steroids. There was no worsening of eye disease. There were no other complications of Radioiodine treatment recorded.
Conclusion
We conclude that with appropriate patient selection and monitoring, radioiodine treatment for benign thyroid disease is a safe and effective treatment option.

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## P484

**Severe myxoedema complicated by peri-orbital oedema, gum oedema, SIADH and ECG changes**

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A 71 year old lady presented to MAU with a recent onset of generalised weakness and a history of facial and periorbital swelling developing over seven years. She had also noticed hoarsening of her voice, cold intolerance, weight gain, constipation and reduced appetite. Admission blood tests showed marked hyponatremia of 113 mmol/l with severe hypothyroidism (TSH 45.24 mIU/l, $fT_4$ 1.1 pmol/l and TPO antibody 358 IU/ml). Subsequent tests confirmed SIADH (serum osmolality of 236 mOsmol/kg, urinary osmolality of 604 mOSmol/kg and urinary sodium 48 mmol/l). Other pituitary hormones were unremarkable (post-menopausal range of FSH and LH, random cortisol of 706 nmol/l and prolactin 159 mIU/l). Initial ECG showed sinus rhythm, rate 56 bpm with biphasic T wave in V2-5. CXR did not reveal any abnormality.
She was started on oral L-thyroxine 50 μg OD, which was increased to 100 μg OD after 4 days. She did not require liothyronine as she responded clinically well with L-thyroxine and given her abnormal ECG there were concerns about potentially provoking a cardiac event. It should be noted that widespread T wave inversions can be seen in myxoedema. Hydrocortisone was not given as she did not meet the clinical criteria for myxoedema coma.
Hyponatremia slowly responded to fluid restriction and resolved completely a month after starting L-thyroxine. Her TSH improved to 12.4 mIU/l after 4 weeks and normalised within 7 weeks of treatment.
On review two months later, her periorbital oedema is much improved (pre and post treatment photos compared), her voice is less hoarse and her gums have shrunk so much that she has had to get new dentures. T wave changes on ECG have resolved and echocardiogram shows normal ventricular function.

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## P485

**Inadequate TSH – resistance to thyroid hormones: two case reports**

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Resistance to thyroid hormones (RTH) is a genetic syndrome characterized by reduced responsiveness of target tissues to thyroid hormones (TH) and accompanied by normal or slightly high TSH values with high serum concentrations of $fT_4$ or $fT_3$. We present two cases of RTH, one pituitary and one generalised resistance.
Case 1
A 31 y.o. male presented with thyrotoxicosis symptoms and a biochemical profile showing increased serum $fT_4$ 28.02 pmol/l (N 12–22) and $T_3$ 209.7 ng/dl (80–200) but normal TSH (4.33 mUI/l); he had a small goitre, increased SHBG and ATPO was negative. His CT scan revealed a pituitary microadenoma of 7/4 mm, but TRH test documented increasing TSH with 10 mUI/l from baseline to a maximum at 20 min. He was diagnosed with RTH syndrome with hyperthyroidism and started on Methimazole and beta blockers, with good clinical and biochemical response at follow-up.
Case 2
A female aged 57 with a small goiter, obesity, dyslipidaemia, glucose intolerance, infiltrated skin was diagnosed with subclinical hypothyroidism – TSH 7.41 mUI/l, normal $fT_4$, and negative ATPO. She was given 50 μg/day L-thyroxine and had normal thyroid profile for one year, when she presented with very high $fT_4$ values (61.96 pmol/l-N 10.6–19.4) and a TSH in the upper limit, with the same clinical and biochemical features, suggestive of hypothyroidism. TRH test confirmed

RTH syndrome, in a generalized form considering the clinical and biochemical profile. She is well under L-thyroxine treatment in the same dose. The genetic determination was not available in the two cases.
Conclusion
Despite rare, RTH syndrome should be considered when there are mismatches between the clinical and the thyroid functional profile.
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## P486

**Management of hypothyroidism in pregnancy with armour thyroid**
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Armour thyroid is desiccated porcine extract which is a historical treatment for hypothyroidism. It contains both L-thyroxine and liothyronine. Each grain (60 mg) contains 38 μg of L-thyroxine and 9 μg liothyronine. Since the 1960's its use has been superseded by L-thyroxine. Due to limited clinical effectiveness studies, it is not licensed for use in the United Kingdom. We present a case of Armour thyroid use in pregnancy at patient's request following refusal to switch to L-thyroxine.
Case
33 year old lady was referred to our joint antenatal-endocrine clinic at 18 weeks gestation. She was initially diagnosed with primary hypothyroidism in 2010 with strongly positive TPO antibodies 6 weeks following her first pregnancy. She was commenced on 125 μg of L-thyroxine. Treatment with L-thyroxine failed to abate her symptoms and as a result she commenced a self-prescription with three grains of armour thyroid in 2011. She had a subsequent pregnancy which ended with a miscarriage at 6 weeks gestation whilst on armour thyroid. In her third pregnancy she increased her dose of armour thyroid to three and a half grains at booking. Her TSH was suppressed throughout gestation at $<0.02$ mU/l (0.20–4.00 mU/l). Her average free $T_4$ was 12.4 pmol/l (9.0–19.0 pmol/l) and free $T_3$ 6.15 pmol/l (2.5–5.7 pmol/l). The entire course pregnancy course was uneventful with foetal normal anatomy and interval scans. She delivered a live male foetus at 40 weeks with an unremarkable baby check and heel prick screen.
Discussion
Armour thyroid is not recommended for treatment of hypothyroidism in the UK, this is more so in the context of pregnancy. There are safety concerns regarding excessive amounts of $T_3$ relative to $T_4$, which is inconsistent with normal physiology. In pregnancy, the potential risk to both mother and foetus are unknown. Moreover compared to L-thyroxine, it is not regarded as a pure preparation of thyroid hormone. Our patient's TSH was suppressed throughout the pregnancy, despite being fully aware of the potential risks. Although the outcome was uneventful, this was an ethico-legal challenge balancing patient expectation against recommended practice. We do not recommend its use in pregnancy.
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## P487

**A case of thyroid hormone resistance with unusually elevated free thyroxine**
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A 21 year old Caucasian female with no known personal or family history of thyroid disease presented with several years history of anxiety and was found to have abnormal thyroid function tests. Results showed TSH: 1.8 mu/l (0.3–5.5), $T_4$: 73 pmol/l (10–22), $T_3$ 12.3 pmol/l (3.1–6.8). Past medical history included chronic anxiety with no regular medications or nutritional supplements. There was no history of recent iodinated contrast administration, illness or amiodarone use. Clinically she was euthyroid with no goitre.
Assay interference was excluded with dual platform analysis: DELFIA and CENTAUR, which showed good agreement with the local Roche assay with respect of the TSH, $T_4$ and $T_3$. The SHBG was normal which made a thyrotoxic state due to a TSH-secreting pituitary adenoma unlikely. Genetic analysis confirmed a diagnosis of thyroid hormone resistance. Family screening was discussed.
Thyroid hormone resistance is a rare but recognised cause of hyperthyroxinaemia with a non-suppressed TSH. Usually levels of free thyroxine ($FT_4$) are mildly elevated. In our patient, the symptoms of anxiety and weight loss led to treatment with carbimazole due to concerns over the markedly elevated $T_4$ levels. This case illustrates that thyroid hormone resistance should be considered in the differential diagnosis with a non-suppressed TSH and markedly elevated serum free thyroxine. The levels of hyperthyroxinaemia are unusual but should not detract from considering the diagnosis of Resistance to Thyroid Hormone Syndrome.
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## P488

**Hypercalcaemia: look beyond the usual**
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We present a case of a 50 year old patient with sepsis and a significant and symptomatic hypercalcaemia of 3.38 mmol/l with a background of MS. The infection responded to treatment however the hypercalcaemia persisted despite appropriate measures. Further investigation showed a suppressed PTH, normal ACE level, electrophoresis and 25(OH) vitamin D level, but found her to be thyrotoxic with TSH suppressed at $<0.01$, free T4 – 37.2 nmol/l, free T3 – 212 nmol/l. She has no previous history of thyroid disease. Treatment of the thyrotoxicosis with PTU resulted in a state of euthyroidism and also led to the resolution of her hypercalcaemia.
Loss of bone mineral density is a common phenomenon in patients with hyperthyroidism however biochemical hypercalcaemia is uncommon and for the patient to be symptomatic even rarer. When it does present it can hinder the detection of hyperthyroidism.
In our case the symptoms of sepsis and hypercalcaemia meant that the clinical signs of thyrotoxicosis were overlooked. It is important to consider thyroid disease as a cause of hypercalcaemia in patients not responding to usual management. It appears in our case that the thyrotoxicosis, immobility from MS and sepsis induced dehydration combined to give her such a significantly raised calcium.
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## P489

**If it feels like myxoedema coma, then it probably is!**
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Introduction
Myxodedema coma comprises a complex endocrinological emergency whereby there is severe clinical hypothyroid state. It is a life threatening yet a potentially reversible condition that may prove rather difficult to recognise due to the complex symptomatology. Very few articles report the specific therapy for myxoedema coma.
The case
Here we present an elderly lady with previous total thyroidectomy, who despite receiving oral thyroxin tablets, her TSH maintains at a marginally increased level with normal $fT_3$ and $fT_4$. She presented to the hospital with a trivial urinary tract infection and dehydration for which she was treated with antibiotics and fluids, but despite all, she still exhibited profound hypothermia, bradycardia with obtunded consciousness levels and signs of a severe hypothyroid crisis. Only two intravenous pulses of Tri-iodothyronine ($T_3$) saw the patient making a remarkable recovery within hours to being able to take Levothyroxine orally and maintain response.
Conclusion
This case describes success with intravenous $T_3$ monotherapy and demonstrates how myxoedema coma can present even with normal serum thyroid hormones; both phenomena are sparsely reported in the literature. We hope to emphasise both the reversibility and the potential life-threatening consequences of myxoedema coma and highlight issues when it presents among multiple co-morbidities posing a wider differential diagnosis; ultimately to raise the clinicians' suspicion to swiftly tailor the examination and treat promptly perhaps, regardless of the initial serum thyroid function tests.
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### Preoperative parathyroid imaging: a retrospective audit

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Introduction
Preoperative ultrasonography or Sestamibi scanning (or both) of the parathyroid glands could facilitate a focused or minimally invasive surgical approach. In this audit, we wanted to find out whether one form of imaging is superior to the other in correctly identifying a parathyroid adenoma, later confirmed on histology.
Methods
Data were collected from consecutive patients who underwent parathyroidectomy for primary hyperparathyroidism, conducted by a single Head and Neck Surgeon at a Teaching Hospital in England between March 2012 and October 2014. Core Patient Database (CPD) Software was used to review the ultrasound (USS) and Sestamibi scans reports and histology results. We excluded patients with secondary causes for hyperparathyroidism; parathyroid cancers and familial/inherited causes for hyperparathyroidism.
Results
57 patients were identified, average age 59 years, 44 females (77.2%) and 13 males (22.8%). A neck USS and Sestamibi scans were both performed in 52 cases. Both scans correctly identified a parathyroid adenoma (confirmed on histology) in 37 cases (71.2%). Only the Sestamibi scan (not USS) correctly identified the parathyroid adenomas in 6 cases (11.5%). Only the USS correctly identified the parathyroid adenomas in six cases (11.5%). While neither scan was able to identify the parathyroid adenoma in five cases (9.6%). Selecting only one modality for imaging, either USS or Sestamibi scan, prior to parathyroidectomy resulted in no adenoma or the wrong adenoma being identified in 21.1% of cases; this risk was reduced to 9.6% when both scans are performed.
Conclusions
Our data support the use of both neck USS and Sestamibi scans prior to parathyroidectomy.

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